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Alternativas de envasado de pera y melón frescos cortados en atmósfera modificada

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RESUMEN

La producción y consumo de productos frescos cortados ha experimentado un notable incremento en los últimos años debido a los cambios en los hábitos de los consumidores. Sin embargo, el desarrollo de productos frescos cortados a partir de frutas se ha visto limitado por la elevada intensidad respiratoria que presentan la mayoría de frutos como respuesta a los daños mecánicos infligidos durante el procesado. La modificación de la atmósfera de los envases mediante sistemas de envasado adecuados limita la intensidad respiratoria de los productos frescos cortados, alargando significativamente su vida útil. No obstante, las reacciones que llevan al deterioro del producto, tales como deshidratación, pardeamiento, pérdida de textura o procesos de anaerobiosis, se inician a menudo antes de que se alcance la atmósfera de equilibrio en el interior de los envases. Esto es debido a que las películas plásticas disponibles comercialmente no presentan las características adecuadas de permeancia a los gases y al agua requeridas para productos frescos cortados con elevada actividad respiratoria.

En base a este planteamiento, y con el fin de mantener las propiedades organolépticas y de seguridad requeridas, en primer lugar se establecieron las condiciones más adecuadas de procesado de melón y pera frescos cortados, evaluando el efecto de distintos antioxidantes en el control del pardeamiento enzimático de pera cortada, así como el estado óptimo de madurez de ambas frutas para su procesado. Seguidamente, se procedió al estudio del efecto de distintas alternativas de envasado en la actividad fisiológica, estabilidad microbiológica, parámetros físico-químicos y propiedades antioxidantes del melón y pera frescos cortados. Se estudió el uso de concentraciones altas de O_2 como alternativa al envasado bajo concentraciones bajas de O_2, además de la aplicación de recubrimientos comestibles.

Para el procesado de melón ‘Piel de Sapo’, se seleccionó un estado de madurez intermedio (13,5 ºBrix) junto con la inmersión en una disolución de cloruro cálcico al 0,5% p/v. De la misma forma, las pérdidas de calidad en pera fresca cortada se minimizaron procesando los frutos en un estado de madurez intermedio, con una firmeza de aproximadamente 43 N, y empleando una disolución de N-acetilcisteína y glutatión al 0,75% p/v.

El efecto inhibidor sobre los microorganismos de concentraciones bajas de O_2 y altas de CO_2 fue similar al observado bajo concentraciones altas de O_2. Sin embargo, el uso de concentraciones altas de O_2 limitó el crecimiento de la flora predominante en la fruta cortada. El uso de concentraciones altas de O_2 indujo un aumento de la actividad respiratoria de la fruta cortada, en comparación con concentraciones bajas de O_2 y altas de CO_2, aunque retrasó la iniciación del metabolismo anaeróbico del fruto. Además, la aplicación de N-acetilcisteína (0,75% p/v) y glutatión (0,75% p/v) en pera inhibió de manera importante la elevada producción de etileno bajo concentraciones altas de O_2.

Las concentraciones altas de O_2 disminuyen el potencial antioxidante de melón y pera frescos cortados aunque estas pérdidas pueden verse compensadas gracias a la reducción de los fenómenos de estrés oxidativo, que se aceleran bajo condiciones de anoxia. Además, la adición de N-acetilcisteína (0,75% p/v) y glutatión (0,75% p/v) favorecieron la síntesis de compuestos fenólicos en pera fresca cortada en condiciones de anoxia de los tejidos, a través de la activación del metabolismo fenilpropanoide. La actividad antioxidante de melón y pera frescos cortados se atribuyó más a su contenido fenólico que al de vitamina C.

Los recubrimientos comestibles a base de pectina o alginato mejoraron la resistencia al vapor de agua de los trozos de fruta, además de disminuir la acumulación de compuestos fenólicos en melón y mantener sus atributos de calidad. En pera fresca cortada, la incorporación de N-acetilcisteína (0,75% p/v) y glutatión (0,75% p/v) en los recubrimientos fue eficaz para el control del pardeamiento y mantenimiento del potencial antioxidante, observándose una disminución de la producción de etileno y del crecimiento microbiano.

RESUM

La producció i consum de productes frescos tallats ha experimentat un creixement notable en els últims anys degut als canvis en l'estil de vida dels consumidors. No obstant, el desenvolupament de productes frescos tallas a partir de fruites s'ha vist limitat per l'elevada intensitat respiratòria que presenten la majoria de fruits com a resposta als danys mecànics produïts durant el processat. La modificació de l'atmosfera dels envasos mitjançant uns sistemes d'envasat adequats limita la intensitat respiratòria dels productes frescos tallats, allargant significativament la seva vida útil. No obstant, les reaccions que donen lloc al deteriorament del producte, com la deshidratació, enfosquiment, pèrdua de textura o processos d'anaerobiosi, s'inicien sovint abans que s'aconsegueixi una atmosfera d'equilibri en l'interior dels envasos. Això es degut a que els materials plàstics disponibles comercialment no presenten les característiques adequades de permeància als gasos i a l'aigua, necessàries per a la conservació de fruita fresca tallada amb elevada activitat respiratòria.

En base a aquest plantejament, i amb l'objectiu de mantenir les propietats organolèptiques i de seguretat necessàries, en primer lloc es van establir les condicions més adequades de processat del meló i pera frescos tallats, avaluant l'efecte de diferents antioxidants pel control de l'enfosquiment en pera tallada, així com l'estat òptim de maduresa pel processat d'ambdues fruites. Seguidament, es va procedir a l'estudi de l'efecte de diferents alternatives d'envasat en l'activitat fisiològica, estabilitat microbiològica, paràmetres fisico-químics i propietats antioxidants del meló i pera frescos tallats. Es va estudiar la utilització de concentracions altes d'O_2 com a alternativa a l'envasat en concentracions baixes d'O_2 i altes de CO_2, a més de l'aplicació de recobriments comestibles.

Pel processat de meló 'Piel de Sapo', es va seleccionar un estat de maduresa intermedi (13,5 ºBrix) junt amb la immersió de la fruita tallada en una solució de clorur de calci al 0,5% p/v. De la mateixa manera, les pèrdues de qualitat en pera fresca tallada es van minimitzar processant els fruits en un estat de maduresa intermedi, amb una fermesa d'aproximadament 43 N, i utilitzant una solució de N-acetilcisteïna i glutatió al 0,75% p/v.

L'efecte inhibidor sobre els microorganismes de les concentracions baixes d'O_2 i altes de CO_2 va ser similar a l'observat sota concentracions altes d'O_2. No obstant, la utilització de concentracions altes d'O_2 va limitar el creixement de la microflora predominant en la fruita tallada. Les concentracions altes d'O_2 provocaren un augment de l'activitat respiratòria de la fruita tallada, en comparació amb concentracions baixes d'O_2 i altes de CO_2, encara que es va aconseguir retardar la iniciació del metabolisme anaeròbic dels fruits. A més, l'aplicació de N-acetilcisteïna i glutatió al 0,75% p/v en pera va inhibir de manera important la elevada producció d'etilè en concentracions altes d'O_2.

Les concentracions altes d'O_2 disminuïren el potencial antioxidant de meló i pera frescos tallats encara que aquestes pèrdues es van veure compensades per la reducció dels fenòmens d'estrès oxidatiu, que s'acceleraren en condicions d'anoxia. A més, l'addició de N-acetilcisteïna i glutatió al 0,75% p/v va afavorir la síntesi de compostos fenòlics en pera fresca tallada en absència d'O_2 i concentracions altes de CO_2, mitjançant l'activació del metabolisme fenilpropanoide. L'activitat antioxidant de meló i pera frescos tallats es va atribuir principalment al seu contingut fenòlic més que al de vitamina C.

El recobriments comestibles a base de pectina o alginat van millorar la resistència al vapor d'aigua dels trossos de fruita, a més de disminuir l'acumulació de compostos fenòlics en meló i mantenir els seus atributs de qualitat. En pera fresca tallada, la incorporació de N-acetilcisteïna i glutatió al 0,75% p/v en els recobriments va ser efectiva en el control de l'enfosquiment i manteniment del potencial antioxidant, observant-se una disminució de la producció d'etilè i del creixement microbià.

ABSTRACT

The increase in popularity of fresh-cut fruits and vegetables is due to a response of a consumer's trend towards fresh-like high-quality products. However, the commercial development of fresh-cut fruits is getting difficult as a result of the reduced shelf-life of these commodities. Modified atmosphere packaging can reduce tissue respiration as well as provide a moisture barrier to the product. However, degradation processes such as dehydration, browning, softening or fermentative reactions often occur before the desired equilibrium of gas concentrations in the packages is reached. Plastic materials with O_2 transmission rates that are high enough to prevent excessive modification of the package headspace atmosphere are still under development.

Firstly, the objective of this research was to determine the optimal conditions for processing of fresh-cut melon and pears, evaluating the effect of different antibrowning agents on enzymatic browning inhibition of fresh-cut pears, as well as the optimal ripeness state of fruits prior to processing. Secondly, the effects of different packaging systems on physiological activity, microbiological stability, physicochemical attributes and antioxidant properties of fresh-cut melon and pears were investigated. The use of high O_2 concentrations was studied as a packaging alternative to low O_2 and high CO_2 concentrations. The investigation concluded with the application of polysaccharide-based edible coatings as a new preservation method for fresh-cut fruits.

The selected processing conditions for fresh-cut melon included a dip of calcium chloride at 0.5% w/v of fruit pieces at an intermediate ripeness state (13.5 ºBrix). For fresh-cut pears, fruit processing in an intermediate ripeness state, with firmness values of 43 N, and the use of a dipping treatment consisting of N-acetylcysteine and glutathione at 0.75% w/v resulted in the best maintenance of fruit quality.

The inhibitory effect on microbial growth of low O_2 and high CO_2 concentrations was similar to that observed with high O_2 concentrations. However, the use of high O_2 atmospheres reduced the growth of the predominant microflora on fresh-cut fruits. High O_2 concentrations increased the physiological activity of fresh-cut fruits compared with low O_2 and high CO_2 concentrations, although it delayed the initiation of anaerobic metabolism in the fruit tissues. In addition, the

application of N-acetylcysteine and glutathione at 0.75% w/v on fresh-cut pears substantially inhibited the high ethylene production under high O_2 atmospheres.

High O_2 concentrations decreased the antioxidant potential of fresh-cut melon and pears, although these losses may be compensated through the reduction of oxidative stress processes, which are specially induced under anoxia conditions. Moreover, the addition of N-acetylcysteine and glutathione at 0.75% w/v accelerated the formation of phenolic compounds on fresh-cut pears under anoxia conditions through the activation of the phenylpropanoid metabolism. Antioxidant activity of fresh-cut melon and pears was related to their phenolic content rather than to vitamin C.

Polysaccharide-based edible coatings based on pectin or alginate increased the water vapour resistance of fruit pieces. Moreover, the use of coatings decreased phenolic compounds accumulation and maintained quality attributes of fresh-cut melon. The incorporation of N-acetylcysteine and glutathione at 0.75% w/v on edible coatings for fresh-cut pears was effective in controlling enzymatic browning and in maintaining antioxidant potential. A decrease in ethylene production and microbial growth was also observed.

ÍNDICE

1.Introducción

1. Introducción

Las frutas son alimentos indispensables en nuestra alimentación. Aportan poca energía y son ricas en fibra, vitaminas y minerales, además de poseer fitonutrientes que ofrecen protección frente a enfermedades degenerativas, dando lugar a una menor mortalidad y a una mayor expectativa y calidad de vida. No obstante, el consumo de frutas y hortalizas en España está estable aunque por debajo de las recomendaciones de las guías alimentarias, a pesar de ser uno de los países con el mayor consumo en Europa. Por tanto, es fundamental conseguir aumentar la ingesta de fruta fresca, ya que es la mejor forma de aprovechar todas sus virtudes y propiedades nutritivas. En este sentido, el movimiento internacional *5 al día* promociona el consumo de frutas y hortalizas en el mundo y está presente en más de 40 países de los 5 continentes. Su nombre se basa en la ración mínima de consumo diario de frutas y hortalizas frescas recomendada por la comunidad científica y médica en una dieta saludable.

1.1. El sector de las frutas y hortalizas frescas cortadas.

Los cambios en los modelos de consumo alimentario están ejerciendo un efecto determinante sobre las áreas de innovación tecnológica y, especialmente, en la producción de alimentos que conserven al máximo las características organolépticas del producto fresco (Schlimme, 1995). Una respuesta a la demanda de este tipo de productos son las frutas y hortalizas frescas cortadas, que abren un nuevo mercado a aquellas personas que, por falta de tiempo o por simple comodidad no tienden a consumir productos en fresco. La introducción en los mercados de los productos frescos cortados es una forma de incrementar el consumo de frutas y hortalizas hasta los niveles recomendables en una dieta saludable.

1.1.1. Producción y consumo

Según datos de AFHORLA (la Asociación Española de Frutas y Hortalizas Lavadas y Listas para su empleo), la comercialización de frutas y hortalizas frescas cortadas llegó a 53.465 toneladas en

2006, un 20% más que en el año 2005. Esta cantidad aumenta continuamente, pero el consumo de cada español aún no llega a 1,5 kilos por persona y año. Muy lejos queda el consumo británico, líder absoluto en Europa, con más de 12 kilos por habitante, el consumo francés que llega a 6 kilos, el holandés, belga o alemán que supera los 3 kilos per capita al año. No obstante, un ciudadano norteamericano llega a consumir más de 30 kilos de frutas y hortalizas frescas cortadas al año.

Según la Revista Mercados (2007), la evolución del mercado norteamericano se debe al desarrollo del espacio expositivo, a la mayor variedad de productos en los supermercados y al aumento de la vida media de los productos de hasta 10-16 días para ensaladas frescas. La realidad norteamericana contrasta con la española o italiana donde la vida media de una ensalada fresca cortada gira en torno a la semana. Esta diferencia de tiempo se debería a la diversa dotación tecnológica y logística, en especial para el mantenimiento de la cadena de frío.

El mercado de frutas frescas cortadas está consolidado en USA, mientras que en países como España su desarrollo es aún muy lento. Según los últimos datos, la comercialización española de frutas y hortalizas cortadas hasta agosto de 2007 aumentó un 13,6% más que en el mismo período de 2006 aunque el 99% de la cantidad comercializada por AFHORLA correspondió a hortalizas (Infoagro, 2007). Se trata de una gama con una complejidad mayor que las hortalizas y al ser España un gran productor de fruta, se pueden encontrar en el mercado frutas frescas de calidad a precios asequibles. La comercialización de la fruta fresca cortada en los mercados requiere el desarrollo y combinación inteligente de tecnologías adecuadas para el procesado que permitan prolongar la vida útil del producto manteniendo las características sensoriales y organolépticas del producto fresco original. No obstante, la clave principal para el éxito de las frutas frescas cortadas es la utilización de materias primas de alta calidad y el mantenimiento de la cadena de frío durante todo el proceso de elaboración, distribución y comercialización del producto (Martín-Belloso et al., 2007).

1.1.2. Aspectos legislativos

En la actualidad no existen legislaciones específicas para productos frescos cortados. En España, este tipo de productos están regulados a través del Real Decreto 3484/2000 por el que se establecen las normas de higiene para la elaboración, distribución y comercio de comidas preparadas. Esta regulación y la mayoría de las existentes en los Estados Unidos y la Unión Europea se refieren exclusivamente a aspectos de seguridad y calidad microbiológica. La legislación española fija un nivel máximo de contaminación de 5×10^7 ufc/g a la fecha de caducidad y prohíbe la presencia de microorganismos patógenos como la Salmonella, mientras que los recuentos de *Escherichia coli* o *Listeria monocytogenes* están restringidos a 1×10^2 ufc/g. Los criterios microbiológicos en materia alimentaria de la Unión Europea (Official Journal of the European Union, 2005) establecen un límite de 1×10^2 ufc/g para *E. coli* como indicativo de la aceptabilidad del proceso de elaboración. Este criterio se aplica independientemente de otras regulaciones específicas referentes a otros microorganismos, y sirve a los fabricantes como herramienta para decidir si un producto está listo para ser comercializado.

La manipulación de los productos frescos cortados puede conllevar riesgos microbiológicos. El sistema de análisis de peligros y puntos de control crítico (APPCC) garantiza la seguridad microbiológica de los alimentos y está enfocado al control no sólo del producto final sino de todo el proceso de elaboración. Su aplicación en productos alimenticios es obligatoria según el reglamento (CE) núm. 852/2004 por la cual se hace expresa la obligación de desarrollar y aplicar sistemas de autocontrol de productos alimenticios y, además, incorpora la posibilidad de desarrollar las guías de prácticas correctas de higiene. Las medidas adoptadas por la UE en materia de seguridad alimentaria y productos alimenticios remiten frecuentemente al Codex Alimentarius para justificar sus prescripciones. En el caso particular de los productos frescos cortados, la Comisión del Codex Alimentarius (CCA) desarrolló un documento titulado "Código de prácticas de higiene para las frutas y hortalizas frescas" (FAO, 2003a) con un anexo sobre frutas y hortalizas frescas precortadas listas para el consumo que pretende la implementación de buenas prácticas de fabricación para ayudar al control de riesgos microbiológicos, físicos, y químicos asociados al procesado de frutas y hortalizas frescas cortadas. El anexo se refiere de manera extensa a los principios generales de higiene de alimentos (FAO, 2003b) pero con especial énfasis en algunos aspectos de control referentes a las instalaciones, programas de capacitación de personal así como

al mantenimiento de documentación y registros adecuados durante el período de fabricación y almacenamiento del producto. La *U.S. Food and Drug Administration* (FDA) elaboró un documento similar, "Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial Hazards on Fresh and Fresh-cut Produce", identificando los riesgos potenciales y revisando los métodos de intervención para reducir los riesgos microbiológicos en productos frescos cortados (FDA 2001).

1.2. Elaboración de frutas frescas cortadas

Los productos frescos cortados son un importante segmento del mercado en rápido y constante crecimiento, que atrae el interés del sector alimentario, incluyendo la industria, distribución y restauración (Wiley, 1994). La definición más amplia para estos productos es la dada por Wiley (1994), el cual define a las frutas y hortalizas mínimamente procesadas como aquellas obtenidas mediante la aplicación de una o varias operaciones unitarias de preparación, tales como pelado, cortado, reducción de tamaño y envasado, incluyendo tratamientos químicos, cuya combinación puede tener un efecto sinérgico. En la Figura 1 se observa el esquema general de preparación de frutas frescas cortadas (Martín-Belloso y Rojas-Graü, 2004). No obstante, según el tipo de fruta que se elabore, se deberá aplicar un tratamiento específico para las distintas operaciones y métodos de conservación (Wiley, 1997).

Cada etapa del proceso de elaboración juega un papel importante en el control de los mecanismos de alteración de las frutas y hortalizas frescas, tales como pérdida de agua, pardeamiento enzimático, ablandamiento por rotura de tejidos, aumento de la tasa respiratoria con la consecuente producción de etileno, presencia de sabores y olores desagradables, así como posibles alteraciones microbianas. Estos fenómenos fisiológicos son responsables de cambios bioquímicos que conllevan la degradación de las propiedades sensoriales de la fruta recién cortada, aunque son numerosos los factores que influyen en los procesos de deterioro. Factores como variedad, estado de madurez en la recolección, manipulación postcosecha, acondicionamiento de la materia prima, así como las condiciones de almacenamiento del producto terminado, intervienen directamente en la calidad final de los productos frescos cortados (Martín-Belloso et al., 2006).

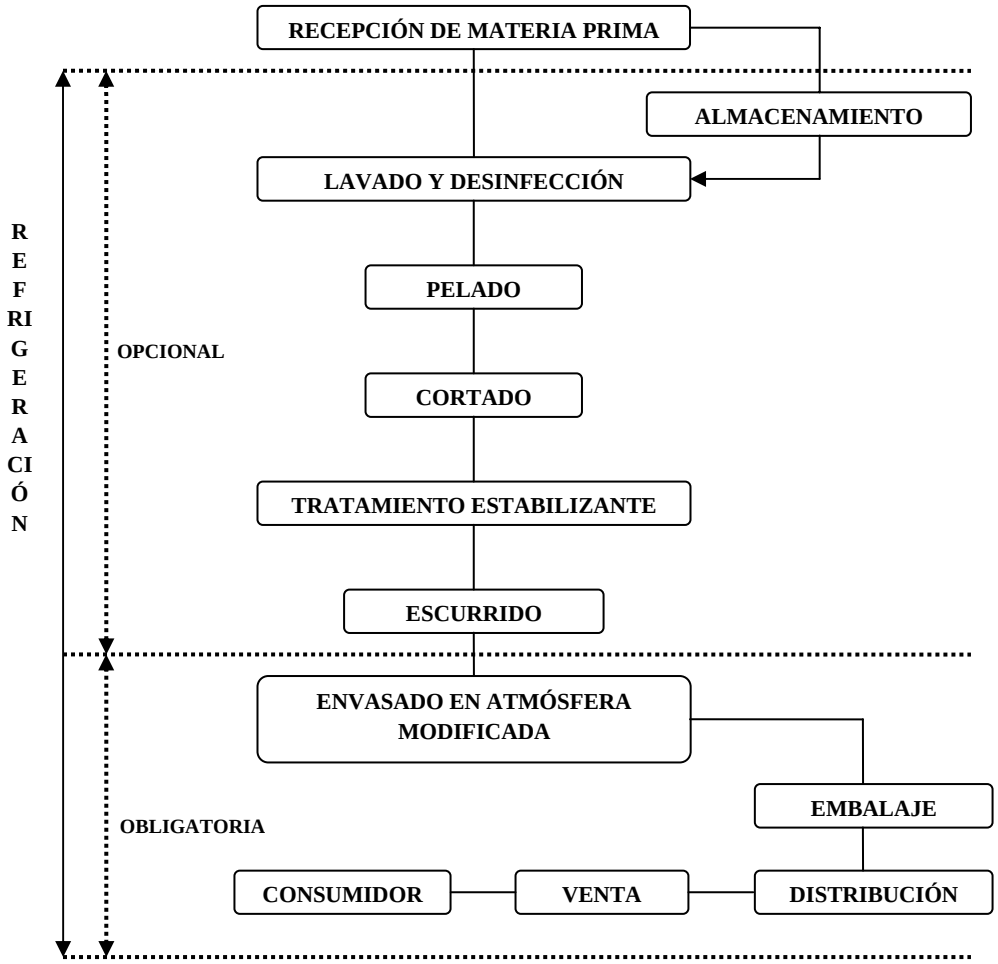


Figura 1. Esquema general de preparación de fruta fresca cortada

La elaboración de productos vegetales frescos cortados comienza por una buena selección de la materia prima. La misma debe recolectarse cuidadosamente, en óptimas condiciones higiénicas y con el adecuado grado de madurez. Este último es uno de los factores más importantes a tener en cuenta tanto en el momento de la recolección como del procesado, ya que tiene una gran influencia sobre la calidad del producto final. En peras, se ha visto que un estado de madurez avanzado del fruto favoreció la pérdida de textura y el pardeamiento enzimático del producto cortado (Soliva-Fortuny et al., 2004a). En cambio, un estado intermedio de madurez podría minimizar y retardar el desarrollo de los procesos fermentativos que limitan la vida útil de manzana fresca cortada, además de mantener las características sensoriales propias de la fruta (Soliva-Fortuny et al.,

2002a). La síntesis de etileno de frutos climatéricos responde al daño mecánico que sufren los tejidos durante el procesado. El grado de madurez de la materia prima tiene una gran importancia en esta producción de etileno. En la Figura 2 se observa que la producción de etileno de peras frescas cortadas parcialmente maduras fue dos veces superior que la de frutos de madurez avanzada (Soliva-Fortuny et al., 2004a).

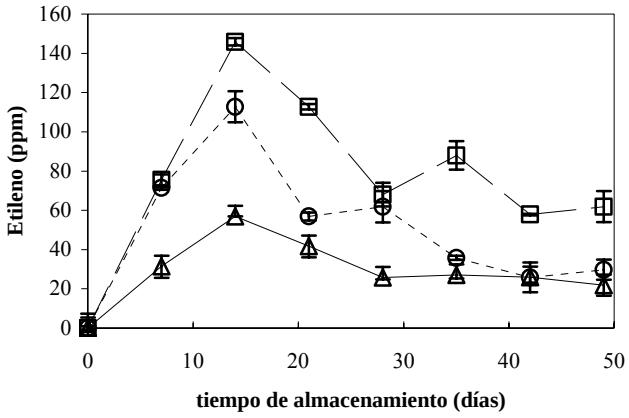


Figura 2. Concentraciones de etileno en el espacio de cabeza de pera fresca cortada procesada en diferentes estados de madurez: precoz (o), intermedio (□), avanzado (△)

La aplicación de técnicas que permitan controlar los factores alterantes en frutas y hortalizas frescas cortadas es actualmente el objetivo principal de muchas investigaciones en el campo de la ciencia y tecnología de los alimentos. En este sentido, deben aplicarse técnicas de conservación, que combinadas o no, puedan mantener o mejorar las características originales del producto, alargando su vida útil sin que se pierdan las características sensoriales y nutricionales, asegurando además su estabilidad microbiológica. Dentro de las más usadas se encuentra el uso de agentes desinfectantes, el envasado en atmósfera modificada que permita controlar los procesos respiratorios típicos de los tejidos vivos, el empleo de agentes antioxidantes para evitar el oscurecimiento superficial, la aplicación de compuestos antimicrobianos para prevenir la proliferación de microorganismos causantes de alteraciones, y más recientemente el empleo de películas comestibles (Martín-Belloso et al., 2007).

La elaboración, distribución y comercio de productos frescos cortados requiere el mantenimiento ininterrumpido de la cadena de refrigeración, desde la producción hasta el punto de venta. Un adecuado control a través de la cadena de frío reduce la proliferación microbiana aunque los microorganismos psicrótrofos podrían crecer lentamente e incluso producir la alteración y la consiguiente reducción de la vida útil del producto. De este modo, para prevenir el crecimiento de microorganismos y el desarrollo de alteraciones microbiológicas en el producto, y así alargar la vida útil del producto final, se debe llevar a cabo un control de la microflora mediante una higiene muy estricta durante la manipulación de la fruta y el almacenamiento bajo atmósfera modificada en refrigeración (Martín-Belloso y Rojas-Graü, 2004). El lavado con agua no suele ser suficiente para evitar la contaminación del producto y es necesaria la desinfección de la materia prima para rebajar la carga microbiana presente en la superficie del tejido vegetal. El cloro se utiliza ampliamente tanto en fruta entera como en fruta fresca cortada a un concentración no superior de 200 ppm (Gorny et al., 2000; Dong et al., 2000). No obstante, la efectividad del cloro puede verse influida por el tipo de producto y la diversidad de microorganismos que se encuentran presentes en la superficie de la fruta (Beuchat, 2000). Además, el cloro puede representar un riesgo para el consumidor ya que se descompone con la consiguiente formación de substancias tóxicas. No obstante, se están estudiado nuevos desinfectantes como el H_2O_2, dióxido de cloro y ácidos para la destrucción de microorganismos. La vida útil de melón se alargó en 4-5 días con un tratamiento de H_2O_2 comparado con un tratamiento con dióxido de cloro (Sapers y Simmons, 1998). Este tratamiento fue también efectivo en la descontaminación de ciruelas y uvas de mesa (Sapers, 1996) y su uso combinado con otros desinfectantes comerciales inactivó *E. coli* (Sapers et al., 1999). Ukuku et al. (2005) consiguieron una inactivación total de *L. monocytogenes* y al menos reducciones de 4 log ufc/mL de *E. coli* en melón tratado con una solución de 1% H_2O_2 + 25 μg/mL nisina + 1% lactato de sodio + 0.5% ácido cítrico. En cambio, un tratamiento con dióxido de cloro, aplicado en una concentración 16 veces superior a la recomendada (80 μg/mL) solo redujo en 2.5 log ufc/mL los recuentos de *E. coli* O157:H7. El lavado de manzanas con una solución de 5% de ácido acético durante 2 min también fue efectivo en la inactivación de *E. coli* O157:H7. La luz ultravioleta representa también una nueva alternativa a los desinfectantes ya que es efectiva en la destrucción microbiana y además, no deja residuos en el producto. Un tratamiento con UV-C de 24 mW/cm^2 consiguió un reducción de 3,3 log ufc/mL de *E. coli* O157:H7 inoculado en manzana (Yaun et al., 2004).

1.3. Cambios microbiológicos, físico-químicos y sensoriales de fruta fresca cortada

Cada etapa del proceso de elaboración juega un papel importante en los mecanismos de alteración del producto final.

1.3.1. Cambios microbiológicos

Debido al pH bajo de la mayoría de frutas, la flora predominante consiste en mohos y levaduras, responsables de la producción de una amplia variedad de enzimas que intervienen en los procesos degradativos de los tejidos vegetales como son las enzimas pécticas. La microflora predominante en frutas de pepita son mohos tales como *Penicillium spp*., *Rhizopus spp*., *Alternaria spp*., y *Trichoderma spp*., y levaduras tales como *Metschnikowia spp*., *Debaryomyces spp*., y *Candida spp*. (Nguyen-the y Carlin, 1994), pero las operaciones de procesado y las condiciones de envasado pueden transformar la flora inicial microbiana. Durante el almacenamiento de manzanas 'Granny Smith' frescas cortadas se observó una sustitución de los mohos presentes inicialmente por bacterias Gram-positivas (Lanciotti et al., 1999).

Las fuentes de contaminación más importantes de fruta entera son las heces animales, y el uso de agua contaminada (Beuchat, 1996). Además, las operaciones mecánicas pueden destruir la barrera natural de protección del vegetal, favoreciendo la liberación hacia el exterior de substancias ricas en nutrientes y dándose así las condiciones idóneas para el crecimiento microbiano. Durante las operaciones de procesado de frutas puede ocurrir el paso de microorganismos patógenos, si están presentes, desde la superficie de la fruta intacta, al tejido interno. Aunque el riesgo de crecimiento de bacterias patógenas es poco importante en fruta de pH bajo (pH < 4), algunas frutas menos ácidas como melones (pH entre 6,2 y 6,7), sandías (pH 5.8 y 6.0) y papayas (pH 4.5 y 6.0) pueden permitir el crecimiento de patógenos (Zhuang et al., 2003). Así, se ha visto una multiplicación de *Salmonella* spp. y *Shigella* spp. en melón cortado a 23 ºC (Fernández-Escartin et al., 1989; Golden et al., 1993). *Escherichia coli* O157:H7 se multiplicó en cubos de melón y sandía a 25 ºC y alta humedad (Del Rosario y Beuchat, 1995). Incluso *Campylobacter jejuni* sobrevivió en sandía y papaya cortada el tiempo suficiente para representar un riesgo al consumidor (Castillo y Escartin, 1994). El tratamiento con luz ultravioleta para la inactivación de microorganismos vegetativos en

melón fresco cortado inoculado con *Clostridium. botulinum* y almacenado bajo una atmósfera pasiva de envasado, favoreció el deterioro microbiano y la formación de toxina botulínica (Larson y Johnson, 1999).

1.3.2. Cambios físico-químicos

El pardeamiento enzimático es una de las principales alteraciones en fruta fresca cortada, en la que los compuestos fenólicos de la fruta son oxidados hasta quinonas mediante reacciones catalizadas por enzimas denominadas genéricamente polifenoloxidasas (PPO). Durante el procesado mínimo de fruta se daña la integridad del tejido vegetal y se produce un incremento de las actividades metabólicas y una descompartimentación de enzimas y substratos que al entrar en contacto reaccionan formando compuestos activos. Estos últimos, a su vez experimentan procesos de polimerización que dan lugar a compuestos coloreados denominados melaninas, produciendo el pardeamiento superficial del producto y disminuyendo así su calidad visual. El grado de pardeamiento que sufren las frutas puede depender de la concentración y tipo de compuestos fenólicos presentes en los frutos, actividad de la PPO, estado de madurez, presencia de oxígeno (O_2) y compartimentación de los enzimas y sustratos (Nicoli et al., 1994; Rocha et al., 1998; Garcia y Barrett, 2002). De acuerdo con Soliva-Fortuny et al. (2002a), la desintegración del cloroplasto en manzanas maduras causa la solubilización de la PPO, incrementando así el pardeamiento de los tejidos. Se ha demostrado que la susceptibilidad al pardeamiento en peras de diferentes variedades es similar, aunque en frutos de madurez avanzada se observó una disminución significativa del contenido de compuestos fenólicos (Amiot et al., 1995). Además, se relacionaron actividades reducidas de la PPO con un menor pardeamiento de peras 'Conference' frescas cortadas (Soliva-Fortuny et al., 2002b).

Otro cambio muy evidente es la pérdida de firmeza debida principalmente a la acción de enzimas proteolíticas y pectolíticas sobre los componentes de la pared celular. Durante las operaciones de procesado y, especialmente, tras el cortado, los tejidos vegetales sufren una pérdida de firmeza debido a la hidrólisis enzimática de la sustancias pépticas que forman parte de la pared celular (Varoquaux, 1991). Enzimas como la pectino-metil-esterasa (PME) y poligalacturonasa (PG)

juegan un papel importante en el ablandamiento de los tejidos. La PME produce la hidrólisis de la pectina, formando metanol y ácido péctico, el cual puede ser despolimerizado por la PG, desestabilizando las estructuras celulares (Alandes et al., 2006; Quiles et al., 2007). No obstante, la actividad enzimática de los tejidos cortados puede depender del estado fisiológico del fruto entero. La maduración de peras de la variedad 'Flor de Invierno', previamente almacenadas en refrigeración durante 7 meses, conllevó un incremento de la actividad pectinmetilesterasa (PME) durante la segunda semana de almacenamiento a 20 ºC. Este incremento de la actividad enzimática causó daño microestructural a diferentes componentes estructurales de la célula. Se observó una disminución de la adhesión entre células y la aparición de exudados que inundaron los espacios intercelulares debido a la permeabilización de las membranas (Varela et al., 2007a). El estrés que sufren los tejidos durante las operaciones de pelado y cortado podría aumentar la permeabilización de las membranas y así el intercambio celular de fluidos con la consecuente inundación de los espacios intercelulares (Soliva-Fortuny et al., 2002c). Este daño celular provoca una descompartimentalización de enzimas y sustratos, causando un aumento de la actividad enzimática y pérdida de fluidos. En pera fresca cortada, se observó una importante pérdida de agua debido a las operaciones de pelado y cortado, y en consecuencia una deshidratación del producto (Gorny et al., 2000).

1.3.3. Cambios sensoriales

La pérdida de calidad sensorial de muchos alimentos se produce antes que el deterioro microbiológico (Salvador et al., 2007). Por tanto, en estos casos, los atributos sensoriales del producto limitan su comercialización. Según Varela et al. (2007a), la aceptabilidad de pera 'Flor de Invierno' disminuye debido a cambios en la textura, más blando, menos crujiente y jugoso, y una disminución de la relación entre acidez / azúcares durante su maduración. Además, en peras y manzanas, se produce un rápido pardeamiento de las superficies cortadas disminuyendo su calidad visual (Rocha et al., 1998; Gorny et al., 2000; 2002). En general, estudios sensoriales realizados en diversas frutas muestran cómo el producto no cumple los requisitos de calidad exigibles para su comercialización tras una semana de almacenamiento. La vida útil de melón 'Cantaloupe' fresco cortado se limitó a 6 días debido al ablandamiento de los tejidos, desarrollo fúngico y olores

desagradables (Chonhenchob et al., 2007). No obstante, se deben considerar aspectos químicos y nutricionales, ya que éstos tienen un efecto directo en los atributos sensoriales del producto.

La calidad sensorial de la fruta viene determinada de manera decisiva por su dulzura. En melón fresco cortado, se ha visto una elevada correlación entre este atributo y el contenido en sólidos solubles (r = 0.61) (Lester, 2006). Los contenidos de glucosa (16,7 g/kg) y fructosa (16,4 g/kg) son mayoritarios en melones de madurez temprana de la variedad 'Piel de Sapo'. En cambio, su contenido disminuye ligeramente durante la maduración, mientras aumenta considerablemente el contenido de sacarosa de 1g/kg a 95,4 g/kg (Villanueva et al., 2004). En melón 'Cantaloupe' fresco cortado, los contenidos de glucosa, fructosa y sacarosa disminuyeron significativamente durante el almacenamiento a 20 ºC, mientras que no se observaron cambios significativos a 4 ºC. Estudios en pera (Senesi et al., 1999) y manzana frescas cortadas demuestran que el almacenamiento refrigerado del producto no afecta al contenido de azúcares (Bett et al., 2001; Rocha et al., 1998). El contenido en ácidos orgánicos de fruta fresca cortada se relaciona con la acidez del producto. El contenido en ácido málico de melón 'Cantaloupe' fresco cortado se mantuvo durante 5 días de conservación a 4 ºC. En cambio, el ácido málico disminuyó considerablemente a 20 ºC siendo el ácido láctico el predominante después de 2 días, debido a la fermentación malo-láctica llevada a cabo por bacterias lácticas (Lamikanra et al., 2000). Durante periodos de conservación prolongados de 5 semanas, se observó una disminución de los niveles iniciales de 3.5 mg de ácido málico/g en manzana fresca cortada independientemente de la temperatura, 5 ºC y 10 ºC (Buta et al., 1999). La calidad sensorial de las frutas frescas cortadas también depende en gran medida de los compuestos volátiles presentes en el fruto. Estudios realizados por Bett et al. (2001) en manzana 'Gala' fresca cortada demostraron que los cambios aromáticos tras 14 días de almacenamiento no eran significativos, no desarrollándose aromas desagradables.

1.3.4. Cambios en el contenido de compuestos antioxidantes

En los últimos años, los compuestos bioactivos presentes en las frutas y vegetales han adquirido importancia debido a su efecto protector contra enfermedades cardiovasculares y cáncer. Su efecto beneficioso se ha atribuido a su actividad antioxidante, cuyo mecanismo de acción es inhibir la iniciación o impedir la propagación de las reacciones de oxidación, evitándose así el daño de oxidativo (Shi et al., 2001). De este modo, el contenido de compuestos fenólicos en la fruta fresca cortada tiene importancia por su contribución a las propiedades antioxidantes de la misma además de por la participación de éstos como substratos del pardeamiento enzimático. El pelado y cortado de la fruta provoca una descompartimentación de los enzimas y sustratos favoreciendo su interacción. Compuestos fenólicos como el ácido clorogénico, ácido cafeico, catequina y epicatequina son fácilmente oxidados por la PPO provocando pardeamiento en los tejidos. En cambio, otros compuestos fenólicos naturales de la fruta como los ácidos cinámico, p-cumárico, ferúlico y sináptico no actúan como sustratos de la PPO e incluso se ha visto que pueden inhibir su actividad en manzana, pera, cereza, pulpa de plátano y uvas (Macheix et al., 1990).

La degradación de compuestos antioxidantes en fruta fresca cortada se puede ver favorecida durante el procesado debido a la exposición al oxígeno (O_2) y la luz (Gil et al., 2002). En manzana fresca cortada, la degradación de compuestos fenólicos durante los 2 primeros días de almacenamiento fue debido a la rápida oxidación de estos compuestos presentes en la superficie del tejido y que se encuentran en contacto directo con el O_2 (Gil et al., 1998). En cambio, la restricción del O_2 en los envases de tomate fresco cortado de diversas variedades contribuyó al mantenimiento del contenido de vitamina C y compuestos fenólicos (Odriozola-Serrano et al., 2007).

El procesado mínimo (pelado y cortado) de la fruta puede afectar al contenido, composición, actividad y biodisponibilidad de los antioxidantes (Robles-Sánchez et al., 2007). Su efecto en la retención de compuestos bioactivos varía según los diferentes frutos. En limones frescos cortados, se observó una buena retención de vitamina C durante 10 días a 5 ºC (Artés-Hernández et al., 2006), mientras que la pérdida fue del 13% en mandarina después de 12 días de conservación a 4 ºC bajo atmósfera modificada (Piga et al., 2002). Además, variaciones en el contenido de vitamina C y flavonoides durante el almacenamiento de fruta fresca cortada también dependen de la

variedad seleccionada (Del Caro et al., 2004). De hecho, estos autores observaron un aumento del contenido de flavonoides en naranjas frescas cortadas 'Shamouti' mientras que en naranjas 'Salustiana' se detectó una disminución significativa durante los 12-15 días de almacenamiento refrigerado. Odriozola-Serrano et al. (2007) observaron niveles mayores de licopeno durante el almacenamiento de tomate fresco cortado de la variedad Bodar (80.5 mg/kg) que en otras tales como Rambo, Durinta, Pitenza, Cencara o Bola (20 – 43.1 mg/kg).

Actualmente se requiere de la evaluación de la capacidad antioxidante para determinar la eficiencia de los antioxidantes naturales, en relación a la protección de productos vegetales contra el daño oxidativo y pérdida de su valor comercial y nutricional (Sánchez-Moreno, 2002). Varios estudios sobre la evaluación de la capacidad antioxidante en frutos han revelado aspectos interesantes en relación al comportamiento de los constituyentes antioxidantes. Eberhardht et al. (2000), en un estudio realizado en manzanas, demostraron que la vitamina C por sí sola aporta menos del 0,4% de la actividad antioxidante total del fruto, sugiriendo que la mezcla compleja de antioxidantes en las frutas proporciona beneficios sobre la salud, principalmente a través de la combinación de efectos aditivos y/o sinérgicos. Cao et al. (1996) y Wang et al. (1996) realizaron estudios en frutas y hortalizas, demostrando que la mayor parte de la actividad antioxidante proviene principalmente del contenido de flavonoides y otros compuestos fenólicos. Por otra parte, estudios en nectarinas, melocotones y ciruelas (Gil et al., 2002) y cítricos (Gorinstein et al., 2004), han demostrado que existe una correlación significativa entre la capacidad antioxidante y los compuestos fenólicos totales. En muchos estudios in vitro, los compuestos fenólicos han demostrado alta actividad antioxidante, incluso mayor que la de vitaminas y carotenoides (Re et al., 1999; Vinson et al., 1995). Los flavonoides se encuentran entre los antioxidantes vegetales más potentes, siendo la quercetina el flavonol con más poder antioxidante (Bravo, 1998). La absorción e incorporación a la circulación sanguínea de antioxidantes fenólicos puede inhibir o regular la oxidación de las lipoproteínas de baja densidad (LDL), retrasando de este modo el proceso aterosclerótico (Vinson et al., 1995).

1.4. Factores de control

1.4.1. Envasado en atmósfera modificada (EAM)

La modificación de la atmósfera interior de los envases es una tecnología de envasado en plena expansión, que permite alargar significativamente la vida útil de productos frescos cortados, es decir, el período durante el cual el alimento mantiene las propiedades organolépticas y de seguridad requeridas para su consumo, bajo unas determinadas condiciones de conservación.

La modificación pasiva de la atmósfera consiste en la utilización de películas plásticas de diferente permeabilidad a los gases, para crear de forma pasiva una atmósfera modificada favorable por efecto de la permeabilidad del envase, la respiración y actividad bioquímica del producto, entre otros factores (Day, 2000). No obstante, cuando la atmósfera modificada de equilibrio no se consigue antes que se activen reacciones que llevan al deterioro del producto, tales como el pardeamiento enzimático o la pérdida de textura, se puede modificar activamente la atmósfera de envasado. En este caso, la atmósfera modificada se consigue mediante la substitución mecánica del aire que rodea el producto por una mezcla adecuada de gases, de tal manera que la atmósfera en el envase va variando con el paso del tiempo en función de las necesidades y respuesta del producto (Day, 2000). Además, la atmósfera modificada se puede conseguir con la incorporación de ciertos aditivos en el envase. Entre ellos se encuentran los absorbedores de O_2, absorbedores/emisores de CO_2, generadores de vapor de etanol y absorbedores de etileno (Brody et al., 2001).

El diseño de un envase en atmósfera modificada depende de variables como las características del producto, su masa, la composición gaseosa recomendada, la permeabilidad a los gases del material de envasado, y su dependencia a la temperatura, y la actividad respiratoria del producto que variará en función de la composición gaseosa y la temperatura (Fonseca et al., 2002). Las propiedades de permeabilidad de las películas poliméricas dependen del tipo de material, de la temperatura ambiente, el grosor del film, de la permeabilidad del gas y de la diferencia de concentración del gas a través del film (Al-Ati y Hotchkiss, 2002).

Cada fruto fresco cortado, en función de su estado fisiológico, requiere un envasado en una atmósfera gaseosa de composición diferente para su conservación. En la Tabla 1 se muestran las concentraciones recomendadas para algunos frutos frescos cortados (Martín-Belloso et al., 2006).

Tabla 1. Composición gaseosa recomendada para la conservación en atmósfera modificada de diferentes frutas frescas cortadas

Producto	Atmósfera	Fuente bibliográfica
Manzana	< 1 kPa O_2	Gil et al. (1998) Soliva-Fortuny et al. (2002a)
Pera	0.5 kPa O_2 2 kPa O_2	Rosen y Kader (1989) Gorny et al. (2000) Soliva-Fortuny et al. (2004b)
Melocotón	2 kPa O_2 + 12 kPa CO_2 0.25 kPa O_2 + 10 kPa CO_2	Palmer-Wright y Kader (1997) Gorny et al. (1999)
Kiwi	2 kPa O_2 + 5 kPa CO_2	Agar et al. (1999)
Melón Cantaloupe	4 kPa O_2 + 10 kPa CO_2	Bai et al. (2001)
Melón Honeydew	2 kPa O_2 + 10 kPa CO_2 5 kPa O_2	Qi et al. (1999) Ayhan y Chism (1998)
Sandía	3 kPa O_2 + 15 kPa CO_2	Cartaxo et al. (1997)
Mango	2 kPa O_2 + 10 kPa CO_2	Nithiya et al. (2001)
Caqui	2 kPa O_2 + 12 kPa CO_2	Palmer-Wright y Kader (1997)
Fresas	1-2 kPa O_2 + 10 kPa CO_2	Watada et al. (1996)
Cítricos	Aire	Palma et al. (2003)

1.4.1.1. Efecto del EAM sobre la estabilidad microbiológica

Contenidos bajos de O_2 en la atmósfera de envasado inhiben el crecimiento de los microorganismos aerobios, mientras que condiciones de anaerobiosis pueden estimular el crecimiento de microorganismos anaerobios psicrótrofos (Farber, 1991). Por tanto, los niveles de O_2 en las atmósferas modificadas para fruta cortada se pretende que sean bajos, no superiores a 5 kPa, aunque es necesario un nivel mínimo de O_2 que evite problemas de anoxia, activación de vías fermentativas y crecimiento de microorganismos anaerobios. Se ha demostrado que concentraciones altas en CO_2 (5-20 kPa) pueden tener actividad antimicrobiana debido principalmente a la reducción del pH y a la interferencia con el metabolismo celular (Brackett, 1997). No obstante, concentraciones excesivas de CO_2 resultan perjudiciales para los tejidos vegetales, desarrollándose olores desagradables (Kader, 1989).

Durante los últimos años, se está demostrando que el empleo de concentraciones superatmosféricas de O_2 (CSO) ($\geq$ 60 kPa) puede ser efectivo en la prevención de reacciones fermentativas de anaerobiosis, y en la inhibición del crecimiento de microorganismos aerobios y

anaerobios en productos frescos (Kader y Ben-Yehosua, 2000). Wszelaki y Mitcham (2000) demostraron la efectividad de CSO (60-100 kPa O_2) en el control microbiológico de fresas durante 14 días de almacenamiento a 5 ºC. No obstante, los resultados sobre los efectos de las altas concentraciones de O_2 en el crecimiento microbiano son contradictorios. Aunque el crecimiento de levaduras se ve generalmente inhibido, algunas cepas no se ven afectadas o su crecimiento incluso se estimula (Van der Steen et al., 2002).

1.4.1.2. Efecto del EAM sobre la actividad fisiológica

El control de la maduración y senescencia de los tejidos vegetales se consigue con la disminución del metabolismo respiratorio y la inhibición de la síntesis y acción del etileno. Durante las operaciones de procesado, la fruta fresca cortada sufre un estrés fisiológico con un aumento notable de las concentraciones de etileno durante las primeras horas posteriores al procesado de la fruta. La reducción de los niveles de O_2 y el enriquecimiento en CO_2 puede reducir la intensidad de la respiración y disminuir o incluso suprimir la producción de etileno (Solomos, 1997a, Gorny et al., 1999, 2002). La inhibición de la síntesis de etileno como consecuencia de concentraciones bajas de O_2 en el interior de los envases ha sido descrita ampliamente, sugiriéndose que el O_2 participa en la conversión del ácido 1-amino-ciclopropano-1-carboxílico (ACC) a etileno (Yang, 1981). En trabajos realizados en pera (Gorny et al., 2002), melocotón y nectarina (Gorny et al., 1999) se observó un efecto sinérgico de las atmósferas bajas en O_2 y niveles elevados de CO_2 en la reducción de la producción de etileno y la tasa de respiración.

Los materiales plásticos disponibles comercialmente aún no ofrecen las características idóneas para el envasado en atmósfera modificada de frutas frescas cortadas. En general, las permeabilidades al O_2 y CO_2 de los materiales plásticos no son suficientemente altas, favoreciendo una excesiva disminución de O_2 y la acumulación de CO_2 en el envase. Aunque las CSO podrían evitar condiciones de anaerobiosis y la acumulación de metabolitos fermentativos, su efecto sobre la actividad fisiológica del producto es contradictorio. Según Kader y Ben-Yehoshua (2000), las CSO podrían estimular, no tener ningún efecto, o reducir la tasa de respiración, dependiendo del

producto, estado de madurez, concentración de O_2, tiempo y temperatura de almacenamiento, y las concentraciones de CO_2 y etileno.

El mantenimiento de la calidad de un producto mínimamente procesado en fresco depende en gran medida de un apropiado diseño del envasado en atmósfera modificada, para el cual resulta muy conveniente lograr un modelo matemático que permita prever la evolución de la tasa respiratoria del producto bajo distintas concentraciones gaseosas de O_2 y CO_2, y diversas temperaturas. La mayoría de estudios han descrito el comportamiento respiratorio de productos frescos cortados a través de cinéticas enzimáticas basadas en la ecuación de Michaelis-Menten, ya que éstas se ajustan bien a los datos experimentales obtenidos para las actividades respiratorias de numerosos productos hortofructícolas (Fonseca et al., 2002). Esta ecuación matemática describe la relación entre la tasa de consumo de O_2 o producción de CO_2 del producto y las concentraciones de O_2 (Ecuación 1). Además, se han introducido diversas modificaciones para describir el efecto inhibidor de las concentraciones de CO_2 sobre la tasa de consumo de O_2 (Peppelenbos y van't Leven, 1996a). Así, el proceso de respiración se simplifica al de una reacción enzimática, inhibida por el propio producto, el CO_2. Entre los diferentes modelos de inhibición enzimática, la inhibición no competitiva (Ecuación 2) parece ser la que mejor describe el efecto del CO_2 en la respiración aeróbica de los frutos (Peppelenbos y van't Leven, 1996a). No obstante, en condiciones anaeróbicas, la tasa de producción de CO_2 se puede calcular a través de la Ecuación 3 donde se considera una inhibición de los procesos fermentativos debido a las concentraciones de O_2 altas y en la que se incluyen dos términos para describir ambos procesos oxidativo y de anaerobiosis (Peppelenbos et al., 1996b). Además, la influencia de la temperatura sobre los parámetros de la ecuación de Michaelis-Menten se puede cuantificar a través de la ecuación de Arrhenius, mediante una relación lineal o del valor de Q_{10}, que mide el incremento de la tasa respiratoria al aumentar la temperatura 10 ºC (Fonseca et al., 2002).

$$R_{O_2} \text{ or } R_{CO_2} = \frac{V_{max} O_2}{K_M + O_2} \qquad \text{(Ecuación 1)}$$

$$R_{O_2} \text{ or } R_{CO_2} = \frac{V_{max} O_2}{\left(K_M + O_2\right) * \left(1 + \frac{CO_2}{Kmn_{CO_2}}\right)} \qquad \text{(Ecuación 2)}$$

$$R_{CO_2} = RQ_{ox} R_{O_2} \frac{Vm_{CO_2\ (f)}}{1 + \frac{O_2}{Kmc_{O_2\ (f)}}} \qquad \text{(Ecuación 3)}$$

dónde, R_{O_2} y R_{CO_2}: consumo de O_2 y producción de CO_2 (mL/kg/h); O_2 y CO_2: concentraciones de gas (kPa), V_{max}: máxima tasa de consumo de O_2 (mL/kg/h), K_M: concentración de O_2 a la cual el consumo de O_2 es un 50% del máximo (kPa), Kmn_{CO_2}: concentración de CO_2 a la cual el consumo de O_2 es inhibido un 50%, RQ_{ox}: coeficiente de respiración, $Vm_{CO_2\ (f)}$: la máxima tasa de producción fermentativa de CO_2 (mL/kg/h) y $Kmc_{O2\ (f)}$: la constante de Michaelis-Menten de inhibición de la producción fermentativa de CO_2 por el O_2.

1.4.1.3. Efecto del EAM sobre las características físico-químicas

En el diseño de los envases se debería considerar la reducción de la pérdida de agua ya que ésta podría provocar cambios de textura, translucidez, y/o deshidratación superficial, aunque sin facilitar la formación en el envase de condensados que podrían favorecer el crecimiento microbiano. Se han usado concentraciones de O_2 del orden de 0,25-5% en combinación con concentraciones de CO_2 en el rango de 10-20% para el mantenimiento de la apariencia visual de muchos frutos frescos cortados tales como melón (Qi et al., 1999; Bai et al., 2001), melocotón (Palmer-Wright y Kader, 1997, Gorny et al., 1999), kivi (Agar et al., 1999) y mango (Rattanapanone et al., 2001). No obstante, el EAM no previene completamente el pardeamiento enzimático y su efecto sobre la textura de los tejidos vegetales es mínimo. Bajas concentraciones de O_2 (0,25 o 0,5 kPa) o elevadas de CO_2 (aire enriquecido con 5, 10 o 20 kPa CO_2) no evitaron el pardeamiento de rodajas de pera Bartlett fresca cortada durante un período de conservación de 8 días (Gorny et al., 2002). De acuerdo con la evolución de la luminosidad en pera y manzana frescas cortadas, concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 aceleraron el pardeamiento de los tejidos en comparación a la atmósfera en ausencia de aire (100 % N_2) durante el período de almacenamiento (Soliva-Fortuny et al., 2001, 2002b). Se ha constatado que el envasado bajo CSO puede ser efectivo en la inhibición del pardeamiento enzimático de algunos productos frescos cortados (Kader y Ben-Yehoshua, 2000; Gómez et al., 2006). No obstante, es necesario el estudio

en profundidad de los mecanismos de inhibición enzimática de las concentraciones altas de O_2 en diferentes frutas frescas cortadas.

1.4.1.4. Efecto del EAM sobre la calidad sensorial

Las condiciones de envasado no afectan significativamente a la evolución de los niveles de azúcares y ácidos orgánicos durante el almacenamiento de fruta fresca cortada (Gil et al., 1998; Lamikanra et al., 2000; González-Aguilar et al., 2000; Bett et al., 2001). En cambio, el perfil de compuestos volátiles puede verse modificado substancialmente debido al a las condiciones de envasado. Manzana 'Gala' fresca cortada envasada en un película de alta barrera no sufrió cambios aromáticos significativos durante 14 días de almacenamiento (Bett et al., 2001). En cambio, diversos estudios han demostrado que el uso de CSO podría afectar a la síntesis y acumulación de algunos compuestos volátiles asociados al metabolismo respiratorio tales como acetaldehído, etanol y acetato de etilo (Solomos et al., 1997b; Whitaker et al., 1998). La presencia de etanol y acetaldehído a bajas concentraciones podría mejorar el aroma de la fruta fresca aunque concentraciones altas podrían inducir olores desagradables (Artés-Hernández et al. 2006). Durante el almacenamiento de fresas en 100 kPa O_2 se observó una mayor producción de metabolitos fermentativos que en aire + 15 kPa CO_2, afectando negativamente a la calidad organoléptica de las fresas (Wszelaki y Mitcham, 2000). No obstante, el uso de películas de permeabilidad alta al CO_2 mejoró el efecto de la aplicación de 95 kPa O_2 en la conservación de las características organolépticas de fresas respecto una atmósfera 3 kPa O_2 + 5 kPa CO_2 y la conservación en aire (Van der Steen et al., 2002).

1.4.1.4. Efecto del EAM sobre el contenido en compuestos antioxidantes

La investigación sobre los efectos del EAM en el contenido de compuestos antioxidantes en fruta fresca cortada es aún incipiente y necesita ser desarrollada en mayor profundidad. En rodajas de caqui y melocotón, se detectaron cambios mínimos en el contenido de carotenoides durante un almacenamiento de 8 días a 5 ºC bajo 2 kPa O_2 + 12 kPa CO_2 (Wright y Kader, 1997). La

disponibilidad de O_2 en la atmósfera de envasado no afectó significativamente al contenido de compuestos fenólicos de rodajas de manzana (Gil et al., 1998). En cambio, la presencia de O_2 en el interior de los envases podría tener un gran impacto en la disminución del contenido de ácido ascórbico (AA) en fruta fresca cortada envasada bajo atmósfera modificada. Los niveles de ácido ascórbico se mantienen constantes bajo condiciones de anoxia mientras que los descensos en el contenido de AA son importantes en otras condiciones. Según trabajos realizados por Soliva-Fortuny et al. (2003a, 2004b), la conservación de manzana y pera fresca cortada en ausencia de aire y en plástico de baja permeabilidad al O_2 previno eficazmente las pérdidas de ácido ascórbico. Agar et al. (1999) estudió la influencia de varias condiciones de envasado sobre el contenido de ácido ascórbico en rodajas de kiwi. El contenido de ácido ascórbico en las rodajas envasadas en 0,5, 2 y 4 kPa O_2 disminuyó alrededor de un 7, 12 y 18 %, respectivamente, después de 12 días de conservación. En cambio, los niveles de ácido ascórbico tras 12 días de almacenamiento en aire combinado con 5, 10, 20 kPa CO_2 disminuyeron 14, 22 y 34% respectivamente, en relación a los niveles iniciales de ácido ascórbico. Altas concentraciones de CO_2 (> 5 kPa) en la atmósfera de envasado podrían ser perjudiciales para la conservación del contenido en ácido ascórbico (Agar et al., 1997; Gil et al., 1998; Wright y Kader, 1997). En cambio, existe muy poca información sobre los efectos de CSO en los compuestos antioxidantes de frutas frescas cortadas. No obstante, estudios en lechuga sugieren que las CSO podrían mejorar la retención de ácido ascórbico y no afectar los niveles de antioxidantes en comparación con el uso de concentraciones de O_2 bajas (Day, 1996).

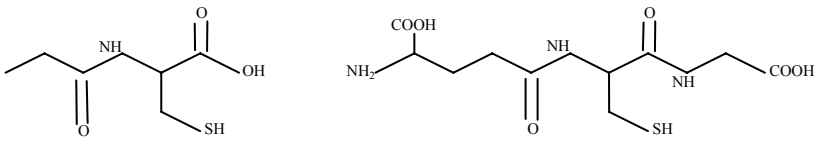
1.4.2. Tratamientos estabilizantes

En general, la inmersión de la fruta troceada en disoluciones de agentes antioxidantes y sales de calcio es el método más utilizado para el control del oscurecimiento y ablandamiento de los tejidos vegetales. La alteración del color es debida al pardeamiento enzimático, en el que los compuestos fenólicos de la fruta son oxidados hasta quinonas mediante reacciones catalizadas por las enzimas PPO. Las enzimas y sus substratos entran en contacto debido a la rotura del tejido durante el procesado y, como consecuencia, se forman compuestos activos que polimerizan dando lugar a melaninas, responsables del pardeamiento superficial del producto (Rocha, 1998). La adición de

antioxidantes retrasa o inhibe la reacción de pardeamiento enzimático, actuando sobre la enzima o su sustrato. El ácido ascórbico, como agente reductor o antioxidante, previene del pardeamiento por la reducción de las *o*-quinonas a difenoles incoloros (Gil et al., 1998; Soliva-Fortuny et al., 2002b). No obstante, este tratamiento no es completamente efectivo en la inhibición del pardeamiento de algunas frutas frescas cortadas, ya que cuando el ácido ascórbico es oxidado completamente a deshidroascórbico, las *o*-quinonas se acumulan en el tejido vegetal y experimentan reacciones de polimerización dando lugar a compuestos coloreados, responsables del oscurecimiento superficial del producto (Nicolas et al., 1994).

Se han aplicado agentes de origen natural como el glutatión, la cisteína y sus derivados tales como la N-acetilcisteína (Figura 3) en productos como la manzana, patata y zumos de fruta como inhibidores del pardeamiento enzimático (Molnar-Perl et al., 1990a, 1990b, Friedman et al., 1992). Estos compuestos, que contienen grupos tiol, han demostrado tener un gran potencial antioxidante. Reaccionan con las o-quinonas que se han formado en la primera fase de la reacción enzimática de pardeamiento para formar productos incoloros o reducen la o-quinonas a o-difenoles (Richard et al., 1991). No obstante, a bajas concentraciones de estos aminoácidos, el exceso de *o*-quinonas podría oxidarlos provocando la regeneración de fenoles y dando lugar a pardeamientos (Vamos-Vigyazo, 1995). El uso de N-acetilcisteína a concentraciones superiores al 0,75% p/v, previno el pardeamiento de manzanas frescas cortadas (Rojas-Graü et al., 2006a).

El compuesto 4-hexilresorcinol ha demostrado tener un efecto inhibidor del oscurecimiento de gambas (McEvily et al., 1992), champiñones (Osuga et al., 1994) y rodajas de manzana (Monsalve-González et al., 1993). Su mecanismo de acción podría estar relacionado con su capacidad de inhibición de la actividad polifenoloxidásica debido a su parecido estructural con los substratos fenólicos (McEvily et al., 1992).



a) b)

Figura 3. Estructuras químicas de: a) N-acetilcisteína b) Glutatión

En numerosos estudios se ha combinado el uso de compuestos antioxidantes y/o sales de calcio para mejorar su efecto en el control del pardeamiento enzimático y mantenimiento de la calidad de las frutas frescas cortadas (Tabla 2) (Martín-Belloso et al., 2006). En manzana 'Fuji' fresca cortada se seleccionó un tratamiento con 1% de cloruro de calcio, 0.5% de ácido ascórbico y 0.1% de ácido propiónico para mantener el color y la textura de las manzanas y la aceptabilidad de las muestras tratadas dentro del rango exigible para su comercialización (Varela et al., 2007b). Los tratamientos combinados de antioxidantes y sales de calcio, además de controlar el pardeamiento enzimático refuerzan las estructuras de las paredes celulares en frutas. En presencia de calcio, el ácido carboxílico liberado por la desesterificación de la pectina por acción de la enzima PME interacciona con el calcio formando pectatos cálcicos que originan sobre la pared celular enlaces químicos más fuertes. Se han estudiado diferentes tipos de sales de calcio para mantener la estructura y textura de la fruta fresca cortada. Aunque el uso de cloruro de calcio fue mayoritario entre los años 1980-1990, se ha comprobado que puede conferir amargor y olores desagradables al producto (Luna-Guzmán y Barrett, 2000). No obstante, en productos de pepita, se ha visto que inmersiones en disoluciones de cloruro de calcio a concentraciones no superiores del 1% ayudan a mantener la firmeza de los tejidos (Soliva-Fortuny et al., 2002c; 2003b). Así, el propionato de calcio o lactato de calcio podrían ser otras fuentes de calcio alternativas. De hecho, su efecto en el mantenimiento de la estabilidad estructural de las paredes celulares de manzana 'Fuji' fresca cortada ha sido probada mediante estudios de microestructura (Alandes et al., 2006; Quiles et al., 2007). En manzana cortada, Alandes et al. (2006) aconsejó el uso de lactato de calcio para contrarrestar el aumento de la actividad de las enzimas PME y PG. El tratamiento con propionato de calcio, en cambio, redujo la actividad enzimática de la PME (Alandes et al., 2006). Esto se explicó debido no solo a la inhibición causada por el propionato sino también por la formación de pectatos cálcicos, que actúan como una barrera natural que dificulta la interacción enzima y sustrato (Quiles et al., 2007).

Tabla 2. Tratamientos estabilizantes recomendados para mejorar la calidad de diferentes frutas frescas cortadas

Producto	Tratamiento estabilizante	Fuente bibliográfica
Manzana	1% AA + 0,5% $CaCl_2$	Soliva-Fortuny et al. (2001)
	0,001 M 4-HR + 0,5 M AIA	Buta et al. (1999)
	0,75% AA + 0,75% $CaCl_2$	Rocha et al. (1998)
	0,01% 4-HR + 0,5% AA	Luo y Barbosa-Cánovas (1996)
	1% AA + 0,2% AC o 0,5% AA	Pizzocaro et al. (1993)
	0,5% AA + 1% $CaCl_2$ + 0,1% AP	Varela et al. (2007b)
	1% NAC+ 1% GSH + 1% LCa	Raybaudi-Massilia et al. (2007)
	4% PCa	Quiles et al. (2007)
Pera	2% AA + 1% CaL + 0,5% Cys	Gorny et al. (2002)
	1% AA + 0,5% $CaCl_2$	Soliva-Fortuny et al. (2002b)
	0,01% 4-HR + 0,5% AA	Dong et al. (2000)
	4% NaE + 0,2% $CaCl_2$ + 100 ppm 4-HR	Sapers and Miller (1998)
Melocotón/ nectarina	2% AA + 1% LCa	Gorny et al. (1999)
Plátano	0,5 M AC + 0,05 M NAC	Moline et al. (1999)
Melón	2,5% LCa	Luna-Guzmán y Barrett (2000)
Sandía	2% $CaCl_2$	Mao et al. (2005)
Mango	0,001 M 4-HR + 0,5 M AIA	González-Aguilar et al. (2000)
	3% $CaCl_2$	Souza et al. (2006)
kivi	1% $CaCl_2$ or 2% LCa	Agar et al (1999)
Aguacate	1% AA + 0,2% Cys	Dorantes-Albarez et al. (1998)

AA: ácido ascórbico, 4-HR: 4-hexilresorcinol; AIA: acido isoascórbico; AC: ácido cítrico; AP: ácido propiónico; LCa: lactato cálcico; Cys: cisteína; NaE: eritorbato sódico; N-Acys: N-acetilcisteína; PCa: propionate de calcio

Para mantener un alimento seguro frente a la contaminación por microorganismos patógenos se recomienda la acidificación del producto. De hecho, el ácido cítrico ha sido ampliamente utilizado para reducir el pH de frutas como naranja (Pao y Petracek, 1997), manzana (Rocha et al., 1998), melocotón, albaricoque y kiwi (Senesi y Pastine, 1996). No obstante, en los últimos años, los esfuerzos en este ámbito se han dirigido a la búsqueda de nuevas alternativas para prevenir el crecimiento fúngico y bacteriano. Así, el uso de agentes antimicrobianos de origen natural podría ser una alternativa a los aditivos sintéticos. Se ha demostrado que algunas substancias naturales como hexanal, hexanol, 2-(E)-hexenal, and 3-(Z)-hexenol, responsables del aroma de algunas frutas y vegetales, tienen actividad antimicrobiana (Gardini et al., 2002). La efectividad del hexanal en la mejora de la calidad de las manzanas frescas cortadas se basa en su actividad antimicrobiana, prevención del pardeamiento y mejora de las propiedades aromáticas del producto (Lanciotti et al., 1999). En manzana 'Fuji' fresca cortada, la adición de ácido málico también

mejoró significativamente la estabilidad microbiológica del producto (Raybaudi-Massilia et al., 2007).

1.4.3. Aplicación de recubrimientos comestibles (RC)

Un RC es definido como una capa delgada de material comestible formado como un revestimiento sobre el alimento. Su uso sobre el tejido vegetal cortado produce una atmósfera modificada en el fruta que retrasa su deterioro y maduración, reduce la pérdida de agua, retarda los cambios de color, mejora la apariencia, disminuye la pérdida de aromas y puede servir como transporte de sustancias tales como antioxidantes y estabilizantes de textura (Olivas y Barbosa-Cánovas, 2005).

Para la formación de un RC se necesita en primer lugar una solución que pueda constituir una matriz estructural con suficiente cohesión (Debeaufort et al., 1998). Existen dos grandes grupos de materiales usualmente empleados en la elaboración de RC para frutas frescas cortadas: polisacáridos y proteínas. Entre los polisacáridos más empleados como base para formar RC en frutas cortadas se encuentran: maltodextrinas, metilcelulosa, carboximetilcelulosa, quitosano, pectina, alginato y gelano (Díaz-Sobac et al., 2001; Turhan et al., 2001; Wong et al., 1994; Krochta y Mulder-Johnston, 1997; Yang y Paulson, 2000; Pavlath et al., 1993; LeTien et al., 2001; Rojas-Graü et al., 2007b). Algunos polisacáridos como las pectinas de grado bajo de metoxilación ($\leq$ 40%), el alginato o el gelano forman geles en presencia de iones de calcio. En presencia de iones de calcio, el alginato experimenta cambios conformacionales dando lugar al modelo de gelación conocido como "caja de huevo", formado por puentes entre cationes divalentes como los del cloruro de calcio y los grupos con carga negativa del ácido gulurónico del alginato (Figura 3). Entre las proteínas como base para la aplicación de RC se incluyen la caseína, proteínas de suero, gluten de trigo, zeína y proteína de soja, entre otras (Avena-Bustillos, y Krotcha, 1993; Gagri et al., 2001; Gontard et al., 1993; Sabato et al., 2001; Pérez-Gago et al., 2003). La elección del tipo de material a utilizar depende del tipo de producto que se quiera recubrir.

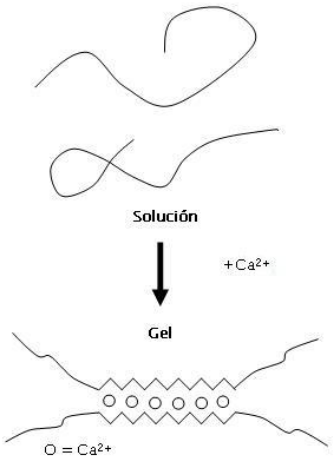


Figura 3. Modelo de gelación conocido como "caja de huevo"

En el caso de frutas cortadas, por ser un producto que continua respirando, se debe prestar especial atención a las propiedades barrera a los gases impuestas por cada recubrimiento. Generalmente, se combinan dos o más materiales con el fin de mejorar el intercambio de gases, la adherencia y las propiedades de permeabilidad a la humedad (Baldwin et al., 1995). Dichas mezclas suelen realizarse mediante emulsión de uno de los componentes, generalmente un lípido, en el resto de los componentes, o mediante un recubrimiento multicapa, donde el recubrimiento se aplica mediante una técnica de laminación, en la cual se realiza la inmersión de la fruta en una primera solución, generalmente la matriz, seguida por una inmersión en otro tipo de solución, ya sea de naturaleza lipídica o cálcica, entre otras (Rojas-Graü et al., 2007a).

Además de los componentes de naturaleza polimérica (matriz) y lipídica, otro componente importante de los RC son los plastificantes. Éstos son moléculas pequeñas de bajo peso molecular, de baja volatilidad y con una naturaleza química similar a la del polímero formador del recubrimiento. Se usan para mejorar la flexibilidad y la funcionalidad de los recubrimientos. Dentro de los agentes plastificantes utilizados más frecuentemente se encuentran: glicerol, polietilénglicol, sorbitol, aceites, ácidos grasos, ceras, etc., siendo el glicerol uno de los más utilizados (Rojas-Graü et al., 2007a). En la Tabla 3 se pueden observar algunos RC empleados en frutas mínimamente procesadas (Rojas-Graü et al., 2007a).

Tabla 3. Algunos recubrimientos comestible utilizados en frutas frescas cortadas

Producto	Matriz del RC	Plastificantes y aditivos	Función del RC	Fuente bibliográfica
Manzana	Quitosano	CAB	Reducción del crecimiento microbiano	Assis y Pessoa (2004)
	Carragenato/CPS +CMC	Glicerol, PEG, AA, AO, AC	Disminución del intercambio gaseoso; mejor calidad sensorial	Lee et al. (2003)
	APS + CAB	Glicerol, AE	Reducción del intercambio gaseoso, pardeamiento y pérdida de agua	Pérez-Gago et al. (2003)
	Puré de manzana + pectina + CAB o aceite vegetal	Glicerol, AA, AC	Reducción de pérdida de humedad y pardeamiento	McHugh y Senesi (2000)
	Alginato, gelano	Glicerol, aceite de girasol, N-cys	Reducción de pérdida de humedad y mantenimiento del color original	Rojas-Graü et al. (2007b)
	Carragenato, pectina, alginato, CM + MGA	AA, AC, $CaCl_2$, NaCl	Disminución de la producción de CO_2 y etileno en un 50 y 90%.	Wong et al. (1994)
Pera	MC + AE	PEG, SP, AA, $CaCl_2$	Reducción del pardeamiento y pérdida de agua; retención del aroma	Olivas et al. (2003)
Fresa	CPS + Caseína + pectina + agar	Glicerol, $CaCl_2$	Reducción del crecimiento fúngico	Vachon et al. (2003)
Papaya	Alginato, gelano	Glicerol, aceite de girasol, AA	Reducción de pérdida de humedad, reducción de pérdidas de AA et alor	Tapia et al. (2005)
Mango	CMC	Lecitina, PEG, AC	Mantenimiento del color	Níspero-Carriedo (1994)
	Quitosano		Reducción de pérdida de agua, mantenimiento del color y sabor original	Chien et al. (2007)

CAB: cera de abeja; CPS: concentrado de proteínas de suero lácteo; CMC: carboximetilcelulosa; PEG: polietilenglicol; AA: ácido ascórbico; AO: ácido oxálico; AC: ácido cítrico; APS: aislado de proteínas de suero lácteo; AE: ácido esteárico; CM: celulosa microcristalina; MGA: monoglicérido acetilado; SP: sorbato de potasio

1.4.3.1. Efecto de los RC sobre la actividad fisiológica

La aplicación de RC en frutas permite la producción de atmósferas modificadas aislando el producto del ambiente que lo rodea (Olivas y Barbosa-Cánovas, 2005). Las coberturas de base polisacárida y proteica reducen la tasa respiratoria y el intercambio de gases gracias a su permeabilidad selectiva al O_2 y CO_2 (Nisperos-Carriedo, 1994; Nussinovitch, 1997; Cisneros-Zevallos y Krochta, 2003). En manzana fresca cortada, el uso de RC compuestos por un bicapa de naturaleza polisacárida y lípidica produjo disminuyó sustancial la actividad respiratoria y producción de etileno (Wong et al., 1994). Lee et al. (2003) observaron una reducción de la tasa respiratoria inicial, de 44.80 a 34.95 mg CO_2 kg^{-1} h^{-1}, en trozos de manzana recubiertos con un concentrado de proteina de suero.

1.4.3.2. Efecto de los RC sobre las características físico-químicas

La característica funcional más importante de los RC para muchas aplicaciones es la resistencia a la migración de humedad (Kester y Fennema, 1986). La deshidratación superficial constituye uno de los principales problemas en el mantenimiento de la calidad de los productos cortados. La naturaleza del RC empleado desempeña aquí un papel muy importante: a mayor hidrofilicidad de los materiales utilizados, mayor permeabilidad al vapor de agua (Martín-Belloso et al. 2005). Los recubrimientos elaborados a partir de polímeros naturales así como aquellos a base de proteínas, muestran una baja resistencia al agua y poseen pobres propiedades barrera como consecuencia de su naturaleza hidrofílica (Yang y Paulson, 2000). Por tanto, la incorporación de lípidos es necesaria para mejorar las propiedades barrera al vapor de agua de este tipo de recubrimientos (García et al., 2000; Yang y Paulson, 2000). La adición de lípidos a una formulación para manzana fresca cortada, basada en puré de manzana y pectina, disminuyó sustancialmente el intercambio gaseoso a través de la matriz (McHugh y Senesi, 2000).

Los agentes estabilizantes de color y textura se aplican normalmente mediante inmersión de la fruta troceada en una disolución. No obstante el uso potencial de los recubrimientos comestibles en frutas frescas cortadas como medio de transporte de aditivos ha sido investigado por varios autores (Baldwin et al., 1996; Lee et al., 2003; Rojas-Graü et al., 2007b). Baldwin et al. (1996) observó un

control más efectivo del pardeamiento enzimático de manzanas troceadas cuando el ácido ascórbico fue incorporado como parte del recubrimiento comestible que por inmersión directa de los trozos en una solución acuosa conteniendo dicho compuesto antioxidante. Rojas-Graü et al. (2007b) demostraron que la incorporación de antioxidantes naturales, tales como N-acetilcisteína o glutatión en un recubrimiento elaborado a partir de polisacáridos, permitía mantener el color original de trozos de manzana 'Fuji' más de 21 días de almacenamiento. Además de compuestos antioxidantes, los recubrimientos comestibles pueden incorporar agentes estabilizantes de la textura. La incorporación de cloruro de calcio (1%) dentro de la formulación de una cobertura de proteína de suero ayudó a mantener la firmeza de trozos de manzana cortada (Lee et al., 2003). Se ha visto que la adición de calcio o de algún ión multivalente en un recubrimiento comestible facilita la formación de enlaces cruzados, contribuyendo a la obtención de recubrimientos estructuralmente más fuertes y estables (Mei et al., 2002).

1.4.3.3. Efecto de los RC sobre la estabilidad microbiológica

La incorporación de agentes antimicrobianos dentro de recubrimientos comestibles constituye una técnica innovadora en el mantenimiento de la seguridad, inocuidad y vida útil de alimentos mínimamente procesados. El crecimiento de microorganismos en la superficie de productos cortados es una de las principales causas de deterioro, pudiendo ser evitado mediante el uso de agentes antimicrobianos. Entre los principales agentes antimicrobianos incorporados en recubrimientos comestibles se encuentran sorbatos, ácidos, bacteriocinas, lisozima y, más recientemente, aceites esenciales (Rojas-Graü, 2006b). Estos últimos pueden ser añadidos a los RC para modificar su sabor, aroma, olor e introducir propiedades antimicrobianas (Cagri et al., 2004). Se ha publicado poco acerca de la incorporación de aceites esenciales en las películas comestibles y RC. Recientemente se ha evaluado la actividad antimicrobiana de diferentes aceites esenciales, incorporados a una película comestible elaborada a partir de puré de manzana, frente *E. coli* O157:H7. Se observó que el aceite de orégano fue el más efectivo frente al crecimiento del microorganismo, observándose una destrucción total de éste a una concentración de 0,1% p/v. En cambio, fueron necesarias concentraciones cinco veces mayores de aceite de canela o hierba de limón para obtener una actividad antimicrobiana similar a la obtenida con aceite de orégano

(Rojas-Graü et al., 2006b). En estudios posteriores, la incorporación de agentes antimicrobianos dentro de la esta película comestible a basé de puré de manzana inhibió significativamente el crecimiento de *L. inocua* en trozos de manzana comparado con las muestras control (sin antimicrobianos), donde se observaron recuentos de 6,2 UFC/g al final del almacenamiento, confirmándose una vez más que el RC no ejerce ningún efecto en el crecimiento de *L. inocua*. La mayor actividad antimicrobiana fue ejercida por los aceites de hierba de limón (1,0 y 1,5% p/p) y de orégano (0,5% p/p), los cuales redujeron el número de colonias de *L. inocua* por debajo del límite de detección (2,0 log UFC/g) e inhibieron totalmente el recuento de microorganismos aerobios psicrófilos (con un límite de detección de 1,0 log UFC/g) y de mohos-levaduras (con un límite de detección de 2,0 log UFC/g) (Rojas-Graü et al., 2007c).

1.4.3.4. Efecto de los RC sobre cambios sensoriales y valor nutritivo

En general, un recubrimiento comestible no debería poseer ningún tipo de sabor o, en su defecto, poseer un sabor bastante suave, de forma que no pueda detectarse durante el consumo del alimento en el cual se aplica (Contreras-Medellin y Labuza, 1981). Se ha visto que un RC basado en alginato y puré de manzana no produce por si mismo un cambio apreciable en las características sensoriales de manzana fresca cortada (Rojas-Graü et al., 2007c). En cambio, estos autores sí detectaron cambios apreciables de las características sensoriales de los trozos de fruta al añadir aceite de orégano, hierba de limón o vainillina dentro del recubrimiento. Por otro lado, se ha demostrado que los RC podrían mejorar la retención de aromas en frutas frescas cortadas. Olivas et al. (2003) observó una mayor producción de hexil acetato y butilacetato durante el almacenamiento de pera fresca cortada recubierta con metilcelulosa y adición de ácido esteárico en comparación con fruta sin recubrir.

Por último, la incorporación de recubrimientos comestibles puede emplearse para mejorar el valor nutricional de los alimentos. En este sentido, Tapia et al. (2007) emplearon una formulación a base de alginato o gelano conteniendo bifidus con el fin de proporcionar propiedades funcionales a los RC de trozos de manzana y papaya. Mei et al. (2002) emplearon un recubrimiento a base de goma xantano para transportar 5% de "Gluconal Cal" (mezcla de lactato de calcio y de gluconato, y

0.2% de acetato de alfa tocoferol (vitamina E). Estos recubrimientos fueron aplicados en zanahorias para mejorar sus propiedades sensoriales y nutricionales.

Bibliografía

Agar, I.T.; Streif, J.; Bangerth, F. 1997. Effect of high CO2 and controlled atmosphere (CA) on the ascorbic and dehydroascorbic acid content of some berry fruits. Postharvest Biology and Technology, 11: 47-55

Agar, I.T.; Massantini, R.; Hess-Pierce, B.; Kader, A.A. 1999. Postharvest CO2 and ethylene production and quality maintenance of fresh-cut kiwifruit slices. Journal of Food Science, 64: 433-440ç

Al-Alti, T.; Hotchkiss, H. 2002. Application of Packaging and Modified Atmosphere to Fresh-cut Fruits and Vegetables. In: Lamikanra, O. (Ed.), Fresh-cut fruits and vegetables science, technology, and Market. CRC Press, Boca Raton, FL, USA, pp. 305-338.

Alandes, L.; Hernando, I.; Quiles, A.; Pérez-Munuera, I.; Lluch, M.A. 2006. Cell wall stability of fresh-cut Fuji apples treated with calcium lactate. Journal of Food Science, 71 (9): 615-620

Amiot, M.J.; Tacchini, M.; Aubert, S.Y.; Leszek, W. 1995. Influence of cultivar, maturity stage and storage conditions on phenolic composition and enzymatic browning of pear fruits. Journal of Agricultural and Food Chemistry, 43(5): 1132-1137.

Artés-Hernández, F.; Rivera-Cabrera, F.; Kader, A.A. 2006. Quality retention and potential shelf-life of fresh-cut lemons as affected by cut type and temperature. Postharvest Biology and Technology, 43(2): 245-254.

Assis, O.B.; Pessoa, J.D. 2004. Preparation of thin films of chitosan for use as edible coatings to inhibit fungal growth on sliced fruits. Scientific Note. Brazilian Journal of Food Technology, 7: 17-22.

Avena-Bustillos, R.J.; Krochta, J.M. 1993. Water vapor permeability of caseinate-based edible films as affected by pH, calcium crosslinking and lipid content. Journal of Food Science, 58: 904-907.

Ayhan, Z.; Chism, G.W.; Richter, E.R. 1998. The shelf life of minimally processed fresh-cut melons. Journal of Food Quality, 21: 29-40.

Bai, J.H.; Saftner, R.A.; Watada, A.E; Lee, Y.S. 2001. Modified atmosphere maintains quality of fresh-cut cantaloupe (Cucumis melo L.). Journal of Food Science, 66: 1207-1211.

Baldwin, E.A.; Níspero-Carriedo, M.O.; Baker, R.A. 1995. Edible coatings for lightly processed fruits and vegetables. HortScience, 30: 35-40.

Baldwin, E.A.; Níspero-Carriedo, M.O.; Chen, X.; Hagenmaier, R.D. 1996. Improving storage life of cut apple and potato with edible coating. Postharvest Biology and Technology, 9: 151-163.

Bett, K.L.; Ingram, D.A.; Grimm, C.C.; Lloyd, S.W.; Spanier, A.M.; Miller, J.M.; Gross, K.C.; Baldwin, E.A.; Vinyard, B.T. 2001. Flavor of fresh-cut gala apples in barrier film packaging as affected by storage time. Journal of Food Quality, 24: 141-156.

Beuchat, L.R. 1996. Pathogenic microorganims associated with fresh produce. Journal of Food Science, 59(2): 204-216.

Beuchat, L.R. 2000. Use of sanitizers in raw fruit and vegetable processing. In: Alzamora, S.M.; Tapia, M.S.; López-Malo, A. (Eds.). Minimally processed fruits and vegetables. Fundamental aspects and applications. An Aspen Publication, Gaithersburg, pp. 63-78.

Brackett, R.E. 1997. Microbiological spoilage and pathogens in minimally processed refrigerated fruits and vegetables. In Wiley, R.C. (Ed.). Minimally processed refrigerated fruits and vegetables. Chapman and Hall, New York, pp. 226-268.

Bravo, L. 1998. Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance. Nutrition Reviews, 56: 317-326.

Brody, A.L.; Strupinsky, E.R.; Kline, L.R. 2001. Active Packaging for Food Applications. Lancaster: Technomic.

Buta, J.G.; Moline, H.E.; Spaulding, D.W.; Wang, C.Y. 1999. Extending storage life of fresh-cut apples using natural products and their derivatives. Journal of Agriculture and Food Chemistry, 47: 1-6.

Cagri, A.; Uspunol, Z., Roser, E. 2004. Antimicrobial edible films and coating. Journal of Food Protection. 67, 833-848.

Cao, G.; Sofic, E.; Prior, R.L. 1996. Antioxidant capacity of tea and common vegetables. Journal of Agricultural and Food Chemistry, 44: 3426-3431.

Cartaxo, C.B.C.; Sargent, S.A.; Huber, D.J.; Chia, M.L. 1997. Controlled atmosphere storage suppresses microbial growth on fresh-cut watermelon. Proceedings of the Florida State Horticultural Society, 110: 252-257.

Castillo, A.; Escartin, E.F. 1994. Survival of Campylobacter jejuni on sliced watermelon and papaya. Journal of Food Protection, 58:105-107.

Chien, P.; Sep, F.; Yang, F. 2007. Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. Journal of Food Engineering, 78: 225-229.

Chonhenchob, V.; Chantarasomboon, Y.; Singh, S.P. 2007. Quality changes of treated fresh-cut tropical fruits in rigid modified atmosphere packaging containers. Packaging Technology and Science, 20: 27-37.

Cisneros-Zevallos, L.; Krochta, J.M. 2003. Whey protein coating for fresh fruits and relative humidity effects. Journal of Food Science, 68 (1): 176-181.

Contreras-Medellin, R.; Labuza, T.P. 1981. Prediction of moisture protection requirements for foods. Cereal Food World, 26: 335-342.

Day, B.P.F. 1996. High oxygen modified atmosphere packaging for fresh prepared produce. Postharvest News and Information, 7(3): 31-34

Day, B.P.F. 2000. Modified atmosphere packaging of fresh fruit and vegetables-An overview. Acta Horticulturae, 553: 585-590.

Debeaufort, F.; Quezada-Gallo, J.A.; Voilley, G. 1998. Edible films and coatings: tomorrow´s packaging: a review. Critical Reviews in Food Science and Nutrition, 38: 299-313.

Del Caro, A.; Piga, A.; Vacca, V.; Agabbio, M. 2004. Changes of flavonoids, Vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage. Food Chemistry, 84, 99-105.

Del Rosario, A.; Beuchat, L.R. 1995. Survival and growth of enterohemorrhagic Escherichia coli O157:H7 in cantaloupe and watermelon. Journal of Food Protection, 58:105-107.

Díaz-Sobac, R.; Beristain, C.; Vernon-Carter, E. 2001. Water vapor permeability of an emulsion coating of maltodextrin and surfactants. Journal of Food Processing and Preservation, 25: 25-34.

Dong, X.; Wrolstad, R.E.; Sugar, D. 2000. Extending shelf life of fresh-cut pears. Journal of Food Science. 65: 181-186.

Dorantes-Alvarez, L.; Parada-Dorantes, L.; Ortiz-Moreno, A.; Santiago-Pineda, T.; Chiralt-Boix, A.; Barbosa-Cánovas, G. 1998. Effect of anti-browning comounds on the quality of minimally processed avocados. Food Science and Technology International, 4: 107-113

Eberhardt, M.V.; Lee, C.Y.; Liu, R.H. 2000. Antioxidant activity of fresh apples. Nature, 405: 903-904.

Farber, J.M. 1991. Microbiological aspects of modified-atmosphere packaging technology—a review. Journal of Food Protection, 54: 58– 70.

Fernández-Escartin, E.F.; Castillo-Ayala, A.C.; Saldana-Lozano, J.S. 1989. Survival and growth of Salmonella and Shigella on sliced fruits. Journal of Food Protection, 52: 471-472.

Fonseca, S.C.; Oliveira, F.A.R.; Frias, J.M.; Brecht, J.K.; Chau, K.V. 2002. Modelling respiration rate of shredded Galega kale for development of modified atmosphere packaging. Journal of Food Engineering, 54: 299-307.

Food Agricultural Organization of the United Nations (FAO). 2003a. Code of hygienic practice for fresh fruits and vegetables CAC/RCP 53. Retrieved February 7, 2006, from http://www.codexalimentarius.net/web/standard_list.do.

Food Agricultural Organization of the United Nations (FAO). 2003b. Recommended international code of practice. General principles of food hygiene. CAC/RCP 1-1969, Rev. 4-2003. Retrieved February 7, 2006, from http://www.codexalimentarius.net/web/standard_list.do.

Food Drug and Administration (FDA). 2001. Analysis and evaluation of preventive control measures for the control and reduction/ elimination of microbial hazards on fresh and fresh-cut produce. Retrieved February 7, 2006, from http://vm.cfsan.fda.gov/~commift3-toc.html.

Friedman, M.; Molnar-Perl, I.; Knighton, D.R. 1992. Browning prevention in fresh and dehydrated potatoes by SH-containing amino acids. Food Additives and Contaminants. 9: 499-503.

Gagri, A.; Ustunol, Z.; Ryser, E.T. 2001. Antimicrobial, mechanical, and moisture barrier properties of low pH whey protein-based edible films containing p-aminobenzoic or sorbic acid. Journal of Food Science, 66: 865-911.

García, M.A.; Martino, M.N.; Zaritzky, N.E. 2000. Lipid addition to improve barrier properties of edible starch-based films and coatings. Journal of Food Science, 65: 941-947.

Garcia, E.; Barrett, D.M. 2002. Preservative treatments for fresh-cut fruits and vegetables. In: Lamikanra, O. (Ed.). Fresh-cut fruits and vegetables science, technology, and market. CRC Press, Boca Raton, FL, USA, pp. 267-303.

Gardini, F.; Lanciotti, R.; Belletti, N.; Guerzoni, M.E. 2002. Use of natural aroma compounds to control microbial growth in foods. In: Mohan, R. (Ed.). Research Advances in Food Science. Global Research Network, Kerala, vol. 3 pp. 63-78.

Gil, M.I.; Gorny, J.R.; Kader, AA. 1998. Responses of 'Fuji' apple slices to ascorbic acid treatments and low-oxygen atmospheres. HortScience, 33: 305-309

Gil, M.I.; Tomás-Barberán, F.A.; Hess-Pierce, B.; Kader, A.A. 2002. Antioxidant capacities, phenolics compounds, carotenoids, and vitamin C contents of nectarine, peach and plum cultivars from California. Journal of Agricultural and Food Chemistry, 50: 4676-4982.

Golden, D.A.; Rhodehamel, E.J.; Kautter, D.A. 1993. Growth of Salmonella spp. in cantaloupe, watermelon y honeydew melons. Journal of Food Protection, 56:194-196.

Gómez, P.A.; Geysen, S.; Verlinden, B.; Artés, F.; Nicolaï, B.M. 2006. Modelling the effect of superatmospheric oxygen concentrations on in vitro mushroom PPO activity. Journal of the Science of Food and Agriculture, 86: 2387-2394.

Gontard, N.; Guilbert, S.; Cuq, J.L. 1993. Water and glycerol as plasticizer affect mechanical and water vapor barrier properties of an edible wheat gluten film. Journal of Food Science, 58: 206-211.

González-Aguilar, G.A.; Wang, C.Y.; Buta, J.G. 2000. Maintaining quality of fresh-cut mangoes using antibrowning agents and modified atmosphere packaging. Journal of Agricultural and Food Chemistry, 48: 4202-4208.

Gorinstein, S.; Leontowicz, H.; Leontowicz, M.; Krzeminski, R.; Gralak, M.; Martín-Belloso, O.; Delgado, L.E.; Haruenkit, R.; Katrich, E.; Park, Y.S.; Jung, S.T.; Trakhtenberg, S. 2004. Fresh Israeli Jaffa blond (Shamouti) orange and Israeli Jaffa red star ruby (Sunrise) grapefruits juices affect plasm lipid metabolism and antioxidant capacity in rats fed added cholesterol. Journal of Agricultural and Food Chemistry, 52: 4853-4859.

Gorny, J.R.; Hess-Pierce, B.; Kader, A.A. 1999. Quality changes in fresh-cut peach and nectarine slices as affected by cultivar, storage atmosphere and chemical treatments. Journal of Food Science, 64: 429-432.

Gorny, J.R.; Cifuentes, R.A.; Hess-Pierce, B.; Kader, A.A. 2000. Quality changes in fresh-cut pear slices as affected by cultivar, ripeness stage, fruit size, and storage regime. International Journal of Food Science and Technology, 65: 541-544.

Gorny, J.R.; Hess-Pierce, B.; Cifuentes, R.A.; Kader, A.A. 2002. Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. Postharvest Biology and Technology, 24: 271-278.

Infoagro, 2007. La comercialización de frutas y hortalizas de IV Gama hasta agosto aumenta un 13,6%. Recuperado 25/10/2007 de http://www.infoagro.com/noticias/2007/10/20071015asp

Kader, A.A.; Ben-Yehoshua, S. 2000. Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. Postharvest Biology and Technology, 20: 1-13.

Kader, A.A.; Zagory, D.; Kerbel, D.L. 1989. Modified atmosphere packaging of fruits and vegetables. Critical Reviews in Food Science and Nutrition. 28: 1-30.

Kester, J.J.; Fennema, O. 1986. Edible films and coatings: a review. Food Technology, 40: 47-59

Krochta, J.M.; Mulder-Johnston, C. 1997. Edible and biodegradable polymer films: challenges and opportunities. Food Technology, 51: 60-74.

Lamikanra, O.; Chen, J.C.; Banks, D.; Hunter, P.A. 2000. Biochemical and microbial changes during the storage of minimally processed cantaloupe. Journal of Agricultural and Food Chemistry. 48 (12): 5955-5961.

Lanciotti, R.; Corbo, M.R.; Gardini, F.; Sinigaglia, M.; Guerzoni, M.E. 1999. Effect of hexanal on the shelf-life of fresh apple slices. Journal of Agricultural and Food Chemistry, 47: 4769-4775

Larson, A.E.; Johnson, E.A. 1999. Evaluation of botulinal toxin production in packaged fresh-cut cantaloupe and honeydew melons. Journal of Food Protection, 62: 948-952.

Lee, J.Y.; Park, H.J.; Lee, C.Y.; Choi, W.Y. 2003. Extending shelf-life of minimally processed apples with edible coatings and antibrowning agents. LWT-Food Science and Technology. 36: 323-329.

Lester, G. 2006. Consumer preference quality attributes of melon fruits. Acta Horticulture, 712 (1): 175-181

LeTien, C.; Vachon, C.; Mateescu, M.A.; Lacroix, M. 2001. Milk protein coatings prevent oxidative browning of apples and potatoes. Journal of Food Science, 66: 512-516.

Luo, Y.; Barbosa-Cánovas, G.V. 1996. Preservation of apple slices using ascorbic acid and 4-hexylresorcinol. Food Science and Technology International. 2: 315-321.

Luna-Guzmán, I.; Barrett, D.M. 2000. Comparison of calcium chloride and calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupes. Postharvest Biology and Technology, 19(1): 61-72.

Macheix, J.; Fleuriet, A.; Billot, J. 1990. Phenolic compounds in fruit processing. Fruit phenolics. CRC Press, Inc. Boca Raton, Florida, USA, pp. 295-357.

Mao, L.; Jeong, J., Que, F.; Huber, D.J. 2005. Physiological properties of fresh-cut watermelon (Citrillus lanatus) in response to 1-methylcyclopropene and post-processing calcium application. Journal of Science and Food Agriculturae, 86: 46-53

Martín-Belloso, O.; Rojas-Grau, M.A. 2004. Factores que afectan a la calidad de las frutas mínimamente procesadas. En: G. González-Aguilar y M.C. Fabiola Cuamea Navarro (Eds.). Estado actual del mercado de frutos y vegetales cortados en Iberoamérica. CIAD, Hermosillo, Sonora, México pp. 17-23.

Martín-Belloso, O.; Soliva-Fortuny, R.C.; Baldwin, E. 2005. Conservación mediante recubrimientos comestibles. En: González-Aguilar, G.; Gardea, A.; Cuamea-Navarro, F. (Eds.). Nuevas tecnologías de conservación de productos vegetales frescos cortados. CIAD, Hermosillo, Mexico, pp. 341-356.

Martín-Belloso, O.; Soliva-Fortuny, R.; Oms-Oliu, G. 2006. Fresh-cut Fruits. In: Hui, Y.H. (Ed.). Handbook of Fruits and Fruit Processing. Blackwell Publishing, Ames, Iowa, USA, pp. 129-144.

Martín-Belloso, O.; Soliva-Fortuny, R.; Oms-Oliu, G. 2007. Influencia de la materia prima y los tratamientos de conservación en la calidad de la fruta fresca cortada. Phytoma, 189: 130-132.

McEvily, A.J.; Iyengar, R.; Otwell, W.S. 1992. Inhibition of enzymatic browning in foods and beverages. Critical Reviews in Food Science and Nutrition, 32: 253-273.

McHugh, T.H.; Senesi, E. 2000. Apple wraps: a novel method to improve the quality and extend the shelf life of fresh-cut apples. Journal of Food Science, 65: 480-485.

Mei, Y.; Zhao, Y.; Yang, J.; Furr, H.C. 2002. Using edible coating to enhance nutritional an sensory qualities of baby carrots. Journal of Food Science, 67: 1964-1968.

Moline, H.E.; Buta, J.G.; Newman, I.M. 1999. Prevention of browning of banana slices using natural products and their derivatives. Journal of Food Quality, 22: 499-511.

Molnar-Perl, I.; Fiedman, M. 1990a. Inhibition of Browning by Sulfur Amino Acids. 2. Fruit juices and Protein Containing Foods. Journal of Agricultural and Food Chemistry, 38: 1648-1651.

Molnar-Perl, I.; Friedman, M. 1990b. Inhibition of browning by sulphur amino acids. 3. Apples and Potatoes. Journal of Agricultural and Food Chemistry, 38: 1652-1656.

Monsalve-González, A.; Barbosa-Cánovas, G.V.; Cavalieri, R.P.; McEvily, A.J.; Iyengar, R. 1993. Control of browning during storage of apple slices preserved by combined methods. 4-hexylresorcinol as browning inhibitor. Journal of Food Science. 58, 797-826.

Nguyen-the, C.; Carlin, F. 1994. The microbiology of minimally processed fresh fruits and vegetables. Critical Reviews in Food Science and Nutrition, 34: 371-401.

Nicolas, J.J.; Richard-Forget, F.C.; Goupy, P.M.; Amiot, M.J.; Aubert, S.Y. 1994. Enzymatic browning reactions in apple and apple products. Critical Reviews in Food Science and Nutrition, 34: 109-157.

Nicoli, M.C.; Anise, M.; Severinc, C. 1994. Combined effects in preventing enzymatic browning reactions in minimally processed fruit. Journal of Food Quality, 17:221-229.

Nisperos-Carriedo, M.O. 1994. Edible coatings and films based on polysaccharides. In: Krochta, J.M.; Baldwin, E.A.; Nisperos-Carriedo, M.O (Eds.). Edible coatings and films to improve food quality. Technomic Publishing Co, Lancaster, pp. 305-355.

Nithiya, R.; Yuen, L.; Tianxia, W.; Watada, A.E. 2001. Quality and microbial changes of fresh-cut mango cubes held in controlled atmosphere. HortScience. 36: 1091-1095.

Nussinovitch, A. 1997. Agricultural uses of hydrocolloids. In: Nussinovitch, A. (Ed.). Hydrocolloid applications: Gum technology in the food and other industries. Blackie Academic and Professional, London, pp. 169-189.

Odriozola-Serrano, I.; Soliva-Fortuny, R.; Martín-Belloso, O. 2007. Effect of minimal processing on bioactive compounds and color attributes of fresh-cut tomatoes. LWT-Food Science and Technology, doi: 10.1016/j.lwt.2007.03.002.

Official Journal of the European Union. 2005. Commision regulation (EC) No 2073/2005 of November 2005 on microbiological criteria for foodstuffs. OJ 22.12.2005, L 338/1.

Olivas, G.I.; Rodríguez, J.J.; Barbosa-Cánovas, G.V. 2003. Edible coatings composed of methylcellulose, stearic acid, and additives to preserve quality of pear wedges. Journal of Food Processing and Preservation, 27: 299-320.

Olivas, G.I.; Barbosa-Cánovas, G.V. 2005. Edible coating for fresh-cut fruits. Critical Reviews in Food Science and Nutrition, 45: 657-670.

Osuga, D.; van der Schaaf, A.; Whitaker, J.R. 1994. Control of polyphenoloxidase activity using a catalytic mechanism. In: Yada, R.Y.; Jackman, R.L.; Smith, J.L. (Eds.). Protein Structure-Function Relationships in foods. New York, Blackie Academic and Professional, pp. 62-88.

Palma, A.; D'Aquino, S.; Agabbio, M. 2003. Physiological response of minimally processed citrus fruit to modified atmosphere. Acta Horticulturae, 604: 789-794.

Palmer-Wright, K.; Kader, A.A. 1997. Effect of controlled-atmosphere storage on the quality and carotenoid content of sliced persimmons and peaches. Postharvest Biology and Technology, 10: 89-97.

Pao, S.; Petracek, P.D. 1997. Shelf life extension of peeled oranges by citric acid treatment. Food Microbiology, 14: 485-491.

Pavlath, A.E.; Wong, D.S.W.; Kumosinski, T.F. 1993. New coatings for cut fruits and vegetables. Chemical Technology, 23: 36-40.

Pérez-Gago, M.B.; Serra, M.; Alonso, M.; Mateos, M.; Del Río, M.A. 2003. Effect of solid content and lipid content of whey protein isolate-beeswax edible coatings on color change of fresh-cut apples. Journal of Food Science, 68: 2186-2191.

Peppelenbos, H.W.; van't Leven, J. 1996a. Evaluation of four types of inhibition for modelling the influence of carbon dioxide on oxygen consumption of fruits and vegetables. Postharvest Biology and Technology, 7: 27-40.

Peppelenbos, H.W.; Tijskens, L.M.M.; van't Leven, J.; Wilkinson, E.C. 1996b. Modelling oxidative and fermentative carbon dioxide production of fruits and vegetables. Biology and Technology, 9: 283-95.

Piga, A.; Agabbio, M.; Gambella, F.; Nicoli, M.C. 2002. Retention of antioxidant activity in minimally processed mandarin and satsuma fruits. LWT-Food Science and Technology, 35: 344-347.

Pizzocaro, F.; Torreggiani, D.; Gilardi, G. 1993. Inhibion of apple polyphenoloxidase (PPO) by ascorbic acid, citric acid and sodium chloride. Journal of Food Processing and Preservation, 17: 21-30

Qi, L.; Wu, T.; Watada, A.E. 1999. Quality changes of fresh-cut honeydew melons during controlled atmosphere storage. Journal of Food Quality, 22: 513-521.

Quiles, A.; Hernando, I.; Pérez-Munuera, I.; Lluch, M.A. 2007. Effect of calcium propionate on the microstructure and pectin methyl-esterase activity in the parenchyma of fresh-cut Fuji apples. Journal of the Science of Food and Agriculture, 87: 511-519.

Rattanapanone, N.; Lee, Y.; Watada, A.E. 2001. Quality and microbial changes of fresh-cut mango cubes held in contolled atmosphere. Hortscience, 36: 1091-1095.

Raybaudi-Massilia, R.M.; Mosqueda-Melgar, J.; Sobrino-López, A.; Soliva-Fortuny, R.; Martín-Belloso, O. 2007. Shelf-life extension of fresh-cut Fuji apples at different ripeness stages using natural substances. Postharvest Biology and Technology, 45(2): 265-275.

Re, R.; Pellegrini, N.; Proteggentee, A.; Pannala, A.; Yang, M.; Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medical, 26: 1231-1237.

Revista Mercados. Agosto Setiembre 2007 nº 72. Movimientos en la cuarta gama de la lechuga a la Macedonia con zumo. Recuperado el 25 octubre, 2007, de http://www.revistamercados.com

Richard, F.; Goupy, P.M.; Nicolas, J.J.; Lacombe, J.M.; Pavia, A.A. 1991. Cysteine as an inhibitor of enzymatic browning. 1. Isolation and characterization of addition compounds formed during

oxidation of phenolics by apple polyphenol oxidase. Journal of Agricultural and Food Chemistry, 39: 841-847.

Robles-Sánchez, M.; Gorinstein, S.; Martín-Belloso, O.; Astiazarán-García, H.; González-Aguilar, G.; Cruz-Valenzuela, R. 2007. Frutos tropicales mínimamente procesados: potencial antioxidnte y su impacto en la salud. Interciencia, 32 (4): 227-232.

Rocha, A.M.C.N.; Brochado, C.M.; Morais, A.M.M.B. 1998. Influence of chemical treatment on quality of cut apple (cv. Joangored). Journal of Food Quality, 21: 13-28.

Rojas-Graü, M.A.; Sobrino-López, A.; Tapia, M.S.; Martín-Belloso, O. 2006a. Browning inhibition in fresh-cut 'Fuji' apple slices by natural antibrowning agent. Journal of Food Science, 79: 59-65.

Rojas-Graü, M.A.; Avena-Bustillos, R.; Friedman, M.; Henika, P.; Martín-Belloso, O.; McHugh, T. 2006b. Mechanical, barrier and antimicrobial properties of apple puree edible films containing plant essential oils. Journal of Agricultural and Food Chemistry, 54: 9262-9267.

Rojas-Graü, M.A.; Tapia, M.S.; Martín-Belloso, O. 2007a. Empleo de recubrimientos comestibles en fruta frescas cortadas: nuevo enfoque de conservación y desarrollo de productos. Alimentaria, 382: 105-118.

Rojas-Graü, M.A.; Tapia, M.S.; Rodríguez, F.J.; Carmona, A.J.; Martín-Belloso, O. 2007b. Alginate and gellan based edible coatings as support of antibrowning agents applied on fresh-cut Fuji apple. Food Hydrocolloids, 21, 118-127.

Rojas-Graü, M.A.; Raybaudi-Massilia, R.M.; Soliva-Fortuny, R.; Avena-Bustillos, R.J.; McHugh, T.H.; Martín-Belloso, O. 2007c. Apple puree-alginate edible coating as carrier of antimicrobial agents to prolong shelf-life of fresh-cut apples. Postharvest Biology and Technology, 45 (2): 254-264.

Rosen, J.C.; Kader, A.A. 1989. Postharvest physiology and quality maintenance of sliced pear and strawberry fruits. Journal of Food Science, 54 (3): 656-659.

Sabato, S.F.; Ouattara, B.; Yu, H.; D'Aprano, G.; Le Tien, C.; Mateescu, M.A.; Lacroix, M. 2001 Mechanical and barrier properties of crosslinked soy and whey protein based films. Journal of Agricultural and Food Chemistry, 49: 1397-1403.

Salvador, A.; Varela, P.; Fiszman, S. 2007. Consumer acceptability and shelf life of 'Flor de Invierno' pears (Pyrus communis L.) under different storage conditions. Journal of Sensory Studies, 22: 243-255.

Sánchez-Moreno, C. 2002. Review: Methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Science and. Technology International, 8: 121-137.

Sapers, G.M. 1996. Hydrogen peroxide as an alternative to chlorine. IFT Annual Meeting: Book of Abstracts. Institute of Food Technology, Chicago, Abstract 59-4.

Sapers, G.M.; Miller, R.L. 1998. Browning inhibition in fresh-cut pears. Journal Food Science, 63: 342-346.

Sapers, G.M.; Simmons, G.F. 1998. Hydrogen peroxide disinfection of minimally processed fruits and vegetables. Food Technology, 52(2): 48-52.

Sapers, G.M.; Miller, R.L.; Mattrazzo, A.M. 1999. Effectiveness of sanitizing agents in inactivating Escherichia coli in Golden Delicious apples. Journal of Food Science, 64(4): 734-737.

Senesi, E.; Pastine, R. 1996. Pre-treatments of ready-to-use fresh-cut fruits. Industrie Alimentari, 35: 1161-1166.

Senesi, E.; Galvis, A., Fumagalli, G. 1999. Quality indexes and internal atmosphere of packaged fresh-cut pears (Abate fetel and Kaiser varieties). Italian Journal of Food Science, 2: 111-120.

Schlimme, D.V. 1995. Marketing Lightly Processed Fruits and Vegetables. Hortscience, 30 (1): 15-17.

Shi, H.; Noguchi, N.; Niky, E. 2001. Natural antioxidants. In: Pokorny, J.; Yanishlieva, N.; Gordon, M. (Eds.). Antioxidants in food practical application. CRC press, Cambridge, UK, pp. 147-148.

Soliva-Fortuny, R.; Grigelmo-Miguel, N.; Odriozola-Serrano, I.; Gorinstein, S.; Martín-Belloso, O. 2001. Browning evaluation of ready-to-eat apples as affected by modified atmosphere packaging. Journal of Agricultural and Food Chemistry, 49: 3685-3690.

Soliva-Fortuny R.; Oms-Oliu, G.; Martín-Belloso, O. 2002a. Effects of ripeness stages on the storage atmosphere, color, and textural properties of minimally processed apple slices. Journal of Food Science, 67: 1958-1963.

Soliva-Fortuny, R.; Biosca-Biosca, M.; Grigelmo-Miguel, N.; Martín-Belloso, O. 2002b. Browning, polyphenol oxidase activity and headspace gas composition during storage of minimally processed pears using modified atmosphere packaging. Journal of the Science of Food and Agriculture, 82: 1490-1496.

Soliva-Fortuny, R.; Grigelmo-Miguel, N.; Hernando, I.; Lluch, M.A. and Martín-Belloso, O. 2002c. Effect of minimal processing on the textural and structural properties of fresh-cut pears. Journal of the Science of Food and Agriculture, 82: 1682-1688.

Soliva-Fortuny, R.; Martín-Belloso, O. 2003a. Microbiological and biochemical changes in minimally processed fresh-cut 'Conference' pears. European Food Research and Technology, 217:4-9.

Soliva-Fortuny, R.; Lluch, M.A.; Quiles, A.; Grigelmo-Miguel, N. and Martín-Belloso, O. 2003b. Evaluation of textural properties and microstructure during storage of minimally processed apples. Journal of Food Science, 68 (1): 312-317.

Soliva-Fortuny, R.; Alòs-Saiz, N.; Espachs-Barroso, A.; Martín-Belloso, O. 2004a. Influence of maturity at processing on quality attributes of fresh-cut Conference pears. Journal of Food Science. 69(7): 290-294.

Soliva-Fortuny, R.; Elez-Martínez, P.; Martín-Belloso, O. 2004b. Microbial and biochemical stability of fresh-cut apples preserved by modified atmosphere packaging. Innovative Food Science and Preserving Technologies, 5: 215-224.

Solomos, T. 1997a. Principles underlying modified atmosphere packaging. In; Wiley, R.C. (Ed.). Minimally processed refrigerated fruits & vegetables. Chapman and Hall, New York, U.S.A., pp 183-225.

Solomos, T.; Whitaker, B.; Lu, C. 1997b. Deleterious effects of pure oxygen on 'Gala' and 'Granny Smith' apples. HortScience, 32: 458.

Souza de, B.S.; O'Hare, T.J.; Durigan, J.F.; Souza de, P.S. 2006. Impact of atmosphere, organic acids, and calcium on quality of fresh-cut 'Kensington' mango. Postharvest Biology and Technology, 42: 161-167

Tapia, M.S.; Rodríguez, F.J.; Rojas-Graü, M.A.; Martín-Belloso, O. 2005. Formulation of alginate and gellan based edible coatings with antioxidants for fresh-cut apple and papaya. IFT Technical Program Abstracts, Paper 36E-43 - Annual Meeting, New Orleans, USA.

Tapia, M.S.; Rojas-Graü, M.A.; Rodríguez, F.J.; Ramírez, J.; Carmona, A.; Martín-Belloso, O. 2007. Alginate- and gellan-based edible films for probiotic on fresh-cut fruits. Journal of Food Science, 72 (4): 190-196

Turhan, K.N.; Sahbaz, A.; Güner, A. 2001. A spectrophotometric study of hydrogen bonding in methylcellulose-based edible films plasticized by polyethylene glycol. Journal of Food Science, 66: 59-62.

Ukuku, D.O.; Bari, M.L.; Kawamoto, S.; Isshiki, K. 2005. Use of hydrogen peroxide in combination with nisin, sodium lactate and citric acid for reducing transfer of bacterial pathogens form whole melon surfaces to fresh-cut pieces. International Journal of Food Microbiology, 104(2): 225-33.

Vachon, C.; Aprano, G.D.; Locroix, M.; Letendre, M. 2003. Effect of edible coating process and irradiation treatment of strawberry fragaria spp. on storage-keeping quality. Journal of Food Science, 68: 608-612.

Vamos-Vigyazo, L. 1995. Prevention of enzymatic browning in fruits and vegetables. A review of principles and practice; ACS symposium Series. Enzymatic Browning Symposium; American Chemical Society, Washington. 600, 49-62.

Van der Steen, L.; Jacxsens, L.; Devlieghere, F.; Debevere, J. 2002. Combining high oxygen atmospheres with low oxygen modified atmosphere packaging to improve the keeping quality of strawberries and raspberries. Postharvest Biology and Technology, 26: 49-58.

Varela, P.; Salvador, A.; Hernando, I.; Pérez-Munuera, I.; Quiles, A.; Lluch, M.A.; Fiszman, S. 2007a. Eating quality of 'Flor de Invierno' pears: chemical and structural aspects. International Journal of Food Science and Technology, 42: 1052-1058.

Varela, P.; Salvador, A.; Fiszman, S.M. 2007b. The use of calcium chloride in minimally processed apples: A sensory approach. European Food Research and Technology, 224: 461-467

Varoquaux, P. 1991. Ready-to-use fresh fruits and vegetables. Revue Générale du Froid, 81 (6): 33-43.

Villanueva, M.J.; Tenorio, M.D.; Esteban, M.A.; Mendoza, M.C. 2004. Compositional changes during ripening of two cultivars of muskmelon fruits. Food Chemistry, 87: 179-185.

Vinson, J.A.; Jang, J.; Dabbagh, Y.A. ; Serry, M.M. ; Cai, S. 1995. Plant polyphenols exhibit lipoprotein bound antioxidant activity using an in vitro oxidation model for heart disease. Journal of Agricultural and Food Chemistry. 43: 2798-2799.

Watada, A.E.; Ko, N.P.; Minott, D.A. 1996. Factors affecting quality of fresh-cut horticultural products. Postharvest Biology and Technology, 9: 115-125.

Wiley, R.C. 1994. Introduction to minimally processed refrigerated fruits and vegetables. In: R.C. Wiley (Ed.). Minimally processed refrigerated fruits and vegetables. Chapman & may, New York, pp. 1-14.

Whitaker, B.D.; Solomos, T.; Harrison, D.J. 1998. Synthesis and oxidation of α-farnesene during high and low O2 storage of apple cultivars differing in scald susceptibility. Acta Hortscience, 464: 165-170.

Wang, H.; Cao, G.; Prior, R.L. 1996. Total antioxidant capacity of fruits. Journal of Agricultural and Food Chemistry, 44: 701-705.

Wong, D.W.S.; Tillin, S.J.; Hudson, J.S.; Pavlah, A.E. 1994. Gas exchange in cut apples with bilayer coatings. Journal of Agricultural and Food Chemistry, 42: 2278-2285.

Wright, K.P.; Kader, A.A. 1997. Effect of slicing and controlled atmosphere storage on the ascorbate content and quality of strawberries and persimmons. Postharvest Biology and Technology, 10: 39-48.

Wszelaki, A.L.; Mitcham, E.J. 2000. Effects of superatmospheric oxygen on strawberry fruit quality and decay. Postharvest Biology and Technology, 20: 125-133.

Yang, S.F. 1981. Biosynthesis of ethylene and its regulation. In: Friend, J.; Rhodes, M.J.C. (Eds.). Recent advances in the biochemistry of fruit and vegetables. Academic Press, London, pp. 89-106.

Yang, L.; Paulson, A.T. 2000. Mechanical and water vapor barrier properties of edible gellan films. Food Research International, 33: 563-570.

Yaun, B.R.; Summer, S.S.; Eifert, J.D.; Marcy, J.E. 2004. Inhibition of pathogens on fresh produce by ultraviolet energy. International Journal of Food Microbiology, 90(1): 1-8.

Zhuang, H.; Barth, M.; Hankinson, T.R. 2003. Microbial safety, quality and sensory aspects of fresh-cut fruits and vegetables. In: Novak, J.S.; Sapers, G.M.; Juneja, V.K. (Eds.). Microbial Safety of Minimally Processed Foods. CRC Press, Boca Raton, London, New York, Washington, D.C., pp. 255-278.

2.Objetivos

2. Objetivos

El objetivo general de esta tesis fue evaluar distintas alternativas de envasado para la conservación de melón y pera frescos cortados. Se estudió el uso de concentraciones altas de O_2 como alternativa al envasado en concentraciones bajas de O_2 y altas de CO_2 o sin modificación de la atmósfera inicial. Además, el envasado de la fruta cortada se combinó con otros tratamientos de conservación tales como agentes antioxidantes y estabilizantes de textura. La investigación se complementó con la aplicación de diferentes recubrimientos comestibles a la fruta cortada como nuevo método de conservación y soporte de sustancias antioxidantes. Con esta finalidad, se plantearon los siguientes objetivos específicos:

→ Determinar el estado óptimo de madurez del melón 'Piel de Sapo' y pera 'Flor de Invierno' para su procesado mínimo.

→ Evaluar el efecto individual y combinado de distintos antioxidantes en el control del pardeamiento de pera 'Flor de Invierno' fresca cortada.

→ Estudiar el efecto de la aplicación de distintas alternativas de envasado en atmósfera modificada en la actividad fisiológica, estabilidad microbiológica, parámetros físico-químicos y propiedades antioxidantes de melón y pera frescos cortados.

→ Evaluar el efecto del uso de recubrimientos comestibles en la actividad respiratoria, estabilidad microbiológica, propiedades físico-químicas, antioxidantes y sensoriales de melón y pera frescos cortados.

3.Publicaciones

Capítulo I

Effect of ripeness on the shelf-life of fresh-cut melon preserved by modified atmosphere packaging

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Abstract

Fresh-cut 'Piel de Sapo' melon was processed at different ripeness stages and stored under modified atmosphere packaging for 35 days at 5 ºC. Raw material firmness and soluble solids content ranged from 6.5 to 3.9 N and 11.1 to 14.3 ºBrix, respectively. The effects of a 2.5 kPa O_2 + 7 kPa CO_2 packaging atmosphere and a dip of 1% ascorbic acid and 0.5% calcium chloride on physiology, microbiological stability as well as color and firmness were evaluated. An intermediate stage of ripeness at processing was the most suitable to extend the shelf-life of fresh-cut 'Piel de Sapo' melon. Green-mature fresh-cut melon reduced CO_2 accumulation and ethanol production. In addition, a treatment with ascorbic acid and calcium chloride in combination with modified atmosphere packaging, contributed to a greater extension of the shelf-life of fresh-cut melon than that reported for fruits stored under non modified atmosphere, slowing down the growth of microbial populations, maintaining the original color and reducing softness. Thus, the shelf-life of green-mature fresh-cut 'Piel de Sapo' melon dipped in an ascorbic acid and calcium chloride solution and packaged under modified atmosphere was about 10 days.

Keywords: fresh-cut melon; fruit ripeness; modified atmosphere packaging; microbial stability; softening; color

Introduction

'Piel de Sapo' melon (*Cucumis melo* L.) is a major commercial cultivar in Spain. The fruits are oval shaped with reticular greenish color skin and white sweet flesh. Like other members of the Cucurbitaceae, 'Piel de Sapo' is chilling injury sensitive. This physiological disorder appears when melons are stored at low temperature. Changes in membrane structure in response to chilling temperatures are considered as the primary events of chilling injury and lead to a loss of permeability and metabolic dysfunction. Secondary reactions include ethylene production, increased respiration or accumulation of toxic compounds such as ethanol and acetaldehyde (Valdenegro et al., 2004a).

Processing of fresh-cut fruits involves wounding stress as a result of mechanical injury when peeling or cutting, leading to an increase in the respiration rates of fresh-cut commodities in comparison to those of the corresponding whole fruits (Watada et al., 1996). Minimal processing damages the tissue integrity, leading to several biochemical deteriorations such as browning, off-flavours and texture breakdown (Varoquaux, 1991). Many factors may affect the physiological response of the fruit, including the cultivar, maturity stage, temperatures or packaging atmospheres.

The stage of ripeness appears to be essential to keep quality of fresh-cut produce and to extend their shelf-life. The more mature the fruit, the shorter the shelf-life of the fresh-cut commodity. Moreover, if the fruit is not sufficiently mature at processing the product will not reach the optimal sensorial attributes (Beaulieu et al., 2004). To our knowledge there are no data available comparing the physiological and microbiological response of fresh-cut melon processed at different stages of maturity.

It has been reported that the modification of package atmospheres, in concomitance with chemical treatments, effectively retards the detrimental phenomena that occur during fresh-cut processing. Low O_2 and/or high CO_2 atmospheres have been shown to reduce respiration, decrease ethylene production and inhibit or delay enzymatic reactions. A storage atmosphere of 2-5 kPa O_2 plus 10-15 kPa CO_2 at 0-5 ºC has been proved to maintain the quality of fresh-cut 'Cantaloupe' or 'Honeydew' melons (Bai et al., 2001; Bai et al., 2003). Fresh-cut 'Amarillo' melon maintained its fresh quality attributes under a passive modified atmosphere (MA) for 10 days at 5 ºC (Aguayo et al., 2003). Temperatures below 7-10 ºC are associated to the occurrence of chilling injury in intact melons. However, fresh-cut produce is recommended to be held at temperature near 5 ºC because

of a significant reduction in the metabolic rate of commodity at low temperatures. In addition, it has been reported lower microbial population and longer shelf-life of fresh-cut produce stored at 5 ºC rather than 10 ºC (Bai et al., 2003).

The addition of chemical agents is the most common way to control browning and softening phenomena. Some blends of additives have been proved to extend the storage life of fresh-cut produce. Dips containing ascorbic acid have been generally used to delay browning on cut fruit surfaces (Soliva-Fortuny et al., 2002a; Soliva-Fortuny et al., 2004). Ascorbic acid (AA) content is regarded as a quality indicator for fresh-cut fruits and vegetables. Because of its antioxidant activity it plays an important role in many metabolic pathways that have a direct impact on the oxidative stability of fruits (Lurie, 2003). On the other hand, treatments with calcium salts have been shown to be effective in preserving fresh-cut melon from softening (Luna-Guzmán et al., 1999). The action of calcium chloride ($CaCl_2$) treatments in fruits and vegetables may be generally attributed to its diffusion into the tissue and its complexion to polygalacturonic acid residues, present in the cell wall and middle lamella, to provide stabilization of membrane systems (Poovaiah, 1986).

In this work, we aimed to determine the effect of ripeness at processing of fresh-cut 'Piel de Sapo' melon preserved by modified atmosphere packaging and dipped in a solution containing ascorbic acid and calcium chloride on respiration rate, ethylene production, anaerobic metabolites, microbiological stability and quality attributes.

Materials and methods

Evaluation of fruit ripeness

'Piel de Sapo' melons, grown in Castilla-La Mancha (Spain), were harvested in August at a slightly under-ripe maturity stage. The fruits were grouped in three lots of 20 each, and stored in a ventilated room at 10ºC. At each storage interval, 3 melons in optimal conditions were removed from each group at random. They were peeled and cut into 8 sections parallel to the longitudinal axis. The rind and seeds were removed, and the pulp was sliced into small portions. Melon pieces were combined and three samples removed for raw fruit characterization, through determination of soluble solids content (Atajo RX-100 refractometer; Atago Company Ltd., Japan), total acidity (AOAC 2000), pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), pulp

color (Minolta CR-400 chroma meter; Konica Minolta Sensing, Inc., Osaka, Japan) and firmness (TA-XT2 Texture Analyzer; Stable Micro Systems Ltd., Surrey, England, UK).

Sample preparation

Melons were sanitized in a 200 ppm NaClO solution for 2 min, rinsed with tap water, and dried by hand. The fruits were sliced and cut to obtain trapezoidal sections. The fruit pieces were dipped for 1 min in a solution of 1% v/v L-ascorbic acid and 0.5% v/v calcium chloride at a product: solution ratio of 1:2. Control pieces were dipped into distilled water. The excess of water was completely drained and then, 100 g of melon pieces were packaged in polypropylene trays. The O_2 and CO_2 permeabilities of film were 110 $cm^3 \cdot m^{-2} \cdot day^{-1} \cdot bar^{-1}$ and 500 $g \cdot m^{-2} \cdot day^{-1} \cdot bar^{-1}$ at 23ºC and 0% RH, respectively (ILPRA Systems España, S.L. Mataró, Spain). The modification of the package atmosphere was carried out by flushing a mixture of 2.5 kPa O_2 + 7 kPa CO_2 (N_2 balanced) in a ratio melon: gas mixture of 1:2 (v/v) and thermosealing with a vacuum packing machine ILPRA Food Pack Basic V/6 (ILPRA Systems. CP. Vigevono, Italia). Trays were stored at 4 ± 1ºC in darkness and analyzed throughout 35 days of storage in duplicate.

Headspace gases analysis

The gas composition of the package headspace was determined by a gas analyzer (Micro-GC CP 2002, Chrompack International, Middelburg, The Netherlands) equipped with a thermal conductivity detector. A sample of 1.7 ml was automatically withdrawn from the headspace atmosphere with a pin-needle connected to the injection system. The determination of the oxygen concentration was carried out by injecting a sample of 0.25 µl to a CP-Molsieve 5Å column (4m x 0.35 mm, df=10 µm) at 60ºC and 100 kPa whereas a 0.33 µl portion was injected to a Pora-PLOT Q column (10m x 0.32 mm, df=10 µm) at 75 ºC and 200 kPa for carbon dioxide, ethylene and ethanol analysis.

Color and firmness evaluation

The color of fresh-cut melon was determined with a Minolta CR-400 chroma meter (Konica Minolta Sensing, Inc., Osaka, Japan). The equipment was set up for a D75 illuminant and 10º observer angle. Ten replicates from two packages were evaluated for each treatment. Three readings were obtained for each replicate by changing the sample position to get uniform color

measurements. CIE L* (lightness), a* (red-green) and b* (yellow-blue) color parameters were obtained through reflectance values. Color changes of processed melon were expressed as chroma (C*) and whiteness index (WI).

$$C^* = \left[(a^*)^2 + (b^*)^2\right]^{\frac{1}{2}} \quad \text{(Eq. 1)}$$

$$WI = 100 - \left[(100 - L^*)^2 + (a^*)^2 + (b^*)^2\right]^{\frac{1}{2}} \quad \text{(Eq. 2)}$$

Firmness evaluation was performed using a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, England, UK) by measuring the maximum penetration force. Cylindrical samples of 2.0 cm high were obtained from the trapezoidal melon pieces and were positioned to be penetrated by a 4 mm diameter rod through their geometric center. The downward distance was set at 10 mm at a rate of 5 mm/s and automatic return. Ten samples from two trays of fresh-cut melon were randomly withdrawn from each treatment to obtain representative readings.

Microbiological stability

The microbiological stability of fresh-cut 'Piel de Sapo' melon was evaluated through the determination of total mesophilic aerobic bacteria and yeast and mold populations. Two replicate counts were obtained each time from two packages at the same experimental condition. The analyses were carried out weekly for 35 days. A sample of 10 g melon was homogenized for 2 min with 90 ml of 0.1% sterile peptone solution with a Stomacher Lab Blender 400 (Seward medical, London, England). Serial dilutions of fruit homogenates were plated on plate count agar (PCA) at 30ºC ± 1ºC for 72 h ± 3 h for mesophilic aerobic bacteria counts (ISO, 1991) and chloramphenicol glucose agar (GCA) at 25ºC ± 1ºC for 5 days for yeast and mold counts (ISO, 1988).

Statistical analysis and mathematical modeling

Statistical analysis was performed using the Statgraphics plus v.5.1 software (Manugistics, Inc., Rockville, MA, USA). Data were analyzed by multifactor analysis of variance. The analysis of covariance was used to decide whether significant differences ($p < 0.05$) existed among treatments. The Duncan multiple range test was applied to determine differences among levels of each factor.

The growth of microorganisms as affected by the studied conditions was modeled according to a modification of the Gompertz equation [Equation 3], which has been proposed to predict the shelf-life of fresh-cut apple slices (Lanciotti et al., 1999).

$$y = k + A\exp\left\{-\exp\left[\left(\frac{\mu_{max} e}{A}\right)\cdot(\lambda - t)+1\right]\right\} \quad \text{(Eq. 3)}$$

where: k, initial count estimated by the model [log (cfu g^{-1})]; A, maximum growth attained at the stationary phase [log (cfu g^{-1})]; μ_{max}, maximal growth rate [Δlog (cfu g^{-1}) day^{-1}]; λ, lag time (days); t, storage time (days).

Results and discussion

Physicochemical characteristics of 'Piel de Sapo' melon

Melon maturity was determined on the basis of the pulp sugar content, total acidity (TA), pH, color and firmness. The soluble solids content (SSC) in melon flesh ranged from 11.1 to 14.3 ºBrix, depending on their physiological stage at processing. The sugar content followed an upward trend through ripening (Table 1). Villanueva et al. (2004) reported SSC of 11.0-15.5 ºBrix in moderately ripe or ripe 'Piel de Sapo' melons. These authors suggested minimum sugar content for this fruit of 8 ºBrix, since the melons are not usually fit for consumption below that level.

Table 1. Physicochemical characteristics of melon before processing [a]

	Ripeness stage		
	Green	*Green-mature*	*Mature*
Soluble solids (ºBrix)	11.1±0.3	13.45±0.07	14.30±0.14
Total acidity (g citric acid / 100 g)	0.302±0.003	0.288±0.009	0.284±0.006
pH	5.880±0.014	5.95±0.08	5.99±0.04
Pulp color			
L*	61.71±0.19	60.2±0.7	59.0±1.5
a*	-1.79±0.07	-1.76±0.09	-1.256±0.009
b*	6.1±0.3	5.9310±0.0007	4.77±0.15
Whiteness index (WI)	61.09±0.13	59.8±0.7	57.74±0.08
Chroma (C*)	6.4±0.3	5.684±0.022	4.93±0.15
Firmness (N)	6.49±1.08	3.97±1.05	3.9±1.4

[a] Mean ± standard deviation

Changes in total acidity, pulp color and flesh firmness indicated composition changes in fruit occurring during ripening process. The ripening process from green to green-mature melon involved a decrease in TA and an increase of pH (Table 1). The pH values in green-mature or

mature stages were about 6, in agreement with those reported by Villanueva et al. (2004) for moderately ripe and fully ripe 'Piel de Sapo' melon. Aguayo et al. (2004) reported lower initial TA in 'Galia' and 'Cantaloupe' than in 'Amarillo' or 'Piel de Sapo' fresh-cut melon.

Table 1 shows that color changes on melon flesh through ripening were due to a decrease in a* and b* values whereas no significant differences in lightness (L*) were detected. The more advanced the fruit maturity at processing, the greater decrease experienced by C* (Eq. 1) and WI (Eq. 2) parameters. This depletion was also reported by Aguayo et al. (2004) as a consequence of translucency injury on fresh-cut melon, which has been found to be related to a physiological disorder exhibited as symptom of senescence. Translucency may be a consequence of an advanced stage of ripeness, treatments that accelerate ripening or high storage temperatures.

The firmness significantly depleted from 6.5 to 3.9 for green and green-mature melons (Table 1). Fruit ripening is characterized by tissue softening, that is associated with the enzymatic degradation of cell wall compounds like pectic substances and neutral detergent fiber (NDF) such as cellulose, hemicelluloses and lignin. A slight decrease of NDF content as well as degradation of pectic substances at final stage of ripening of 'Piel de Sapo' melon has been reported by Villanueva et al. (2004).

Package atmosphere composition

Oxygen and carbon dioxide partial pressures in trays headspace throughout storage of fresh-cut melon are shown in Figures 1 and 2. The oxygen consumption of fresh-cut melon was influenced neither by the fruit ripeness condition at processing nor by dips of 1% AA and 0.5% $CaCl_2$. On the other hand, the composition of packaging atmosphere significantly affected O_2 concentration inside packages through storage ($p<0.001$). The O_2 concentration in packages that were initially flushed with 2.5 kPa O_2 + 7 kPa CO_2 depleted to levels below 1 kPa O_2 beyond 14 days storage in all the assayed conditions and treatments (Fig. 1A and B). On the other hand, O_2 levels progressively decreased under non MA but concentrations above 1kPa were maintained throughout storage (Fig. 1C and D).

The stage of ripeness at processing had a significant effect on CO_2 accumulation inside the packages throughout storage ($p<0.001$). Thus, the more advanced the state of ripeness at processing, the higher the CO_2 production (Fig. 2). Mature melon evolved more CO_2 than green and green-mature melons, especially beyond 17 days of storage. Mature melon packaged under

non MA and without dipping treatment exhibited a maximum CO_2 accumulation of 45.5 kPa at the end of storage (Fig. 2D). Enhanced CO_2 production may be due to microbial growth and a general deterioration of the tissue of fresh processed melon (Villanueva et al., 2004). A dipping treatment of 1% AA and 0.5% $CaCl_2$ was found to slow down the CO_2 accumulation under non MA packaging ($p<0.001$). According to these results, a low temperature calcium treatment during processing of fresh-cut 'Cantaloupe' melon has been shown to effectively retard CO_2 production (Luna-Guzmán et al., 1999; Lamikanra and Watson, 2004).

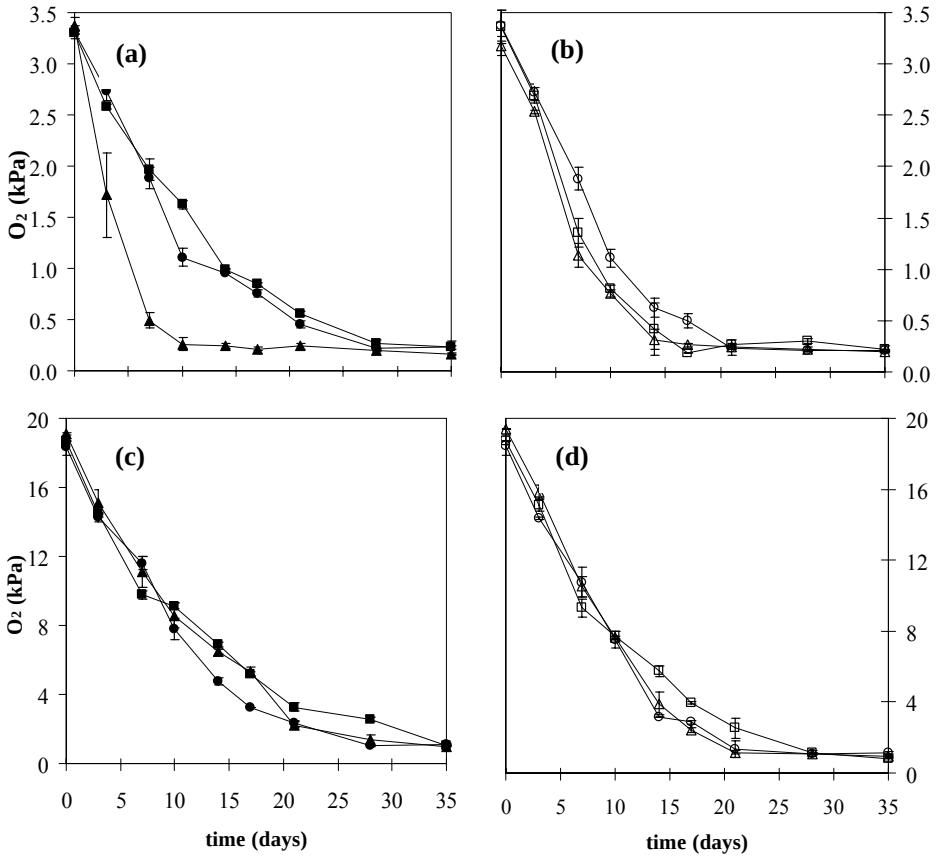

Figure 1. Package headspace O_2 concentrations of fresh-cut 'Piel de Sapo' melon processed at different ripeness stages. (a), (b): packaged under 2.5 kPa O_2 + 7 kPa CO_2 conditions; (c), (d): packaged under non MA. (a), (c): dipped into a 1% ascorbic acid and 0.5% $CaCl_2$ solution (• green, ■ mature-green, ▲mature); (b), (d): control (o green, □ mature-green, Δ mature).

Low O_2 levels may cause a risk of anaerobic fermentation and thus, minimum 1 kPa O_2 and maximum 15 kPa CO_2 have been suggested as safe levels to prevent toxicity or anoxia inside packages of fresh-cut melon (Bai et al., 2003). An atmosphere composition of 4 kPa O_2 and 12-13 kPa CO_2 was reached in packages of fresh processed 'Amarillo' melon stored under non MA at 5 ºC for 14 days. This packaging condition was effective to keep sensorial quality and microbial safety and to avoid loss of weight and translucency (Aguayo et al., 2004). Similar O_2 and CO_2 concentrations were reached in packages of fresh-cut 'Piel de Sapo' melon stored under non MA after 14 days of storage at 5 ºC (Fig. 1C and D).

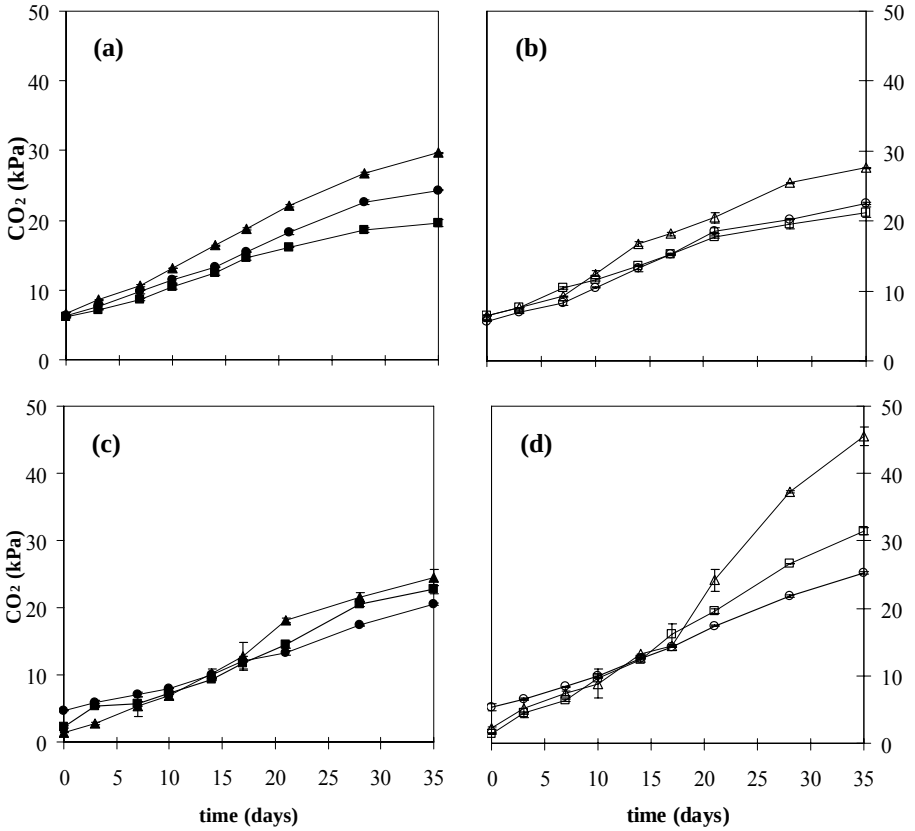

Figure 2. Package headspace CO_2 concentrations of fresh-cut 'Piel de Sapo' melon processed at different ripeness stages. (a), (b): packaged under 2.5 kPa O_2 + 7 kPa CO_2 conditions; (c), (d): packaged under non MA. (a), (c): dipped into a 1% ascorbic acid and 0.5% $CaCl_2$ solution (● green, ■ mature-green, ▲mature); (b), (d): control (o green, □ mature-green, Δ mature).

Fresh-cut 'Piel de Sapo' melon evolved small amounts of ethylene thus indicating a low physiological activity. Neither the stage of ripeness at processing nor the dipping treatment affected significantly ethylene levels inside the packages ($p>0.05$). On the other hand, ethylene production inside packages stored under non MA was higher than under 2.5 kPa O_2 + 7 kPa CO_2 conditions ($p<0.05$). Ethylene levels reached values of 0.5 ppm and 0.8 ppm in packages stored under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere and non MA, respectively (Fig. 3). Melon cultivars such as 'Cantaloupe' have exhibited a climacteric pattern of ethylene production during ripening (Hadfield et al., 1995). However, 'Piel de Sapo' melon shows a low physiologic activity and has been reported to exhibit a non climacteric ripening pattern (Valdenegro et al., 2004b).

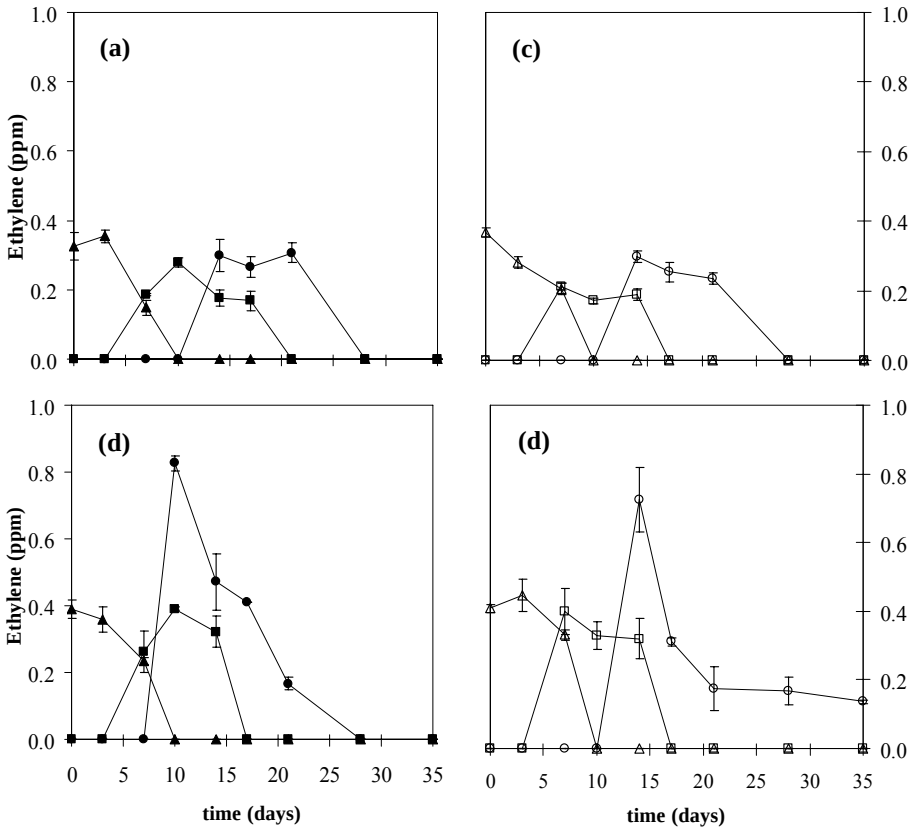

Figure 3. Package headspace ethylene concentrations of fresh-cut 'Piel de Sapo' melon processed at different ripeness stages. (a), (b): packaged under 2.5 kPa O_2 + 7 kPa CO_2 conditions; (c), (d):

packaged under non MA. (a), (c): dipped into a 1% ascorbic acid and 0.5% $CaCl_2$ solution (• green, ■ mature-green, ▲mature); (b), (d): control (o green, □ mature-green, Δ mature).

Ethanol content in packages of green fresh-cut 'Piel de Sapo' melon increased throughout time, reaching maximum concentrations ranging from 80 to 125 ppm (Fig. 4).

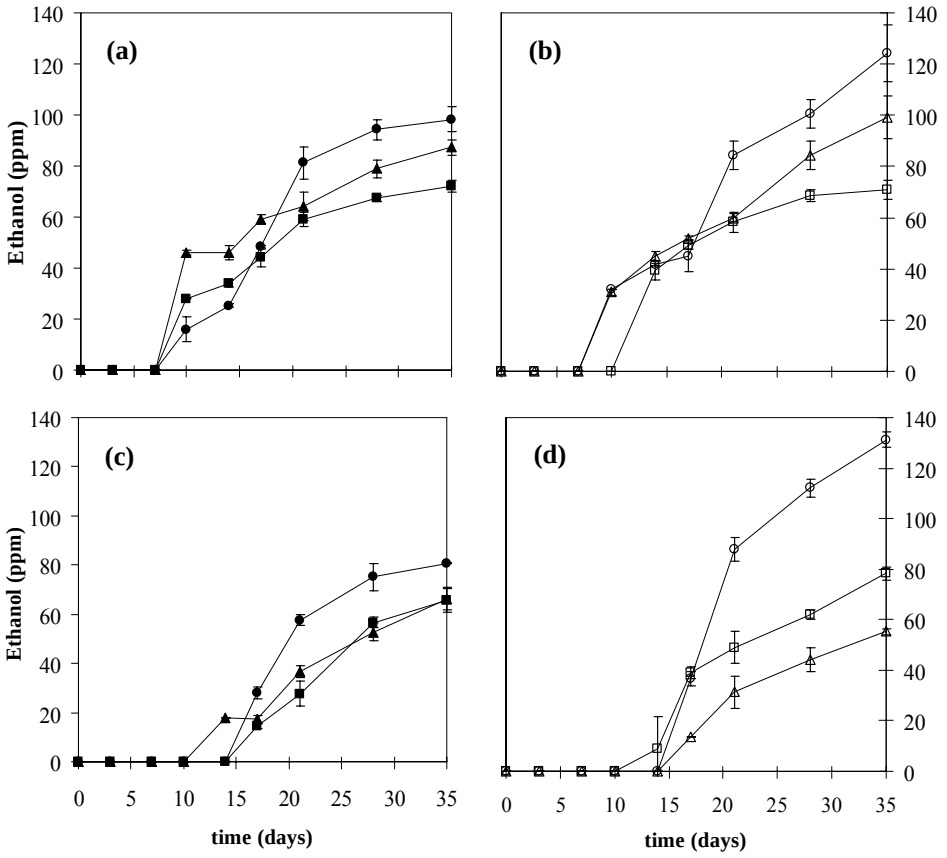

Figure 4. Package headspace ethanol concentrations of fresh-cut 'Piel de Sapo' melon processed at different ripeness stages. (a), (b): packaged under 2.5 kPa O_2 + 7 kPa CO_2 conditions; (c), (d): packaged under non MA. (a), (c): dipped into a 1% ascorbic acid and 0.5% $CaCl_2$ solution (• green, ■ mature-green, ▲mature); (b), (d): control (o green, □ mature-green, Δ mature).

Irrespective of packaging conditions or dipping treatment, fermentative pathways were triggered with higher intensity in green melons, which suggests that slightly under-ripe fruits are more

susceptible to undergoing anaerobic metabolism. The enhanced increase of ethanol content in green ripeness stage beyond the 21 day of storage could be due to a stress response that may lead to a loss of membrane integrity. In fact, physiological disorders and membrane damage in fruits are attributed to the effects of stressful levels of too-low oxygen or too-high carbon dioxide (Lester, 2003). Green-mature fresh-cut melon exhibited the least accumulation of ethanol at the end of storage. These results agreed with those reported by Soliva-Fortuny et al. (2004) in fresh-cut 'Conference' pears. Moreover, ethanol accumulation further increased under 2.5 kPa O_2 + 7 kPa CO_2 atmospheres in comparison to non MA, regardless of the dipping treatment ($p<0.001$). Ethanol production was avoided under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere during the first 10 days of storage. On the other hand, packages of fresh-cut melon stored under initial non MA were kept 14 days without ethanol accumulation. Anaerobic metabolism may be promoted by a low availability of O_2 and high accumulation of CO_2 in packages preserved under initial 2.5 kPa O_2 + 7 kPa CO_2 atmospheres. Lakakul et al. (1999) pointed out the importance of maintaining O_2 level just above the fermentation threshold and keeping CO_2 concentration below the range that causes injury to fruits.

Color and firmness evolution

Color evolution on fresh-cut 'Piel de Sapo' throughout storage was mostly due to L* and WI depletion (Table 2). Aguayo et al. (2004) reported that WI decreased when translucency injury increased on fresh processed melon, as a consequence of a physiological disorder characterized by dark and glassy flesh. A dip containing AA and $CaCl_2$ was effective ($p<0.001$) to reduce translucency in packages of green-mature and mature melon. On the other hand, translucency was not observed on cut surfaces of green melons throughout storage. It seems that translucency is a symptom of senescence attributed to advanced stages of ripeness (Bai et al., 2001). In addition, greater percentage of translucency was observed on fresh-cut 'Piel de Sapo' melon stored under 2.5 kPa O_2 + 7 kPa CO_2 atmospheres than under non MA conditions. The deterioration of fresh-cut 'Honeydew' or 'Cantaloupe' melon packaged under MA has been found to be primarily due to the development of translucency (Bai et al., 2001, 2003; Aguayo et al., 2003, 2004, O'Connor-Shaw et al., 1994).

Table 2. Changes in lightness and whiteness index of fresh-cut 'Piel de Sapo' melon processed at different ripeness stages and packaged under 2.5 kPa O_2 + 7 kPa CO_2 and non MA over 35 days at 4 ºC

2.5%O_2+7%CO_2

	Green				Green- Mature				Mature			
	AA + $CaCl_2$		Control		AA + $CaCl_2$		Control		AA + $CaCl_2$		Control	
Days	L*	WI	L*	WI	L*	WI	L*	WI	L*	WI	L*	WI
0	62.35a*	62.05a	54.39a	54.25a	51.55a	51.47a	44.82a	44.63a	54.17a	54.08a	59.52a	59.25a
7	63.35a	62.95a	53.82a	53.74a	52.15a	52.07a	45.21a	45.07a	53.07a	52.97a	59.36a	59.12a
14	63.74a	63.32a	54.65a	54.58a	52.11a	52.03a	46.69ab	46.50ab	54.57a	54.47a	59.16a	58.91a
28	62.90a	62.51a	54.69a	54.63a	52.55a	52.47a	48.69bc	48.45bc	53.70a	53.66a	59.80a	59.53a
35	62.30a	61.90a	54.25a	54.16a	52.63a	52.55a	50.28c	50.10c	53.88a	53.85a	55.91b	55.69b

Non MA

	Green				Green-Mature				Mature			
	AA + $CaCl_2$		Control		AA + $CaCl_2$		Control		AA + $CaCl_2$		Control	
Days	L*	WI	L*	WI	L*	WI	L*	WI	L*	WI	L*	WI
0	60.67a	60.38a	61.45a	61.20a	58.23a	57.96a	53.97ab	53.70ab	60.77a	60.39a	59.64ab	59.37ab
7	61.73a	61.43a	59.54a	59.26a	58.04a	57.78a	51.71a	51.47a	61.10a	60.68a	57.86a	57.61a
14	61.00a	60.75a	57.88a	57.63a	57.47a	57.19a	55.39b	55.13b	60.35a	59.91a	60.18b	59.91b
28	59.80a	59.48a	59.55a	59.33a	60.42a	60.17a	60.49c	60.18c	60.44a	60.00a	63.08c	62.80c
35	57.37a	57.07a	59.41a	59.16a	60.09a	59.85a	63.61d	63.31d	59.16a	58.70a	63.42c	63.11c

MA, modified atmosphere, AA, ascorbic acid; L*, lightness; WI, whiteness index

* Data are the means of ten replications

Different letters in the same column indicate that mean values are significantly different by Duncan's multiple-range test ($p < 0.05$)

Softening of fresh-cut melon differed significantly among ripeness conditions ($p<0.001$). Green fresh-cut melon underwent a dramatic decrease in firmness (Table 3) as a consequence of physiological stress. Such loss of firmness could be correlated with an enhanced accumulation of ethanol in green melon packages (Figure 4). Packaging with 2.5 kPa O_2 + 7 kPa CO_2 atmospheres in concomitance with dipping treatment reduced softening of green and green-mature melons ($p<0.05$) but could not further delay senescence in mature melon. Recent studies have reported the effectiveness of a dip of 1% AA and 0.5% $CaCl_2$ combined with packaging under a low O_2 atmosphere to maintain firmness of fresh-cut apples and pears during several weeks (Soliva-Fortuny et al., 2002b, 2004). Restricted oxygen concentrations appear to inhibit many biochemical reactions which may lead to a loss of cellular integrity. In addition, calcium salts interact with pectin to form a cross-linked polymer network that increases mechanical strength, thus delaying senescence and controlling physiological disorders in fruits and vegetables (Soliva-Fortuny et al., 2003a). Calcium chloride (1 – 5%) dips for 1-5 min provided a firming effect on fresh-cut 'Cantaloupe' melon stored at 5 ºC (Luna-Guzmán et al., 1999).

Table 3. Changes in firmness (N) of fresh-cut 'Piel de Sapo' melon processed at different ripeness stages and packaged under 2.5 kPa O_2 + 7 kPa CO_2 or non MA over 28 days at 4 ºC

2.5%O_2+7%CO_2						
	Green		*Green-mature*		*Mature*	
Days	AA + $CaCl_2$	Control	AA + $CaCl_2$	Control	AA + $CaCl_2$	Control
0	4.17±0.08a*	4.30±0.03a	3.356±0.024a	3.37±0.06a	3.49±0.14a	3.41±0.08a
7	3.37±0.14b	3.403±0.109b	3.45±0.19a	3.42±0.05a	3.0±0.4b	3.36±0.05a
14	3.06±0.08c	3.03±0.07c	2.96±0.09b	2.76±0.06b	2.61±0.09c	2.79±0.03b
28	2.19±0.08d	2.39±0.16d	3.06±0.15b	2.35±0.12c	2.31±0.12d	2.10±0.05c
35	2.26±0.07d	1.79±0.25e	3.01±0.14b	2.50±0.09c	2.31±0.12d	2.17±0.18c
Non MA						
	Green		*Green-mature*		*Mature*	
Days	AA + $CaCl_2$	Control	AA + $CaCl_2$	Control	AA + $CaCl_2$	Control
0	4.21±0.04a	4.18±0.04a	3.38±0.06a	3.348±0.002a	3.93±0.07a	2.77±0.07a
7	2.72±0.04b	2.152±0.003b	3.29±0.13a	2.7±0.6b	3.39±0.04b	2.78±0.09a
14	2.64±0.13b	2.146±0.016c	3.19±0.08a	2.79±0.22b	3.04±0.07c	2.70±0.14a
28	2.49±0.08c	1.39±0.05d	2.47±0.14b	1.82±0.21c	2.81±0.07d	2.7±0.3a
35	2.453±0.006c	1.07±0.05e	2.241±0.014c	1.638±0.202c	2.619±0.006e	2.4±0.2b

MA, modified atmosphere, AA, ascorbic acid

Different letters in the same column indicate that values are significantly different by Duncan's multiple-range test ($p< 0.05$)

* Data are the means of ten replications

Microbial stability and shelf life modeling

Initial counts of aerobic mesophilic microorganisms on just processed green or green-mature 'Piel de Sapo' melons were approximately 3 log (cfu g^{-1}) whereas mature melon loads were about 6 log (cfu g^{-1}). Too-soft initial texture of raw material may be responsible for the appearance of exudates on fruit cut surface at processing, which contain some compounds that may be used to native or exogenous microorganisms to grow on the product surface rapidly (Soliva-Fortuny et al., 2003b). On the other hand, populations of yeasts and molds for all ripeness conditions ranged from 1 to 2.5 log (cfu g^{-1}) at the beginning of storage. Our results for green-mature fresh-cut melon were similar to those found by Bai et al. (2001) for fresh-cut 'Cantaloupe' melons at a similar stage of ripeness. These authors reported initial bacteria counts of 3.2 log (cfu g^{-1}) under 4 kPa O_2 + 10 kPa CO_2 atmospheres and non MA. Under these packaging conditions, yeast and molds counts were less than 1.9 log (cfu g^{-1}).

Packaging atmosphere and ripeness state at processing affected significantly to microbiological counts during refrigerated storage ($p<0.05$). The more advanced the stage of ripeness, the higher the microbiological counts (Fig. 5). Tables 4 and 5 show the kinetic constants estimated by a modification of the Gompertz model (Zwietering et al., 1990) (Eq. 3) to describe the growth of aerobic mesophilic microorganisms and fungi on fresh-cut melon. The maximal growth rate of mesophilic aerobic microorganisms (μ_{max}) was higher than that of yeasts and molds except for mature fresh-cut melon (Figs. 4 and 5). Bai et al. (2001) reported that bacterial populations in fresh-cut 'Cantaloupe' stored under a 4 kPa O_2 + 10 kPa CO_2 atmosphere or non MA exhibited faster growth than yeasts and molds.

Low oxygen atmospheres generally inhibit the growth of aerobic microorganisms (Bai et al., 2001). A low O_2 atmosphere combined with a dipping treatment reduced the proliferation of aerobic mesophilic microorganisms on green or green-mature fresh-cut 'Piel de Sapo' melon ($p<0.05$). Counts were particularly low on green fresh-cut melon for 14 days (Fig. 5A). On the other hand, melon processed at the most advanced ripeness exhibited the highest counts under all conditions, exceeding counts of 6 log (cfu g^{-1}) at the beginning of storage (Fig. 5). The United States and most European countries have regulations relative to fresh-cut produce, which limit the counts of aerobic microorganisms to 6 log (cfu g^{-1}) at expiration date (Martín-Belloso et al., 2006). Table 4 shows that a dipping treatment combined with a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere on green or green-mature melons reduced the maximal growth rate (μ_{max}) and the counts attained at

the stationary phase (A). The lag phase period (λ) of aerobic mesophilic microorganisms on green and green-mature fresh-cut melon was around 7-10 days.

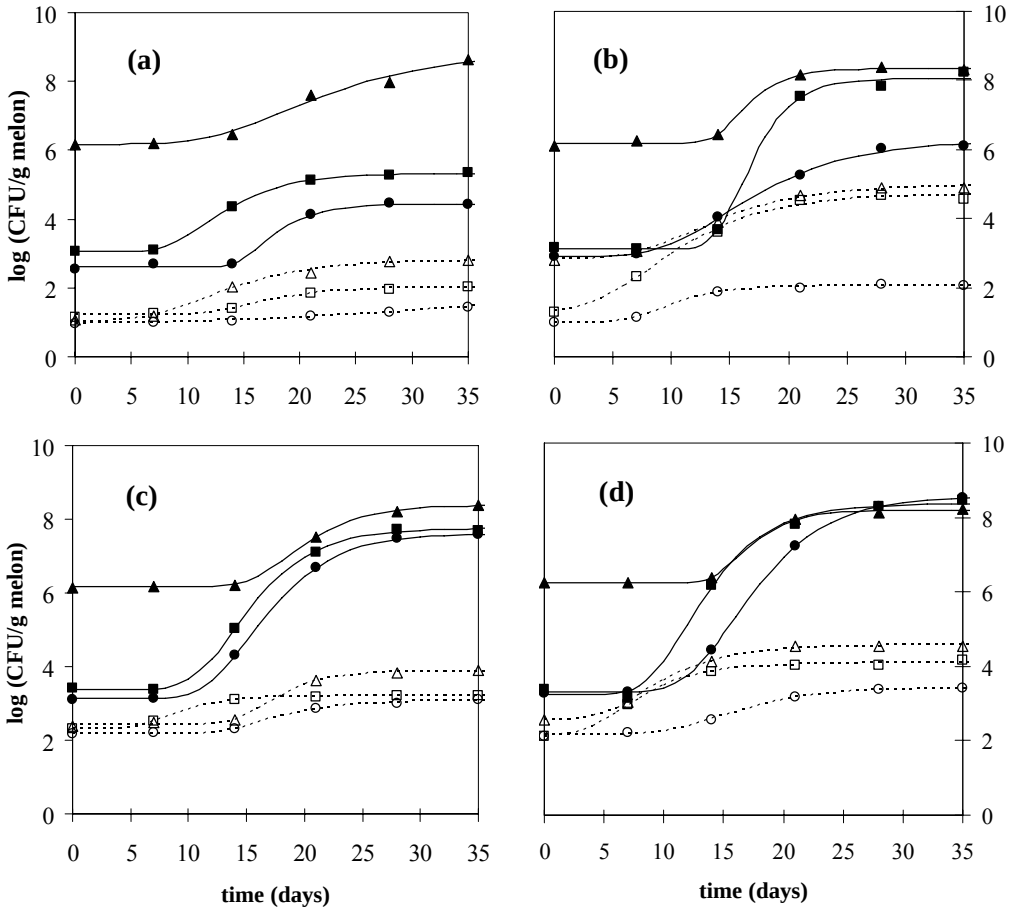

Figure 5. Microbial growth on fresh-cut 'Piel de Sapo' melon processed at different ripeness stages: Aerobic mesophilic microorganisms (• green, ■ mature-green, ▲mature); Yeast and moulds (o green, □ mature-green, Δ mature). (a), (b): packaged under 2.5 kPa O_2 + 7 kPa CO_2; (c), (d): packaged under non MA. (a), (c): dipped into 1% ascorbic acid and 0.5% $CaCl_2$ solution (• green, ■ mature-green, ▲mature); (b), (d): control melon (o green, □ mature-green, Δ mature).

Yeasts and molds were less numerous than bacteria on fresh-cut 'Piel de Sapo' melon throughout storage (Fig. 5), which was also reported on fresh-cut 'Cantaloupe' (Bai et al., 2001) and on other

fresh-cut produce (Nguyen-The and Cartin, 1994). Initial counts grew slightly on green melons compared to fruits processed at more advanced ripeness ($p<0.05$) (Fig. 5). Low O_2 atmospheres combined with AA and calcium treatment led to a significant reduction of maximal growth rate of fungi in comparison with the other studied conditions (Table 5). However, the counts attained at the stationary phase were not significantly affected by the different packaging conditions. The lag phase of fresh-cut melon dipped in a solution of AA and calcium chloride was lengthened to a minimum of 5 days whereas that of untreated samples was less than 5 days, regardless of the atmosphere conditions (Table 5). Without adding calcium, the decrease of membrane strength and integrity would cause a decompartmentalization of enzymes and substrates leading to a rise in fluids and solute exchanges and thus, promoting the proliferation of microorganisms.

Conclusions

Ripeness stage at processing is a limiting factor for the shelf-life of fresh-cut 'Piel de Sapo' melon. Green-mature fresh-cut melon exhibited lower rate of CO_2 and ethanol accumulation than mature melon. Low O_2 atmospheres in combination with AA and $CaCl_2$ treatments significantly reduced CO_2 accumulation and ethylene production as well as reduced the maximal growth rate of bacteria, yeasts and molds, except for fully ripe melon which exhibited the highest initial counts. The dipping treatment combined with packaging under 2.5 kPa O_2 + 7 kPa CO_2 atmospheres was also effective to prevent the development of translucency and softening of green or green-mature fresh-cut melon. In addition, low O_2 atmospheres prevented the production of fermentative metabolites like ethanol for 10 days. Therefore, for commercial purposes, a shelf-life of 10 days is suggested for green-mature fresh-cut 'Piel de Sapo' melon dipped in 1% AA and 0.5% $CaCl_2$ solution and packaged under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere.

Acknowledgements

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Table 4. Kinetic constants estimated by a modification of the Gompertz model in order to describe the microbial growth of aerobic mesophilic microorganisms of fresh-cut 'Piel de Sapo' melon, processed at different ripeness stage and packaging conditions

MA packaging	*Aerobic mesophilic microorganisms*									
	AA + $CaCl_2$					Control				
	k	A	μ_{max}	λ	R^2	k	A	μ_{max}	λ	R^2
2.5%O_2+7%CO_2										
Green	2.61±0.21	1.8±0.3	0.26±0.15	14.2±3.3	99.43	2.8±0.3	3.4±0.6	0.22±0.08	8±4	99.69
Green-mature	3.05±0.08	2.28±0.11	0.22±0.04	7.8±1.3	99.97	3.1±0.5	4.9±0.1	0.7±0.4	13.49±2.02	99.49
Mature	6.17±0.6	2.64±0.25	0.15±0.015	11.59±2.09	96.436	6.18±0.24	2.1±0.3	0.3±0.26	13.4±1.8	99.48
non MA										
Green	3.13±0.06	4.498±0.108	0.41±0.03	11.2±0.4	99.99	3.3±0.09	5.29±0.15	0.4±0.04	11.55±0.48	99.98
Green-mature	3.39±0.23	4.4±0.4	0.44±0.15	10.1±1.9	99.87	3.2±0.8	5.1±0.3	0.5±0.6	8±8	99.14
Mature	6.16±0.08	2.23±0.16	0.23±0.06	14.9±1.8	99.93	6.25±0.14	1.94 ±0.21	0.29±0.11	13.9±1.6	99.78

MA, modified atmosphere; AA, ascorbic acid

k, initial counts estimated by the model [log (cfu g^{-1})]; A, maximum growth attained at the stationary phase [log (cfu g^{-1})]; μ_{max}, maximal growth rate [Δlog (cfu g^{-1}) day^{-1}]; λ: lag time (days)

Table 5. Kinetic constants estimated by a modification of the Gompertz model in order to describe the microbial growth of yeasts and moulds of fresh-cut 'Piel de Sapo' melon, processed at different ripeness stage and packaging conditions

MA packaging	*Yeasts and moulds*									
	AA + $CaCl_2$					Control				
	k	A	μ_{max}	λ	R^2	k	A	μ_{max}	λ	R^2
2.5%O₂+7%CO₂										
Green	0.97±0.04	0.8±0.7	0.020±0.003	12±4	99.76	0.99±0.21	1.06±0.26	0.12±0.08	6±3	98.95
Green-mature	1.20±0.14	0.81±0.24	0.07±0.06	11±5	98.57	1.3±0.8	3.44±1.13	0.22±0.15	3±7	98.78
Mature	1.0±0.4	1.8±0.5	0.12±0.08	6±1	98.76	2.8±0.3	2.1±0.4	0.14±0.06	6±4	99.56
non MA										
Green	2.18±0.14	0.89±0.24	0.09±0.07	12±4	98.77	2.16±0.19	1.3±0.3	0.10±0.08	10±6	98.98
Green-mature	2.30±0.03	0.89±0.04	0.12±0.02	5.3±0.5	99.96	2.1±0.3	1.9±0.3	0.2±0.14	3±4	99.44
Mature	2.42±0.18	1.45±0.28	0.18±0.11	14±3	99.31	2.54±0.22	2.02±0.27	0.18±0.07	4.4±2.2	99.67

MA, modified atmosphere; AA, ascorbic acid

k, initial counts estimated by the model [log (cfu g^{-1})]; A, maximum growth attained at the stationary phase [log (cfu g^{-1})]; μ_{max}, maximal growth rate [Δlog (cfu g^{-1}) day^{-1}]; λ: lag time (days)

References

Aguayo, E.; Allende, A.; Artés, F. 2003. Keeping quality and safety of minimally fresh processed melon. European Food Research and Technology, 216: 494-499.

Aguayo, E.; Escalona, V.H.; Artés, F. 2004. Metabolic behaviour and quality changes of whole and fresh processed melon. *Journal of Food Science*, 69 (4): 148-155.

Bai, J.H.; Saftner, R.A.; Watada, A.E.; Lee, Y.S. 2001. Modified atmosphere maintains quality of fresh-cut cantaloupe (*Cucumis melo L.*). *Journal of Food Science*, 66 (8): 1207-1211.

Bai, J.; Saftner, R.A.; Watada, A.E. 2003. Characteristics of fresh-cut honeydew (*Cucumis xmelo L.*) available to processors in winter and summer and its quality maintenance by modified atmosphere packaging. *Postharvest Biology and Technology*, 28: 349-359.

Beaulieu, J.C.; Ingram, D.A.; Lea, J.M.; Bett-Garber, K.L. 2004. Effect of harvest maturity on the sensory characteristics of fresh-cut cantaloupe. *Journal of Food Science*, 69 (7): 250-254.

Hadfield, K.A.; Rose, J. K. C.; Bennett, A.B. 1995. The respiratory climacteric is present in Charentais (*Cucumis melo* cv. Reticulatus FI Alpha) melons ripened on or off the plant. *Journal of Experimental Botany*, 46: 1923-1925.

ISO 4833, 1991. Methods for Microbiological Examination of Food and Animal Feeding Stuffs. Enumeration of Micro-organisms. Colony Count Technique at 30 ºC. Geneva, Switzerland: International Standards Organisation

ISO 7954, 1988. General guidance for enumeration of yeasts and moulds. Colony count technique at 25 ºC. Geneva, Switzerland: International Standards Organisation

Lakakul, R.; Beaudry, R.; Hernandez, R.J. 1999. Modeling respiration of apple slices in modified-atmosphere packages. *Journal of Food Science*, 64 (1): 105-110.

Lamikanra, O.; Watson, M.A. 2004. Effect of Calcium Treatment Temperature on Fresh-cut Cantaloupe Melon during Storage. *Journal of Food Science*, 69(6): 468-472.

Lanciotti, R.; Corbo, M.R.; Gardini, F.; Sinigaglia, M.; Guerzoni, M.E. 1999. Effect of hexanal on the shelf-life of fresh apple slices. *Journal of Agricultural and Food Chemistry*, 47 (11): 4769-4776.

Lester, G.E. 2003 Oxidative stress affecting fruit senescence. In: Hodges, D.M. (Ed.). *Postharvest oxidative stress in horticultural crops*. Food Products Press, New York, USA, pp. 113-129.

Luna-Guzmán, I.; Cantwell, M.; Barreto, D. 1999. Fresh-cut cantaloupe: effects of $CaCl_2$ dips and heat treatments on firmness and metabolic activity. *Postharvest Biology and Technology*, 17: 201-213.

Luna-Guzmán, I.; Barrett, D.M. 2000. Comparison of calcium chloride and calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupes. *Postharvest Biology and Technology*, 19: 61-72.

Lurie, S. 2003. Antioxidants. In: Hodges, D.M. (Ed.). *Postharvest oxidative stress in horticultural crops*. Food Products Press, New York, pp. 131-150.

Martín-Belloso, O.; Soliva-Fortuny, R.; Oms-Oliu, G. 2006. Fresh-cut fruits. In: Hui, Y.H. (Ed.). *Handbook of fruits and fruit processing*. Blackwell Publishing, Ames, Iowa, USA, pp. 129-144.

Nguyen-the, C.; Carlin, F. 1994. The microbiology of minimally processed fresh fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, 34: 371-401.

O'Connor-Shaw, R.E.; Roberts, R.; Ford A.L. and Nottingham, S.M. 1994. Shelf-life of minimally processed Honeydew, kiwifruit, papaya, pineapple, and Cantaloupe. *Journal of Food Science*, 59: 1202-1206.

Poovaiah, B.W. 1986. Role of calcium in prolonging storage life of fruits and vegetables. *Food Technology*, 40(5): 86-9.

Soliva-Fortuny, R.C.; Oms-Oliu, G., Martín-Belloso, O. 2002a. Effects of ripeness stages on the storage atmosphere, colour, and textural properties of minimally processed apple slices. *Journal of Food Science*, 67(5): 1958-1963.

Soliva-Fortuny, R.C.; Grigelmo-Miguel, N.; Hernando, I.; Lluch, M.A.; Martín-Belloso, O. 2002b. Effect of minimal processing on the textural and structural properties of fresh-cut pears. *Journal of the Science of Food and Agriculture*, 82: 1682-1688.

Soliva-Fortuny, R.C.; Lluch, M.A.; Quiles, A.; Grigelmo-Miguel, N.; Martín-Belloso, O. 2003a. Evolution of textural properties and microstructure during storage of minimally processed apples. *Journal of Food Science*, 68 (1): 312-317.

Soliva-Fortuny, R.; Martín-Belloso, O. 2003a. Microbiological and biochemical changes in minimally processed fresh-cut 'Conference' pears. *European Food Research and Technology*, 217:4-9.

Soliva-Fortuny, R.C.; Alós-Saiz, N.; Espachs-Barroso, A.; Martín-Belloso, O. 2004. Influence of ripeness at processing on quality attributes of fresh-cut 'Conference' pears. *Journal of Food Science*, 69 (7): 244-248.

Valdenegro, M.; Flores, F.B.; Romojaro, F.; Ramírez, M.; Martínez-Madrid, M.C. 2004a. Susceptibility of Spanish Melon fruits to Chilling Injury during Cold Storage. *Acta Horticulturae*, 682: 1219-1225.

Valdenegro, M.; Ramírez, M.; Flores, F.B.; Romojaro, F. 2004b. Influencia de 1-MCP y etileno sobre los daños por frío en melón español durante el almacenamiento. In: Instituto Nacional de Investigação Agrária (Ed.), Maturação e Pós-colheita 2004 – Frutos e Hortícolas, 6-9 Octubro 2004. Instituto Nacional de Investigação Agrária, Oeiras, Portugal, pp. 519-521.

Varoquaux, P. 1991. Ready-to-use fresh fruits and vegetables. *Revue Générale du Froid*, 81 (6): 33-43.

Villanueva, M.J.; Tenorio, M.D.; Esteban, M.A.; Mendoza, M.C. 2004. Compositional changes during ripening of two cultivars of muskmelon fruits. *Food Chemistry*, 87: 179-185.

Watada, A.E.; Ko, N.P.; Minott, D.A. 1996. Factors affecting quality of fresh-cut horticultural products. *Postharvest Biology and Technology*, 9: 115-125.

Zwietering, M.H.; Jongenburger, I.; Rombouts, F.M.; Riet, K. van't. 1990. Modelling of the bacterial growth curve. *Applied and Environmental Microbiology*, 56(6): 1875-1

Capítulo II

Modeling changes of headspace gas concentrations to describe the respiration of fresh-cut melon under low or superatmospheric oxygen atmospheres

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Abstract

Packages of fresh-cut 'Piel de Sapo' melon were stored under 2.5 kPa O_2 + 7 kPa CO_2, 21 kPa O_2, and 70 kPa O_2 atmospheres for 35 days at 4 ºC. A mathematical procedure was tested to model changes of in-package O_2 and CO_2 concentrations throughout storage, in order to predict the respiratory activity of the commodity. The relationships between respiratory activity and quality parameters of fresh-cut 'Piel de Sapo' melon were also assessed. A 70 kPa O_2 atmosphere reduced CO_2 production rate during 14 days, as well as prevented ethanol production during three weeks of storage. On the other hand, fermentative pathways were triggered under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere. Although 70 kPa O_2 levels involved a high O_2 consumption and a decrease in the soluble solids content, the use of superatmospheric O_2 atmospheres are proposed to reduce CO_2 production rates, avoid fermentative reactions and, maintain firmness and chewiness of fresh-cut 'Piel de Sapo' melon for two weeks of storage.

Keywords: fresh-cut melon, modified atmosphere packaging, superatmospheric O_2 levels, modeling, quality

Introduction

Minimal processing operations, such as peeling, coring and slicing are critical, because they limit the shelf life of fruit products due to physiological stress caused by physical damage or wounding (Varoquaux and Wiley, 1997). Wounding stress results in increased respiration rates of fresh-cut produce, and a metabolic activation that promotes biochemical reactions related to changes in color, flavor, texture and nutritional quality (Soliva-Fortuny and Martín-Belloso, 2003). However, these responses depend on the physiological status of the raw product, processing, storage, temperature and modified atmosphere conditions (Rocculi, et al., 2006).

Low temperature is essential for maintaining quality of fresh-cut fruits. Commercially, fresh-cut fruits are handled and transported below 5 ºC to reduce their metabolic rate. However, 'Piel de Sapo' melon (*Cucumis melo L.*) is sensitive to chilling injury. This physiological disorder appears when melons are stored at very low temperature. Microbiological spoilage and loss of firmness are the main causes of quality loss in fresh-cut melon stored in air conditions at chilling temperature (Aguayo et al., 2003). The use of dips, containing calcium salts, combined to modified atmosphere packaging (MAP) have been shown to preserve fresh-cut melon from softening, thus extending the shelf-life of the product (Oms-Oliu et al., 2006). Gas mixtures consisting of low O_2 concentrations (2 to 5 kPa) and high CO_2 levels (7 to 15 kPa) have been recommended for fresh-cut melons (O'Connor-Shaw et al., 1996; Bai et al., 2001, Oms-Oliu et al., 2006). Decreasing O_2 and increasing CO_2 concentrations in the package atmosphere are effective in maintaining visual quality and reducing microbial growth of fresh-cut fruits. Due to respiratory O_2 uptake and CO_2 evolution of packaged produce, and gas transfer from the packaged films, a proper combination of product characteristics and film permeability results in the evolution of an appropriate atmosphere within packages (Del Nobile et al., 2006). However, packaging films currently available for fresh-cut produce do not have sufficient O_2 and CO_2 transmission rates and, as a consequence, too low O_2 levels and/or excessive amounts of CO_2 in package headspace are often detrimental to fresh-cut fruit. Since the traditional MAP technology does not provide the desired materials, the application of superatmospheric O_2 concentrations has been suggested to overcome these limitations (Allende et al., 2004). Superatmospheric O_2 levels ($\geq$ 70 kPa O_2) could be effective to prevent anaerobic fermentation reactions, inhibit microbial growth and maintain product quality (Day 1996, 2000).

Low levels of O_2 combined to high CO_2 concentrations can potentially reduce respiration rates (Kader et al., 1989), whereas superatmospheric O_2 atmospheres may stimulate, have no effect, or

reduce rates of respiration, depending on the commodity, maturity and ripeness stage, O_2 concentration, storage time and temperature, and concentrations of CO_2 and ethylene present in the atmosphere (Kader and Ben-Yehoshua, 2000).

Most respiratory models reported in the literature to describe the respiration rate of fresh-cut produce are based on Michaelis-Menten type enzyme kinetics and valid only under aerobic conditions. These models can be used under steady-state (Lakakul et al., 1999) or non-steady conditions, in the early period of storage (Lee et al., 1996; Del Nobile et al., 2006; Rocculi et al., 2006). However, to our knowledge, models associated with the non-steady state of the headspace conditions of fresh-cut fruit packages have not yet been developed to describe both aerobic and anaerobic respiration.

Our first objective was to model changes of in-package O_2 and CO_2 concentrations throughout storage, in order to calculate the respiration rate and respiratory quotient of fresh-cut 'Piel de Sapo' melon packaged under low or superatmospheric oxygen conditions. Secondly, we aimed to investigate relationships between respiratory activity and quality parameters of fresh-cut melon throughout storage.

Materials and Methods

Sample preparation

'Piel de Sapo' melons (*Cucumis melo* L) harvested in Valencia (Spain) were stored in a ventilated room at 10 ºC prior to processing. Melons at commercial ripeness were sanitized in a 200 ppm NaClO solution for 2 min, rinsed with tap water, and hand dried. Melons were sliced and cut to obtain trapezoidal sections. Melon pieces were dipped for 1 min in a solution of 0.5% w/v calcium chloride, according to previous studies (Oms-Oliu et al., 2006). Once the excess of water was completely drained, 100 g of fruit were packaged in polypropylene trays and sealed with a plastic film. The O_2 and CO_2 permeance of the film were 110 $cm^3 \cdot m^{-2} \cdot day^{-1} \cdot bar^{-1}$ and 500 $cm^3 \cdot m^{-2} \cdot day^{-1} \cdot bar^{-1}$ at 23 ºC and 0% RH, respectively (ILPRA Systems España, S.L. Mataró, Spain). The initial in-package O_2 and CO_2 concentrations were 2.5 kPa O_2 + 7 kPa CO_2, 21 kPa O_2 (air) and 70 kPa O_2, in a ratio product: gas mixture of 1:2. The trays were thermosealed with a vacuum packing machine ILPRA Food Pack Basic V/6 (ILPRA Systems. CP. Vigevono, Italia) and stored at 4 ±

1ºC in darkness. Two trays were taken at each sampling time to perform analyses throughout 35 days of storage.

Headspace gas analysis

The gas composition of the packages headspace was determined with a gas analyzer (Micro-GC CP 2002, Chrompack International, Middelburg, The Netherlands) equipped with a thermal conductivity detector. An aliquot of 1.7 ml was automatically withdrawn through an adhesive rubber septum with a sampling needle directly connected to the injection system. The determination of the oxygen concentration was carried out by injecting a sample of 0.25 µl a CP-Molsieve 5Å column (4m x 0.35 mm, df=10 µm) at 60ºC and 100 kPa, whereas a portion of 0.33 µl was injected to a Pora-PLOT Q column (10m x 0.32 mm, df=10 µm) at 75 ºC and 200 kPa for carbon dioxide and ethanol determination. Two trays were taken at each time to perform the analysis and 2 readings were carried out for each package.

Mathematical modelling

Different models were fitted to the experimental data and it was found that Weibull model (Eq. 1) and Logistic model (Eq. 2) best fitted O_2 and CO_2 concentrations, respectively.

$$n_{O_2(t)} = n_{O_2(i)} \times e^{-\left(\frac{t}{\tau}\right)^{\beta}} \qquad \text{(Eq. 1)}$$

where $n_{O_2(t)}$ are the in-package moles of O_2 at time t, $n_{O_2(i)}$ are the in-package moles of O_2 at time zero, τ (day) is the scale factor and β (dimensionless) is a constant, that determines the shape of the rate function.

$$n_{CO_2(t)} = \frac{A_{max}}{1 + e^{\left[k \times \left(t_{1/2} - t\right)\right]}} \qquad \text{(Eq. 2)}$$

where $n_{CO_2(t)}$ are the in-package moles of CO_2 at time t, A_{max} are the maximum in-package moles of CO_2, k (day^{-1}) is the accumulative velocity and $t_{1/2}$ (day) is the required time to reach half of the maximum in-package moles of CO_2, the inflection point.

The respiration rates were calculated using a mass balance on the O_2 and CO_2 concentrations in the package headspace as follows:

$$R_{O_2} = \left[-\frac{d\left(n_{O_2\,(t)}\right)}{dt} + \left[S \times P_{O_2} \times \left(y^{e}_{O_2} - y_{O_2(t)}\right)\right]\right] / m \quad \text{(Eq. 3)}$$

$$R_{CO_2} = \left[\frac{d\left(n_{CO_2\,(t)}\right)}{dt} + \left[S \times P_{CO_2} \times \left(y_{CO_2\,(t)} - y^{e}_{CO_2}\right)\right]\right] / m \quad \text{(Eq. 4)}$$

where R_{O_2} (O_2 mol kg^{-1} day^{-1}) and R_{CO_2} (CO_2 mol kg^{-1} day^{-1}) are the O_2 consumption rate and CO_2 production rate, respectively, $n_{O_2\,(t)}$ and $n_{CO_2\,(t)}$ are the in-package moles of O_2 and CO_2 at time t, respectively, S (m^2) is the area of package surface, the P_{O_2} (O_2 mol m^{-2} day^{-1} kPa^{-1}) and P_{CO_2} (CO_2 mol m^{-2} day^{-1} kPa^{-1}) are, respectively, O_2 and CO_2 permeance of film, $y_{O_2(t)}$ and $y_{CO_2(t)}$ (kPa) are the O_2 and CO_2 partial pressures in the package headspace at time t and, $y^{e}_{O_2}$ and $y^{e}_{CO_2}$ (kPa) are the external O_2 and CO_2 partial pressures and m (kg) is the mass product.

The accumulative terms of the balances Eq. (3) and (4) can be obtained from the first derivatives of the adjusted Eq. (1) and (2).

$$\frac{d\left(n_{O_2\,(t)}\right)}{dt} = n_{O_2\,(i)} \times e^{-\left(\frac{t}{\tau}\right)^{\beta}} \times \left[-\frac{\beta}{\tau}\left(\frac{t}{\tau}\right)^{\beta-1}\right] \quad \text{(Eq. 5)}$$

$$\frac{d\left(n_{CO_2\,(t)}\right)}{dt} = \frac{A_{max}}{\left[1+e^{\left[k\times\left(t_{1/2}-t\right)\right]}\right]^{2}} \times \left[-k \times e^{\left[k\times\left(t_{1/2}-t\right)\right]}\right] \quad \text{(Eq. 6)}$$

The respiration data obtained from the Eq. (3) and (4) were used to estimate the respiratory quotient,

$$RQ = \frac{R_{CO_2}}{R_{O_2}} \quad \text{(Eq. 7)}$$

Determination of Color and Texture parameters

Melon color was directly measured with a Minolta Chroma Meter model CR-400 (Konica Minolta Sensing, Inc., Osaka, Japan). The equipment was set up for a D65 illuminant and 10º observer

angle. Five fruit pieces from each of two replicate packages were evaluated for each treatment at each sampling time. CIE L* (lightness), a* (red-green) and b* (yellow-blue) color parameters were obtained through reflectance values. Color changes were measured through whiteness index (WI) (Eq. 8). According to previous studies (Oms-Oliu et al., 2006), color changes of fresh-cut 'Piel de Sapo' melon were shown to be mostly due to WI depletion.

$$WI = 100 - \left[(100 - L^*)^2 + (a^*)^2 + (b^*)^2\right]^{\frac{1}{2}} \quad \text{(Eq. 8)}$$

Firmness evaluation was performed using a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, England, UK) by measuring the maximum penetration force. Cylindrical samples of 2.0 cm high obtained from trapezoidal melon pieces were positioned to be penetrated through their geometric center by a 4 mm diameter rod. The downward distance was set at 10 mm at a rate of 5 mm/s and automatic return. Evaluation of chewiness was performed by compressing the samples in a 2-cycle test to 30% of the original height with 2.54 cm diameter cylindrical aluminium probe. Chewiness is defined as hardness x cohesiveness x springiness and its values were calculated from the texture variables obtained from force and area measurements (Bourne, 1982) as follow: hardness, peak force during the first compression cycle (N); cohesiveness, ratio of the positive force area during the second compression cycle to that during the first compression; springiness, height recovered by the sample during the time that elapses between the end of the first compression cycle and the start of the second cycle. Two trays were taken at each sampling time to perform the analyses, and 5 fruit pieces from each replicate were randomly withdrawn to carry out repetitions.

Determination of soluble solids, acidity and pH

The soluble solids content and pH of the juice obtained from a 40 g sample were determined at 20 ºC with a temperature-compensated refractometer Atago RX-1000 (Atago, Japan) and using a CRISON 2001 pH-meter (Crison, Barcelona, Spain), respectively. A portion of 20 g of the crushed sample was diluted into 100 mL distilled water and titrated with 0.1 N NaOH up to pH 8.1 to measure titratable acidity.

A pair of trays was taken at each sampling time and two replicate analyses were carried out for each tray.

Statistical analysis

Experimental data were fitted to the models by non-linear regression procedures of Statistica 7.0 software (Statsoft, Tulsa, OK, USA). Estimated parameters are given with their respective confidence intervals, product of the standard error of the estimates by the Student-t-adjusted at the degree of freedom. Fitting accuracy of the models was evaluated through the analysis of R^2 coefficients and the accuracy factor A_f. The higher the R^2 value, the better is the adequacy of the model to describe the data (Neter, Kutner, Nachtsheim & Wasserman, 1996). The accuracy factor was proposed by Ross (1996) to evaluate the performance of models. Eq. (9) computes A_f from J experimental observations of O_2 and CO_2 concentrations (n) and their respective J predicted values by the fitted model. For this factor, the nearer the A_f value to the unit, the better the accuracy.

$$A_f = 10^{\frac{\sum_{j=1}^{j=J}\left|\log\left(\frac{\text{predicted } n_j}{\text{observed } n_j}\right)\right|}{J}} \qquad j = 1, 2, 3, \ldots, J \qquad \text{(Eq. 9)}$$

The analyses of variance were carried out with Statistica 7.0 software (Statsoft, Tulsa, OK, USA). Duncan's multiple range test was employed to determine differences between means at a 5% significance level. All analyzed parameters were subjected to principal component analysis (PCA) to evaluate relationships among them. PCA is a multivariate statistical technique based on the calculation of linear combinations between the variables that explain the most variance of the data. As a result, data can be reduced to a set of new variables called principal components (PCs). The loadings of the PC define the direction of greatest variability.

Results and discussion

Respiratory activity of fresh-cut 'Piel de Sapo' melon

Fig. 1 shows the results of fitting the mathematical models described by Eq. (1) and (2) to O_2 and CO_2 headspace gas partial pressures during storage of fresh-cut melon packaged under different modified atmospheres. The experimental O_2 and CO_2 concentrations as a function of storage time were best adjusted to the Weibull model Eq. (1) and to a Logistic function Eq. (2), respectively.

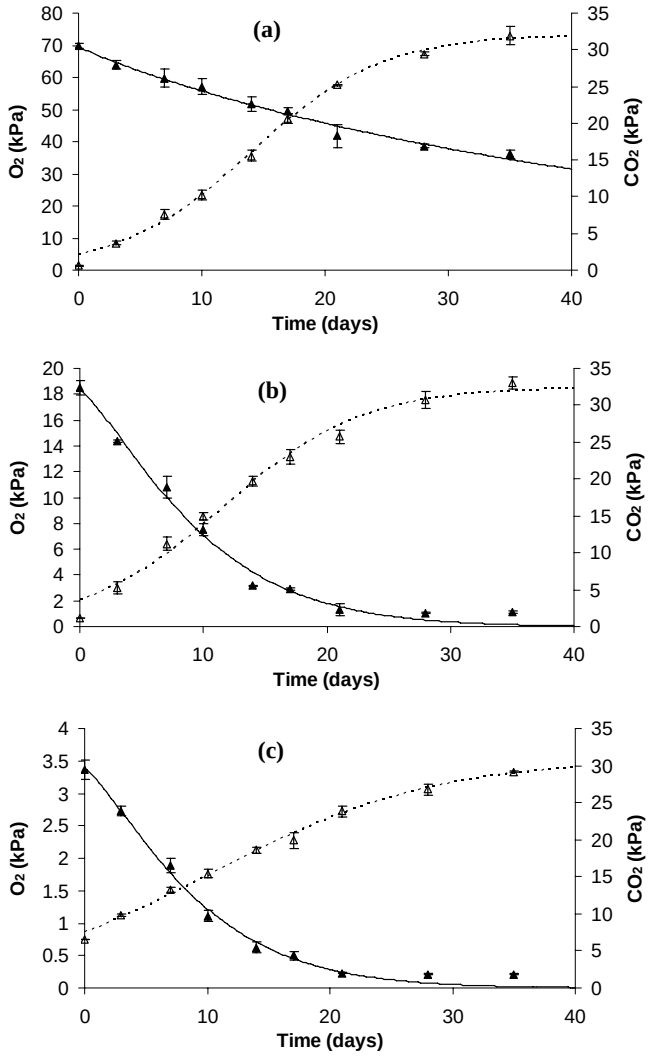

Figure 1. O_2 and CO_2 concentrations in the package headspace of fresh-cut 'Piel de Sapo' melon packaged under different modified atmospheres and fit of Weibull model and Logistic model to O_2 and CO_2 experimental data, respectively: 70 kPa O_2 (a), 21 kPa O_2 (b); 2.5 kPa O_2 + 7 kPa CO_2 (c). Data shown are mean ± standard deviation

Fig. 2 shows the fair agreement between predicted values and experimental concentrations for O_2 and CO_2, where it is visible that the trend is not biased. The differences in the model parameters were statistically significant at $p < 0.001$. The computed parameters, the determination coefficients (R^2) and the accuracy factors (A_f) of the Weibull model when relating O_2 concentration with time are given in Table 1. The fitted model yielded high R^2-values (0.984-0.995) and exhibited good accuracy (A_f =1.066-1.186). Both τ and β resulted to be significantly ($p< 0.05$) dependent on modified atmosphere conditions. In the present study, τ-values were very high for a 70 kPa O_2 compared to the values obtained for 21 kPa O_2 and 2.5 kPa + 7 kPa CO_2 atmospheres. In addition, β-values, related to the shape of the curve, were lower than 1 under superatmospheric O_2 atmospheres, which implies a concave depletion of O_2 concentrations, whereas values were slightly higher or similar to 1 for packages stored under low O_2 and air concentrations, respectively. Values equal to 1 correspond to an exponential function and higher than 1 to a convex curve. Thus, the shape curve of O_2 consumption within packages of fresh-cut 'Piel de Sapo' melon depends on initial O_2 concentration.

The model parameters (A_{max}, k and T) of the Logistic function, which best described CO_2 accumulation of fresh-cut 'Piel de Sapo' melon packages, are shown in Table 2. The model fitted well the experimental data ($R^2 \geq 0.981$-0.997) with accuracy factors of 1.068-1.113. A_{max}-values resulted to be significantly ($p < 0.05$) non-depending on MAP conditions. On the other hand, differences were detected for k-values among the different packaging atmospheres. Thus, k-values were significantly ($p\leq 0.05$) lower under low O_2 atmospheres than under superatmospheric O_2 and air concentrations. In 10-15 days of storage, packages of fresh-cut 'Piel de Sapo' melon reached a fifty percent of the maximum CO_2 accumulation in all atmosphere conditions. As can be observed in Fig. 1, CO_2 concentrations did not increase above 15 kPa during 10 days of storage, regardless initial atmosphere conditions. Bai et al. (2003) reported maximum levels of 15 kPa CO_2 to prevent toxicity inside packages of fresh-cut melon.

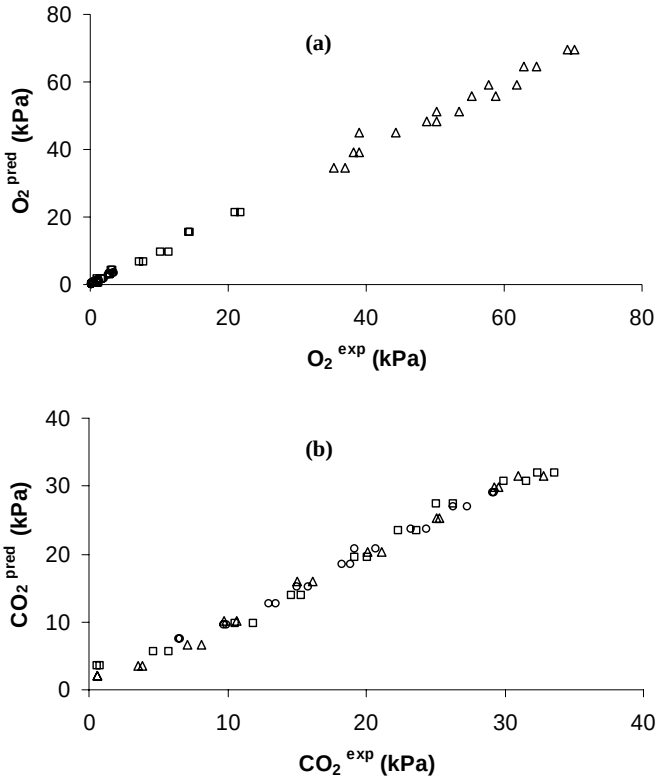

Figure 2. Relationship between experimental (exp) and predicted (pred) concentration values for O_2 (a) and CO_2 (b) in the package headspace of fresh-cut 'Piel de Sapo' melon stored under 70 kPa O_2 (△), 21 kPa O_2 (□) or 2.5 kPa O_2 + 7 kPa CO_2 (○) atmospheres

Table 1. Kinetic constants of Weibull distribution function [(Eq. 1)] for headspace O_2 concentrations in packages of fresh-cut melon [a].

Modified atmosphere	$n_{O_{2(i)}} \times 10^2$ (moles)	τ (days)	β	R^2	A_f
2.5 kPa O_2 + 7 kPa CO_2	0.0658±0.0017	9.8±0.4	1.27±0.09	0.995	1.186
21 kPa O_2	0.412±0.011	8.8±0.4	1.02±0.07	0.985	1.123
70 kPa O_2	1.3±0.3	51±4	0.91±0.09	0.984	1.066

R^2: determination coefficient; A_f: accuracy factor

[a] Values ± confidence interval at $p \leq 0.05$

Table 2. Kinetic constants of Logistic function [(Eq. 2)] for headspace CO_2 concentrations in packages of fresh-cut melon [a].

Modified atmosphere	$A_{max} \times 10^2$ (moles)	k ($days^{-1}$)	T (days)	R^2	A_f
2.5 kPa O_2 + 7 kPa CO_2	0.600±0.016	0.109±0.007	10.4±0.6	0.996	1.068
21 kPa O_2	0.629±0.019	0.179±0.016	11.7±0.6	0.981	1.113
70 kPa O_2	0.623±0.012	0.188±0.009	14.0±0.3	0.997	1.105

R^2: determination coefficient; A_f: accuracy factor

[a] Values ± confidence interval at $p \leq 0.05$

Fig. 3 shows the changes of O_2 consumption and CO_2 production rates with time of fresh-cut 'Piel de Sapo' melon. This dependence was estimated by fitting Eq. (3) and Eq. (4) to O_2 and CO_2 partial pressures measured inside packages. The accumulative terms of these equations are obtained from the first derivative of the adjusted Eq. (1) and Eq. (2). As shown in Fig. 3a, O_2 consumption rate of fresh-cut melon stored under a 70 kPa O_2 atmosphere was far higher than that of melon pieces packaged under 2.5 kPa O_2 + 7 kPa CO_2 and 21 kPa O_2, respectively. The O_2 consumption rate of the product packaged under initial air or low O_2 concentrations ranged from 0.013-0.004 mol kg^{-1} day^{-1} whereas de O_2 consumption rate under superatmospheric O_2 levels ranged from 0.034-0.010 mol kg^{-1} day^{-1}. According to the respiratory pattern, O_2 consumption rates increased under initial 21 kPa O_2 to reach steady values after 15 days, whereas rates decreased throughout storage under superatmospheric O_2 levels up to values similar to those observed under other atmospheres by the end of storage. On the other hand, under low O_2 atmospheres, O_2 consumption rates were maintained constant during storage. A linear model fitted well ($R^2 \geq 0.901$) the dependence of O_2 consumption with headspace O_2 concentrations. As it can be observed in Fig. 4a, O_2 consumption rate increased with decreased O_2 concentration when headspace O_2 levels ranged from 0 to 21 kPa whereas respiration rates decreased with decreased O_2 concentrations under superatmospheric O_2 levels. Respiration is widely assumed to be slowed down by decreasing availability of O_2 as a consequence of reduction of overall metabolic activity (Fonseca et al., 2002). However, as can be observed in Fig. 3b, a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere enhanced the CO_2 production rates at the early stage of storage (7-10 days) in comparison to 21 kPa O_2 and 70 kPa O_2 atmospheres. Beyond the third week of storage, CO_2 production rate of fresh-cut 'Piel de Sapo' melon was higher under initial 21 kPa O_2 and 70 kPa O_2 than 2.5 kPa O_2 + 7 kPa CO_2 concentrations, reaching maximum values around 0.030 mol CO_2

day^{-1} kg^{-1}. The stimulation of CO_2 production under 2.5 kPa O_2 + 7 kPa CO_2 atmospheres at early stages of storage may be explained in terms of O_2 and CO_2 injury of tissues. Lakakul et al. (1999) pointed out the importance of maintaining O_2 level just above the fermentation threshold and keeping CO_2 concentration below the range that causes injury to fruits. A rapid depletion of O_2 and accumulation of CO_2 may be due to a low packaging film gas transmission that strongly affected the changes of in-package atmospheres. A linear model well fitted ($R^2 \geq 0.988$) the dependence of CO_2 production rates with headspace CO_2 concentrations. An increase of CO_2 production rates with increased headspace CO_2 concentrations was observed under all tested conditions, detecting respiration rates from 0.03 to 0.003 mol kg^{-1} day^{-1} (Fig. 4b).

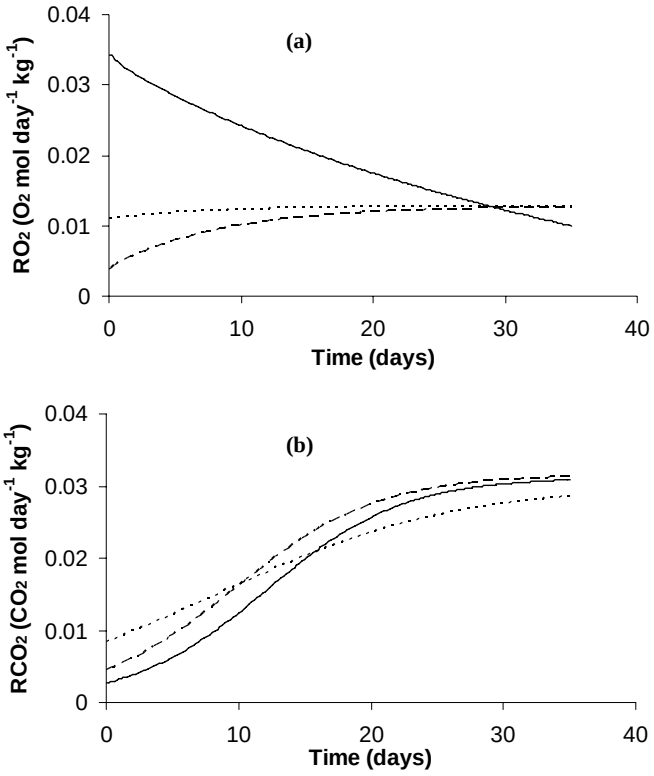

Figure 3. O_2 consumption rates (a) and CO_2 production rates (b) in the package headspace of fresh-cut 'Piel de Sapo' melon stored under different modified atmospheres as predicted by Eq. (3) and Eq. (4): 2.5 kPa O_2 + 7 kPa CO_2 (·····); 21 kPa O_2 (– – –); 70 kPa O_2 (——)

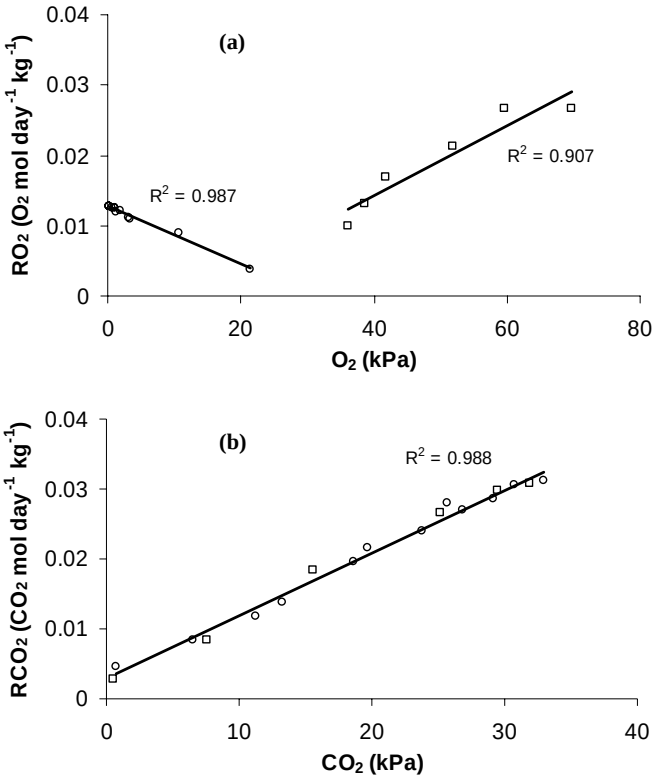

Figure 4. Relationship between O_2 consumption rates and O_2 concentrations (a) and CO_2 production rates and CO_2 concentrations (b) in the package headspace of fresh-cut melon 'Piel de Sapo' melon stored under 70 kPa O_2, 21 kPa O_2 or 2.5 kPa O_2 + 7 kPa CO_2 atmospheres: (○) O_2 concentrations ranging from 0 to 21 kPa, (□) superatmospheric O_2 concentrations

Initial respiratory quotient (RQ) of fresh-cut 'Piel de Sapo' melon stored under 21 kPa O_2 and 2.5 kPa O_2 + 7 kPa CO_2 atmospheres was 0.8 and 1.2, respectively. On the other hand, under 70 kPa concentrations, excessively low RQ (0.08) was observed at early stage of storage (Fig. 5), which may be related to oxidative processes in tissue at the beginning of storage. Therefore, respiratory activity of fresh-cut produce should not be expressed through O_2 consumption rate.

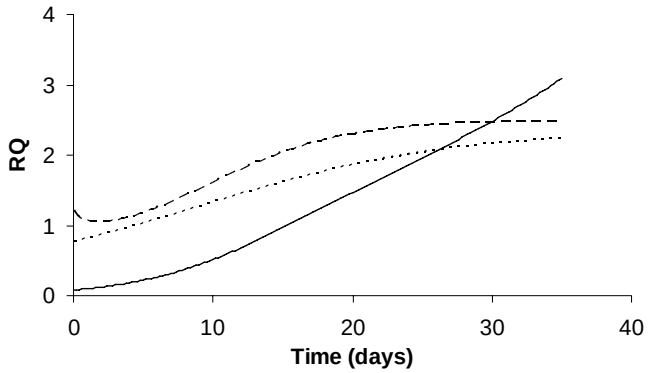

Figure 5. Changes of respiration quotient (RQ) during storage of fresh-cut 'Piel de Sapo' melon stored under different modified atmospheres as predicted by means of Eq. (7): 2.5 kPa O_2 + 7 kPa CO_2 (·····); 21 kPa O_2 (– – -); 70 kPa O_2 (——)

Average RQ values reported in the literature regarding aerobic respiration are ranging from 0.7 to 1.3 (Kader et al., 1989). In fermentative metabolism, ethanol production involves decarboxylation of pyruvate to CO_2 without O_2 uptake (Fonseca et al., 2002) and the RQ is much greater than 1.0. Values of RQ above 1.3 were detected in fresh-cut 'Piel de Sapo' melon after 10-14 days of storage under initial 2.5 kPa O_2 + 7 kPa CO_2 or 21 kPa concentrations. Under these conditions, fresh-cut melon produced ethanol beyond the first and second week of storage, respectively (Fig. 6). Ethanol production was also detected beyond the third week of storage under 70 kPa O_2 storage atmospheres. The stress that the cell undergoes under high O_2 levels may affect synthesis and accumulation of some volatile compounds associated with respiratory metabolism, including fermentative metabolites such as acetaldehyde, ethanol and ethyl acetate (Wszelaki and Mitcham, 2000). Moreover, RQ values correlated well with CO_2 production rates and headspace CO_2 concentrations (Fig. 7). The predicted RQ as a function of the calculated CO_2 production rates and experimental headspace CO_2 concentrations were well adjusted to a linear model and exhibited high $R^2 \geq 0.809$. Beaudry (1993) observed that RQ increased under high CO_2 concentrations in blueberry fruit. Our results suggest that CO_2 production rates and CO_2 concentrations had the greatest effect on the RQ rather than O_2 consumption rate or headspace O_2 concentrations.

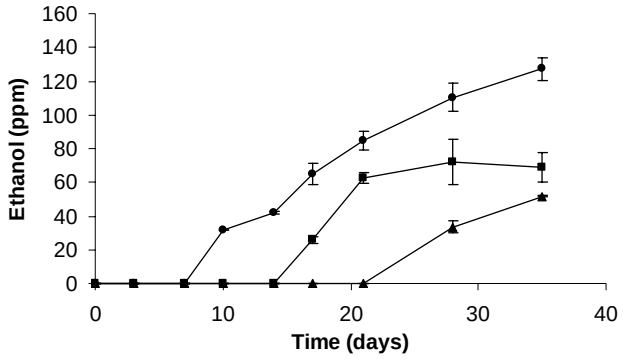

Figure 6. Ethanol concentrations in the package headspace of fresh-cut 'Piel de Sapo' melon stored under different modified atmospheres: (●) 2.5 kPa O_2 + 7 kPa CO_2, (■) 21 kPa O_2, (▲) 70 kPa O_2. Data shown are mean ± standard deviation

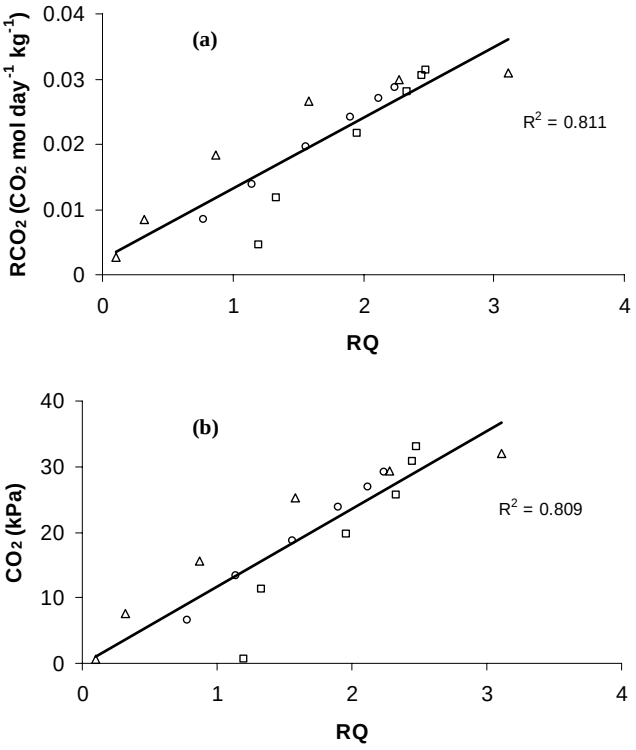

Figure 7. Relationship between respiration quotient (RQ) values and CO_2 production rates (a) and CO_2 concentrations (b) in the package headspace of fresh-cut 'Piel de Sapo' melon stored under 70 kPa O_2 (△), 21 kPa O_2 (□) or 2.5 kPa O_2 + 7 kPa CO_2 (○) atmospheres

Relationship between respiratory activity and quality parameters of fresh-cut 'Piel de Sapo' melon

Data on some physico-chemical characteristics of fresh-cut 'Piel de Sapo' melon as affected by different modified atmosphere conditions are shown in Table 3. The means obtained for each variable were subjected to principal component analysis (PCA) to evaluate relationships among the studied parameters. Two principal components (PC1 and PC2) were calculated, accounting for a 72.49% of the variability in the original data for fresh-cut melon (Fig. 8). The main component (PC1) explained 51.96% of the total variance. This variation was mainly described by the variables CO_2 production rates, headspace CO_2 concentrations, RQ and ethanol. Results show that there was an outstanding negative correlation between WI and O_2 consumption rate or headspace O_2 concentrations due to the opposed position in PCA plot. Initial WI values were the lowest for packages stored under superatmospheric O_2 concentrations (Table 3), which consumed a great amount of oxygen compared to other conditions. On the other hand, less O_2 consumption of both 2.5 kPa O_2 + 7 kPa CO_2 and 21 kPa O_2 storage atmospheres was related to higher WI values during three weeks of storage. Beyond that period, a decrease in WI was detected in fresh-cut melon packaged under 2.5 kPa O_2 + 7 kPa CO_2 atmospheres, indicating development of translucency caused by the fermentative decay. Aguayo et al. (2003) reported a decrease of WI when translucency injury increased on fresh-processed 'Amarillo' melon, as a consequence of chilling injury. On cantaloupe melon, this physiological disorder was associated with an accumulation of ethanol, acetaldehyde and ethyl acetate (Flores et al., 2004). The accumulation of these fermentative metabolites under low O_2 and high levels of CO_2 can be the cause of many anaerobic off-odors (Arvanitoyannis et al., 2005).

Fig. 8 shows a positive correlation among O_2 consumption rates, headspace O_2 concentrations and texture of fresh-cut melon. A progressive softening was observed under 21 kPa O_2 or 2.5 kPa O_2 + 7 kPa CO_2 storage atmospheres (Table 3). This could be due to the total flooding of intercellular gaps under anoxic conditions, as it was also observed in fresh-cut 'Conference' pears stored under initial 2.5 kPa O_2 + 7 kPa CO_2 atmospheres. As a consequence, pear tissue structure was seriously damaged (Soliva-Fortuny et al., 2002). These authors related the loss of hardness, springiness, gumminess and chewiness of fresh-cut pears with a decrease in firmness. Our results show that both firmness and chewiness values of fresh-cut 'Piel de Sapo' melon stored under superatmospheric O_2 concentrations were best maintained in spite of the high O_2 respiration rates (Table 3). The effects of superatmospheric O_2 concentrations on firmness of fresh-cut 'Piel de

Sapo' melon were previously investigated (Oms-Oliu et al., 2007). The use of high O_2 concentrations was proposed to prevent anaerobic conditions during storage, thus reducing wounding stress and deteriorative changes such as softening, off-odor development and taste deterioration.

Table 3. Effects of different packaging conditions on physico-chemical characteristics of fresh-cut 'Piel de Sapo' melon during 35 days of storage at 4 ºC

2.5 kPa O_2 + 7 kPa CO_2						
Days	WI	F	C	TA	pH	SSC
0	64.3b	3.4a	2.8a	0.27a	5.7a	12.5a
7	67.3ab	2.9a	1.8b	0.28a	5.5c	11.9b
14	65.2ab	2.9a	1.7b	0.28a	5.6b	11.5c
21	69.2a	2.9a	1.5b	0.28a	5.7a	11.5c
28	57.9c	2.1b	1.0c	0.27a	5.7a	5.7d
35	55.2c	1.9b	0.8c	0.27a	5.7a	5.6e
21 kPa O_2						
Days	WI	F	C	TA	pH	SSC
0	63.9c	3.4a	2.9a	0.27b	5.7a	12.6a
7	68.7a	2.6b	1.6b	0.27b	5.6b	12.2a
14	65.5bc	1.9c	1.3b	0.28b	5.4c	12.6a
21	69.5a	1.9c	1.3b	0.30a	5.2d	11.7b
28	69.4a	1.9c	1.1b	0.30a	5.1d	5.1c
35	68.3a	1.9c	1.1b	0.31a	5.1d	5.2c
70 kPa O_2						
Days	WI	F	C	TA	pH	SSC
0	62.7a	3.4a	3.3a	0.28c	5.7a	12.9a
7	60.7a	3.4a	2.5ab	0.25d	5.7a	9.9b
14	62.8a	3.5a	2.5ab	0.30bc	5.2bc	9.9b
21	61.8a	3.9a	2.2b	0.31ab	5.1d	9.8b
28	61.2a	3.5a	2.1b	0.32ab	5.2cd	5.2c
35	62.1a	3.1a	2.0b	0.33a	5.1dcd	5.1c

WI: whiteness index; F: firmness (N); C: chewiness (N); TA: titratable acidity (g citric acid /100 g); SSC: soluble solids content (ºBrix)

Values within a column followed by the same letter indicate that mean values are not significantly different by Duncan's multiple-range test ($p \leq 0.05$)

Titratable acidity of fresh-cut melon stored under initial 21 kPa O_2 or 70 kPa O_2 atmospheres significantly increased throughout the storage (Table 3). Aguayo et al. (2003) also observed an increase in acidity on fresh-cut 'Amarillo' melon after 14 days of storage. Titratable acidity values were negatively correlated to pH values (Fig. 8) but neither of these parameters was related to respiratory activity of fresh-cut melon. A decrease in pH values could be due to lactic acid production because of the proliferation of lactic acid bacteria as it has been observed in fresh-cut cantaloupe melon (Lamikanra et al., 2000). However, some authors have related the increase in acidity with concentration of acids when fruits suffer dehydration (Brackmann et al., 2006).

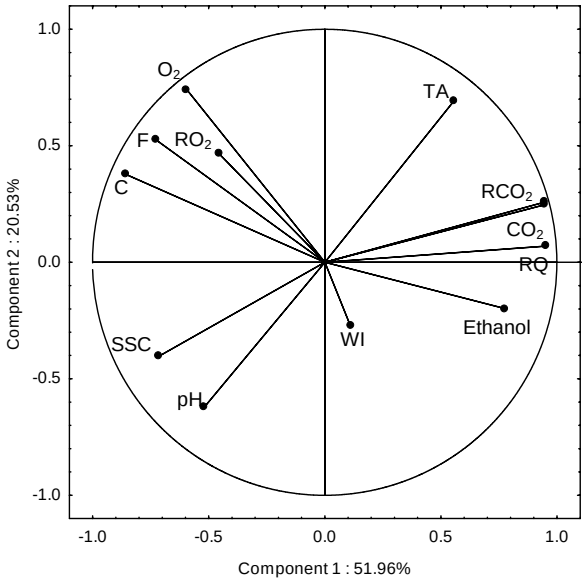

Figure 8. PCA plot of fresh-cut 'Piel de Sapo' melon packaged under different modified atmospheres for all analysed parameters: headspace O_2 and CO_2 concentrations (O_2 and CO_2), O_2 consumption rate (RO_2), CO_2 production rate (RCO_2), respiratory quotient (RQ), ethanol, whiteness index (WI), firmness (F), chewiness (C), titratable acidity (TA), soluble solids content (SSC)

Flavor, sweetness and texture are factors that consumer greatly appreciated in melons. Fruit sweetness was highly correlated with soluble solids concentrations (r = 0.61) (Lester, 2006). In our study, a strong depletion of soluble solids content was observed in packages stored under

superatmospheric O_2 levels (Table 3). A decrease in sweetness scores of fresh-cut 'Cantaloupe' melon was attributed to higher consumption rates of carbohydrates due to an increased respiratory activity (Brackmann et al., 2006; Boynton et al., 2006). However, soluble solids content could not be correlated with respiratory activity of fresh-cut melon (Fig. 8). Thus, the strong depletion of soluble solids content observed in all storage conditions after three weeks may be due to the microbial growth rather than increased respiration rates. Lactic acid bacteria ferment glucose, fructose, and sucrose during growth while producing lactic acid (Boynton et al., 2006). On fresh-cut 'Cantaloupe' melon, limited shelf-life of 6 days was due primarily to flesh softening, visible fungi and off-odor development (Chonhenchob et al., 2007). In our study, no signs of microbial alteration were detected on fresh-cut melon during the two first weeks and general appearance was not dramatically affected by storage under modified atmosphere. Previous studies reported a microbiological shelf-life from 7 to 14 days for fresh-cut 'Piel de Sapo' melon stored under MAP (Oms-Oliu et al., 2007).

Conclusions

The respiratory activity of fresh-cut 'Piel de Sapo' melon stored under 70 kPa O_2 atmospheres was adequately described through CO_2 production rates. Under superatmospheric O_2 concentrations, initial high O_2 consumption rates were threefold higher than under 2.5 kPa O_2 + 7 kPa CO_2 atmospheres, as a consequence of oxidative processes. Low permeability of the packaging film accounted for a rapid depletion of O_2 and accumulation of CO_2 in packages of fresh-cut melon stored under 2.5 kPa O_2 + 7 kPa CO_2 atmospheres. Low O_2 and CO_2 enriched atmospheres seem to stimulated CO_2 production rates and values of respiratory quotient above 1.3 after 10-14 days were related to anaerobic metabolism of fresh-cut melon. Superatmospheric O_2 levels delayed the deteriorative changes related to the initiation of anaerobic pathways such as softening, off-odors and off-flavors.

Nomenclature

$n_{O_2(t)}$	in-package moles of O_2 at time t (mol)
$n_{O_2(i)}$	in-package moles of O_2 at time zero (mol)
τ	scale factor (day)
β	constant
t	time (day)
$n_{CO_2(t)}$	in-package moles of CO_2 at time t (mol)
A_{max}	maximum in-package moles of CO_2 (mol)
k	accumulative velocity (day^{-1})
$t_{1/2}$	time to reach half of the maximum in-package moles of CO_2 (day)
R_{O_2}	O_2 consumption rate (O_2 mol kg^{-1} day^{-1})
R_{CO_2}	CO_2 production rate (CO_2 mol kg^{-1} day^{-1})
S	area of package surface (m^2)
P_{O_2}	O_2 permeability of film (O_2 mol m^{-2} day^{-1} kPa^{-1})
P_{CO_2}	CO_2 permeability of film (CO_2 mol m^{-2} day^{-1} kPa^{-1})
$y_{O_2(t)}$	O_2 partial pressure in the package headspace at time t (kPa)
$y_{CO_2(t)}$	CO_2 partial pressure in the package headspace at time t (kPa)
$y^e_{O_2}$	external O_2 partial pressure (kPa)
$y^e_{CO_2}$	external CO_2 partial pressure (kPa)
m	mass product (kg)

Acknowledgements

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References

Aguayo, E.; Allende, A.; Artés, F. 2003. Keeping quality and safety of minimally fresh processed melon. *European Food Research and Technology*, 216: 494-499.

Allende, A.; Luo, Y.; McEvoy, J.L.; Artés, F.; Wang, C.Y. 2004. Microbial and quality changes in minimally processed baby spinach leaves stored under super atmospheric oxygen and modified atmosphere conditions. *Postharvest Biology and Technology*, 33: 51-59.

Arvanitoyannis, I.S.; Khah, E.M.; Christakou, E.C.; Bletsos, F.A. 2005. Effect of grafting and modified atmosphere packaging on eggplant quality parameters during storage. *International Journal of Food Science and Technology*, 40: 311-322.

Bai, J.; Saftner, R.A.; Watada, A.E.; Lee, Y.S. 2001. Modified atmosphere maintains quality of fresh-cut cantaloupe (Cucumis melo L.). *Journal of Food Science*, 66 (8): 1207-1211.

Bai, J.; Saftner, R.A.; Watada, A.E. 2003. Characteristics of fresh-cut honeydew (Cucumis xmelo L.) available to processors in winter and summer and its quality maintenance by modified atmosphere packaging. *Postharvest Biology and Technology*, 28: 349-359.

Beaudry, R.M. 1993. Effect of carbon dioxide partial pressure on blueberry fruit respiration and respiratory quotient. *Postharvest Biology and Technology*, 3: 249-258.

Boynton, B.B.; Welt, B.A.; Sims, C.A.; Balaban, M.O.; Brecht, J.K.; Marshall, M.R. 2006. Effects of low-dose electron beam irradiation on respiration, microbiology, texture, color, and sensory characteristics of fresh-cut Cantaloupe stored in modified-atmosphere packages. *Journal of Food Science*, 71: 149-155.

Brackmann, A.; Eisermann, A.C.; Giehl, R.F.H.; Faban, E.B.; Medeiros, S.L.P.; Steffens, C.A. 2006. Qualidade de melões (Cucumis melo L. var. cantalupensis Naud.), híbrido Torreon, produzidos em hidroponia e armazenados em embalagens de polietileno. *Ciência Rural*, 36: 1143-1149.

Chonhenchob, V.; Chantarasomboon, Y.; Singh, S.P. 2007. Quality changes of treated fresh-cut tropical fruits in rigid modified atmosphere packaging containers. *Packaging Technology and Science*, 20: 27-37.

Day, B. 1996. High oxygen modified atmosphere packaging for fresh prepared produce. *Postharvest News and Information*, 7: 31-34.

Day, B. 2000. Novel MAP for freshly prepared fruit and vegetable products. *Postharvest News and Information*, 11: 27-31.

Del Nobile, MA.; Baiano, A.; Benedetto, A.; Massignan, L. 2006. Respiration rate of minimally processed lettuce as affected by packaging. *Journal of Food Engineering*, 74(1): 60-69.

Flores, F.B.; Martínez-Madrid, M.C.; Amor, M.B.; Pech, J.C.; Latché, A.; Romojaro, F. 2004. Modified atmosphere packaging confers additional chilling tolerance on ethylene-inhibited cantaloupe Charentais melon fruit. *European Food Research and Technology*, 219, 614-619.

Fonseca, S.C.; Oliveira, F.A.R.; Brecht, J.K. 2002. Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. *Journal of Food Engineering*, 52: 99-119.

Kader, A.A.; Zagory, D.; Kerbel, E.L. 1989. Modified atmosphere packaging of fruits and vegetables. *CRC Critical Reviews in Food Science and Nutrition*, 28 (1): 1-30.

Kader, A.A.; Ben-Yehoshua, S. 2000. Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. *Postharvest Biology and Technology*, 20: 1-13.

Lakakul, R.; Beaudry, R.M.; Hernandez, R.J. 1999. Modeling respiration of apple slices in modified atmosphere packages. *Journal of Food Science*, 64(1): 105-110.

Lamikanra, O.; Chen, J.C.; Banks, D.; Hunter, P.A. 2000. Biochemical and microbial changes during the storage of minimally processed Cantaloupe. *Journal of Agricultural and Food Chemistry*, 48: 5955-5961.

Lee, D. S.; Song, Y.; Yam, K.L. 1996. Application of an enzyme kinetics based respiration model to permeable system experiment of fresh produce. *Journal of Food Engineering*, 27: 297-310.

Lester, G. 2006. Consumer preference quality attributes of melon fruits. *Acta Horticulturae*, 712 (1): 175-181.

Neter, J.; Kutner, M.H.; Nachtsheim, C.J.; Wasserman, W. 1996. Applied linear regression models. Chicago, USA: The McGraw-Hill Co., Inc.

O'Connor-Shaw, R.E.; Roberts, R.; Ford, A.L.; Nottingham, S.M. 1996. Changes in sensory quality of sterile cantaloupe dice stored in controlled atmosphere. *Journal of Food Science*, 61(4): 847-851.

Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso, O. 2006. Effect of ripeness on the shelf-life of fresh-cut melon preserved by modified atmosphere packaging. *European Food Research and Technology*, doi: 10.1007/s00217-006-0415-9.

Oms-Oliu, G.; Raybaudi-Massilia, R.M.; Soliva-Fortuny, R.; Martín-Belloso, O. 2007. Effect of superatmospheric and low oxygen modified atmospheres on shelf-life extension of fresh-cut melon. *Food Control*, doi: 10.1016/j.foodcont.2007.03.009.

Rocculi, P.; Del Nobile, M.A.; Romani, S.; Baiano, A.; Dalla Rosa, M. 2006. Use of a simple mathematical model to evaluate dipping and MAP effects on aerobic respiration of minimally processed apples. *Journal of Food Engineering*, 76: 334-340.

Ross, T. 1996. Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Bacteriology*, 81: 501-508.

Soliva-Fortuny, R.C.; Grigelmo-Miguel, N.; Hernando, I.; Lluch, M.A.; Martín-Belloso, O. 2002. Effect of minimal processing on the textural and structural properties of fresh-cut pears. *Journal of the Science of Food and Agriculture*, 82: 1682-1688.

Soliva-Fortuny, R.C.; Martín-Belloso, O. 2003. New advances in extending the shelf-life of fresh-cut fruits: a review. *Trends in Food Science & Technology*, 14 (9): 341-353.

Varoquaux, P.; Wiley, RC. 1997. Biological and biochemical changes in minimally processed refrigerated fruits and vegetables. In R.C. Wiley (Ed.). *Minimally processed refrigerated fruits and vegetables*, Chapman, New York, pp. 226-268.

Wszelaki, A.L.; Mitcham, E.J. 2000. Effects of superatmospheric oxygen on strawberry fruit quality and decay. *Postharvest Biology and Technology*, 20: 125-133.

Capítulo III

Effect of superatmospheric and low oxygen modified atmospheres on shelf-life extension of fresh-cut melon

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Food Control (2007), 19 (2): 191-199

Abstract

The physiological, physicochemical and microbiological quality of fresh-cut 'Piel de Sapo' melon packaged under 2.5 kPa O_2 + 7 kPa CO_2, 70 kPa O_2 or non modified atmospheres was studied. Initial low O_2 levels combined with moderate CO_2 concentrations reduced in-package ethylene concentration whereas high O_2 levels avoided anaerobic metabolism. Both 2.5 kPa O_2 + 7 kPa CO_2 and 70 kPa O_2 atmospheres significantly reduced the growth of microorganisms for 14 days of storage at 5 ºC. Rhodotorula mucilaginosa was initially the dominant yeast, and prevailed during the subsequent storage of fresh-cut 'Piel de Sapo' melon although high O_2 levels as well as low O_2 and high CO_2 conditions were found to have a certain inhibitory effect on its growth. Therefore, a 70 kPa O_2 atmosphere prevented fermentation and significantly improved the quality of fresh-cut melon, while preserving its microbiological stability.

Keywords: fresh-cut melon; high oxygen; modified atmosphere packaging

Introduction

'Piel de Sapo' melon (*Cucumis melo* L.) is the most widely available melon in Spanish markets. Like other members of the Cucurbitaceae, 'Piel de Sapo' is chilling injury sensitive, when stored at low temperatures, which limits its storability and causes significant postharvest losses (Wang, 1989). Thus, fresh-cut processing may be a strategy to reduce losses in quantity and quality during storage of perishable whole fruits. However, good sensory quality is required for fresh-cut products and the fruit processing industry requires the development of preservation techniques capable of keeping safe shelf-life and preserving the original organoleptic fresh-like characteristics of fresh-cut melon.

Low O_2 atmospheres combined with adequate CO_2 concentrations have been used to extend the shelf-life of fresh-cut fruits, helping to reduce respiration and ethylene production, inhibiting or delaying enzymatic reactions and preserving the product from quality losses. Atmosphere modification can also substantially delay the growth of most aerobic spoilage microorganisms. However, under certain conditions, the growth of some anaerobic psychrotrophic pathogens might be allowed or even stimulated (Soliva-Fortuny and Martín-Belloso, 2003a). Initial concentrations of 2-5 kPa O_2 combined with 5-10 kPa CO_2 have been suggested for fresh-cut melon quality maintenance (Qi et al., 1999; Ayhan and Chism, 1998; Bai et al., 2001; Bai et al., 2003).

However, excessively low O_2 levels and/or excessive amounts of CO_2 in the package headspace are often detrimental to the fruit shelf-life because anaerobic respiration is induced, leading to fermentation processes, and the subsequent production of undesirable metabolites (Zagory and Kader, 1988; Soliva-Fortuny et al., 2002). Respiration quotient of fresh-cut butter lettuce exposed to 0-10 kPa O_2 increased above 1 due to fermentation whereas under aerobic conditions ($\geq$ 20 kPa O_2) the respiration quotient was below 1 (Escalona et al., 2006). High CO_2 concentrations inhibit several enzymes of the Krebs's cycle including succinate dehydrogenase, which would either trigger anaerobic respiration or result in accumulation of succinic acid, which is potentially toxic to the fruit tissue (Varoquaux, 1991). Besides, high CO_2 levels above 20 kPa may be responsible for physiological disorders, which can be visually assessed through accelerated browning and necrosis in the flesh tissue (Gorny et al., 2002).

The use of elevated O_2 atmospheres ($\geq$ 70 kPa O_2) has been suggested as an effective method to inhibit the growth of bacteria, yeasts and molds (Amanatidou et al., 1999; Jacxsens et al., 2001; Van der Steen et al., 2002). However, the impact of high O_2 atmospheres on microorganisms

associated with fresh-cut fruits and vegetables differs greatly among commodities and the effect may be dependent on storage temperature (Poubol and Izumi, 2005) or CO_2 accumulation in the headspace environment (Jacxsens et al., 2001; Allende et al., 2004). A 100 kPa O_2 atmosphere inhibited mycelial growth of *B. cinerea* and subsequent fruit decay of strawberries at 5 ºC (Wszelaki and Mitcham, 2000). Consistently, an initial high O_2 of ≥ 70 kPa O_2 retarded the growth of molds (Van der Steen et al., 2002) and yeasts (Jacxsens et al., 2003) on strawberries and raspberries.

On the other hand, exposure to elevated oxygen levels may stimulate, reduce or have no effect on respiration, production of fermentative metabolites, enzymatic browning and loss of overall sensory quality, depending on the commodity, O_2 concentration, storage time and temperature, and CO_2 and C_2H_4 concentrations (Kader and Ben-Yehoshua, 2000). Treatments with high oxygen partial pressures about 100 kPa have been reported to have some positive effects on enzymatic browning inhibition in fresh-cut potatoes (Limbo and Piergiovanni, 2006).

This work aims to overcome the major problems associated with packaging of fresh-cut melon, such as off-odor appearance, development of translucency, loss of firmness and microbial spoilage. The objective was to determine the effects of package atmospheres with different oxygen content on physiological, physicochemical and microbiological quality of fresh-cut 'Piel de Sapo' melon.

Materials and Methods

Sample preparation

'Piel de Sapo' melon (*Cucumis melon L.*) were purchased at a local supplier at a commercial stage of ripeness (Table 1) and stored in a ventilated room at 10 ºC prior to processing. The fruits were cleaned, peeled and the flesh was cut into trapezoidal sections. The fruit pieces were dipped for 1 min in a solution of 0.5% v/v calcium chloride at a product/solution ratio of 1:2. The excess of water was completely drained and then, 100 g were packaged in polypropylene trays. The O_2 and CO_2 permeability of the film were 110 $cm^3 \cdot m^{-2} \cdot day^{-1} \cdot bar^{-1}$ and 500 $cm^3 \cdot m^{-2} \cdot day^{-1} \cdot bar^{-1}$, respectively, at 23ºC and 0% RH (ILPRA Systems España, S.L. Mataró, Spain). The packages were packaged under non modified atmosphere (non MA) or flushed with a mixture of 2.5 kPa O_2 + 7 kPa CO_2 (N_2 balanced) or 70 kPa O_2 (N_2 balanced) in a ratio product / gas mixture of 1:2. The trays were thermosealed with a vacuum packaging machine ILPRA Food Pack Basic V/6 (ILPRA

Systems. CP. Vigevono, Italia). Trays were stored in darkness at 4 ± 1ºC and analyzed throughout 28 days of storage in duplicate.

Table 1. Physicochemical characteristics of fresh melon before processing

Soluble solids (ºBrix)	13.4±0.1
Total acidity (g citric acid / 100 g)	0.29±0.01
pH	5.95±0.08
Pulp color	
L*	70.2±0.7
a*	-2.8±0.1
b*	20.9±0.6
WI [a]	63.5 ±0.5
Firmness (N)	3.97±1.05

Mean ± standard deviation

[a] WI: whiteness index

Headspace gas analysis

The gas composition of the packages headspace was determined with a gas analyzer (Micro-GC CP 2002, Chrompack International, Middelburg, The Netherlands) equipped with a thermal conductivity detector. A little adhesive septum was stuck to the plastic that seals the tray to reinforce the place where the gas sample is taken, thus avoiding leakage of gas. A sample of 1.7 ml was automatically withdrawn from the headspace atmosphere through a needle connected to the injection system. The determination of the oxygen concentration was carried out by injecting a sample of 0.25 µl to the a CP-Molsieve 5Å column (4m x 0.35 mm, df=10 µm) at 60ºC and 100 kPa whereas a portion of 0.33 µl was injected to a Pora-PLOT Q column (10m x 0.32 mm, df=10 µm) at 75 ºC and 200 kPa for carbon dioxide, ethylene acetaldehyde and ethanol determination. Two trays were taken at each time to perform the analysis and 2 readings were carried out for each package.

The variation of headspace atmosphere composition in packages was also expressed through the following equation (Eq. 1):

$$\frac{CO_2\ accumulated}{O_2\ depleted} = \frac{CO_{2t} - CO_{2i}}{O_{2i} - O_{2t}} \qquad \text{(Eq. 1)}$$

where: O_{2i} and CO_{2i} are the initial oxygen and carbon dioxide concentrations, and O_{2t} and CO_{2t} are the oxygen and carbon dioxide concentration at a given time, t.

Color and Firmness evaluation

The color of fresh-cut melon was determined with a Minolta CR-400 chromameter (Konica Minolta Sensing, Inc., Osaka, Japan). The equipment was set up for a D75 illuminant and 10º observer angle. Five fruit pieces from each of two replicate packages were evaluated for each treatment at each sampling time. CIE L* (lightness), a* (red-green) and b* (yellow-blue) values were measured using reflectance measurements. Color changes of fresh-cut processed melon were also measured through whiteness index (WI) (Eq. 2) according to other authors such as Aguayo et al. (2003), Aguayo et al. (2004) and Oms-Oliu et al. (2007).

$$WI = 100 - \left[(100 - L^*)^2 + (a^*)^2 + (b^*)^2\right]^{\frac{1}{2}} \quad \text{(Eq. 2)}$$

Firmness evaluation was performed using a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, England, UK) by measuring the maximum penetration force. Cylindrical samples of 2.0 cm high were obtained from trapezoidal melon pieces and were positioned to be penetrated by a 4 mm diameter rod through their geometric center. The downward distance was set at 10 mm at a rate of 5 mm/s and automatic return. Two trays were taken at each sampling time to perform the analyses, and 5 fruit pieces from each replicate were randomly withdrawn to carry out repetitions.

Microbial counts

Total aerobic psychrophilic microorganisms and yeast and mold populations were evaluated during storage of fresh-cut 'Piel de Sapo' melon. Two counts were obtained each time from each of two replicate packages. The analyses were carried out twice a week during the first two weeks and then, weekly up to 28 days. In sterile conditions, 10 g of melon sample were homogenized for 2 min with 90 ml of 0.1% sterile peptone water with a Stomacher Lab Blender 400 (Seward medical, London, England). Serial dilutions of fruit homogenates were poured in plate count agar (PCA; Biokar Diagnostics. Beauvais, France) at 7ºC ± 1ºC for 7 days for psychrophilic aerobic bacteria counts (ISO 4833, 1991) and chloramphenicol glucose agar (GCA) at 25ºC ± 1ºC for 5 days for yeast and mold counts (ISO 7954, 1988).

Predictive modeling

The Gompertz equation has been often used to calculate the shelf-life of numerous fresh-cut fruits and vegetables (Lanciotti et al., 1999; Soliva-Fortuny and Martín-Belloso, 2003b; Soliva-Fortuny et al., 2004; Corbo et al., 2004). Experimental data of this study were used to test the re-parameterized Gompertz equation (Eq. 2) proposed by Corbo et al. (2006) in order to include shelf-life as a parameter of the equation. The model was tested for psychrophilic aerobic bacteria counts, taking into account the maximum acceptable contamination value at the end of the microbiological shelf-life according to the Spanish legislation.

$$y = \log(10^7) - A\exp\left\{-\exp\left\{\left[\left(\mu_{max}e\right)\cdot\left[\frac{(\lambda - SL)}{A}\right]\right]+1\right\}\right\} + A\exp\left\{-\exp\left\{\left[\left(\mu_{max}e\right)\cdot\left[\frac{(\lambda - t)}{A}\right]\right]+1\right\}\right\}$$

(Eq. 3)

where: $\log(10^7)$ is the recommended limit of acceptability for aerobic microorganisms population, according to the Spanish legislation (RD 3484/2000) [$\log(CFU\ g^{-1})$]; A, the maximum growth attained at the stationary phase [$\log(CFU\ g^{-1})$]; μ_{max}, the maximal growth rate [$\Delta\log(CFU\ g^{-1})\ day^{-1}$]; λ, the lag time (days); t, the storage time (days); SL, the estimated shelf life parameter (days).

Isolation and identification of spoilage microorganisms on fresh-cut melon

Three types of yeasts were isolated as prevalent spoilage flora from fresh-cut melon packages stored under MA packaging at 4ºC for their identification. Colonies were isolated and purified on glucose chloramphenicol agar (GCA) (Biokar Diagnostics. Beauvais, France) and incubated for 24 hours. The yeast strains purified were kept on extract malt agar slants (EMA) (Biokar Diagnostics. Beauvais,France) at 4 ºC until their identification. Yeasts were identified with an API biochemical kit (api® 20C AUX, bioMérieux, Marcy l'Etoile, France). The three yeasts isolated from fresh-cut 'Piel de Sapo' melon stored under 2.5 kPa O_2 + 7 kPa CO_2, air and 70 kPa O_2 atmospheres were *Rhodotorula mucilaginosa*, *Candida famata* and *Candida ciferii*.

Strain, inoculums preparation and fresh-cut melon inoculation

Rhodotorula mucilaginosa isolated as the dominant yeast strain associated with spoilage of fresh-cut melon packed under MA and stored at 4ºC in the Food Technology Department of Lleida University, Lleida, Spain, was used to evaluate the effect of different modified atmosphere

packaging conditions on the inactivation of main spoilage yeasts of fresh-cut melon. Stock culture of *Rhodotorula mucilaginosa* was grown in malt extract broth (EMB) at 30ºC for 24h and 80 rpm. Concentration was then adjusted to10^5 CFU ml^{-1} using saline peptone (0.1% peptone, Biokar Diagnostics. Beauvais, France. + NaCl, Scharlau Chemie, S.A. Barcelona, Spain). Fifty grams of fresh-cut melon processed as in point 2.1 were inoculated by uniformly spreading 500 µl of *Rhodotorula mucilaginosa* diluted stock culture (10^5CFU ml^{-1}) over its entire upper surface using a sterile micropipette. Trays of inoculated fresh-cut melon were then packaged under 2.5 kPa O_2 + 7 kPa CO_2, 70 kPa O_2 and non MA conditions as it is indicated in point 2.1. Non inoculated fresh-cut melon, packaged under the same atmosphere conditions, was used as a control. A total of 84 trays (42 inoculated and 42 without inoculated) containing 50 g of fresh-cut melon were stored at 4ºC for 28 days. A pair of trays of inoculated and non-inoculated fresh-cut melon packaged under each atmosphere condition was analyzed at 0, 3, 7, 10, 14, 21 and 28 days.

Statistical analysis

Statistical analysis was performed using the Statgraphics plus v.5.1 software (Manugistics, Inc., Rockville, MA, USA). Data were analyzed by multifactor analysis of variance. The analysis of covariance provided significant differences throughout the time within fresh-cut melon stored under the conditions tested. The statistical package was used to apply the Duncan multiple range test in order to find significant differences between packaging conditions.

Results and discussion

Atmosphere composition in packages of fresh-cut melon

The concentrations of O_2 and CO_2 in the trays headspace of fresh-cut melon packaged under 2.5 kPa O_2 + 7 kPa CO_2, 70 kPa O_2 or non MA are shown in Fig. 1. The O_2 levels inside high O_2 atmosphere packages decreased slowly during the first 14 days, thus remaining above 60 kPa (Fig. 1A), and underwent a more marked decrease during the subsequent days up to levels of approximately 40 kPa. O_2 levels in packages stored under non MA decreased continuously throughout storage, reaching values below 10 kPa after 10-15 days and only slightly above 1 kPa at the end of storage. On the other hand, an initial 2.5 kPa O_2 + 7 kPa CO_2 atmosphere strongly induced critical O_2 concentrations below 1 kPa at day 14, when values stabilized (Fig. 1A). On the

other hand, CO_2 production increased in packages of fresh-cut 'Piel de Sapo' melon up to 10 kPa during 14 days of storage and no substantial differences were detected among packaging conditions during this period. However, CO_2 levels inside packages flushed with high O_2 atmospheres reached partial pressures of near 30 kPa after 28 days of storage and almost trebled the amount of CO_2 accumulated in packages with initial low O_2 concentrations (Fig. 1B). The relationship between CO_2 accumulation and O_2 depletion was kept constant through the storage under both non MA or high O_2 packaging conditions, whereas it increased through 28 days in samples packaged under 2.5 kPa O_2 + 7 kPa CO_2, indicating induced anaerobic respiration (Fig. 2). This increase of anaerobic respiration and, in turn, of respiration quotient, is generally an indicative of a switch to fermentative reactions (Wills et al., 1998). Our results are therefore in accordance with those of Bai et al. (2003), who suggested a minimum of 1 kPa O_2 and a maximum of 15 kPa CO_2 to ensure safety of fresh-cut Cantaloupe melon in order to prevent anaerobic conditions that cause fermentative decay.

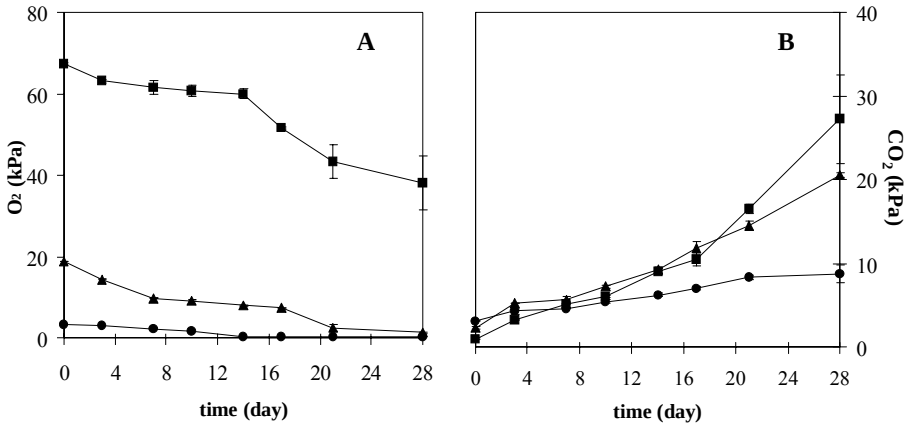

Figure 1. O_2 and CO_2 concentrations in the package headspace of fresh-cut 'Piel de Sapo' melon packaged under 2.5 kPa O_2 + 7 kPa CO_2 (•),70 kPa O_2 (■) or non MA (▲) during 28 days at 4ºC. Data shown are mean ± standard deviation.

The inhibition of ethylene production under absence or low O_2 conditions has been reported by many authors. Low O_2 atmosphere reduced dramatically ethylene production in honeydew melon cubes (Qi et al., 1999). In this study, fresh-cut ´Piel de Sapo´ melon produced more ethylene under 70 kPa O_2 than under 2.5 kPa O_2 + 7 kPa CO_2 or non MA ($p \leq 0.05$) (Fig. 3). However, C_2H_4

levels did not exceed 1 ppm under high O_2 concentrations, suggesting that 'Piel de Sapo' melon is a fruit with low physiological activity (Valdenegro et al., 2004).

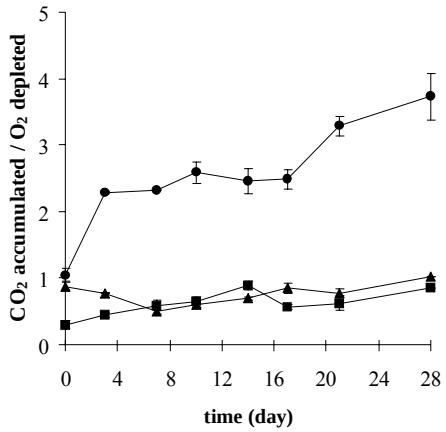

Figure 2. Relationship between CO_2 accumulated and O_2 withdrawn from the package headspace of fresh-cut 'Piel de Sapo' melon packaged under 2.5 kPa O_2 + 7 kPa CO_2 (●), under 70 kPa O_2 (■) or under non MA (▲) during 28 days at 4ºC. Data shown are mean ± standard deviation.

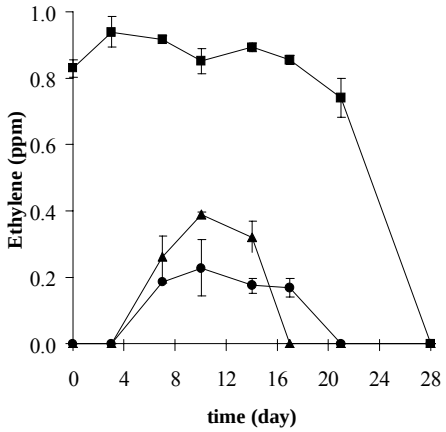

Figure 3. Ethylene concentrations in the package headspace of fresh-cut 'Piel de Sapo' melon packaged under 2.5 kPa O_2 + 7 kPa CO_2 (●), under 70 kPa O_2 (■) or under non MA (▲) during 28 days at 4ºC. Data shown are mean ± standard deviation.

Acetaldehyde and ethanol production was triggered under 2.5 kPa O_2 + 7 kPa CO_2 atmosphere in comparison to 70 kPa O_2 or non MA conditions. An increase in acetaldehyde levels was observed in packages stored under low O_2 levels during the first week, reaching maximum values about 15 ppm (Fig. 4A). The tissue could have initiated anaerobic respiration, by which glucose is converted to pyruvate by glycolysis and then, pyruvate metabolized to acetaldehyde. However, these high amounts began to decrease without significant amounts of ethanol being accumulated in the package atmosphere, suggesting that the initial peak production could be a consequence of the wounding response. Subsequently, the decrease in acetaldehyde content could continue due to the shift to fermentative anaerobic conditions mediating its final conversion to ethanol. Ethanol began to accumulate beyond day 7 in packages stored under initial 2.5 kPa O_2 + 7 kPa CO_2 conditions and beyond the third week storage under initial air atmospheres, as a consequence of a drop of O_2 concentrations inside the packages below 2 kPa levels (Fig. 1A and 4B). Low O_2 atmospheres seem to promote the production of anaerobic metabolites due to anaerobic metabolism (Wszelaki et al., 2000). In packages with initial 70 kPa O_2 conditions, ethanol was detected during the last week of storage (Fig. 4B). Thus, the application of high O_2 levels in packages of fresh-cut fruits and vegetables could be particularly effective in preventing anaerobic fermentative reactions promoted by low O_2 atmospheres (Allende et al., 2004).

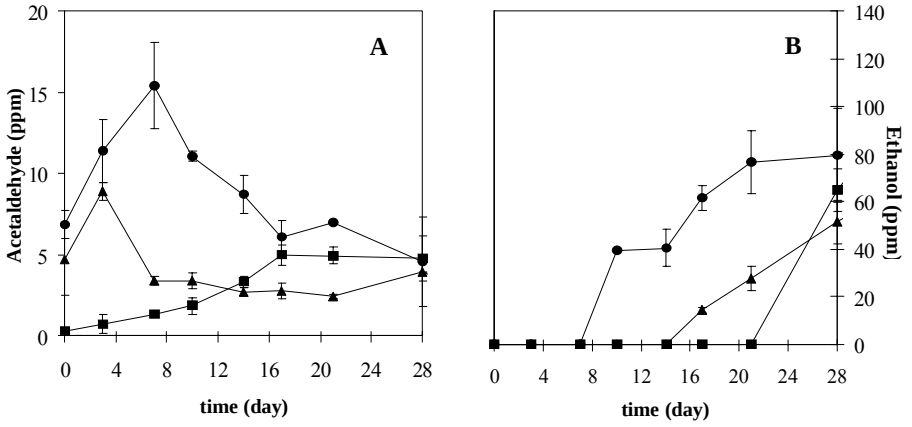

Figure 4. Acetaldehyde and ethanol concentrations in the package headspace of fresh-cut 'Piel de Sapo' melon packaged under 2.5 kPa O_2 + 7 kPa CO_2 (•), under 70 kPa O_2 (■) or under non MA (▲) during 28 days at 4ºC. Data shown are mean ± standard deviation.

Color and firmness evolution

Significant differences on L* and WI values among packaging atmosphere conditions were detected (Table 2). On the other hand, MA did not affect significantly a* and b* values. L* and WI values were significantly lower on fresh-cut melon packaged under 70 kPa O_2 atmospheres compared to other conditions. During the two first weeks, both 2.5 kPa O_2 + 7 kPa CO_2 and non MA atmospheres best maintained initial color of fresh-cut 'Piel de Sapo' melon. Nevertheless, a decrease of L* and WI parameters was detected on fresh-cut melon packaged under 2.5 kPa O_2 + 7 kPa CO_2 conditions at the end of storage, indicating a strong development of translucency caused by the fermentative decay. Aguayo et al. (2004) associated the decrease of WI with an increase in translucently injury in fresh-processed melon. Development of translucency has been found to be the principal visual change of deterioration in fresh-cut melon packaged under MA (O'Connor-Shaw et al., 1994; Bai et al., 2001, 2003; Aguayo et al., 2003; Aguayo et al., 2004).

Firmness of fresh-cut 'Piel de Sapo' melon decreased slightly under low O_2 levels and non MA, whereas it was maintained at values similar to those of the fresh fruit under high O_2 atmospheres. Thus, firmness values of samples stored under 70 kPa O_2 were the highest throughout all the storage period, although differences among modified atmospheres were not statistically significant during the first 10 days of storage (Table 3). According to these results, loss of firmness, strong off-odor and deterioration of taste have been reported on fresh-cut produce due to a combination of extremely low O_2 and high CO_2 in packages (Van der Steen et al., 2002; Allende et al., 2004).

Table 2. L* and WI values of fresh-cut 'Piel de Sapo' melon packaged under 2.5 kPa O_2 + 7 kPa CO_2, 70 kPa O_2 and non MA during 28 days at 4 ºC

	2.5 kPa O_2 + 7 kPa CO_2		Non MA [a]		70 kPa O_2	
Days	L*	WI [b]	L*	WI	L*	WI
0	71.8±2.3abA	65.0±1.7abA	70±3cA	63.9±2.4bA	70±4aA	62.7±1.8aA
2	75±5aA	67±3aA	76±3abA	69±4aA	66±6aB	60±6aB
4	74±3aA	66±3abA	75.1±2.5bA	67±3aA	65±8aB	60±7aB
7	75±6aA	67±5aA	75±6abA	69±3aA	66±5aB	60±4aB
9	75±3aA	67±3aA	76.2±1.9abA	68.2±1.5aA	65±11aB	60±8aB
11	76±6aA	67±6aA	78.7±2.1aA	70±3aA	67±8aB	61±6aB
14	74±3aA	66±2abA	75±3bA	68±3aA	65.6±2.5aB	59.7±1.8aB
21	74±3aB	66±2abB	77.1±1.9abA	69±3aA	67±4aC	62±3aC
28	70±3bB	63±3bB	77±4abA	69±3aA	68±3aB	62±3aB

Mean ± standard deviation

Values within a column followed by the same small letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$)

Values within the same line followed by the same capital letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$)

[a] MA: modified atmosphere, [b] WI: whiteness index

Table 3. Firmness values (N) of fresh-cut 'Piel de Sapo' melon packaged under 2.5 kPa O_2 + 7 kPa CO_2, 70 kPa O_2 and non MA during 28 days at 4 ºC

Days	2.5 kPa O_2 + 7 kPa CO_2	Non MA [a]	70 kPa O_2
0	3.4±1.2aA	3.1±0.4aA	3.4±0.9abcA
2	3.1±0.3abA	2.8±0.4abA	3.1±0.4abA
4	2.4±0.5bcA	2.2±0.9bcA	2.6±0.9bA
7	2.8±0.3abA	2.7±0.6abA	3.0±0.8abcA
9	3.0±0.9abA	1.8±0.4cB	2.8±0.7bcA
11	3.1±0.6abB	2.4±0.5abcB	3.9±1.2aA
14	2.5±0.5bcB	1.9±0.4cB	3.5±1.3abcA
21	2.8±0.4abA	2.1±0.5bcB	3.1±1.2abcA
28	2.1±0.4cB	2.3±0.8bcB	3.5±0.8abcA

Mean ± standard deviation

Values within a column followed by the same small letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$)

Values within the same line followed by the same capital letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$)

[a] MA: modified atmosphere

Effect of MA on microbial growth of fresh-cut melon

Initial aerobic psychrophilic and yeast and mold counts on fresh-cut 'Piel de Sapo' melon were about 1.2-1.5 log (CFU g^{-1}) and 1.5-1.8 log (CFU g^{-1}), respectively (Fig. 5). The proliferation of

aerobic psychrophilic microorganisms through storage was more rapid than that of yeasts and molds under all packaging conditions. Low O_2 and moderate CO_2 atmospheres have been shown to reduce slightly the microbial growth of fresh-cut cantaloupe or honeydew melons (Bai et al., 2001; 2003). High O_2 concentrations have been found to cause damage to microorganisms by intracellular generation of reactive oxygen species (ROS) that damage cell components and reduce cell viability when oxidative stresses overwhelm cellular protection system (Kader et al., 2000; Jacxsens et al., 2001; Poubol et al., 2005; Escalona et al., 2006). However, different organisms vary greatly in their sensitivity to O_2 partial pressure and some of them could have developed strategies, such as the induction of other enzymes that decompose ROS, to avoid their lethal damage (Kader et al., 2000).

Yeast populations were prevalent in fresh-cut 'Piel de Sapo' melon compared to molds throughout the storage, but their growth was significantly affected by the packaging atmosphere composition. Thus, fresh-cut melon was more rapidly spoiled by yeasts under initial non MA than under 2.5 kPa O_2 + 7 kPa CO_2 or 70 kPa O_2 atmospheres. However, yeast and mould counts strongly increased on fresh-cut melon stored under high O_2 levels during the third week of storage, when O_2 levels decreased below 60 kPa. Exposure to high O_2 concentrations during the first days of storage did not inhibit microbial growth but caused a prolongation of the lag phase and a reduction in the growth rate. Amanatidou et al. (1999) agreed that minimally processed vegetables packaged under high O_2 alone (80 to 90 kPa O_2, balance N_2) exhibited more pronounced lag phase at higher O_2 concentration. In agreement, Van der Steen et al. (2002) reported a slight inhibitory effect of high O_2 atmospheres on the microbial growth of packaged strawberries and raspberries. The spoilage of products seems to become detectable for consumers when yeast counts on fresh-cut fruits reach levels above 5 log (CFU g^{-1}) (Jacxsens et al., 1999). Therefore, yeast and mould populations on fresh-cut 'Piel de Sapo' melon packaged under 2.5 kPa O_2 + 7 kPa CO_2 atmosphere remained at an acceptable level [< 5 log (CFU g^{-1})] throughout storage, whereas a 70 kPa O_2 atmosphere had an inhibiting effect on yeast growth for two weeks but exceeded the level of acceptability beyond day 21.

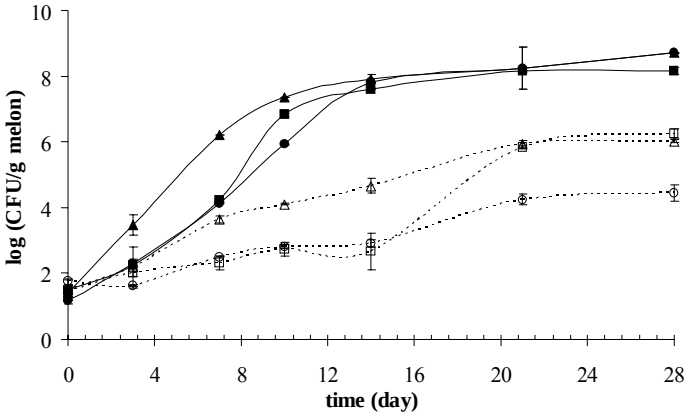

Figure 5. Microbial growth on fresh-cut 'Piel de sapo' melon during 28 days at 4ºC. Aerobic psychrotrophic microorganisms: (●) packaging under 2.5 kPa O_2 + 7 kPa CO_2, (■) packaging under 70 kPa O_2, (▲) packaging under non MA; Yeast and molds (o) packaging under 2.5 kPa O_2 + 7 kPa CO_2, (□) packaging under 70 kPa O_2, (Δ) packaging under non MA. Data shown are mean ± standard deviation.

Maximum aerobic psychrophilic counts of 8 log (CFU g^{-1}) were attained beyond the day 14, regardless the atmosphere conditions. However, 2.5 kPa O_2 + 7 kPa CO_2 and 70 kPa O_2 initial atmospheres were found to decrease bacterial growth for two weeks ($P \leq 0.001$). During the first week storage, differences on bacterial counts were not detected among both high and low O_2 atmospheres. Jacxsens et al. (2001) found no difference in aerobic psychrotrophic growth in chicory endives between conventional (3 kPa O_2 and 5 kPa CO_2) and superatmospheric O_2 (95 kPa O_2 and 5 kPa CO_2) packaging after 1 week storage. On the other hand, these authors reported slightly higher counts at 7 days storage under low O_2 conditions than under superatmospheric packaging for both grated celeriac and mushroom slices. The growth of mesophilic and psychrophilic bacteria on fresh processed lettuce packaged under 80 kPa O_2 was lower than under non MA for 8 days (Allende et al., 2003).

The Gompertz equation was re-parameterized to include the shelf-life as a fitting parameter among the parameters of the equation according to Corbo et al. (2006). By fitting Eq. (3) to the growth of aerobic psychrophilic microorganisms it was possible to determinate shelf-life of fresh-cut melon and its confidence interval. The Gompertz parameters and shelf-life values obtained according to

this approach are listed in Table 4. The lag phase for the growth of aerobic psychrophilic microorganisms on fresh-cut melon was very short for all packaging conditions. The maximal growth rates of bacterial growth (μ_{max}) and maximum counts attained at the stationary phase (A) were similar for all conditions. Packaging under 2.5 kPa O_2 + 7 kPa CO_2 prolonged the microbiological shelf-life of the fresh-cut melon from 10-14 days approximately whereas non MA limited shelf-life of fresh-cut melons to 7-10 days.

Table 4. Kinetic constants estimated by a modification of the Gompertz model in order to describe the microbial growth of aerobic psychrotrophic microorganisms of fresh-cut 'Piel de Sapo' melon packaged under different modified atmosphere conditions. Parameters parameters obtained by fitting re-parameterized Gompertz model (Eq. 3)

Aerobic psychrotrophic microorganisms					
MA conditions [a]	A [b]	μ_{max} [c]	λ [d]	SL [e]	R^2
2.5% O_2+7% CO_2	7.6±1.8	0.62±0.23	1.9±3.9	12.4±2.1	98.9
70% CO_2	7.6±2.5	0.62±0.12	-0.9±4.4	11.1±1.5	99.5
Non modified atmosphere	8±3	0.76±0.15	-1±4	9.0±1.2	99.5

Mean ± standard deviation
[a] MA: modified atmosphere
[b] A: maximum growth attained at the stationary phase [log (CFU g^{-1})]
[c] μ_{max}: maximal growth rate [Δlog (CFU g^{-1}) day^{-1}]
[d] λ: lag time (days)
[e] SL: estimated shelf life parameter (days)

Rhodotorula mucilaginosa, *Candida famata* and *Candida ciferii* were isolated as the most important spoilage microorganisms from fresh-cut 'Piel de Sapo' melon packaged under modified atmosphere conditions. Table 5 shows a clear predominance of *Rhodotorula mucilaginosa* of around a 90% of the total yeast population, once melon was processed. However, its prevalence decreased throughout the storage time and its survival was affected by packaging atmospheres. The reduction was of 40%, 30% and 20% for 70 kPa O_2, 2.5 kPa O_2 + 7 kPa CO_2 and non MA, respectively. Yeast genera found in fresh-cut 'Piel de Sapo' melon were similar to those detected by Poubol et al. (2005) on 'Nam Dokmai' mango cubes stored under high O_2 atmospheres. These authors also found the identified yeast genera to be sensitive to high O_2 levels.

Table 5. Survival percentage of *Rhodotorula mucilaginosa* related to total yeast growth on fresh-cut melon packaged under 2.5 kPa O_2 + 7 kPa CO_2, 70 kPa O_2 and non MA and stored at 4 ºC for 28 days

Days	2.5 kPa O_2 + 7 kPa CO_2	Non MA [a]	70 kPa O_2
0	86.2 ± 0.9aA	88.1 ± 0.7aA	94 ± 8aA
3	66.3 ± 3.2bB	87.2 ± 1.8aA	94 ± 8aA
7	67.6 ± 6bB	88.2 ± 1.4aA	55.9± 2.4bB
10	67 ± 4 bB	83 ± 4abA	58 ± 4bB
14	61 ± 6bB	77 ± 5bcA	39.6 ± 0.2cC
21	62.7 ± 1.2bB	74 ± 3cA	38 ± 5cC
28	63 ± 4bB	74 ± 2cA	32.5 ± 0.1cC

Mean ± standard deviation

Values within a column followed by the same small letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$)

Values within the same line followed by the same capital letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$)

[a] MA: modified atmosphere

Effect of MA on the survival of Rhodotorula mucilaginosa on fresh-cut melon

The potential effect of modified atmospheres to reduce or inhibit the growth of *Rhodotorula mucilaginosa* was assessed through 28 days of storage. Yeast inoculation on the fresh-cut fruit surface was carried out before flushing the packages with different initial atmospheres at a final concentration of approximately 3 log (CFU g^{-1}). This concentration of inoculum was chosen to have observable microbial growth on the fresh-cut product throughout storage, provided that the maximum populations attained on the fresh-cut product did not surpass 6 log (CFU g^{-1})

Table 6 shows differences in the growth pattern of *R. mucilaginosa* on fresh-cut melon packaged under 2.5 kPa O_2 + 7 kPa CO_2, 70 kPa O_2 or non MA conditions. Counts of inoculated and control samples stored under non MA increased throughout storage, exhibiting the highest levels by the end of storage. Exposure to low O_2 levels reduced the growth rate of *R. mucilaginosa*. Initial counts of *R. mucilaginosa* on control and inoculated samples packaged under 2.5 kPa O_2 + 7 kPa CO_2 atmospheres increased slightly after 7 days of storage. On the other hand, initial counts of *R. mucilaginosa* in inoculated and control samples under 70 kPa O_2 levels were maintained for 10 days and appeared to decrease during the following days. Therefore, final counts on fresh-cut 'Piel de Sapo' melon stored under high O_2 levels were < 2 log (CFU g^{-1}) according to the minimum detection level of the pour plate method used in this study, 2 log (CFU g^{-1}).

Table 6. Growth of *Rhodotorula mucilaginosa* (expressed as log CFU g^{-1}) throughout storage of fresh-cut 'Piel de Sapo' melon packaged under 2.5 kPa O_2 + 7 kPa CO_2, 70 kPa O_2 and non MA and stored at 4 ºC for 28 days

Inoculated samples			
Days	2.5 kPa O_2 + 7 kPa CO_2	Non MA [a]	70 kPa O_2
0	3.1±0.3fA	3.13±0.02fA	3.12±0.09abA
3	3.45±0.09eB	4.09±0.05eA	3.14±0.09abC
7	3.69±0.06dB	4.55±0.14dA	3.61±0.09aB
10	4.51±0.11cB	6.44±0.02cA	3.50±0.06aC
14	4.61±0.02bcB	7.08±0.06bA	2.6±0.8bcC
21	4.74±0.01efB	7.23±0.07bA	< 2cC
28	4.79±0.01fB	7.5±0.3aA	< 2cC
Control samples			
Days	2.5 kPa O_2 + 7 kPa CO_2	Non MA [a]	70 kPa O_2
0	2.09±0.12dA	2.3±0.4eA	2.23±0.09aA
3	2.12±0.01dAB	2.45±0.21dA	1.84±0.21bB
7	2.45±0.21cdAB	3.4±0.8cA	2.09±0.12abB
10	3.02±0.13bA	3.8±0.6bA	2.29±0.16aB
14	2.6±0.3cB	5.0±0.9aA	< 2abC
21	4.46±0.04aB	5.11±1.03aA	< 2abC
28	3.69±0.11aB	5.6±0.5aA	< 2abC

Mean ± standard deviation
Values within a column followed by the same small letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$)
Values within the same line followed by the same capital letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$)
[a] MA: modified atmosphere

The total yeast counts in samples without inoculation and stored under low and high O_2 atmospheres (Fig. 5) increased beyond two weeks storage, reaching maximum levels of 4 and 6 log (CFU g^{-1}) by day 28, respectively. Thus, such increase of total yeast counts detected under these conditions after 14 days of storage could be due to the proliferation of other yeasts which are less sensitive to high O_2 levels. Growth of *C. lambica* has shown to be clearly reduced by high O_2 concentrations *in vitro* experiments (Jacxsens et al., 2001). O_2 concentrations of 80 kPa resulted in a stimulation of both *C. guilliermondii* and *C. sake* growth at 8 ºC, but the combination of 80 kPa O_2 and 20 kPa CO_2 almost completely blocked the growth of *C. guilliermondii*. Similar growth characteristics were observed for *C. sake*, although to a lesser extent (Amanatidou et al., 1999).

However, the effect of high O_2 atmospheres in *in vivo* experiments may not to be as pronounced as in *in vitro* experiments (Jacxsens et al., 2001).

Conclusions

A 70 kPa O_2 atmosphere prevented anaerobic fermentation whereas fermentative pathways were triggered under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere. Anaerobic respiration was found to become predominant when O_2 concentration decreased below 2 kPa. In addition, high O_2 levels preserved the initial color and firmness of fresh-cut melon better than low O_2 concentrations or non MA.

In relation to microbiological stability, the shelf-life of fresh-cut melon was 10-14 days for both 2.5 kPa O_2 + 7 kPa CO_2 and 70 kPa O_2 conditions. Yeasts appeared to be the spoiling microorganisms of fresh-cut 'Piel de Sapo' melon packaged under modified atmosphere. Exposure to low and high O_2 levels clearly reduced the yeast growth rate. Moreover, 70 kPa O_2 and 2.5 kPa O_2 + 7 kPa CO_2 atmospheres were shown to have an inhibitory effect on the growth of *R. mucilaginosa*, the most prevalent microorganism on fresh-cut 'Piel de Sapo' melon.

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References

Aguayo, E.; Allende, A.; Artés, F. 2003. Keeping quality and safety of minimally fresh processed melon. *European Food Research and Technology*, 216: 494-499.

Aguayo, E.; Escalona, V.H.; Artés, F. 2004. Metabolic behaviour and quality changes of whole and fresh processed melon. *Journal of Food Science*, 69(4): 148-155.

Allende, A.; Del Carmen Barba, M.; Artés, F. 2003. Effect of High O_2 and CO_2 atmosphere on microbial and sensorial quality of fresh processed 'Lollo Rosso' Letucce. *Acta Horticulturae*, 599: 97-101.

Allende, A.; Luo, Y.; McEvoy, J.L.; Artés F.; Wang, C.Y. 2004. Microbial and quality changes in minimally processed baby spinach leaves stored under superatmospheric oxygen and modified atmosphere conditions. *Postharvest Biology and Technology*, 33: 51-59.

Amanatidou, A.; Smid, E.J.; Gorris, L.G.M. 1999. Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable-associated microorganisms. *Journal of Applied Microbiology*, 86: 429-438.

Ayhan, Z.; Chism, G.W. 1998. The shelf life of minimally processed fresh-cut melons. *Journal of Food Quality*, 21: 29-40.

Bai, J.H.; Saftner, R.A.; Watada, A.E.; Lee, Y.S. 2001. Modified atmosphere maintains quality of fresh-cut cantaloupe (Cucumis melo L.). *Journal of Food Science*, 66 (8): 1207-1211.

Bai, J.; Saftner, R.A.; Watada, A.E. 2003. Characteristics of fresh-cut honeydew (Cucumis xmelo L.) available to processors in winter and summer and its quality maintenance by modified atmosphere packaging. *Postharvest Biology and Technology*, 28: 349-359.

Corbo, M.R.; Altieri, C.; D'Amato, D.; Campaniello, D.; Del Nobile, M.A.; Sinigaglia, M. 2004. Effect of temperature on shelf-life and microbial population of lightly processed cactus pears fruit. *Postharvest Biology and Technology*, 31: 93-104.

Corbo, M.R.; Del Nobile, M.A.; Sinigaglia, M. 2006. A novel approach for calculating shelf-life of minimally processed vegetables. *International Journal of Food Microbiology*, 106: 69-73.

Escalona, V.H.; Verlinden, B.E.; Geysen, S.; Nicolaï, B.M. 2006. Changes in respiration of fresh-cut butterhead lettuce under controlled atmospheres using low and superatmospheric oxygen conditions with different carbon dioxide levels. *Postharvest Biology and Technology*, 39: 48-55.

Gorny, J.R.; Hess-Pierce, B.; Cifuente, R.A.; Kader, A.A. 2002. Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest Biology and Technology*, 24: 271-278.

Jacxsens, L.; Devlieghere, F.; Falcato, P.; Debevere, J. 1999. Behavior of Listeria monocytogenes and Aeromonas spp. of fresh-cut produce packaged under equilibrium modified atmosphere. *Journal of Food Protection*, 62: 1128-1135.

Jacxsens, L.; Devlieghere, F.; Van der Steen, C.; Debevere, J. 2001. Effect of high oxygen atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. *International Journal of Food Microbiology*, 71: 197-210.

Jacxsens, L.; Devlieghere, F.; Van der Steen, C.; Siro, I.; Debevere, J. 2003. Application of ethylene absorbers in combination with high oxygen atmospheres for the storage of strawberries and raspberries. *Acta Horticulturae*, 600: 311-318.

Kader, A.A.; Ben-Yehoshua, S. 2000. Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. *Postharvest Biology and Technology*, 20: 1-13.

Lanciotti, R.; Corbo, M.R.; Gardini, F.; Sinigaglia, M.; Guerzoni, M.E. 1999. Effect of hexanal on the shelf-life of fresh apple slices. *Journal of Agricultural and Food Chemistry*, 47 (11): 4769-4776.

Limbo, S.; Piergiovanni, L. 2006. Shelf life of minimally processed potatoes Part 1. Effects of high oxygen partial pressures in combination with ascorbic and citric acids on enzymatic browning. *Postharvest Biology and Technology*, 39: 254-264.

O'Connor-Shaw, R.E.; Roberts, R.; Ford, A.L.; Nottingham, S.M. 1994. Shelf-life of minimally processed Honeydew, kiwifruit, papaya, pineapple, and Cantaloupe. *Journal of Food Science*, 59: 1202-1206.

Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso, O. 2007. Effect of ripeness on the shelf-life of fresh-cut melon preserved by modified atmosphere packaging. *European Food Research and Technology*, 225: 301-311

Poubol, J.; Izumi, H. 2005. Physiology and Microbiological Quality of Fresh-cut mango cubes as affected by high-O_2 controlled atmospheres. *Journal of Food Science*, 70(6): 286-291.

Qi, L.; Wu, T.; Watada, A.E. 1999. Quality changes of fresh-cut honeydew melons during controlled atmosphere storage. *Journal of Food Quality*, 22: 513-521.

Soliva-Fortuny, R. C.; Oms-Oliu, G.; Martín-Belloso, O. 2002. Effects of ripeness stages on the storage atmosphere, colour, and textural properties of minimally processed apple slices. *Journal of Food Science*, 67(5): 1958-1963.

Soliva-Fortuny, R. C.; Martín-Belloso, O. 2003a. New advances in extending the shelf-life of fresh-cut fruits: a review. *Trends in Food Science and Technology*, 14: 341-353.

Soliva-Fortuny, R.C.; Martín-Belloso, O. 2003b. Microbiological and biochemical changes in minimally processed fresh-cut Conference pears. *Food Research Technology*, 217: 4-9.

Soliva-Fortuny, R.C.; Elez-Martínez, P.; Martín-Belloso, O. 2004. Microbiological and biochemical stability of fresh-cut apples preserved by modified atmosphere packaging. *Innovative Food Science and Emerging Technologies* 5: 215-224.

Qi, L.; Wu, T.; Watada, A.E. 1999. Quality changes of fresh-cut honeydew melons during controlled atmosphere storage. *Journal of Food Quality*, 22: 513-521.

Valdenegro, M.; Flores, F.B.; Romojaro, F.; Ramírez, M.; Martínez-Madrid, M.C. 2004. Susceptibility of Spanish Melon fruits to Chilling Injury during Cold Storage. *Acta Horticulturae*, 682: 1219-1225.

Van der Steen, C.; Jacxsens, L.; Devlieghere, F.; Debevere, J. 2002. Combining high oxygen atmospheres with low oxygen modified atmosphere packaging to improve the keeping quality of strawberries and raspberries. *Postharvest Biology Technology*, 26: 49-58.

Varoquaux, P. 1991. Ready-to-use fresh fruits and vegetables. *Revue Generale du Froid*, 81: 33-43.

Wang, C.Y. 1989. Chilling injury of fruits and vegetables. *Food Reviews International*, 5: 209-236.

Wills, R.; McGlasson, B.; Graham, D.; Joyce, D. 1998. Physiology and Biochemistry. In *Postharvest an Introduction to the physiology and Handling of Fruit, Vegetables and Ornamentals*. CAB International, New York, pp. 33-59.

Wszelaki, A.L.; Mitcham, E.J. 2000. Effects of superatmospheric oxygen on strawberry fruit quality and decay. *Postharvest Biology and Technology*, 20: 125-133.

Zagory, D.; Kader, A.A. 1988. Modified atmosphere packaging of fresh produce. *Food Technology*, 42: 70-77.

Capítulo IV

The role of peroxidase on the antioxidant potential of fresh-cut 'Piel de Sapo' melon packaged under different modified atmospheres

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Abstract

The effects of different initial in-package O_2 and CO_2 concentrations (2.5 kPa O_2 + 7 kPa CO_2, 10 kPa O_2 + 7 kPa CO_2, 21 kPa O_2, 30 kPa O_2 and 70 kPa O_2) on peroxidase activity, vitamin C content, total phenolics and antioxidant capacity of fresh-cut 'Piel de Sapo' melon have been investigated for 14 days at 4 °C. The radical scavenging activity of fresh-cut melon strongly increased after 9 days storage related to a synthesis of phenolic compounds, especially under 2.5 kPa O_2 + 7 kPa CO_2 atmospheres. Low O_2 levels best maintained vitamin C and phenolic content during the storage. However, stressful too-low O_2 and high CO_2 levels induced an important increase of peroxidase activity under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere, which was directly related to changes of vitamin C throughout storage. Therefore, 70 kPa O_2 atmospheres are proposed to prevent anaerobic conditions during storage of fresh-cut melon and thus, reduce wounding stress and deteriorative changes related to high peroxidase activity in tissue.

Keywords: fresh-cut melon, modified atmosphere packaging, phenolic compounds, peroxidase, vitamin C, antioxidant capacity

Introduction

Consumption of fruits and vegetables is associated with a lowered risk of cancer, diabetes, cardiovascular and neurological diseases (Del Caro et al., 2004; Kaur and Kappor, 2001). This protection is due to the presence of antioxidant constituents in fruits and vegetables such as vitamins and phenolic compounds. Their biological properties result from their capacity of decreasing oxidative damage and sequestering reactive oxygen species (ROS), which could initiate cascade reactions that result in the production of hydroxyl radical and other destructive species such as lipid peroxides (Lurie, 2003).

'Piel de Sapo' melon (*Cucumis melo L.*) is a Spanish cultivar, whose fruits are oval shaped with reticular greenish color skin and white sweet flesh. Melon antioxidant properties are mainly due to its high amounts of beta carotenoids of 4700 μg / 100 g (Souci et al., 2000) and moderate vitamin C content of 25 – 42 mg / 100 g (Moreiras, Carvajal et al., 2001; Li et al., 2006).

Due to cutting operations, a great number of cells are disrupted, which causes the release of enzymes and their substrates and promotes the increase of oxidative enzyme-catalysed processes. Wounding and physiological stress may stimulate peroxidase (POD), whose activity in fresh-cut cantaloupe melon could be a response to increased oxidative stress in the cut fruit (Lamikanra and Watson, 2001). POD can oxidize both mono- and diphenols in the presence of small amounts of hydrogen peroxide, which is frequently the oxidizing agent, although, in some cases, O_2 may also activate the enzyme (Robinson, 1991). POD is involved in multiple deteriorative changes affecting flavor, texture, color and nutrition in processed fruits and vegetables. It appears to be a relationship between residual POD activity and the development of off-flavors and off-odors in foods (Bett-Garber et al., 2005).

Reduced O_2 and high CO_2 levels have been proved to effectively control enzymatic browning, firmness and decay of fresh-cut fruits (Soliva-Fortuny and Martín-Belloso, 2003). However, problems associated with the development of off-odors, physiological and microbiological decay, browning and softening may appear when O_2 level is too low and CO_2 is accumulated in packages (Allende et al., 2004). Thus, high O_2 atmospheres ($\geq$ 70 kPa O_2) have been suggested as an effective method to inhibit the growth of microorganisms and prevent undesired anoxic fermentation (Amanitidou et al., 1999; Jacxsens et al., 2001; Van der Steen et al., 2001; Allende et al., 2004). Previous works have been focused on assessing the effects of different modified atmosphere packaging systems on visual, sensory and microbial quality of fresh-cut fruits but their

impact on nutritional and antioxidant properties has been neglected up to date and scarce information is available, especially in fresh-cut melon. Therefore, the purpose of the present work was to study the effect of modified atmosphere packaging (MAP) on vitamin C, phenolics and antioxidant activity as affected by peroxidase activity in fresh-cut 'Piel de Sapo' melon.

Materials and Methods

Sample preparation

'Piel de Sapo' melons (*Cucumis melo* L), harvested in Valencia (Spain), were stored in a ventilated room at 10 ºC prior to processing. Melons at commercial ripeness were sanitized in a 200 ppm NaClO solution for 2 min, rinsed with tap water, and dried by hand. The fruits were sliced and cut to obtain trapezoidal sections. At this stage a physicochemical characterization was carried out (Table 1). Calcium dips have been proved to be effective as firming agents in fresh-cut melon (Luna-Guzmán et al., 1999; Luna-Guzmán and Barrett, 2000). Thus, melon pieces were dipped for 1 min in a solution of 0.5% w/v calcium chloride at a product: solution ratio of 1:2. Once the excess of water was completely drained, 100 g of fruit were packaged in polypropylene trays. The trays were sealed using a digitally controlled compensated vacuum ILPRA Food Pack Basic V/6 system (ILPRA Systems. CP., Vigevono, Italy). The modification of package atmosphere was carried out by flushing a mixture of 2.5 kPa O_2 + 7 kPa CO_2, 10 kPa O_2 + 7 kPa CO_2, 21 kPa O_2, 30 kPa O_2 or 70 kPa O_2 in a ratio product: gas mixture of 1:2. The O_2 and CO_2 permeability of the sealing film were 110 $cm^3 \cdot m^{-2} \cdot day^{-1} \cdot bar^{-1}$ and 500 $cm^3 \cdot m^{-2} \cdot day^{-1} \cdot bar^{-1}$ at 23 ºC and 0% RH, respectively (ILPRA Systems España, S.L. Mataró, Spain). The trays were stored at 4 ± 1ºC in darkness for 14 days. A pair of trays was taken at each sampling time and two replicates analyses were carried out for each tray.

Table 1. Physicochemical characteristics of unprocessed melon

Soluble solids (ºBrix)	12.45 ± 0.7
Total acidity (g citric acid / 100 g)	0.276 ± 0.005
pH	5.71 ± 0.05
Total phenolic compounds (mg gallic acid / 100 g)	15.4 ± 2.3
Total vitamin C (mg vitamin C / 100 g)	45.2 ± 3.5
Pulp color	
L*	73.8±3.9
a*	-3.11±0.22
b*	19.21±1.9
Firmness (N)	3.3±0.7

Values are the mean of three independent determinations ± standard deviation

Total phenolic compounds

The amount of total phenolic compounds in fresh-cut melon was determined according to the Folin-Ciocalteu procedure (Singleton et al., 1999) with some modifications. Fresh-cut melon was ground and centrifuged at 22100 x g for 15 min at 4 ºC with a Centrifuge AVANTI^{TM} J-25 (Beckman Instruments Inc., Fullerton, CA, USA) and then, filtered through a Whatman no 1 filter. An aliquot of 0.5 ml of the supernatant was added to 0.5 ml of Folin-Ciocalteu solution. After 3 min, 10 ml of saturated sodium carbonate solution were added and brought up to 25 ml with distilled water. The absorbance of the blue color that developed was read at 725 nm after 1 hour in darkness conditions. Concentrations were determined by comparing the absorbance of the samples with standards. Results were expressed as milligrams of gallic acid in 100 g of fresh-cut melon.

Enzyme extraction

Peroxidase enzyme was extracted as described by Elez-Martínez et al. (2006). A portion of 50 g of processed melon was blended and mixed with 50 g of 0.2 M L^{-1} sodium phosphate buffer (pH = 6.5). The homogenate was centrifuged at 6000 x g for 15 min at 4ºC with a Centrifuge AVANTI^{TM} J-25 (Beckman Instruments Inc., Fullerton, CA, USA). The resulting supernatant was collected and filtered through a Whatman No 1 paper.

Peroxidase assay

Peroxidase activity was assayed spectrophotometrically in a buffer consisting of 0.02 M Na_2HPO_4 and 0.08 M NaH_2PO_4, 20 mM guaiacol, 4 mM H_20_2, enzyme extract (150 µl), pH 6 in a total volume of 3 ml (Civello et al., 1995). The changes in absorbance due to oxidation of guaiacol at 470 nm and 25 ºC were recorded using a CECIL CE 2021 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK). The absorbance of each replicate was measured in triplicate. The initial reaction rate was estimated from the linear portion of the plotted curve. One unit of POD activity was defined as a change in absorbance at 470 nm min^{-1} ml^{-1} of enzymatic extract. Activity values were given as a percentage of the activity at the beginning of storage (RA, %).

Vitamin C content

The determination of the vitamin C concentration in fresh-cut melon was performed by HPLC-UV. The extraction procedure and the chromatographic conditions were based on a previous study carried out by Odriozola-Serrano et al. (2007). A portion of 25 g of fruit was added to 25 ml of a solution containing 45 g of metaphosforic acid and 7.2 g of DL-1, 4-dithiothreitol (DTT) per liter. The mixture was stirred and centrifuged at 22100 x g for 15 min at 4ºC with a Centrifuge AVANTI^{TM} J-25 (Beckman Instruments Inc., Fullerton, CA, USA). The supernatant was vaccum-filtered through Whatman No 1 paper. The sample were passed through a Millipore 0.45 µm membrane and injected into the HPLC system.

The HPLC system was equipped with a 600 Controller and a 486 Absorbance Detector (Waters, Milford, MA, USA) working at 245 nm. Duplicates of 20 µl of each extract were injected into a reverse-phase C18 Spherisorb® ODS2 (5µm) stainless steel column (250 mm x 4.6 mm) (Waters, Milford, MA, USA), used as stationary phase. A 0.01% solution of sulphuric acid adjusted to pH 2.6 was used as the mobile phase. The flow rate was fixed at 1 ml min^{-1} at room temperature. Results were expressed as milligrams of vitamin C in 100 g of fresh-cut melon.

Antioxidant capacity

The antioxidant capacity of fresh-cut melon was studied through the determination of free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, according to the procedure described by Elez-Martínez and Martín-Belloso (2007). Samples of melon were centrifuged at 6000 x g for 15 min at 4 ºC with a Centrifuge AVANTI^{TM} J-25 (Beckman Instruments Inc.,

Fullerton, CA, USA) and filtered through a Whatman No 1 paper. Aliquots of 0.01 ml of the supernatant were mixed with 3.9 ml of methanolic DPPH of 0.025 g l^{-1} and 0.090 ml of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. The absorption of the samples was measured on a CECIL CE 2021 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK) at 515 nm against blank of methanol without DPPH. Results were expressed as a percentage decrease with respect to the absorption value of a reference DPPH solution.

Headspace gas analysis

The gas composition of the package headspace was analyzed with a micro-GC CP 2002 gas analyzer (Chrompack International, Middelburg, The Netherlands) equipped with a thermal conductivity detector. An aliquot of 1.7 ml was automatically withdrawn through an adhesive rubber septum with a sampling needle directly connected to the injection system. The determination of the O_2 concentration was carried out by injecting a sample of 0.25 μl to a CP-Molsieve 5Å column (4m x 0.35 mm, df=10 μm) at 60ºC and 100 kPa whereas a 0.33 μl portion was injected to a Pora-PLOT Q column (10m x 0.32 mm, df=10 μm) at 75 ºC and 200 kPa for CO_2. Initial gas compositions were determined within the next 15 min after packaging, thus corresponding to the values at time 0 showed in Table 2. Two trays were taken at each sampling time to perform the analysis and 2 readings were carried out for each package.

Statistical analysis

Significance of the results and statistical differences were analyzed using the Statgraphics plus v.5.1 software (Manugistics, Inc., Rockville, MA, USA). Data were analyzed by multifactor analysis of variance. Duncan multiple range test was applied to determine differences among means, with a level of significance of 0.05. Principal component analysis (PCA) was conducted on data to obtain an overview of correlations among variables. PCA is a multivariate statistical technique based on the calculation of linear combinations between the variables that explain the most variance of the data. As a result, data can be reduced to a set of new variables called principal components (PCs). The correlation matrix is used to standardize the variables which are not measured on the same scale. The loadings of the PC define the direction of greatest variability and the score values represent the projection of each object onto PC.

Results and Discussion

POD activity of fresh-cut melon

POD activity of fresh-cut 'Piel de Sapo' melon was related to the availability of O_2 inside packages (Fig. 1). Enzymatic activity in fresh-cut melon packaged under initial 2.5 kPa O_2 + 7 kPa CO_2 conditions was maintained or even increased throughout storage, whereas under initial 70 kPa O_2 atmospheres it decreased progressively reaching 56% inactivation at 14 day storage. POD activity in samples stored under other packaging conditions decreased steadily throughout storage, depending on the packaging atmosphere. Hence, the higher the O_2 availability, the greater the decrease in POD activity throughout storage. The increase of POD activity under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere after one week of storage may be a response to membrane damage because of the stress caused by too-low O_2 and too-high CO_2 levels (Lester, 2003). On the other hand, the observed reduction in POD activity under 70 kPa O_2 atmospheres could be related to a decrease in tissue damage. The application of high O_2 treatments has been reported to prevent oxidative injury of fresh produce (Lu and Toivonen, 2000; Amanatidou et al., 2000; Allende et al., 2004). The oxidative stress is associated with the accumulation of ROS such as superoxide (O_2^-) and H_2O_2 (hydrogen peroxide), which is particularly reactive and can cause lipid peroxidation and a subsequent loss of membrane integrity. Cantos et al. (2002) reported that the possible limiting substrate for the POD reaction could be H_2O_2 and thus, the application of high O_2 atmospheres to prevent the oxidative stress may explain the decrease in POD activity.

Lamikanra and Watson (2000) suggested that relatively high POD activity levels could substantially limit the commercial shelf-life of 'Cantaloupe' melon. Thus, levels of residual enzymatic activity under 70 kPa O_2 atmospheres, lower than those found using other conditions, could contribute to reduce deteriorative quality changes of fresh-cut 'Piel de Sapo' melon. However, other metabolic processes related to quality maintenance of the commodity could take place under high O_2 atmospheres. For instance, fresh-cut 'Piel de Sapo' melon produced more ethylene under 70 kPa O_2 than under 2.5 kPa O_2 + 7 kPa CO_2 or 21 kPa O_2 storage atmospheres (Oms-Oliu et al., 2007). In addition, the exposure to elevated O_2 levels may have different effects on respiration, production of fermentative metabolites and sensory quality, depending on the commodity, O_2 concentrations, storage time and temperature.

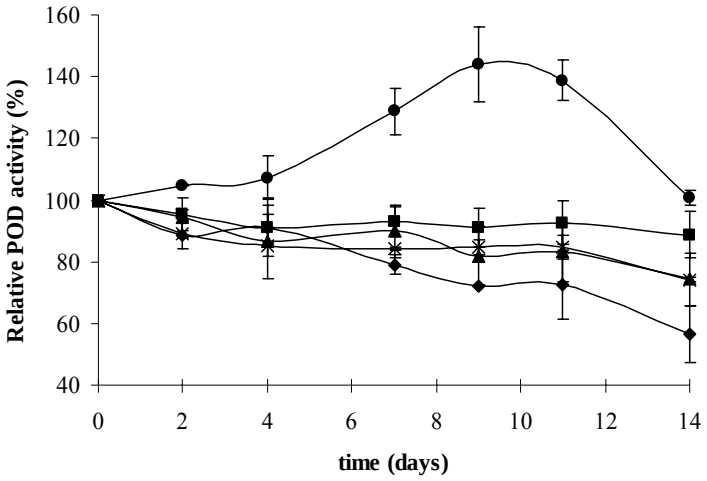

Figure 1. Evolution of relative POD activity (%) of fresh-cut 'Piel de Sapo' melon stored under modified atmosphere during 14 days at 4 ºC. Atmosphere conditions: ● 2.5 kPa O_2 + 7 kPa CO_2, ■ 10 kPa O_2 + 7 kPa CO_2, ▲21 kPa O_2, × 30 kPa O_2, ♦ 70 kPa O_2. Data shown are mean ± standard deviation

Vitamin C content

Table 1 reported a moderate vitamin C concentration of unprocessed 'Piel de Sapo' melon (41.7 – 48.7 mg 100 g^{-1} fw). However, vitamin C depleted due to minimal processing and initial concentrations detected in fresh-cut melon at day 0 were lower than that of unprocessed fruit. During the preparatory steps of minimal processing, the natural protection of fruit (the peel) is removed and hence, they become highly susceptible to oxidation. Fig. 2A shows that initial values of vitamin C detected in fresh-cut melon stored under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere were quite higher than the amount observed under packaging conditions which contained higher initial O_2 concentrations. As expected, ascorbic acid oxidation was greatly favored by the presence of O_2 and thus, a marked decrease in vitamin C content was observed in samples stored under 10 kPa O_2 + 7 kPa CO_2, air, 30 and 70 kPa O_2 atmospheres at day 0. The strongest depletion in vitamin C was detected in fresh-cut melon packaged under ≥ 30 kPa O_2 atmospheres, reaching the lowest content after 14 days of storage.

A marked decrease in vitamin C content was observed in samples stored under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere from the beginning of storage despite the restriction in O_2 concentrations

inside packages. This initial storage atmosphere induced too-low O_2 and high CO_2 concentrations in packages of fresh-cut melon during storage which could contribute to high oxidative stress in fresh-cut melon and, in turn, an increase in peroxidase activity and vitamin C oxidation. Lamikanra et al. (2000) demonstrated the relative affinity of cantaloupe POD for ascorbic acid over guaiacol, and the ability of ascorbic acid in dip solutions to prevent the oxidation of carotenoids in cut cantaloupe melon pieces stored at 4 ºC. High CO_2 levels could also provoke a cytoplasm acidification with the consequent impairment of mitochondrial function that could result in oxidative damage, which could be overcame by ascorbate peroxidase, enzyme that catalyzes ascorbic acid oxidation (Tudela et al., 2002; Pinto et al., 2001).

Total phenolic compounds

Unprocessed 'Piel de Sapo' melon contained small amounts of phenolic compounds (15.4 – 20 mg gallic acid 100 g^{-1} fw) (Table 1). Although no data are available related to total phenolic compounds in 'Piel de Sapo' melon, Lamikanra et al. (2001) reported a low content in 'Cantaloupe' melon (5.16 mg gallic acid 100 g^{-1} fw). Fig. 2B shows the evolution of total phenolic compounds of fresh-cut 'Piel de Sapo' melon stored under different atmospheres. The initial phenolic content was maintained or slightly decreased for 4-7 days, but then the values rose, reaching a maximum accumulation at 9 day storage. Beyond this day, the phenolic content decreased and reached final values about 9.2 – 11.1 mg gallic acid 100 g^{-1} fw in all packaging conditions. A 2.5 kPa O_2 + 7 kPa CO_2 atmosphere induced a higher production of phenolic compounds compared to other atmosphere conditions, which may be related to an enhanced oxidative stress induced by too-low O_2 and high CO_2 concentration inside packages (Fig. 2B). Accumulation of phenolic compounds is a physiological response to infections or injuries (Amanatidou et al., 2000). Wounding may stimulate phenylalanine ammonia lyase (PAL, E.C. 4.3.1.5) activity during minimal processing with consequent further production of the phenolic compounds (Saltveit, 1997). The PAL activation of the phenylpropanoid metabolism could be elicited through induced reactive oxygen species (Reyes et al., 2006).

Phenolic content was best maintained in fresh-cut 'Piel de Sapo' melon stored under 2.5 kPa O_2 + 7 kPa CO_2 and 10 kPa O_2 + 7 kPa CO_2 atmospheres for 4 days. On the other hand, melon initially packaged under 21, 30 and 70 kPa O_2 atmospheres exhibited lower phenolic content throughout storage, especially under a 70 kPa O_2 atmosphere. These conditions induced a strong depletion of

phenolic content at day 0 compared to 2.5 kPa O_2 + 7 kPa CO_2 and 10 kPa O_2 + 7 kPa CO_2 atmospheres (Fig. 2B). Thus, the phenolic content of just-processed fresh-cut melon packaged under 2.5 kPa O_2 + 7 kPa CO_2 and 10 kPa O_2 + 7 kPa CO_2 amospheres was about 16-20 mg gallic acid 100 g^{-1} fw whereas concentration was about 13 mg gallic acid 100 g^{-1} fw under 70 kPa O_2. Cocci et al. (2006) reported that the O_2 availability in the package headspace of fresh-cut apples stored under 21 kPa O_2 could have led to a stronger degradation of the functional compounds such as phenolic compounds.

Antioxidant capacity

Fig. 2C shows the evolution of the antioxidant capacity of fresh-cut 'Piel de Sapo' melon through the DPPH radical scavenging method. Antioxidant capacity of fresh-cut melon decreased during the first 7 days of storage then underwent a marked increase at 9 day storage and finally continued to decrease beyond that time under all packaging atmospheres. A similar change pattern has been reported for some cultivars of fresh-cut citrus segments stored under 21 kPa O_2, where the antioxidant activity decreased at the beginning of the storage period and later increased, after 12 days of storage (Del Caro et al., 2004). At 9 day storage, the increase in antioxidant capacity of fresh-cut melon may be related to an increase in total phenolic compounds (Fig. 2B and 2C). The most marked increase in phenolic compounds was observed in fresh-cut 'Piel de Sapo' melon stored under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere (Fig. 2B). It has been proved that activity of the phenylpropanoid pathway increases under stressful conditions and phenolic compounds are synthesized and accumulated (Kang and Saltveit, 2002).

Low O_2 atmospheres, 2.5 kPa O_2 + 7 kPa CO_2 and 10 kPa O_2 + 7 kPa CO_2, induced an enhanced antioxidant capacity of fresh-cut melon throughout storage compared to 21, 30 and 70 kPa O_2 atmospheres (Fig. 2C). After 14 days of storage, the radical scavenging capacity was greater in samples stored under low O_2, while those stored under 70 kPa O_2 levels showed the lowest antioxidant capacity. According to Martínez-Sánchez et al. (2006), 21 kPa O_2 induced a particularly marked decrease in antioxidant capacity of leaves of wild rocket compared to samples stored under 5 kPa O_2 + 5 kPa CO_2 or 5 kPa O_2 + 10 kPa CO_2 atmospheres, which could be due to losses in some antioxidant constituents and total phenolic compounds.

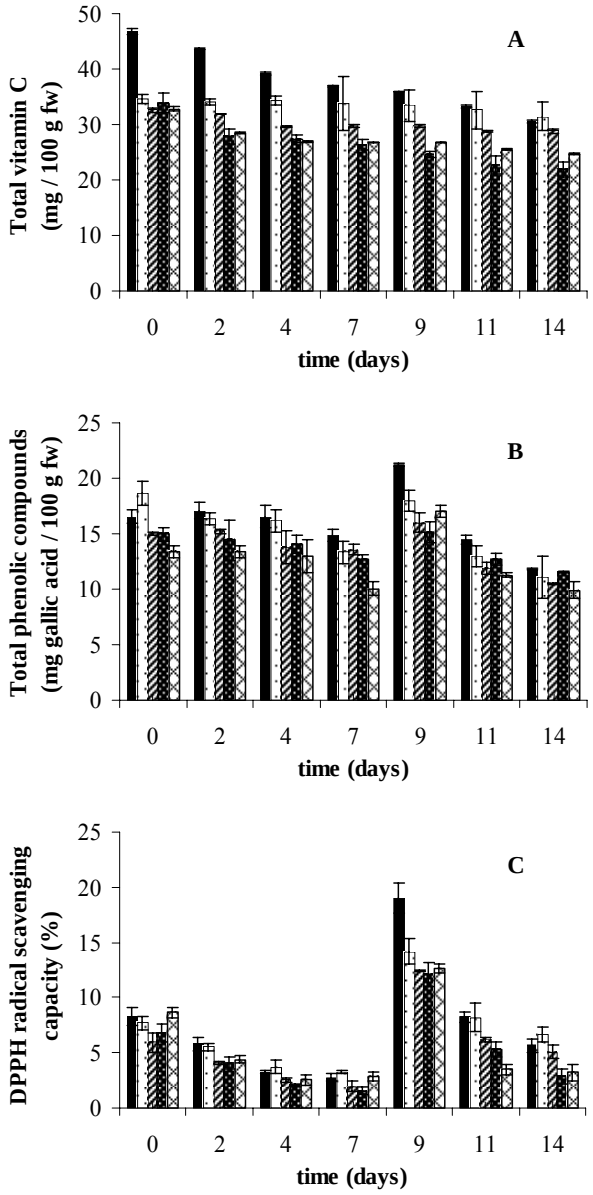

Figure 2. Total Vitamin C, total phenolic compounds and DPPH radical scavenging capacity of fresh-cut 'Piel de Sapo' melon stored under modified atmosphere during 14 days at 4 ºC. Atmosphere conditions: ■ 2.5 kPa O_2 + 7 kPa CO_2, ⊡ 10 kPa O_2 + 7 kPa CO_2, ▨ 21 kPa O_2, ▩ 30 kPa O_2, ⊠ 70 kPa O_2. Data shown are mean ± standard deviation

In our study, a decrease in phenolic compounds and vitamin C content during the first 7 days of storage led to a decrease in antioxidant capacity. In addition, the increase in antioxidant activity was probably due to the synthesis of phenolic compounds due to the induced stress metabolism. Thus, the evolution of total phenolic compounds and vitamin C content showed a significant correlation with the antioxidant capacity throughout storage. Other authors reported a correlation between antioxidant capacity and ascorbic acid content of citrus fruit segments, confirming that ascorbic acid is the main antioxidant in fruits of the *Citrus* genus (Del Caro et al., 2004). However, changes in the antioxidant properties of fresh-cut mandarin were not entirely related to ascorbic acid concentration (Piga et al., 2002). Antioxidant capacity of fruits and vegetables is known to depend on a wide number of compounds (Viña and Chaves, 2003). In this regard, (Chu et al., 2000) have indicated that several phytochemicals, such as flavonoids, phenolic acids, aminoacids, ascorbic acid, tocopherols and pigments, might contribute to the total antioxidant activity.

Principal Component Analysis

In order to globally interpret the results for fresh-cut Piel de Sapo' melon packaged under different conditions, principal component analysis (PCA) was used. PCA was performed on all samples and variables (antioxidant capacity, POD activity, total phenolic compounds, vitamin C, O_2 and CO_2 in-package concentrations) to obtain an overview of the samples. This multivariate technique processed the main information on a limited number of variables. Three principal components (PC1, PC2 and PC3) were calculated. They account for 83.5% of the total variation of the data set (Fig. 3).

The most important component (PC1) explained 45.48% of the total variance. This variation was mainly described by the variables total phenolic compounds, vitamin C, POD activity and headspace O_2 concentration. PC2 explained 21.3% of the total variance and it is mainly composed of CO_2 concentration. Finally, PC3 explained only 16.71% of variance and this variation was mostly described by antioxidant capacity. Fig. 3A and 3B show the loading plots of PC1 vs. PC2 and PC3, respectively. Variables that appear close together in this plot correlated positively. As can be seen in Fig. 3A, there is a close relationship between POD activity, vitamin C and total phenolic compounds, demonstrating a relative affinity of POD enzyme for both substrates. In addition, CO_2 accumulation inside packages does not seem to affect to bioactive compounds (total phenolic compounds and vitamin C), antioxidant capacity and POD activity of fresh-cut 'Piel de

Sapo' melon. As expected, a good correlation was observed between vitamin C and O_2 concentration inside packages (Fig. 3B). Thus, the lower the O_2 concentration, the greater the vitamin C content. The radical-scavenging capacity and total phenolic compounds were found to be similarly loaded on PC3, which indicated that both total phenolics and antioxidant capacity are closely related. By the contrary, antioxidant capacity did not appear to be well correlated with vitamin C content (Fig. 3B).

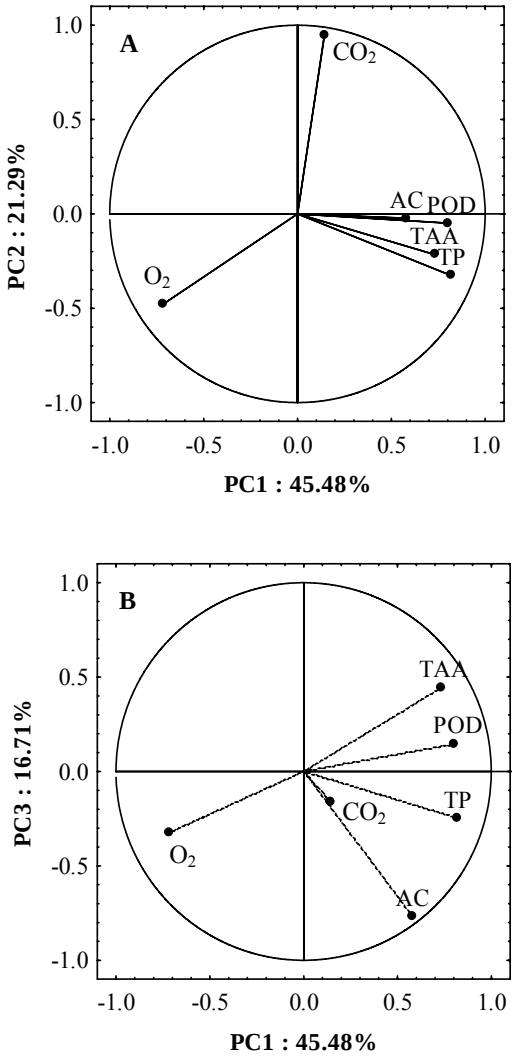

Figure 3. PCA plot of fresh-cut 'Piel de Sapo' melon stored under modified atmosphere for 14 days at 4 ºC: antioxidant capacity (AC), total vitamin C concentration (TAA), total phenolic

compounds (TP), peroxidase activity (POD), oxygen concentration (O_2) and carbon dioxide concentration (CO_2)

The scores of PC1 vs. PC3 are plotted in Fig. 4 using different labels for the different packaging conditions. The main information in the data set was given by PC1, which explained 45.48% of total variance and described differences between samples packaged under low O_2 levels and high O_2 levels. It can be observed that a majority of the samples packaged under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere are situated in the right part of the score plot whereas those samples packaged under a 70 kPa O_2 were situated in the left part of the plot. Thus, fresh-cut 'Piel de Sapo' melon flushed with 2.5 kPa O_2 + 7 kPa CO_2 atmospheres scored higher on vitamin C and POD activity than packages contained higher O_2 concentrations. A minor portion of the total variation explained by PC3 (16.71%) described differences in antioxidant activity between samples stored under different atmosphere conditions. Therefore, it can be also deduced from Fig. 4 that samples corresponding to day 9, located at the bottom of the plot, are those with higher antioxidant capacity due to phenolic compounds synthesis.

Conclusions

The degradation of the initial content of vitamin C and total phenolic compounds was highly promoted by the presence of O_2. Thus, low O_2 levels best maintained vitamin C and total phenolic compounds whereas CO_2 concentration did not seem to affect bioactive compounds. However, an enhanced oxidative stress induced by too-low O_2 and high CO_2 concentrations stimulated the synthesis of phenolic compounds and in turns, the increase of POD activity and loss of vitamin C. Total phenolic compounds and vitamin C content seem to be correlated to POD activity, suggesting that such compounds reacts as substrates of the enzyme. The results obtained in this study conducted with 'Piel de Sapo' melon suggest that 70 kPa O_2 storage atmospheres may decrease wounding stress and reduce deteriorative changes related to high POD activity in tissue. Eventually, phenolic compounds appeared to contribute in a greater manner than vitamin C to the antioxidant capacity of fresh-cut melon.

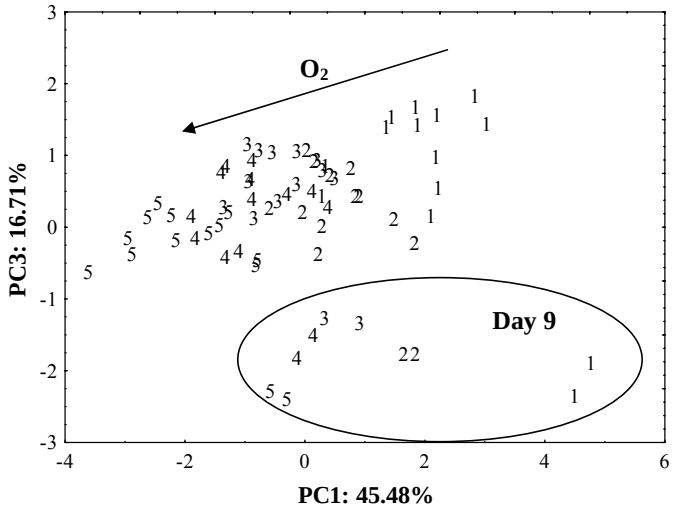

Figure 4. Score plot of PC1 vs. PC3 of all sample labels for the different packaging conditions of fresh-cut 'Piel de Sapo' melon: 2.5 kPa O_2 + 7 kPa CO_2 (1), 10 kPa O_2 + 7 kPa CO_2 (2), 21 kPa O_2 (3), 30 kPa O_2 (4), 70 kPa O_2 (5)

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References

Allende, A.; Luo, Y.; McEvoy, J.L.; Artés, F.; Wang, C.Y. 2004. Microbial and quality changes in minimally processed baby spinach leaves stored under superatmospheric oxygen and modified atmosphere conditions. *Postharvest Biology and Technology*, 33: 51-59.

Amanitidou, A.; Smid, E.J.; Gorris, L.G.M. 1999. Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable-associated microorganisms. *Journal of Applied Microbiology*, 86: 429-438.

Amanatidou, A.; Slump, RA.; Gorris, L.G.M.; Smid, E.J. 2000. High oxygen and high carbon dioxide modified atmsopheres for shelf-life extension of minimally processed carrots. *Journal of Food Science*, 65: 61-66.

Bett-Garber, K.L.; Lamikanra, O.; Lester G.E.; Ingram, D.A.; Watson, M.A. 2005. Influence of soil type and storage conditions on sensory qualities of fresh-cut cantaloupe. *Journal of the Science of Food and Agriculture*, 85: 825-830.

Cantos, E.; Tudela, J.A.; Gil, M.I.; Espín, J.C. 2002. Phenolic compounds and related enzymes are not rate-limiting in browning development of fresh-cut potatoes. *Journal of Agricultural and Food Chemistry*, 50: 3015-3023.

Civello, P.M.; Martínez, G.A.; Chaves, A.R.; Anon, M.C. 1995. Peroxidase from strawberry fruit (Fragaria ananassa Duch.): Partial purification and determination of some properties. *Journal of Agricultural and Food Chemistry*, 43: 2596-2601.

Cocci, E.; Rocculi, P.; Romani, S.; Dalla Rosa, M. 2006. Changes in nutritional properties of minimally processed apples during storage. *Postharvest Biology and Technology*, 39: 265-271.

Chu, Y.-H.; Chang, Ch.-L.; Hsu, H.-F. 2000. Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture*, 80: 561-566.

Del Caro, A.; Piga, A.; Vacca, V.; Agabbio, M. 2004. Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage. *Food Chemistry*, 84: 99-105.

Elez-Martínez, P.; Aguiló-Aguayo, I.; Martín-Belloso, O. 2006. Inactivation of orange juice peroxidase by high-intensity pulsed electric fields as influenced by process parameters. *Journal of the Science of Food and Agriculture*, 86: 71-81.

Elez-Martínez, P.; Martín-Belloso, O. 2007. Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Food Chemistry*, 102: 201-209.

Jacxsens, L.; Devlieghere, F.; Van der Steen, C.; Debevere, J. 2001. Effect of high oxygen atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. *International Journal of Food Microbiology*, 71: 197-210.

Kang, H-M.; Saltveit, M.E. 2002. Antioxidant capacity of lettuce leaf tissue increases after wounding. *Journal of Agricultural and Food Chemistry*, 50: 7536-7541.

Kaur, C.; Kapoor, H.C. 2001. Antioxidants in fruits and vegetables-the millennium's health. *International Journal of Food Science and Technology*, 36: 703-725.

Lamikanra, O.; Watson, M.A. 2000. Cantaloupe melon peroxidase: characterization and effects of food additives on activity. *Nahrung*, 44: 168-172.

Lamikanra, O.; Watson, M.A. 2001. Effects of Ascorbic Acid on Peroxidase and Polyphenoloxidase Activities in Fresh-cut Cantaloupe Melon. *Journal of Food Science*, 66: 1283-1286.

Lester, G.E. 2003. Oxidative stress affecting fruit senescence. In: Hodges, M. (Ed.). *Postharvest Oxidative Stress in Horticultural Crops*. Food Products Press, New York, pp. 113-129.

Li, Z.; Yao, L.; Yang, Y.; Li, A. 2006. Transgenic approach to improve quality traits of melon fruit. *Scientia Horticulturae*, 108: 268-277.

Lu, C.; Toivonen, P.M.A. 2000. Effect of 1 and 100 kPa O_2 atmospheric pretreatments of whole 'Spartan' apples on subsequent quality and shelf life of slices stored in modified atmosphere packages. *Postharvest Biology and Technology*, 18: 99-107.

Luna-Guzmán, I.; Cantwell, M.; Barreto, D. 1999. Fresh-cut cantaloupe: effects of $CaCl_2$ dips and heat treatments on firmness and metabolic activity. *Postharvest Biology and Technology*, 17: 201-213.

Luna-Guzmán, I.; Barrett, D.M. 2000. Comparison of calcium chloride and calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupes. *Postharvest Biology and Technology*, 19: 61-72.

Lurie, S. 2003. Antioxidants. In: Hodges, M. (Ed.). *Postharvest oxidative stress in horticultural crops*. Food Products Press, New York, pp. 131-150.

Martínez-Sánchez, A.; Marín, A.; Llorach, R.; Ferreres, F.; Gil, M.I. 2006. Controlled atmosphere preserves quality and phytonutrients in wild rocket (Diplotaxis tenuifolia). *Postharvest Biology and Technology*, 40: 26-33.

Moreiras, O.; Carvajal, A.; Cabrera, L.; Cuadrado, M. 2001. *Tablas de Composición de Alimentos.* Ediciones Pirámide, Madrid.

Odriozola-Serrano, I.; Hernández-Jover, T.; Martín-Belloso, O. 2007. Comparative evaluation of UV-HPLC methods and reducing agents to determine vitamin C in fruits. *Food Chemistry*, 105 (3): 1151-1158

Oms-Oliu, G.; Raybaudi-Massilia Martínez, R.; Soliva-Fortuny, R.; Martín-Belloso, O. 2008. Effect of superatmospheric and low oxygen modified atmospheres on shelf-life extension of fresh-cut melon. *Food Control*, 19(2): 191-199

Piga, A.; Agabbio, M.; Gambella, F.; Nicoli, M.C. 2002. Retention of Antioxidant activity in minimally processed mandarin and satsuma fruits. *Lebensmittel-Wissenschaft und-Technologie*, 35: 344-347.

Pinto, E.; Lentheric, I.; Vendrell, M.; Larrigaudiere, C. 2001. Role of fermentative and antioxidant metabolisms in the induction of core browning in controlled-atmosphere stored pears. *Journal of the Science of Food and Agriculture*, 81, 364-370.

Reyes, L.F.; Villarreal, J.E.; Cisneros-Zevallos, L.C. 2006. The increase in antioxidant capacity alter wounding depends on the type of fruit or vegetable tissue. *Food Chemistry*, 101: 1254-1262.

Robinson, D.S. 1991. Peroxidases and catalases in foods. In Robinson, D.S.; Eskin, N.A.M. (Eds.). *Oxidative enzymes in Foods*. Elvesier Applied Science, New York, pp. 1-45.

Saltveit, M.E. 1997. Physical and physiological changes in minimally processed fruits and vegetables. In: Tomás-Barberán, F.A.; Robin, R.D (Eds.). *Phytochemistry of Fruits and Vegetables*. Oxford University Press, Oxford, pp. 205-220.

Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299: 152-178.

Soliva-Fortuny, R. C.; Martín-Belloso, O. 2003. New advances in extending the shelf-life of fresh-cut fruits: a review. *Trends in Food Science and Technology*, 14: 341-353.

Souci, S.W.; Fachmann, W.; Kraut, H. 2000. *Food Composition and Nutrition Tables, 6th ed*. In: Scherz, H.; Senser, F. (Eds). CRC Press, London

Tudela, J.A.; Espín, J.C.; Gil, M.I. 2002. Vitamin C retention in fresh-cut potatoes. *Postharvest Biology and Technology*, 26, 75-84.

Van der Steen, C.; Jacxsens, L.; Devlieghere, F.; Debevere, J. 2001. Combining high oxygen atmospheres with low oxygen modified atmosphere packaging to improve the keeping quality of strawberries and raspberries. *Postharvest Biology and Technology*, 26, 49-58.

Viña, S.Z.; Chaves, A.R. 2003. Texture changes in fresh-cut celery during refrigerated storage. *Journal of the Science and Food Agriculture*, 83: 1308-1314.

Capítulo V

Using polysaccharide-based edible coatings to enhance quality and antioxidant properties of fresh-cut melon

G. Oms-Oliu, R. Soliva-Fortuny, O. Martín-Belloso

LWT-Food Science and Technology, en revisión

Abstract

The effect of alginate, pectin and gellan-based edible coatings on the shelf-life of fresh-cut 'Piel de Sapo' melon was investigated. Gas exchange, antioxidant properties, color, firmness, sensory quality and microbial growth of fresh-cut melon were studied during 15 days at 4ºC. Gellan, pectin and alginate coatings increased the water vapor resistance of fresh-cut melon, thus preventing dehydration. They also had an inhibitory effect on ethylene production although O_2 and CO_2 diffusion through coated melon tissue was not avoided. Calcium chloride used as a crosslinking agent helped to maintain fruit firmness. Edible coatings themselves did not improve microbiological stability of fresh-cut 'Piel de Sapo' melon packaged under passive modified atmosphere. Pectin or alginate could reduce the wounding stress induced in fresh-cut melon, which triggered an accumulation of total phenolic compounds and other compounds with antioxidant properties. In addition, pectin-based coating seemed to best maintain sensory attributes.

Keywords: fresh-cut melon, modified atmosphere packaging, phenolic compounds, peroxidase, vitamin C, antioxidant capacity

Introduction

The development of translucency has been found to be the principal visual change of deterioration in fresh-cut melon stored under modified atmosphere packaging (MAP) (Bai et al., 2001; Aguayo et al., 2004; Oms-Oliu et al., 2006). In addition, MAP often results in surface dehydration, or contrarily, promotes the formation of water condensates. Edible coatings can provide an additional protection for fresh-cut fruits and be complementary to MAP. Appropriate edible coating formulations may reduce gas exchange rates and water loss as well as represent and excellent way of incorporating additives to control reactions that are detrimental to quality (Baldwin, Nisperos-Carriedo, & Baker, 1995).

Alginate, derived from a marine brown algae (*Phaeophyceae*), gellan, secreted by the bacterium *Sphingomonas elodea* (formerly referred to as *Pseudomonas elodea*) and pectin, extracted from apple waste or from the peel of citrus fruits are common polysaccharides used as gelling agents in food industry. These polysaccharides are of interest as a potential coating component because of its unique colloidal properties. Alginate, gellan or low methoxyl pectin gel forming properties are mainly due to their capacity to form strong gels or insoluble polymers in the presence of multivalent metal cations like calcium (Mancini and McHugh, 2000; Yang and Paulson, 2000; Rhim, 2004). The gelling mechanism involves interactions between calcium ions and carboxylic groups, forming a three-dimensional cross-linked network. That interaction is produced by mixing the components and casting them as films, or by pouring the cation solution onto a previously cast and dried film (Rhim, 2004).

Polysaccharide based coatings are expected to be a good oxygen barrier due to their tightly packed, ordered hydrogen-bonded network structure although they do not behave well as moisture barriers because of their hydrophilic nature (Nisperos-Carriedo, 1994; Yang and Paulson, 2000). In addition, plasticizers like glycerol, added to increase coating flexibility by reducing the internal hydrogen bonds between polymers chains and increasing intermolecular spacing, generally increase film permeability to oxygen and moisture transmission (Rojas-Graü et al., 2007a). Therefore, lipid incorporation, in small quantities, may be necessary to improve water vapor barrier properties of hydrophilic nature coatings. The addition of sunflower oil with essential fatty acids was shown to improve the barrier properties of alginate and gellan-based edible coatings for fresh-cut 'Fuji' apples (Rojas-Graü et al., 2007a). The addition of a lipid to coating formulations

for fresh-cut apples, based on apple purée and pectin, also remarkably diminished the gas permeation through the edible matrix (McHugh and Senesi, 2000).

In the present work, the objective was to compare the effectiveness of alginate, gellan or pectin based coatings in preserving quality of fresh-cut 'Piel de Sapo' melon. Effects of the coatings on gas exchange, antioxidant properties, sensory and microbial quality were evaluated for 15 days at 4 ºC.

Materials and methods

Materials

'Piel de Sapo' melons (*Cucumis melon L.*) harvested in Valencia (Spain) were stored in a ventilated room at 10 ºC prior to processing. Food grade sodium alginate (Keltone ® LV, ISP, San Diego, CA, USA), food grade deacytaled gellan gum (Kelcogel®, CPKelco, Chicago, IL, USA) and low-methoxyl pectin, esterified potassium salt from citrus fruit, extent of labeling: ~ 30% esterified (Sigma-Aldrich Chemic, Steinhein, Germany) were used as the carbohydrate biopolymers for coating formulations. Glycerol (Merck, Whitehouse Station, NJ, USA) was added as plasticizer. Calcium chloride (Sigma-Aldrich Chemic, Steinhein, Germany) was used to induce crosslinking reaction. A 0.025% (w/v) of sunflower oil (La Española, Spain) with the following composition: 11 g monosaturated, 30 g monounsaturated and polyunsaturated 57.4g: 35 g *omega*-3 and 55-60 g *omega*-6, was used as the lipid source when emulsion films were prepared.

Preparation of the film forming solutions and dipping solutions

Film forming solutions were prepared by dissolving sodium alginate (2g/100 ml water), gellan (0.5g/100 ml water) or pectin (2g/100 ml water) powders in distilled water and heating at 70 ºC while stirring until the solution became clear. Glycerol was added as plasticizer at 1.5 g/100 alginate or pectin solution and 0.6 g/100 ml gellan solution, respectively. Film-forming solutions were emulsified with sunflower oil (0.025 g/100 ml solution) which was dispersed using and Ultra Turrax T25 (IKA ® WERKE, Germany) with a S25N-G25G device, for 5 min at 24,500 rpm, and degassed under vacuum. The concentrations of coating ingredients used in these formulations were set up according to Rojas-Graü et al. (2007a). The viscosities of alginate, pectin and gellan solutions were 52.92, 19.84 and 11.91 mPa·s, respectively. For the crosslinking of carbohydrate polymers, a 2% w/v calcium chloride was also prepared.

Fruit Coating

Melons at commercial ripeness were sanitized in a 200 ppm NaClO solution for 2 min, rinsed with tap water, and dried prior to cutting operations. The fruits were sliced and cut to obtain trapezoidal sections (18.1 cm^2). The melon pieces were introduced in a wire basket and dipped into the polysaccharide solutions for 2 min. The excess of coating material was allowed to drip off for 1 min before submerging the basket again for 2 min in the calcium chloride solution. This latter dipping was necessary to form a solid polymeric matrix upon the fruit pieces through the crosslinking of carbohydrate polymers with calcium ions. Then, 100 g fruit were placed in polypropylene trays in a product: air ratio of 1:2 (v/v). Trays were thermosealed using a packaging machine ILPRA Food Pack Basic V/6 (ILPRA Systems. CP. Vigevono, Italia). The O_2 and CO_2 permeances of sealing film were 110 cm^3/m^2/day/bar and 500 cm^3/m^2/day/ bar at 23ºC and 0% RH, respectively (ILPRA Systems España, S.L. Mataró, Spain). The packages were stored at 4 ± 1ºC in darkness up to random withdrawal for analysis.

Water vapor resistance (WVR)

The procedure for determination of WVR of coatings was carried out according to the method described by Rojas-Graü et al. (2007a). Samples were equilibrated for 24 h in desiccators maintained at 98.9% RH with a 0.6 molal solution of NaCl at room temperature. Then, samples were placed in sealed chambers equilibrated at 33% RH with saturated $MgCl_2 \cdot 6H_2O$ (Panreac Quimica SA, Barcelona, Spain) at 25 ºC. Weight was read for 4h and the slope of the curve of weight loss vs. time in g/s was estimated by linear regression analysis. WVR was calculated using a modified Fick's first law equation:

$$ds/dt = (A\Delta C / R), \qquad \text{(Eq. 1)}$$

where ds/dt is the rate of gas exchange in g/s (slope); A is the exposed area of the fruit pieces (11.6 cm^2) considered as the upper surface plus the lateral area of melon trapezoidal sections; R is the resistance of the coating to water diffusion (s/cm); ΔC is the concentration of gas (ml/cm^3) inside and outside the fruit piece at time *t*. For WPR, ΔC = (Pi-Pa) / RcT, where Rc is the gas constant (3.46 L mmHg/ K g), T is the temperature in degrees Kelvin and (Pi-Pa) is the difference in water vapor pressure (mmHg) inside and outside the fruit tissue, being Pi = a_w of the fruit x P_0 (water

vapor pressure of liquid water at 25 ºC) and Pa = partial water vapor pressure in the environment with 33.3% RH at 25 ºC.

Control tests with uncoated melon were performed to determine the resistance factor of the uncoated fruit to water vapor. Water activity of the samples was measured with an Acqualab CX-2 (Decagon Devices Inc., Pullman, WA). Results were expressed as a percentage increase with respect to the WVR value of uncoated samples.

Package headspace and internal gas composition

A gas analyser (Micro-GC CP 2002, Chrompack International, Middelburg, The Netherlands), equipped with a thermal conductivity detector, was used to analyse the package headspace gas composition. A sample of 1.7 mL was automatically withdrawn from the headspace atmosphere, and a portion of 0.25 µl was injected to a CP-Molsieve 5Å column (4m x 0.35 mm, df=10 µm) at 60 ºC and 100 kPa for O_2 quantification. A portion of 0.33 µl was injected to a Pora-PLOT Q column (10m x 0.32 mm, df=10 µm) at 75 ºC and 200 kPa for CO_2 and ethylene determinations. Two trays were taken at each time to perform the analysis and 2 readings were carried out for each package.

The internal gas composition of fresh-cut melon was determined according to the procedure proposed by Soliva-Fortuny et al. (2007). A sample of 100 g was placed in a saturated de-gassed NaCl solution; the internal gas content was vacuumed and collected in a bell placed at the top of the system. The gas sample was withdrawn from the headspace atmosphere through a rubber septum and injected into a Micro-GC CP 2002, Chrompack International, Middelburg, The Netherlands) for oxygen, carbon dioxide and ethylene quantification. Chromatographic conditions for gas analyses were the same as outlined previously. Two trays were taken at each sampling time to perform replicate analyses.

Vitamin C content

The determination of the vitamin C concentration in fresh-cut melon was performed by HPLC-UV. The extraction procedure and the chromatographic conditions were based on a previous study carried out by Odriozola-Serrano et al. (2007). A portion of 25 g of fruit was added to 25 ml of a solution containing 45 g/l of metaphosforic acid and 7.2 g/l of DL-1, 4-dithiothreitol (DTT). The mixture was stirred and centrifuged at 22100 x g for 15 min at 4ºC (Centrifuge AVANTI^{TM} J-25,

Beckman Instruments Inc., Fullerton, CA, USA). The supernatant was vaccum-filtered through Whatman No 1 paper. The sample was passed through a Millipore 0.45 μm membrane and injected into the HPLC system.

The HPLC system was equipped with a 600 Controller and a 486 Absorbance Detector (Waters, Milford, MA) working at 245 nm. Duplicates of 20 μl of each extract were injected into a reverse-phase C18 Spherisorb® ODS2 (5μm) stainless steel column (250 mm x 4.6 mm) (Waters, Milford, MA), used as stationary phase. A 0.01% solution of sulphuric acid adjusted to pH 2.6 was used as the mobile phase. The flow rate was fixed at 1 ml/min at room temperature. Results were expressed as percentage decrease with respect to vitamin C content of fresh-cut melon at day 0.

Total phenolic compounds

The amount of total phenolic compounds in fresh-cut melon was determined according to the Folin-Ciocalteu procedure (Singleton et al., 1999) with some modifications. A sample of 50 g was ground and centrifuged at 22100 x g for 15 min at 4 ºC (Centrifuge AVANTI^{TM} J-25, Beckman Instruments Inc., Fullerton, CA, USA) and then, filtered through a Whatman no 1 filter. An aliquot of 0.5 ml of the supernatant was added to 0.5 ml of Folin-Ciocalteu solution. After 3 min, 10 ml of saturated sodium carbonate solution were added and brought up to 25 ml with distilled water. The absorbance of the blue color that developed was read at 725 nm after 1 hour in darkness conditions. Phenolic concentrations were determined by comparing the absorbance of the samples with standards. Results were expressed as milligrams of gallic acid in 100 g of fresh-cut melon. Two trays were taken at each sampling time to perform replicate analyses throughout 15 days of storage.

Antioxidant capacity

The antioxidant capacity of fresh-cut melon was studied through the determination of free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, according to the procedure described by Elez-Martínez and Martín-Belloso (2007). Fruit samples were centrifuged at 22100 x g for 15 min at 4 ºC (Centrifuge AVANTI^{TM} J-25, Beckman Instruments Inc., Fullerton, CA, USA) and filtered through a Whatman No 1 paper. Aliquots of 0.01 ml of the supernatant were mixed with 3.9 ml of methanolic DPPH of 0.025 g/l and 0.090 ml of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. The absorption of the

samples was measured with a CECIL CE 2021 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK) at 515 nm against a blank of methanol without DPPH. Results were expressed as a percentage decrease with respect to the absorption value of a reference DPPH solution.

Microbiology analyses

Total aerobic psychrophilic microorganisms and yeast and mold populations were evaluated throughout storage. In sterile conditions, 10 g of sample were homogenized for 2 min with 90 ml of 0.1% sterile peptone water with a Stomacher Lab Blender 400 (Seward medical, London, England). Serial dilutions of fruit homogenates were poured in plate count agar (PCA; Biokar Diagnostics. Beauvais, France) at 7ºC ± 1ºC for 7 days for psychrophilic aerobic bacteria counts (ISO 4833, 1991) and chloramphenicol glucose agar (GCA) at 25ºC ± 1ºC for 5 days for yeast and mold counts (ISO 7954, 1988). Two replicate analyses for each tray were carried out periodically for 15 days.

Sensory analyses

Sensory analyses of coated or uncoated melon pieces stored under passive modified atmospheres were carried out at 1 and 7 days of storage to evaluate consumer acceptability of fresh-cut melon. Thirty consumers, aged between 20 and 65 years old, were recruited among students and personnel of the Department of Food Technology, University of Lleida.

The panelists evaluated the acceptability of odor, color, taste, firmness and overall preference of the samples on a 10 cm non-structured linear scale, where 0 indicated extreme dislike and 10 indicated extreme like. Results were compared with those for freshly processed control samples.

Color and firmness evaluation

The color of fresh-cut melon was determined with a Minolta CR-400 chromameter (Konica Minolta Sensing, Inc., Osaka, Japan). The equipment was set up for a D65 illuminant and 10º observer angle. Five fruit pieces from each of two replicate packages were evaluated for each treatment at each sampling time. CIE L* (lightness), a* (red-green) and b* (yellow-blue) values were measured using reflectance measurements. Color changes of fresh-cut processed melon were also measured through whiteness index (WI), according to previous works (Aguayo et al., 2003; Aguayo et al., 2004; Oms-Oliu et al., 2006).

$$WI = 100 - \left[(100 - L^*)^2 + (a^*)^2 + (b^*)^2\right]^{\frac{1}{2}} \quad \text{(Eq. 2)}$$

The procedure for firmness evaluation was set up according to previous studies carried out on alginate or gellan coated fresh-cut 'Fuji' apples (Rojas-Graü et al., 2007b, Rojas-Graü et al., 2007c). The maximum penetration force was measured using a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, England, UK). Cylindrical samples of 2.0 cm high were obtained from trapezoidal melon pieces and were positioned to be penetrated by a 4 mm diameter rod through their geometric center. The downward distance was set at 10 mm at a rate of 5 mm/s and automatic return. Two trays were taken at each sampling time to perform the analyses, and 5 fruit pieces from each replicate were randomly withdrawn to carry out repetitions.

Statistical analysis

Significance of the results and statistical differences were analyzed using The Statgraphics Plus v.5.1 Windows package (Manugistics, Inc., Rockville, MA, USA). Analysis of variance (ANOVA) was performed to compare mean values for different coatings and control samples. The Duncan multiple range test was applied to determinate differences among means at a 5% significance level.

Results and Discussion

Water vapor resistance of coatings (WVR)

Previous studies have demonstrated the potential use of polysaccharide as coatings for fresh-cut fruits. In previous works carried out in our laboratory, micrographs of an apple cross-section coated with 2% w/v alginate or 0.5% w/v gellan coatings showed that both coatings were homogeneous, covering the whole surface of apple pieces and showing good adherence. Thickness values for alginate or gellan coatings were 132.45 μm and 155.75 μm, respectively (Rojas-Graü et al., 2007a). In addition, swelling ratio (water gained / total solids) and water solubility (soluble solids / total solids) values appear to indicate adequate potential of these films for coating high moisture products like fresh-cut fruits (Tapia et al., 2007). Table 1 shows the increase in WVR of fresh-cut 'Piel de Sapo' melon pieces after coating with 2% w/v alginate, 2% w/v pectin or 0.5% w/v gellan formulations containing glycerol and sunflower. Gellan coating conferred more than 2-fold increase in WVR, while WVR was nearly 2 times higher in alginate or pectin coatings than in

uncoated samples. The increase in WVR observed in coated fresh-cut melon when compared to uncoated samples can be attributed to the addition of sunflower oil, as lipids demonstrated to improve the water barriers properties of hydrophilic alginate or gellan-based coatings (Rojas-Graü et al., 2007a). In agreement with our results, Tapia et al. (2007) observed that gellan coatings exhibited better water barrier properties than alginate coatings for both the papaya and apple fruit pieces. These authors suggested that the nature of each polymer and the higher concentration used for alginate (2% w/v) in relation to that of gellan (0.5% w/v) might explain the lower WVR of alginate coatings. Water vapour transfer generally occurs through the hydrophilic portion of the film and therefore depends on the hydrophilic-hydrophobic ratio of the film constituents (Hernández, 1994). Thus, the behaviour observed in coatings containing different polysaccharide molecules might be due to differences in the hydrophobic / hydrophilic balance of each molecule. Differences in WVR between alginate and pectin coatings were not observed in our study, which is in accordance with the observations of other authors. In fresh-cut apples, the effect of an alginate/lipid bilayer coating on the increase of WVR was not significantly different, on the basis of Duncan's multiple-range test, to that reported by a pectin/lipid bilayer coating (Wong et al., 1994). Surface microstructure of the films has been suggested to be partially responsible for differences in WVP of films (Yang and Paulson, 2000). At the same concentration, surface characteristics of low methoxyl pectin- or alginate-based coatings on the cut fruit surface could be very similar. The interactions between blocks of galacturonic and guluronic acid residues for pectin and alginate, respectively, and calcium ions are described by the same egg box model (Mancini and McHugh, 2000).

Table 1. Effect of polysaccharide based coatings on the resistance of fresh-cut 'Piel de Sapo' melon to water vapor

Coating	resistance ($s \cdot cm^{-1}$)
Gellan	23.35 ± 1.3a
Alginate	20.6 ± 0.9bc
Pectin	19.3 ± 1.4c
Uncoated	10.48 ± 0.25d

Mean ± standard deviation

Values within a column followed by the same small letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$)

Gas exchange of fresh-cut 'Piel de Sapo' melon

The effects of polysaccharide-based edible coatings on respiratory activity and ethylene production were investigated. Physical stresses, such as cutting and slicing, are expected to cause an increase in respiration and ethylene production. Figure 1 shows that there was a decrease in O_2 concentration and an accumulation of CO_2 in the internal atmosphere of fresh-cut melon. Uncoated melon exhibited the least modification of internal atmosphere compared to gellan-, pectin- or alginate-coated pieces at the end of storage. On the other hand, the internal amount of O_2 in gellan-coated samples decreased steeply beyond 1 week of storage and the accumulated CO_2 in these samples was the highest after 15 days. However, these changes may be due to respiratory activity of gellan-coated melon rather than to coating gas barrier properties.

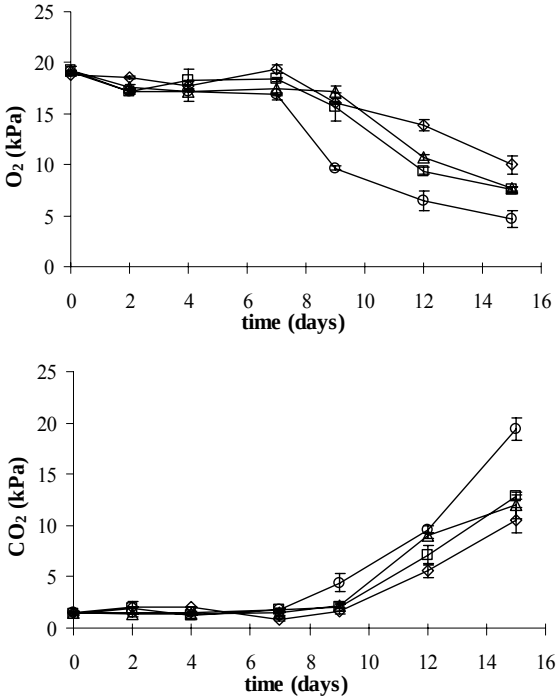

Figure 1. Changes in internal atmosphere of fresh-cut 'Piel de Sapo' melon coated with gellan (○), alginate (□), pectin (△) or uncoated (◇) during 15 days at 4 ºC. Data shown are mean ± standard deviation.

Gellan, pectin and alginate based formulations do not seem to be good barriers to O_2 or CO_2 since a significant modification of package headspace atmosphere was observed throughout storage of

coated fresh-cut melon with internal atmosphere similar to package headspace (Fig. 1 and 2). Changes in gas concentrations observed in package headspace atmosphere were due to the gas diffusion through melon tissue as well as to the low gas permeance of the sealing film.

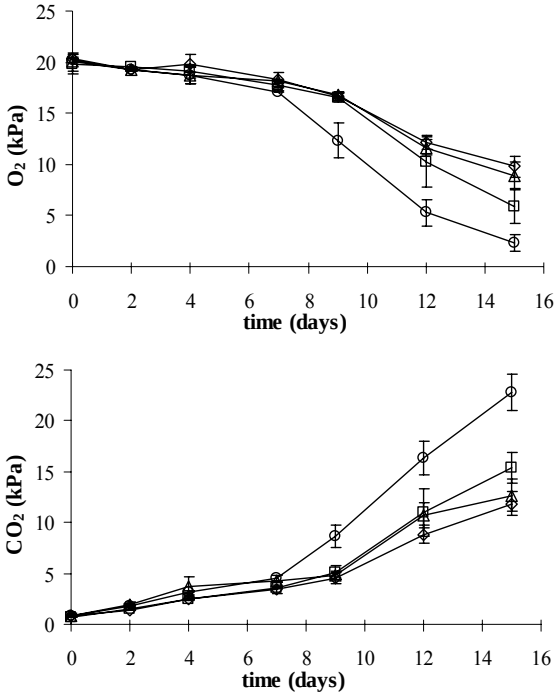

Figure 2. Changes in package headspace atmosphere of fresh-cut 'Piel de Sapo' melon coated with gellan (○), alginate (□), pectin (△) or uncoated (◇) during 15 days at 4 ºC. Data shown are mean ± standard deviation.

Fig. 3 shows the increase in the amount of ethylene evolved in fresh-cut 'Piel de Sapo' melon in both the internal and package headspace atmospheres. In internal fruit atmosphere, coated samples exhibited lower ethylene accumulation compared with the uncoated (Fig. 3a). Ethylene peak concentrations varied from 1.6 to 6.4 ppm in coated samples, whereas a peak concentration of 13.2 ppm was reached in uncoated melon pieces (Fig. 3a). According to Li and Barth (1998), the increase in ethylene production may be attributed to the biosynthesis of wounding ethylene. Thus, edible coatings may serve as a protective layer for the damaged surface of fresh-cut produce and reduce wounding response.

Concentrations in the package headspace remained at fairly constant low levels for both coated and uncoated samples (Fig. 3b). Previous studies in uncoated fresh-cut 'Piel de Sapo' melon also reported small amounts of ethylene in package headspace (Oms-Oliu et al., 2006). In both coated and uncoated fruit pieces, ethylene seems to be accumulated in melon rather than diffusing through the tissue. Thus, coatings did not show good ethylene barrier. Contrarily, in fresh-cut 'Fuji' apples, gellan- and alginate-based coatings reduced significantly ethylene accumulation in the package headspace compared to the uncoated samples (Rojas-Graü et al., 2007b). Some authors have suggested that the reduction of the respiration rate and ethylene production of coated fresh-cut apples may be due to calcium ions contained in the film forming solution rather than the effect of oxygen barrier properties of coatings (Lee, Park, Lee, & Choi, 2003). Calcium ions (Ca^{2+}) are involved in the regulatory function of many enzyme actions in cellular and physiological processes (Wong et al., 1994).

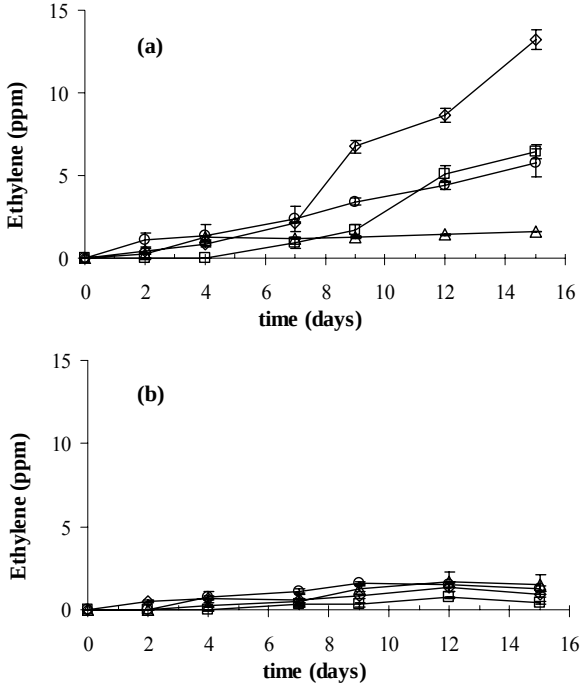

Figure 3. Ethylene changes in internal (a) and package headspace (b) of fresh-cut 'Piel de Sapo' melon coated with gellan (○), alginate (□), pectin (△) or uncoated (◇) during 15 days at 4 ºC. Data shown are mean ± standard deviation.

Antioxidant potential of fresh-cut 'Piel de Sapo' melon

Fig. 4 shows changes in the total vitamin C retention (%) of coated and uncoated fresh-cut melon during 15 days storage. Vitamin C retention was significantly affected by the coating composition and storage time ($p \leq 0.05$). Although vitamin C of both coated and uncoated samples decreased throughout storage, the use of gellan-based edible coatings significantly reduced the loss of vitamin C in fresh-cut melon pieces. After 15 days of storage, vitamin C retention of fruit coated with gellan, pectin and alginate were 85%, 79% and 75%, respectively, whereas uncoated samples maintained 77% of initial vitamin C content. Thus, the gellan formulation was much more effective in reducing the vitamin C loss of fresh-cut melon than other coatings. This fact may be due to a substantial decrease in the amount of O_2 in the package headspace and melon tissue of gellan-coated samples, compared to alginate, pectin or uncoated melon pieces beyond 1 week of storage (Fig. 1 and 2). Keeping oxygen away delays the deteriorative oxidation reactions of vitamin C. In fact, previous studies conducted in fresh-cut 'Piel de Sapo' melon showed that the lower the package headspace O_2 concentration, the greater the vitamin C content (Oms-Oliu et al., 2008).

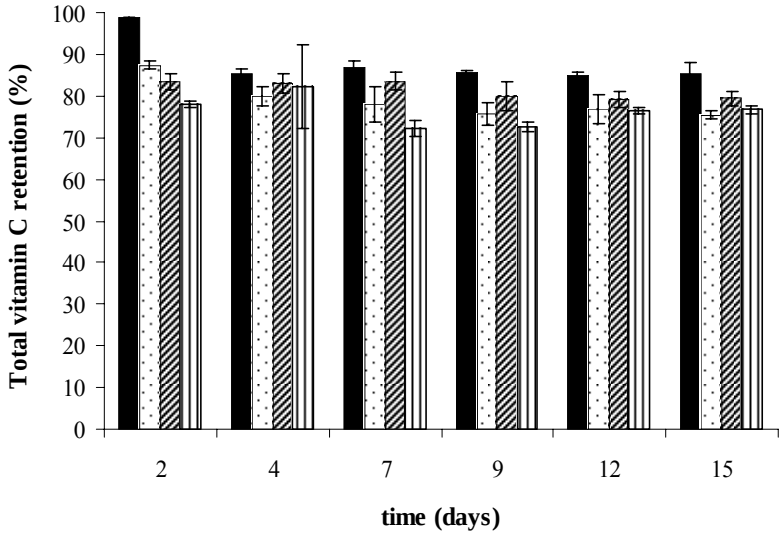

Figure 4. Total vitamin C retention of fresh-cut 'Piel de Sapo' melon coated with gellan (■), alginate (⊡), pectin (▨) or uncoated (▥) during 15 days at 4 ºC. Data shown are mean ± standard deviation

Fig. 5 shows changes in total phenolic compounds of fresh-cut 'Piel de Sapo' melon coated with alginate, gellan, pectin or uncoated fruit pieces. The initial phenolic content was maintained or slightly decreased during the first week of storage, but then it increased up to 20-25 mg gallic acid 100 g^{-1} fw at 12-day storage. The accumulation of phenolic compounds shown in both the coated and uncoated samples may be promoted by phenylalanine ammonia-lyase (PAL) activity. Puschmann et al. (2007) observed an increase in PAL activity during storage of both starch chitosan-coated baby carrots and uncoated fruit. PAL activation has been observed in response to several stresses including CO_2 treatment (Ke and Salveit, 1989). Previous studies showed that a 2.5 kPa O_2 + 7 kPa CO_2 active modified atmosphere induced a greater production of phenolic compounds during storage of fresh-cut 'Piel de Sapo' melon than atmospheres with a higher O_2 content, which was related to oxidative stress induced by too-low and high CO_2 concentrations inside packages (Oms-Oliu et al., 2008). Therefore, the enhanced phenolic accumulation in melon pieces coated with gellan may be due to the substantial modification of both fruit and package headspace atmospheres.

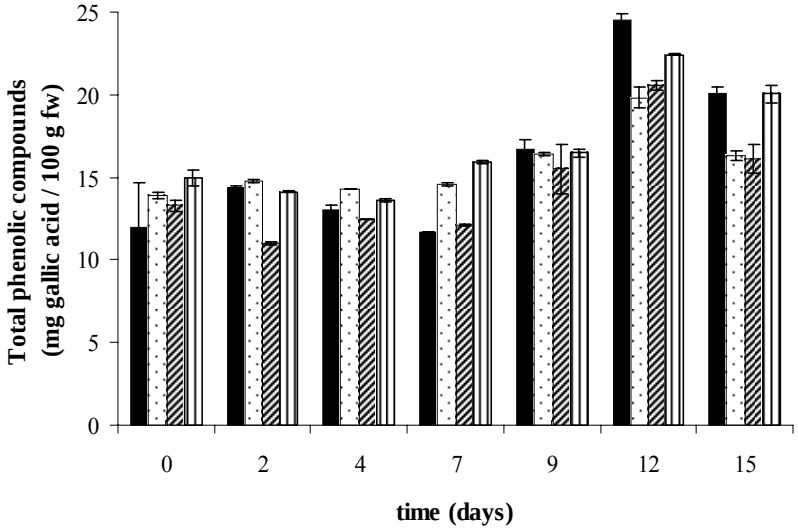

Figure 5. Total phenolic compounds of fresh-cut 'Piel de Sapo' melon coated with gellan (■), alginate (⬚), pectin (▨) or uncoated (▥) during 15 days at 4 ºC. Data shown are mean ± standard deviation

In our study, the increase in phenolic compounds was related to the enhancement of antioxidant capacity of fresh-cut melon (Fig. 6). Some authors have also related the accumulation of phenolic compounds to an increase in antioxidant activity (Reyes and Cisneros-Zevallos, 2003). The most substantial rise in antioxidant capacity was observed in fresh-cut melon coated with gellan, which also underwent the highest accumulation of phenolic compounds. Previous studies in fresh-cut 'Piel de Sapo' melon showed the greater contribution of phenolic compounds than vitamin C to the antioxidant capacity (Oms-Oliu et al., 2008). However, antioxidant capacity comes from other several different phytochemicals (Ederhardt et al., 2000).

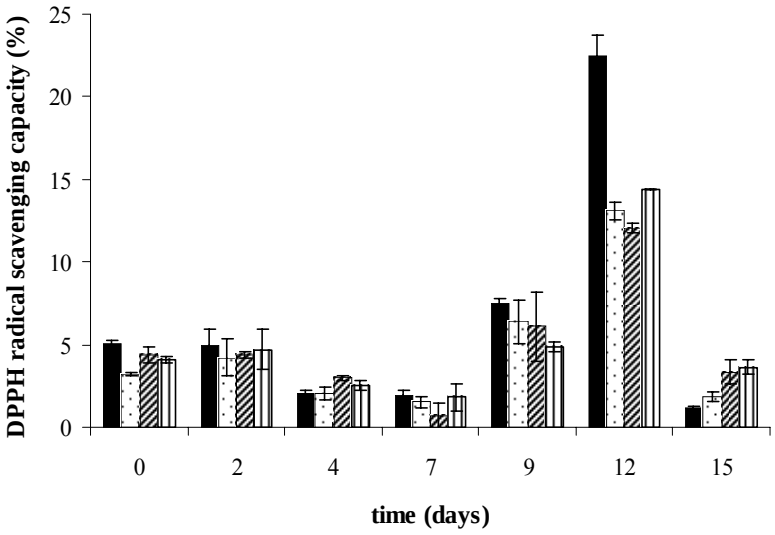

Figure 6. DPPH radical scavenging capacity of fresh-cut 'Piel de Sapo' melon coated with gellan (■), alginate (⬚), pectin (▨) or dipped into distilled water (▥) during 15 days at 4 ºC. Data shown are mean ± standard deviation

Microbiological stability of fresh-cut 'Piel de Sapo' melon

Table 2 shows no significant differences in yeast and mold counts ($p > 0.05$) between coated and uncoated melon pieces. On the other hand, edible coatings significantly affected the growth of aerobic psychrophilic microorganisms ($p \leq 0.05$). The growth of psychrophilic aerobic microorganisms on gellan coated samples was the highest, reaching counts of 8 log CFU/g at the end of storage. On the contrary, the counts on both uncoated and alginate- or pectin-coated

samples exceeded 7 log CFU/g at the end of storage. Rojas-Graü et al. (2007c) indicated that an alginate coating itself did not reduce the physhrophilic aerobic bacteria or yeast and mold counts on fresh-cut 'Fuji' apples. The Spanish regulation for hygienic processing, distribution and commerce of prepared meals (RD 3484/2000) indicates a limit for aerobic counts of 7 log CFU/g at expiration date. The psychrophilic aerobic microorganism counts for both coated and uncoated samples exceeded this limit of 7 log CFU/g during the second week of storage (Table 2).

Table 2. Growth of aerobic psychrophilic microorganisms, yeast and moulds (expressed as log CFU g^{-1}) throughout storage of fresh-cut 'Piel de Sapo' melon coated with gellan, alginate, pectin or dipped into distilled water during 15 days at 4 ºC.

aerobic psychrophilic microorganisms				
Days	Gellan	Alginate	Pectin	Control
0	1.5aA	1.6aA	1.5aA	1.5aA
3	3.0bB	2.6aAB	2.3bA	2.4bA
7	4.7cA	4.5bA	4.6cA	4.1cA
15	8.0dB	7.5cA	7.6dA	7.9dA
yeast and moulds				
Days	Gellan	Alginate	Pectin	Control
0	2.5aA	2.5aA	2.5bA	2.5aA
3	2.5aAB	2.9aB	2.0aA	2.9bB
7	4.5bB	4.5bB	4.8cB	3.9cA
15	5.7cA	5.1cA	5.7dA	5.8dA

Values within a column followed by the same small letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$)
Values within the same line followed by the same capital letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$)

Sensory, firmness and color changes of fresh-cut 'Piel de Sapo' melon

Changes in sensory parameters including odor, color, taste, firmness and overall preference of coated and uncoated fresh-cut 'Piel de Sapo' melon during 7 days of storage are shown in Fig. 7. Consumer testing was not carried out beyond the first week of storage for microbiological reasons, since previous studies showed that the shelf-life of fresh-cut 'Piel de Sapo' melon stored in filled air packages was limited to 7-10 days (Oms-Oliu et al., 2007).

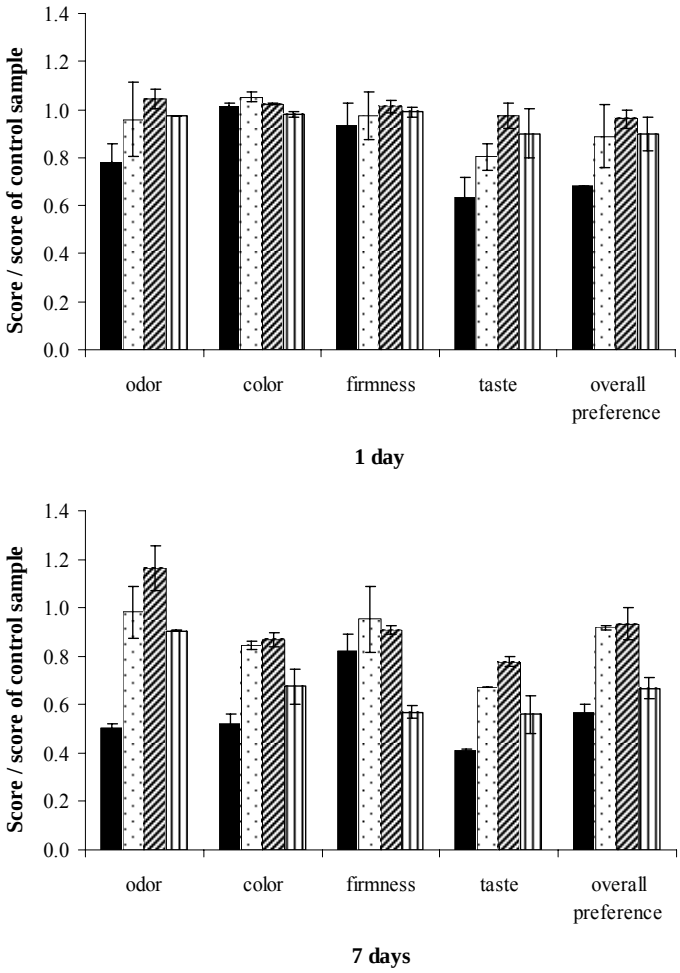

Figure 7. Sensory characteristics of fresh-cut 'Piel de Sapo' melon coated with gellan (■), alginate (⊡), pectin (▨) or dipped into distilled water (▥) after 1 day and 7 days of storage at 4 ºC.

Alginate-, pectin-coated or uncoated fresh-cut melon scored higher than gellan-coated fruit pieces for the overall preference attribute at day 1 ($p \leq 0.05$). Initially, fresh-cut melon coated with gellan had lower scores for odor and taste attributes than alginate-, pectin-coated or uncoated samples ($p \leq 0.05$). On the other hand, color and firmness scores were high (> 0.9) for coated and uncoated samples (Fig. 7a). After one week of storage, pectin coating seemed to best maintain quality attributes (> 0.7) whereas fresh-cut melon coated with gellan scored the lowest for odor, color and

taste (< 0.6) (Fig. 7b). Edible coatings seem to help to maintain firmness of fresh-cut melon ($p \leq 0.05$). Values for coated samples were > 0.8, while those of uncoated samples < 0.6 (Fig. 7b).

Gellan coated samples, which scored the lowest for color after 1 week of storage (Fig. 7b), underwent a substantial decrease in WI values after 1 week of storage (Fig. 8a). This phenomenon may be associated with an increase in translucency injury of fresh-cut melon. It seems that translucency was a symptom of senescence related to the excessive modification of package headspace atmosphere in fresh-cut melon coated with gellan (Fig. 1 and 2). In addition, WI values of uncoated fresh-cut melon were higher than those of coated samples during the first days of storage (Fig. 8a). This may be due to higher dehydration of surface tissue in uncoated samples. According to Li and Barth (1998), it is possible that edible coatings reduced dehydration by physically reducing the air-filled surface tissue.

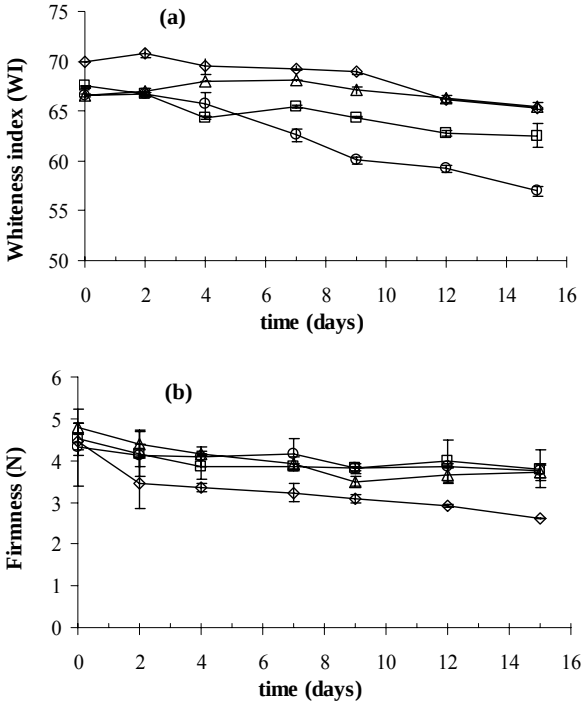

Figure 8. Changes in whiteness index (a) and firmness (b) of fresh-cut 'Piel de Sapo' melon coated with gellan (○), alginate (□), pectin (△) or uncoated (◇) during 15 days at 4 ºC. Data shown are mean ± standard deviation.

Firmness of fresh-cut 'Piel de Sapo' melon was best maintained with the use of the coatings (Fig. 8b). Sensory results agree with instrumental measurements as can be observed in Fig. 7b. In fresh-cut fruits coated with alginate or gellan, the use of calcium chloride for crosslinking the polymers has been reported to prevent the produce from softening (Rojas et al., 2007b; Olivas et al., 2007). As far as we know, the effect of calcium salts on the firmness of coated fresh-cut melon is not yet reported although it has been extensively used in fresh-cut fruits by several authors. Calcium dips for 1-5 min provided a firming effect on uncoated fresh-cut 'Cantaloupe' melon stored at 5 ºC (Luna-Guzmán et al., 1999). Therefore, alginate, pectin or gellan may prevent from fruit softening due to the calcium contained in their formulations. King and Bolin (1989) established that calcium chloride can be used as firming agent for fruit tissues since it reacts with pectic acid in the cell wall to form calcium pectate, which strengthens molecular bonding between constituents of the cell wall. In addition, softening is often associated with water loss. Thus, Rojas et al. (2007a) found that alginate or gellan edible coatings applied to fresh-cut apples were effective in controlling moisture loss, preventing loss of turgor, when the formulation contained 0.025 ml sunflower oil / 100 ml film forming solution. However, Olivas et al. (2007) reported that the effect of calcium in keeping the texture of apple slices is probably higher than the effect of alginate coatings in preventing water loss, since softening of apples can be attributed more to cell wall degradation than to a reduction in turgor pressure.

Conclusions

Edible coatings improved some of the quality attributes of fresh-cut 'Piel de Sapo' melon. Gellan, pectin or alginate increased the water vapour resistance in comparison with uncoated fresh-cut melon, prevented desiccation and best maintained fruit firmness throughout storage. On the other hand, polysaccharide coatings did not provide sufficient barrier to gas diffusion. Edible coatings themselves did not reduce microbiological growth of fresh-cut melon. Phenolic compounds were produced in fresh-cut melon after 1 week as stress response. The antioxidant properties of the synthesized compounds temporally increased the antioxidant activity of fresh-cut melon. Gellan prevented the loss of vitamin C although the excessively atmosphere modification enhanced the synthesis of phenolic compounds as well as dramatically affected the general appearance of fresh-cut melon. On the contrary, the use of pectin- or alginate-based formulations may reduce wounding stress of fresh-cut 'Piel de Sapo' melon and best maintained quality attributes of the

commodity. In conclusion, alginate could be recommended for commercial purposes because of the high economic cost of low methoxyl pectins for industrial applications.

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References

Aguayo, E.; Allende, A.; Artés, F. 2003. Keeping quality and safety of minimally fresh processed melon. *European Food Research and Technology*, 216: 494-499.

Aguayo, E.; Escalona, V.H.; Artés, F. 2004. Metabolic behaviour and quality changes of whole and fresh processed melon. *Journal of Food Science*, 69(4): 148-155.

Bai, J.H.; Saftner, R.A.; Watada, A.E.; Lee, Y.S. 2001. Modified atmosphere maintains quality of fresh-cut cantaloupe (*Cucumis melo L.*). *Journal of Food Science*, 66 (8): 1207-1211.

Baldwin, E.A.; Nisperos-Carriedo, M.O.; Baker, R.A. 1995. Edible coatings for lightly processed fruits and vegetables. *Hortscience*, 30: 35-38.

Baldwin, E.A.; Nisperos, M.O.; Chen, X.; Hagenmaier, R.D. 1996. Improving storage life of cut apple and potato with edible coating. *Postharvest Biology and Technology*, 9 (2): 151-163.

Eberhardt, M.V.; Lee, C.Y.; Liu, R.H. 2000. Antioxidant activity of fresh apples. *Nature*, 405 (6789): 903-904.

Elez-Martínez, P.; Martín-Belloso, O. 2007. Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Food Chemistry*, 102: 201-209.

Hernández, E. 1994. Edible coatings from lipids and resins. In: J.M. Krochta, E.A. Baldwin, and M.O. Nisperos-Carriedo (Eds.), *Edible coatings and films to improve food quality*. Technomic Publishing Co, Lancaster, pp. 279-303.

Ke, D.; Salveit, M.E. 1989. Carbon dioxide-induced brown stain development as related to phenolic metabolism in Iceberg lettuce. *Journal of the American Society for Horticultural Science*, 114: 789-794.

King, A.D.; Bolin, H.R. 1989. Physiological and microbiological storage of stability of minimally processed fruits and vegetables. *Food Technology*, 43: 132-139.

Lee, J.Y.; Park, H.J.; Lee, C.Y.; Choi, W.Y. 2003. Extending shelf-life of minimally processed apples with edible coatings and antibrowning agents. *LWT-Food Science and Technology*, 36: 323-329.

King, A.H. 1983. Brown seed extracts (Alginates). *Food Hydrocolloids*, 2: 115-188.

Li, P.; Barth, M.M. 1998. Impact of edible coatings on nutritional and physiological changes in lightly-processed carrots. *Postharvest Biology and Technology*, 14 (1): 51-60.

Luna-Guzmán, I.; Cantwell, M.; Barreto, D. 1999. Fresh-cut cantaloupe: effects of CaCl2 dips and heat treatments on firmness and metabolic activity. *Postharvest Biology and Technology*, 17 (3): 201-213.

Martín-Belloso, O.; Soliva-Fortuny, R.; Oms-Oliu, G. 2007. Fresh-cut fruits. In: Hui, Y.H. (Ed.), *Handbook of food products manufacturing. Principles, Bakery, Beverages, Cereals, Cheese, Confectionary, Fats, Fruits, and Functional Foods*. John Wiley & Sons, Inc., New Yersey, pp. 879-899.

Mancini, F.; McHugh, T.H. 2000. Fruit-alginate interactions in novel restructured products. *Nahrung*, 44(3): 152-157.

McHugh, T.H.; Krochta, J.M. 1994a. Sorbitol-vs glycerol-plasticized whey protein edible films: Integrated oxygen permeability and tensile property evaluation. *Journal of Agricultural and Food Chemistry*, 42(4): 841-845.

McHugh, T.H., Krochta, J.M. 1994b. Milk-protein-based edible films and coatings. *Food Technology*, 48(1): 97-103.

McHugh, T.H.; Senesi, E. 2000. Apple wraps: A novel method to improve the quality and extend the shelf life of fresh-cut apples. *Journal of Food Science*, 65(3): 480-485.

Nisperos-Carriedo, M.O. 1994. Edible coatings and films based on polysaccharides. In: Krochta, J.M.; Baldwin, E.A.; Nisperos-Carriedo, M.O. (Eds.), *Edible coatings and films to improve food quality*. Technomic Publishing Co, Lancaster, pp. 305-335.

Nussinovitch, A. 1997. Agricultural uses of hydrocolloids. In: Nussinovitch, A. (Ed.), *Hydrocolloid applications: Gum technology in the food and other industries*, Blackie Academic and Professional, London, pp. 169-189.

Odriozola-Serrano, I.; Hernández-Jover, T.; Martín-Belloso, O. 2007. Comparative evaluation of UV-HPLC methods and reducing agents to determine vitamin C in fruits. *Food Chemistry*, 105: 1151-1158.

Olivas, G.I.; Mattinson, D.S.; Barbosa-Cánovas, G.V. 2007. Alginate coatings for preservation of minimally processed 'Gala' apples. *Postharvest Biology and Technology*, 45(1): 89-96.

Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso, O. 2006. Effect of ripeness on the shelf-life of fresh-cut melon preserved by modified atmosphere packaging. *European Food Research and Technology*, 225: 301-311.

Oms-Oliu, G.; Raybaudi-Massilia Martínez, R.M.; Soliva-Fortuny, R.; Martín-Belloso, O. 2007. Effect of superatmospheric and low oxygen modified atmospheres on shelf-life extension of fresh-cut melon. *Food Control*, 19(2): 191-199.

Oms-Oliu, G.; Odriozola-Serrano, I.; Soliva-Fortuny, R.; Martín-Belloso, O. 2008. The role of peroxidase on the antioxidant potential of fresh-cut 'Piel de Sapo' melon packaged under different modified atmospheres. *Food Chemistry*, 106 (3): 1085-1092

Puschmann, R.; Simões, A.; Moreira, S.; Soares, N.; Carnelossi, M.; Gil. M. 2007. Calidad y actividad de la fenilalanina amonioliasa (PAL) en minizanahoria con recubrimiento comestible antimicrobiano. V. In Grupo de Postrecolección y Refrigeración. UPCT (Ed.), *Congreso iberoamericano de tecnología postcosecha y agroexportaciones*. Grupo de Postrecolección y Refrigeración, Cartagena, UPCT Universidad Politécnica de Cartagena, pp. 616-624.

Reyes, L.F.; Cisneros-Zevallos, L. 2003. Wounding stress increases the phenolic content and antioxidant capacity of purple-flesh potatoes. *Journal of Agricultural and Food Chemistry*, 51: 5296-5300.

Rhim, J.W. 2004. Physical and Mechanical properties of water resistant sodium alginate films. *Lebensmittel-Wissenschaft und-Technologie*, 37(3): 323-330.

Rojas-Graü, M.A.; Tapia, M.S.; Rodríguez, F.J.; Carmona, A.J.; Martín-Belloso, O. 2007a. Alginate and gellan based edible coatings as support of antibrowning agents applied on fresh-cut Fuji apple. *Food Hydrocolloids*, 21(1): 118-127.

Rojas-Graü, M.A.; Tapia, M.S.; Martín-Belloso, O. 2007b. Using polysaccharide-based edible coatings to maintain quality of fresh-cut Fuji apples. *Lebensmittel-Wissenschaft und-Technologie*, 41 (1): 139-147

Rojas-Graü, M.A.; Raybaudi-Massilia, R.M.; Soliva-Fortuny, R.C.; Avena-Bustillos, R.J.; McHugh, T.H.; Martín-Belloso, O. 2007c. Apple puree-alginate edible coating as carrier of antimicrobial agents to prolong shelf-life of fresh-cut apples. *Postharvest Biology and Technology*. 45(2): 254-264.

Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299: 152-178.

Soliva-Fortuny, R.C.; Ricart-Coll, M.; Elez-Martínez, P.; Martín-Belloso, O. 2007. International atmosphere, quality attributes and sensory evaluation of MAP packaged fresh-cut Conference pears. *International Journal of Food Science and Technology*, 42: 208-213.

Sothornvit, R.; Krochta, J.M. 2000. Plasticizer effect on oxygen permeability of B-lactoglobulin films. *Journal of Agricultural and Food Chemistry*, 48: 6298-6302.

Tapia, M.S.; Rojas-Graü, M.A.; Rodríguez, E.J.; Ramírez, J.; Carmona, A.; Martín-Belloso. 2007. Alginate- and gellan-based edible films for probiotic coatings on fresh-cut fruits. *Journal of Food Science*, 72(4): 190-196.

Wong, D.W.S.; Camirand, M.W.; Pavlath, A.E. 1994. Development of edible coatings for minimally processed fruits and vegetables. In: Krochta, J.M.; Baldwin, E.A.; Nisperos-Carriedo, M.O. (Eds.), *Edible coatings and films to improve food quality*, Technomic Publishing Co., Lancaster, pp. 65-88.

Capítulo VI

Effect of ripeness at processing on fresh-cut 'Flor de Invierno' pears packaged under modified atmosphere conditions

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Abstract

The effects of ripeness at processing and packaging conditions on respiration, microbiological stability as well as colour and firmness of fresh-cut 'Flor de Invierno' pears were evaluated throughout storage. Although a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere led to an inhibition of ethylene synthesis and carbon dioxide production compared to non modified atmosphere packaging, the more advanced the ripeness stage at processing, the higher the physiological activity and thus, the higher production of carbon dioxide, ethylene and ethanol. A 2.5 kPa O_2 + 7 kPa CO_2 atmosphere inhibited bacterial growth, yeast and mould proliferation in mature-green pears but fast microbial growth was observed especially on ripe pears, under both packaging conditions. In conclusion, the shelf-life of ripe pears was reduced by increased respiration response to processing and accelerated microbial spoilage. Instead, pears processed in a partially-ripe stage were suitable for conservation while gathering desired sensory attributes. Therefore, for commercial purposes, a shelf-life of 10 days was suggested for partially-ripe fresh-cut pears packaged under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere.

Keywords: fresh-cut pears, fruit ripeness, modified atmosphere packaging, microbial stability, fruit quality

Introduction

The market for fresh-cut fruits has undergone a fast growth within the food industry because of the demand for healthy ready-to-eat products. However, these commodities often have a short shelf-life since minimal processing operations damage the tissue integrity of fruit, causing an increase in physiological activity and leading to biochemical changes such as browning, off-flavour development and softening. Important factors that could affect the shelf-life of fresh-cut fruits include cultivar, maturity state at processing, modified atmosphere (MA) packaging and storage temperature (Gorny et al., 2000; Soliva-Fortuny et al., 2002a, 2004).

In Europe, 'Conference', 'Blanquilla' and 'Abate Fetel' are major pear varieties that have familiar oval shape with rounded bottom and a narrow top. Although the flesh of the pear is white, juicy and sweet flavoured, they can quickly become soft, especially when they are tree-ripened. Therefore, winter varieties such as 'Flor de Invierno' could have interesting characteristics for fresh-cut processing. The peel has a greenish background colour that turns slightly yellow when ripen, and fruit has a crispy juicy white flesh that exhibits outstanding firmness retention throughout its storage.

The use of MA to preserve fresh-cut fruits involves reduced oxygen (O_2) levels, below 5%, and/or elevated carbon dioxide (CO_2) levels, above 3% (Kader and Saltveit, 2003). MA delays senescence and associated biochemical and physiological changes, slowing respiration, ethylene production rate, softening, and compositional changes (Sapers and Miller, 1998; Dong et al., 2000; Gorny et al., 1999, 2002).

Respiration involves the oxidative breakdown of complex substrate molecules normally present in plant cells such as starch, sugars, and organic acids to simpler molecules as CO_2 and H_2O. In general, there is an inverse relationship between respiration rate and postharvest shelf-life of fresh-cut fruits. The beneficial effect of MA packaging can be attributed to a reduction in the rate of ethylene production since oxygen is involved in the conversion of 1-amino-cycloprane-1-carboxylic acid (ACC) to ethylene (Yang, 1984). Besides, MA can have direct or indirect effects on microbial safety and stability of fresh-cut fruits. Thus, low O_2 atmospheres can provide a significant decrease on bacteria, yeast and mould counts under MA conditions (Bai et al., 2001; Soliva-Fortuny et al., 2003a). However, an adequate O_2 concentration inside packages is required to maintain aerobic respiration, since too low O_2 levels may trigger anaerobic metabolism with the production of undesirable compounds and other physiological disorders (Soliva-Fortuny et al.,

2003b). The oxygen concentration at which anaerobic respiration begins depends on several factors, such as species, cultivar, maturity and temperature (Wills et al., 1998). In addition, ripeness stage may influence the fruit susceptibility to elevated CO_2 damage, which induces anaerobiosis and tissue injury. Thus, ripe fruit may tolerate higher levels of CO_2 than unripe fruit (Kader and Saltveit, 2003).

MA can also slow the activity of cell wall degrading enzymes that cause fruit softening, as well as retard surface browning although MA packaging cannot effectively prevent browning of fresh-cut fruits (Soliva-Fortuny and Martín-Belloso, 2003b). Low O_2 and/or elevated CO_2 improved the visual quality of fresh-cut fruits such as melon and mango (Ayhan and Chism, 1998; Qi et al., 1999; Rattanapanone et al., 2001). However, Soliva-Fortuny et al. (2002b) and Gorny et al. (2002) detected browning on fresh-cut 'Conference' and 'Bartlett' pears stored in low O_2 and / or high CO_2 atmospheres.

The objective of this study was to determine the effect of ripeness at processing on the shelf-life of fresh-cut 'Flor de Invierno' pears packaged under MA conditions. The effect of MA packaging on physiological activity and microbiological stability as well as colour and firmness as affected by the ripeness stage at processing was determined through storage time.

Materials and methods

Pears

'Flor de Invierno' pear cultivar (*Pyrus communis L.*) was supplied by a local distributor (ACTEL, Lleida, Spain). Three physiological stages were selected in order to study the effect of ripeness at processing on the shelf-life of fresh-cut pears. Fruits were stored at 4 ºC for one month to ensure ripening capacity and were divided into three portions. One was immediately processed whereas the other portions were held at 20 ºC to reach the desired ripeness stages and then be processed. The ripeness stage was characterised by measuring the maximum force necessary to penetrate the flesh of a whole fruit with a 8-mm diameter probe. The selected maturities corresponded to those shown in table 1 and were determined by sampling 10 fruits. When firmness values fell within a selected range, the pears were subjected to a physicochemical characterisation before processing operations.

A physicochemical characterisation of whole pears was carried out by sampling 10 fruits (Table 1): soluble solids content (Atajo RX-100 refractometer; Atago Company Ltd., Japan), total acidity

(AOAC 2000), pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), colour (Minolta CR-400 chroma meter; Konica Minolta Sensing, Inc., Osaka, Japan), firmness (Mechanical Fruit Firmness Tester, QA Supplies, LLC., Norfolk, Virginia, USA), internal gas composition (Micro-GC CP 2002 gas analyser; Chrompack Intl., Middelburg, The Netherlands) (Soliva-Fortuny *et al*., 2005).

Sample preparation

Pears were sanitised in a 200 ppm NaClO solution for 2 min, rinsed with tap water, and dried by hand. The fruits were peeled, the core tissue was completely removed and the remaining tissue was cut into wedges. The excess of juice was completely drained and then, 100 g of pear wedges were packaged in polypropylene trays. The O_2 and CO_2 permeabilities of the film were 110 $cm^3 \cdot m^{-2} \cdot day^{-1} \cdot bar^{-1}$ and 500 $cm^3 \cdot m^{-2} \cdot day^{-1} \cdot bar^{-1}$ at 23ºC and 0% RH, respectively (ILPRA Systems España, S.L. Mataró, Spain). Packages were vacuumed and flushed with a mixture of 2.5 kPa O_2 + 7 kPa CO_2 (balanced N_2) in a ratio product / gas mixture of 1:2 and then heat-sealed with a vacuum compensated machine (ILPRA Food Pack Basic V/6, ILPRA Systems. CP. Vigevono, Italia). Pear wedges packaged into non modified atmospheres (21 kPa O_2) were just sealed. Trays were stored at 4 ± 1 ºC in darkness and analysed throughout 35 days of storage. Two trays were taken at each sampling time to perform the analyses, and 5 fruit pieces from each replicate were randomly withdrawn to carry out repetitions.

Headspace gas analysis and internal gas composition

A gas analyser (Micro-GC CP 2002, Chrompack International, Middelburg, The Netherlands), equipped with a thermal conductivity detector, was used to analyse the gas composition in packages. A sample of 1.7 mL was automatically withdrawn from the headspace atmosphere, and a portion of 0.25 µl was injected to a CP-Molsieve 5Å column (4m x 0.35 mm, df=10 µm) at 60 ºC and 100 kPa for O_2 quantification. A portion of 0.33 µl was injected to a Pora-PLOT Q column (10m x 0.32 mm, df=10 µm) at 75 ºC and 200 kPa for carbon dioxide, ethylene and ethanol determinations. Two trays were taken at each time to perform the analysis and 2 readings were carried out for each package.

For internal gas composition determinations, a sample of 50 g of fresh-cut pear was collected and placed in a saturated NaCl solution, the internal gas content was vacuumed and a sample injected

into a Micro-GC Micro-GC CP 2002, Chrompack International, Middelburg, The Netherlands) for oxygen, carbon dioxide, ethylene, acetaldehyde and ethanol quantification. Chromatographic conditions were the same as outlined previously. Internal gas composition of raw material was carried out sampling 10 fruits for each ripeness stage.

Colour and Firmness evaluation

The colour of fresh-cut pears was determined with a Minolta CR-400 chroma meter (Konica Minolta Sensing, Inc., Osaka, Japan), equipped with a D_{75} light source and the observer at 10º. Two trays were taken at each time to perform the analysis and five fruit pieces were evaluated for each package. Three readings were obtained for each replicate by changing the position of the pear wedges in the optical glass cell to get uniform colour measurements. Colour values of CIE L* (lightness), a* (red-green) and b* (yellow-blue) were measured through reflectance values. Hue angle (h^0) was calculated by Eq. 1.

$$h^o = \arctan\frac{b^*}{a^*} \qquad \text{(Eq. 1)}$$

Firmness evaluation was performed using a TA-XT2 Texture Analyser (Stable Micro Systems Ltd., Surrey, England, UK) by measuring the maximum penetration force. Fruit wedges were cut in rectangular shaped samples 2.0 cm high and were penetrated by a 4 mm diameter rod. The downward distance was set at 10 mm at a rate of 5 mm/s and automatic return. Samples were placed so that the rod penetrated their geometric centre. Two trays were taken at each sampling time to perform the analyses, and 5 fruit pieces from each replicate were randomly withdrawn to carry out repetitions.

Microbiological stability

The microbiological stability of fresh-cut pears through storage time was evaluated by the determination of total mesophilic aerobic bacteria, yeast and mould populations. Two replicate counts were obtained at each time from each two packages at the same experimental condition. The analyses were carried out once a week for five weeks. In sterile conditions, 10 g of pear tissue were added to 90 ml of 0.1% sterile peptone solution in a sterile bag and blended for 2 min in a Stomacher Lab Blender 400 (Seward medical, London, England). Serial dilutions of fruit

homogenates were plated on plate count agar (PCA) at 30 ºC ± 1 ºC for 72 h ± 3 h for mesophilic aerobic bacteria counts (ISO 4833, 1991) and chloramphenicol glucose agar (GCA) at 25 ºC ± 1 ºC for 5 days for yeast and mould counts (ISO 7954, 1988).

Mathematical models and statistical analysis

Statistical analysis was performed using the Statgraphics plus v.5.1 software (Manugistics, Inc., Rockville, MA, USA). Data were analysed by multifactor analysis of variance. A test of mean comparisons according to Duncan multiple range test was applied, with a 0.05 level of significance.

The growth of microorganisms was modelled according to a modification of the Gompertz equation (Eq. 2) (Zwietering et al., 1990), proposed by Lanciotti et al. (1999) to model microbial growth on fresh-cut apple slices.

$$y = k + A\exp\left\{-\exp\left[\left(\frac{\mu_{max} e}{A}\right)\cdot(\lambda - t)+1\right]\right\} \quad \text{(Eq. 2)}$$

where: k, initial count estimated by the model [log (CFU g^{-1})]; A, maximum growth attained at the stationary phase [log (CFU g^{-1})]; μ_{max}, maximal growth rate [Δlog (CFU g^{-1}) day^{-1}]; λ, lag time (days); t, storage time (days).

Results and Discussion

Physicochemical characteristics of 'Flor de Invierno' pear

Table 1 shows some physicochemical and physiological attributes of 'Flor de Invierno' pears at different ripeness stages. In relation to physiological development, the ripeness process in climacteric fruit-type vegetables reflects enhanced metabolic activities that occur at the transition from the later maturity states of the fruit to its senescence phase, which took place during the storage of harvested pears. Thus, the more advanced the ripeness stage, the higher the soluble solids content and the lower the acidity (Table 1). Ripeness involved changes on the skin and pulp colour of pear due to chlorophyll degradation, which involves greenness loss, and the synthesis and/or revelation of pigments ranging from yellow to red. An increase of a* and decrease of h^0 values was observed on ripe pears (Table 1). Moreover, softness increased as pears ripened, decreasing the value of the maximum penetration force (Table 1). In general, physicochemical

changes related to physiological decay in fruits are consequence of a metabolic and chemical reorganization which take place when fruit ripens, leading to an increase in respiration quotient, sweetness, softness and changes in aroma, colour and nutritional value (Gorny et al., 2002). A progressive increase in ethylene production was observed in the internal atmosphere of tissue as ripeness process advanced (Table 1). During ripening, changes in colour, aroma, texture, flavour, and other attributes related to the product physiology are consequence of ethylene production at the onset of the climacteric phase (Pech et al., 2003).

Table 1 Physicochemical and physiological characteristics of the flesh of 'Flor de Invierno' pear before processing [a]

	Ripeness stage		
	Mature-green	Partially-ripe	Ripe
Soluble solids (ºBrix)	13.1[c]	14.3[b]	14.9[a]
Total acidity (g citric acid / 100 g)	0.3[a]	0.3[a]	0.3[a]
pH	4.0[b]	4.1[b]	4.2[a]
Skin colour			
L*	68.6[a]	71.3[a]	70.5[a]
a*	-8.4[b]	-5.9[b]	0.9[a]
b*	41.9[a]	40.6[a]	38.8[a]
h^0	101.1[a]	97.6[a]	88.7[b]
Pulp colour			
L*	73.9[a]	76.0[a]	74.8[a]
a*	-1.8[c]	-1.0[b]	-0.4[a]
b*	15.4[a]	10.1[a]	12.6[a]
h^0	96.8[a]	95.5[a]	92.0[b]
Firmness (N)	65.2[a]	43.3[b]	36.1[c]
Internal gas composition			
O_2 (%)	20.4[a]	15.9[c]	19.9[b]
CO_2 (%)	0.5[c]	0.8[b]	1.3[a]
C_2H_4 (ppm)	-	0.2[b]	0.4[a]

Values within the same row followed by the same superscript capital letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$)

Physiological parameters evolution

The oxygen consumption of fresh-cut 'Flor de Invierno' pears was not influenced by ripeness stage of raw material, whereas the composition of packaging atmosphere significantly affected the O_2 concentration. During the first two weeks, the oxygen levels were significantly higher under non MA packaging than under an initial 2.5 kPa O_2 + 7 kPa CO_2 atmosphere (Fig. 1A and B). In fact, fresh-cut pears packaged under a low O_2 atmosphere underwent a strong decreased in O_2 concentration within the first week, promoting anaerobic respiration and as a consequence, an

accumulation of fermentative metabolites such as acetaldehyde, ethanol and ethyl acetate inside packages. The O_2 consumption of fresh-cut 'Conference' pears was also substantially triggered during the first 7 days of storage under a initial 2.5 kPa O_2 + 7 kPa CO_2 atmosphere (Soliva-Fortuny et al., 2002b). In our study, pears in non MA packaging had O_2 equilibrium values about 1 kPa beyond the third week storage regardless the ripeness stage. In contrast, Soliva-Fortuny et al. (2004) reported a strong decrease in O_2 levels in packages of fresh-cut 'Conference' pear packaged under non MA conditions during the first 14 days of storage, reaching values below 1 kPa during the third week of storage. As Fig. 1C and D show, CO_2 concentration rose during storage time although the evolution was significantly influenced by the ripeness stage and packaging conditions ($P < 0.05$). Beyond the third week of storage, CO_2 production rose dramatically under all packaging conditions although partially-ripe pears exhibited less CO_2 accumulation regardless the initial atmosphere composition. The increase of CO_2 at prolonged storage could be partially attributed to the proliferation of anaerobic microorganisms due to conditions of anoxia in the package headspace, which is in agreement with the results reported by Soliva-Fortuny and Martín-Belloso (2003a). These authors reported a high proliferation of anaerobic microorganims in fresh-cut pears stored under anoxia conditions beyond the first two weeks of storage. CO_2 levels inside packages of fresh-cut 'Flor de Invierno' pears were significantly lower under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere than under non modified conditions, beyond the second week of storage. Thus, partially-ripe pears packaged under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere evolved less CO_2, but even under these conditions CO_2 levels reached values above 20 kPa at the third week of storage (Fig. 1C). Soliva-Fortuny et al. (2004) also reported less CO_2 production in partially-ripe fresh-cut 'Conference' pears packaged under either non MA or 100% N_2 initial packaging conditions. Absolute CO_2 concentration in package headspace atmosphere of ´Flor de Invierno' pears were about 20-30 kPa CO_2 at 21 days storage time whereas 'Conference' pears reached levels up to 40-50 kPa CO_2 (Soliva-Fortuny et al., 2002b). Gorny et al. (2002) reported severe injury on fresh-cut 'Bartlett' caused by air plus 10 or 20 kPa CO_2 atmospheres, which accelerated tissue browning and necrosis compared to the air control. According to our results, O_2 and CO_2 levels in packages of fresh-cut 'Flor de Invierno' pears packaged under a initial 2.5 kPa O_2 + 7 kPa CO_2 atmosphere provided adequate environment for 10-14 days, regardless to ripeness stage of fruit. Beyond that period, high concentrations of CO_2 may have detrimental effects on the physiology of fresh-cut tissue.

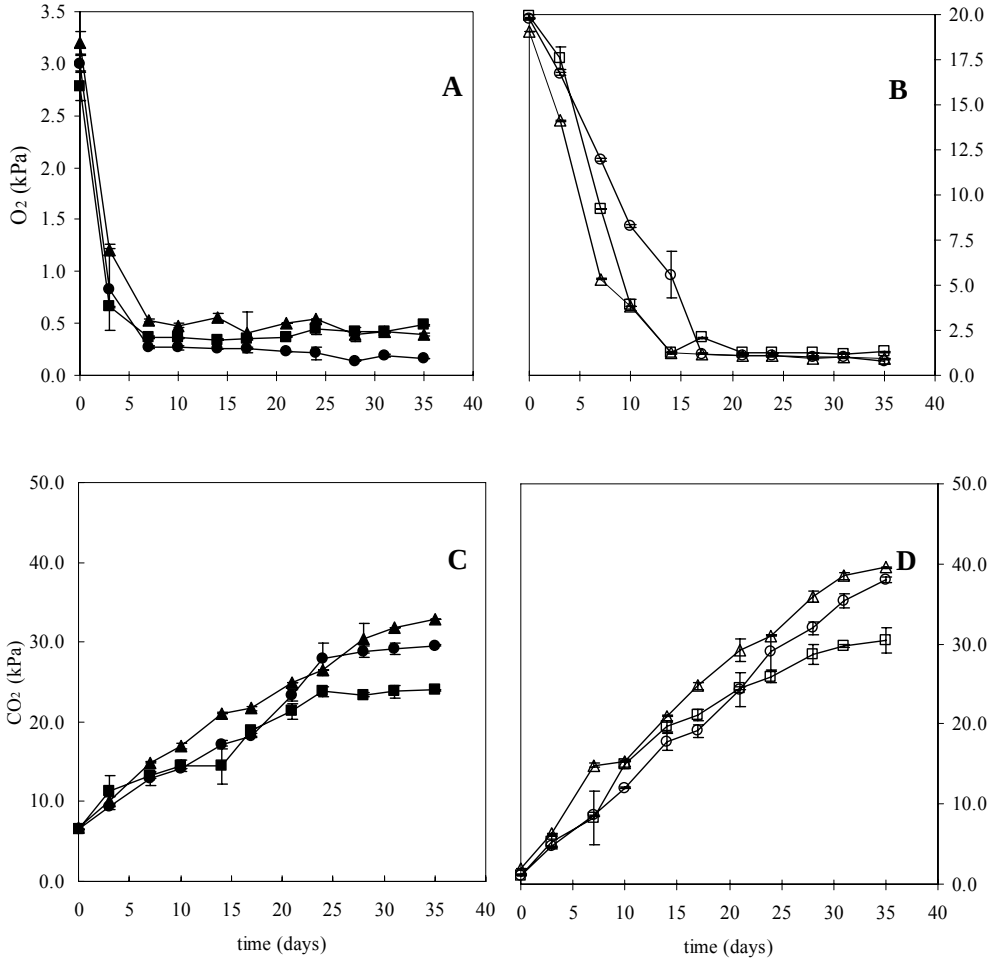

Figure 1. O_2 and CO_2 concentrations in the package headspace of fresh-cut 'Flor de Invierno' pears processed at different ripeness stages and packaging conditions. A, C: packaging under 2.5 kPa O_2 + 7 kPa CO_2 (• mature-green, ■ partially-ripe, ▲ripe); B, D: packaging under non modified atmosphere (o mature-green, □ partially-ripe, Δ ripe). Data shown are mean ± standard deviation

Ethylene production may be induced as a wounding response caused by mechanical stress during processing (Pech et al., 2003). The ripeness stage and packaging conditions affected significantly ($P < 0.05$) the evolution of ethylene concentration throughout storage time, reaching higher values

on ripe fresh-cut pears and maximum ethylene levels under non MA conditions (Fig. 2). Under initial non MA packaging, in both mature-green and partially-ripe fresh-cut pears, ethylene production increased during the two first weeks and then levels of ethylene inside the packages did not significantly change. On the other hand, ethylene concentration rose significantly in ripe fresh-cut 'Flor de Invierno' pears during approximately 21 days storage up to maximum levels and then, ethylene accumulation depleted through time (Fig. 2B).

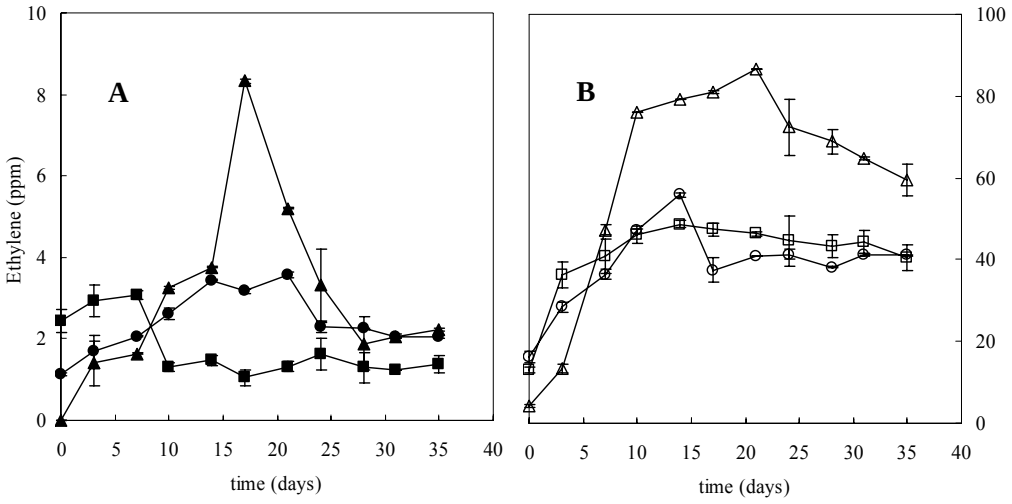

Figure 2. Ethylene concentrations in the package headspace of fresh-cut 'Flor de Invierno' pears processed at different ripeness stages and packaging conditions. A: packaging under 2.5 kPa O_2 + 7 kPa CO_2 (● mature-green, ■ partially-ripe, ▲ripe); B: packaging under non modified atmosphere (o mature-green, □ partially-ripe, Δ ripe). Data shown are mean ± standard deviation

Ethylene concentrations were notably higher under initial non MA conditions than under 2.5 kPa O_2 + 7 kPa CO_2 atmospheres ($P < 0.05$) (Fig. 2). Low ethylene production could be related to a synergistic effect of low O_2 concentrations and high CO_2 concentrations on the inhibition of ethylene synthesis (Soliva-Fortuny et al., 2003b). An initial 2.5 kPa O_2 + 7 kPa CO_2 atmosphere slowed down ethylene production, being 10-fold lower than under initial non MA (Fig. 2). A complete inhibition of ethylene production was reported on fresh-cut apples and pears packaged in O_2 absence (Soliva-Fortuny et al., 2002b, 2004). In addition, low O_2 atmospheres could act synergistically with elevated CO_2 levels to reduce ethylene production (Soliva-Fortuny et al.,

2003b). In general, ethylene production was significantly inhibited under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere compared to non MA ($P < 0.05$). Besides, ripe pears packaged under a low O_2 atmosphere exhibited maximum ethylene levels on the climacteric peak (Fig. 2A).

Ethanol production, as a result of anaerobic respiration, is often associated with off-flavours and its presence might be detrimental to quality (Day, 1994). Anaerobic respiration led to CO_2 accumulation in packages of fresh-cut 'Flor de Invierno' pears. However, in our study, a strong accumulation of ethanol in package headspace was not detected in spite of high-CO_2 atmospheres because carbon dioxide may have an inhibitory effect on accumulation of acetaldehyde, ethanol and ethyl acetate. Gunes et al. (2001) reported that CO_2 levels of 15-30 kPa resulted in about a 50% reduction in acetaldehyde, ethanol and ethyl acetate concentrations in tissues under both anaerobic and low O_2 atmospheres compared with slices kept in CO_2-free atmospheres. Their data suggest that CO_2 might provide a mechanism to reduce accumulation of fermentation products, and that employing high concentrations of CO_2 can increase the tolerance of slices to low O_2 levels. In addition, other works reporting high accumulation of carbon dioxide under anaerobic conditions in packages containing fresh-cut pome fruits, have shown little correlation between ethanol and CO_2 concentrations, indicating that production of final fermentative metabolites is greatly conditioned by the extrinsic factors such as the cultivar, ripeness state or physiological condition (Soliva-Fortuny et al., 2002a, 2004; Rojas-Graü et al., 2007). The ripeness stage of 'Flor de Invierno' pears at processing affected the ethanol concentration evolution in packages through storage ($P < 0.05$), showing higher ethanol production in ripe pears for both packaging conditions. In fact, very low O_2 and high CO_2 levels in ripe pears propitiated a drastic increase of ethanol concentrations during the third week of storage (Fig. 3). According to Pesis (2005), the increase in anaerobic respiration of over-mature fruit could be due to the reduction in mitochondrial activity in tissues as a consequence of membrane damage, making the cells unable to produce enough energy. Therefore, anaerobic respiration begins in the cytoplasm without requirement of a membrane-bound organelle. Soliva-Fortuny et al. (2004) agreed that an advanced ripeness stage of fresh-cut 'Conference' pear enhanced ethanol accumulation, under either initial non MA or O_2 restrictive conditions. On the other hand, ethanol levels in partially-ripe fresh-cut pears packaged under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere were not detectable for 7 days and remained low up to 10-14 days ensuring absence of off-flavour during marketable period.

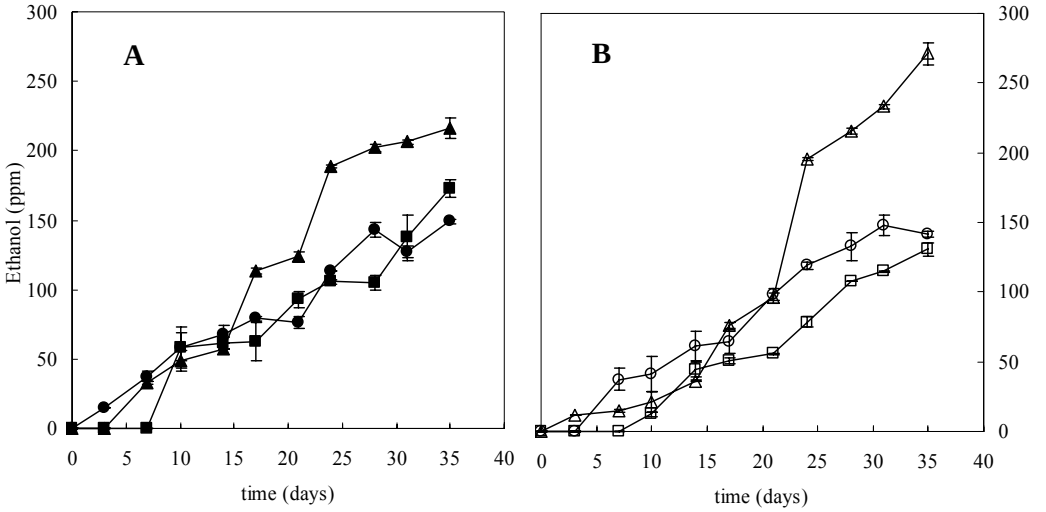

Figure 3. Ethanol concentrations in the package headspace of fresh-cut 'Flor de Invierno' pears processed at different ripeness stages and packaging conditions. A: packaging under 2.5 kPa O_2 + 7 kPa CO_2 (• mature-green, ■ partially-ripe, ▲ripe); B: packaging under non modified atmosphere (o mature-green, □ partially-ripe, Δ ripe). Data shown are mean ± standard deviation

Colour and firmness evolution

As a consequence of mechanical operations during processing, 'Flor de Invierno' pear tissues developed browning rapidly thereafter, especially on ripe pears (Fig. 4). L* and h^0 initial values below 70 and 92º, respectively, were measured after processing. According to Oms-Oliu et al. (2006), a decrease of h^0 values to around 90º indicated browning on fresh-cut 'Flor de Invierno' pears. Soliva-Fortuny et al. (2002b, 2004) reported an increase in the susceptibility to browning of ripe 'Conference' pears, detecting most of the changes in colour during the first hour of storage. Moreover, L* and h^0 initial values were better preserved in ripe fresh-cut pears through 35 days (Fig. 4). According to Amiot et al. (1995), significant decrease in phenolic substrates of polyphenol oxidase enzymes could occur with delayed harvest times, resulting in less browning susceptibility.

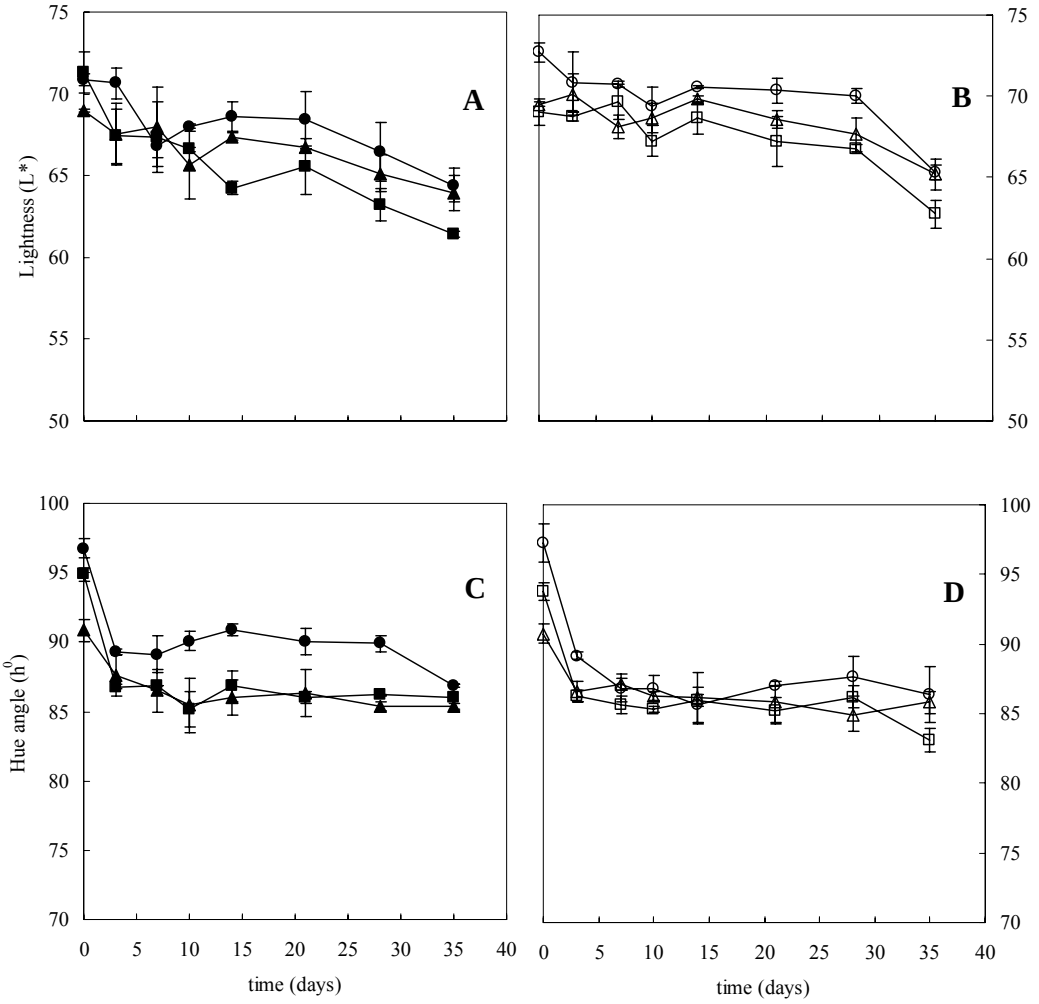

Figure 4. Evolution of colour throughout storage of fresh-cut 'Flor de Invierno' pears processed at different ripeness stages and packaging conditions. A: packaging under 2.5 kPa O_2 + 7 kPa CO_2 (• mature-green, ■ partially-ripe, ▲ripe); B: packaging under non modified atmosphere (o mature-green, □ partially-ripe, Δ ripe). Data shown are mean ± standard deviation

A 2.5 kPa O_2 + 7 kPa CO_2 atmosphere was not effective to control enzymatic browning of 'Flor de Invierno' fresh-cut pears whereas the ripeness stage at processing significantly affected colour evolution ($P < 0.05$). Most of the changes in colour were reflected by L* and h^0 decrease

throughout storage. Thus, the greatest decrease in h^0 and L* values was observed on partially-ripe pear wedges (Fig. 4). This has been suggested to be due to dissolution of polyphenol oxidase that would increase browning oversensitivity. The latent forms of the enzyme might be effectively activated during storage due to cell disruption and subsequent decompartmentalisation of enzymes, substrates and other substances present in cell vacuoles (Soliva-Fortuny et al., 2002b).

In general, 'Flor de Invierno' fresh-cut pears maintained their initial firmness through storage time (Fig. 5). Thus, atmosphere modification was not found to have any significant effect on the evolution of firmness of pear wedges. In agreement with these results, Gorny et al. (2002) did not find any relationship between the oxygen concentration and firmness on fresh-cut 'Bartlett' pear. On the contrary, softening of pear wedges differed considerably among ripeness stages ($P < 0.05$). Thus, both mature-green and partially-ripe pears preserved their initial firmness contrast to ripe pears, which underwent softening after the two first weeks (Fig. 5). These results agree with those reported by Soliva-Fortuny et al. (2004) for 'Conference' fresh-cut pears. Dong et al. (2000) also found that firmness retention of 'Bosc' and 'Barlett' fresh-cut pears was higher for under-ripe fruits. Gorny et al. (2000) found similar results for 'Bosc' and 'Anjou' varieties.

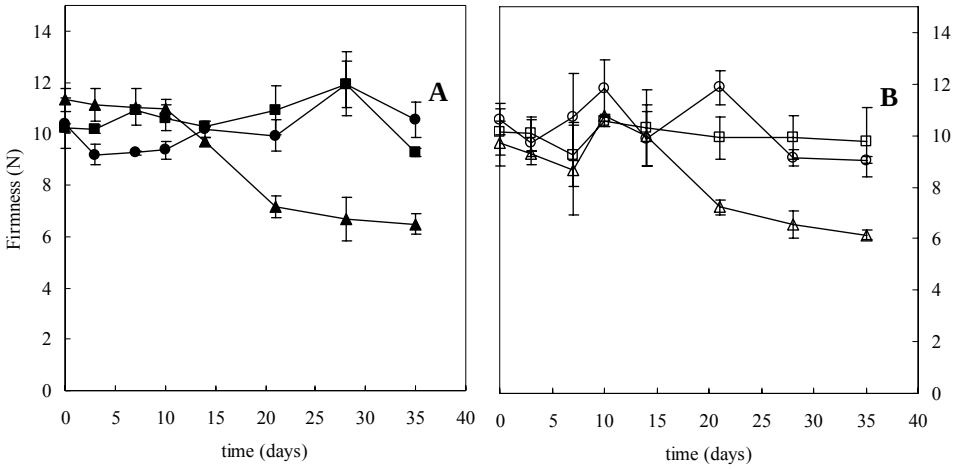

Figure 5. Evolution of firmness (N) throughout storage of fresh-cut 'Flor de Invierno' pears processed at different ripeness stages and packaging conditions. A: packaging under 2.5 kPa O_2 + 7 kPa CO_2 (● mature-green, ■ partially-ripe, ▲ripe); B: packaging under non modified atmosphere (o mature-green, □ partially-ripe, Δ ripe). Data shown are mean ± standard deviation

Microbial stability and shelf-life modelling

Initial counts of aerobic mesophilic microorganisms in processed pears were approximately 2 log (CFU g^{-1}) whereas initial yeast and mould loads ranged from 1 to 1.5 log (CFU g^{-1}) approximately. Although mould strains are predominant on fresh fruit, mechanical operations such as washing, peeling, and cutting could cause the substitution of the initial native microflora. On fresh-cut 'Granny Smith' apples, a quick replacement of the initial yeast and mould populations for gram-positive bacteria was detected as a consequence of processing (Lamikanra and Watson, 2000).

In general, populations of yeasts and moulds were less numerous than mesophilic aerobic microorganisms during refrigerated storage (Fig. 6). Microbial development was significantly affected by both the packaging conditions and the ripeness stage at processing ($P < 0.05$). Hence, advanced ripeness stages went with higher microbial counts throughout storage. On the other hand, during the two first weeks of storage, the development of mesophilic aerobic microorganisms, yeasts and moulds was lower under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere than under non MA. Differences between atmosphere conditions were not detected on partially-ripe and ripe fresh-cut pears beyond that period (Fig. 6).

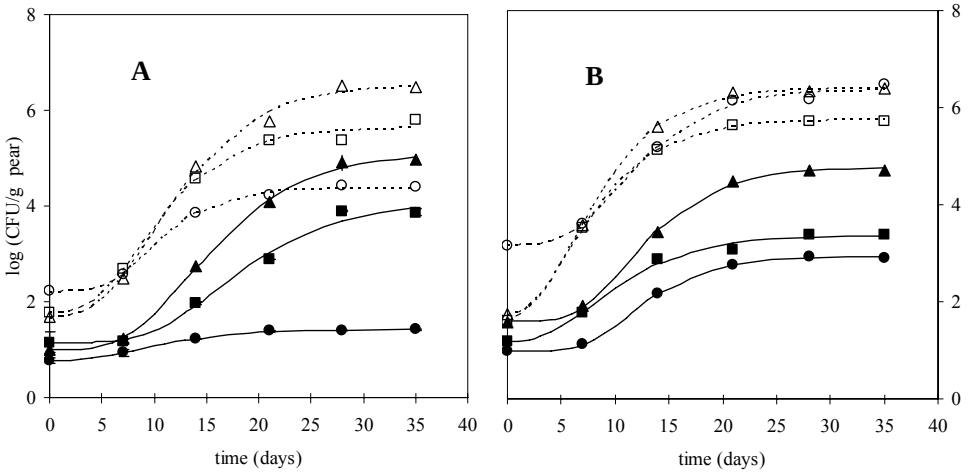

Figure 6. Evolution of microbial growth on fresh-cut 'Flor de Invierno' pears processed at different ripeness stages and packaging conditions, according to Gompertz model. Aerobic mesophilic microorganisms (o mature-green, □ partially-ripe, Δ ripe); Yeast and moulds (• mature-green, ■ partially-ripe, ▲ripe). A: packaging under 2.5 kPa O_2 + 7 kPa CO_2; B: packaging under non modified atmosphere

Table 2 shows the kinetic constants estimated by a modification of the Gompertz model (Eq. 2), which describes the microbial growth of aerobic mesophilic microorganisms, yeasts and moulds on fresh-cut 'Flor de Invierno' pears. In general, maximal growth rate (μ_{max}) of mesophilic aerobic microorganisms was higher than that of yeasts and moulds, for all experimental conditions. These results agreed with those obtained on fresh-cut melon (Bai et al., 2001) or pears and apples (Soliva-Fortuny et al., 2003a, 2004). These authors reported an enhanced effect of MA on the inhibition of yeasts and moulds in comparison with bacteria. In fact, on fresh-cut pears and apples, aerobic bacteria were not inhibited by low O_2 and/or high CO_2 levels atmospheres and, even in small O_2 concentrations, bacterial populations exhibited a fast growth throughout time (Soliva-Fortuny et al., 2003a, 2004).

Table 2. Kinetic constants estimated by a modification of the Gompertz model in order to describe the microbial growth of aerobic mesophilic microorganisms, yeasts and moulds of fresh-cut 'Flor de Invierno' pears, processed at different ripeness stages and packaging conditions. Gompertz parameters: k, initial count estimated by the model [log (CFU g^{-1})]; A, maximum growth attained at the stationary phase [log (CFU g^{-1})]; μ_{max}, maximal growth rate [Δlog (CFU g^{-1}) day^{-1}]; λ, lag time (days)

MAP conditions	k	A	μ_{max}	λ	R^2
Aerobic mesophilic microorganisms					
2.5 kPa O_2+7 kPa CO_2					
Mature-green	2.2±0.1	2.2±0.1	0.08±0.01	5.4±0.3	0.994
Partially-ripe	1.7±0.3	3.9±0.4	0.11±0.03	4.0±0.8	0.989
Ripe	1.6±0.3	4.9±0.3	0.13±0.02	4.6±0.4	0.995
Non modified atmosphere					
Mature-green	3.2±0.3	3.2±0.3	0.09±0.02	5.5±0.4	0.986
Partially-ripe	1.5±0.3	4.2±0.3	0.13±0.03	1.5±0.9	0.995
Ripe	1.6±0.3	4.7±0.4	0.15±0.03	2.2±0.7	0.994
Yeasts and moulds					
2.5 kPa O_2+7 kPa CO_2					
Mature-green	0.8±0.1	0.7±0.1	0.02±0.01	3.8±1.7	0.985
Partially-ripe	1.1±0.2	3.0±0.5	0.06±0.02	8.8±2.7	0.982
Ripe	1.0±0.2	4.2±0.3	0.09±0.01	7.1±1.4	0.997
Non modified atmosphere					
Mature-green	1.0±0.1	1.9±0.1	0.07±0.01	7.1±1.3	0.996
Partially-ripe	1.2±0.2	2.2±0.2	0.06±0.02	3.3±1.9	0.989
Ripe	1.6±0.3	3.2±0.5	0.09±0.03	5.9±2.8	0.976

Significant level: $P < 0.05$, mean ± confidence interval

As can be seen in Table 2, the μ_{max} of aerobic mesophilic microorganisms was slightly higher under non MA than under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere. However, the counts attained at the stationary phase (A) were significantly different only on mature-green fresh-cut pears. Besides, the μ_{max} and the A values increased as ripeness stage advanced, under both packaging conditions. Nevertheless, aerobic mesophilic microorganisms counts remained at an acceptable level fixed at 5.10^7 CFU g^{-1}, consistent with an acceptable quality of fresh-cut produce, according to Spanish legislation (BOE 2000). The lag phase period (λ) was lengthened under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere compared to non MA on partially-ripe and ripe fresh-cut 'Flor de Invierno' pears, while similar λ value, about 5 days, was detected under both packaging conditions on mature-green fresh-cut pears.

Low O_2 levels only inhibited the mould and yeast development on mature-green fresh-cut pears. Hence, the μ_{max} was significantly lower under 2.5 kPa O_2 + 7 kPa CO_2 atmospheres than under non MA and, as a consequence, counts at the stationary phase achieved lower values under low O_2 atmospheres. On the other hand, μ_{max} and A were similar on partially-ripe and ripe 'Flor de Invierno' pears, under both packaging conditions. Besides, λ values were not significantly different for all conditions and the effect of ripeness stage or packaging conditions were not significant.

Conclusions

An advanced ripeness stage at processing could be a limiting factor on the shelf-life of 'Flor de Invierno' fresh-cut pears. More advanced ripeness stage was associated with higher production of carbon dioxide, ethylene or ethanol. In general, firmness was preserved during 35 days storage, but softening was detected in advanced ripe fruits beyond the day 21. According to a modified Gompertz model, bacterial population exhibited faster growth than yeasts and moulds as well as greater maximal growth rate of microorganisms was detected as ripeness advanced.

In relation to the effect of a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere packaging on shelf- life of fresh-cut pears, low O_2 and high CO_2 initial levels inhibited ethylene synthesis and evolved less CO_2 from the second week of storage. However, low O_2 atmospheres did not prevent effectively cut surface browning, which occurred during the few first hours after processing, whereas firmness was preserved throughout refrigerated storage.

Modified atmosphere packaging inhibited mould and yeast proliferation in mature-green pears but could not delay microbial growth in partially-ripe and ripe pears. Thus, the effect of a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere depended on the ripeness stage at processing, which was decisive to prevent microbiological decay of fresh-cut 'Flor de Invierno' pears. The more advanced the ripeness stage, the faster the product spoilage. Therefore, a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere packaging could be recommended for keeping quality and safety of fresh-cut 'Flor de Invierno' pears processed in a partially-ripe state for about 10 days.

Acknowledgements

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References

Amiot, M.J.; Tacchini, M.; Aubert, S.Y.; Oleszek, W. 1995. Influence of cultivar, maturity stage, and storage conditions on phenolic composition and enzymatic browning of pear fruits. *Journal of Agricultural Food Chemistry*, 423: 1132-1137.

AOAC. 2000. Fruits and Fruits Products. In: Association of Official Analytical Chemists (Eds.), *Official Methods of Analysis of AOAC international*. AOAC International, Washington, pp. 11.

Ayhan, Z.; Chism, G.W.; Richter, E.R. 1998. The shelf life of minimally processed fresh-cut melons. *Journal of Food Quality*, 21: 29-40.

Bai, J.H.; Saftner, R.A.; Watada, A.E.; Lee, Y.S. 2001. Modified atmosphere maintains quality of fresh-cut cantaloupe (*Cucumis melo L.*). *Journal of Food Science*, 66: 1207-1211.

BOE. 2000. Normas de higiene para la elaboración, distribución y comercio de comidas preparadas. Real Decreto 3484/2000, pp. 1435-1441.

Day, B.P.F. 1994. Modified atmosphere packaging and active packaging of fruits and vegetables. In: Ahvenainen, R., Mattila-Sandholm, T.; Ohlsson, T. (Eds), *Minimal processing of foods*, VTT Symposium series 142, Majvik, 14-15 April 1994.

Dong, X.; Wrolstad, R.E.; Sugar, D. 2000. Extending shelf life of fresh-cut pears. *Journal of Food Science*, 65: 181-185.

Gorny, J.R.; Hess-Pierce, B.; Kader, A.A. 1999. Quality changes in fresh-cut peach and nectarine slices as affected by cultivar, storage atmosphere and chemical treatments. *Journal of Food Science*, 64: 429-432.

Gorny, J.R.; Cifuentes, R.A.; Hess-Pierce, B.; Kader, A.A. 2000. Quality changes in fresh-cut pear slices as affected by cultivar, ripeness stage, fruit size, and storage regime. *Journal of Food Science*, 65: 541-544.

Gorny, J.R.; Hess-Pierce, B.; Cifuente, R.A.; Kader, A.A. 2002. Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest Biology and Technology*, 24: 271-278.

Gunes, G.; Watkins, C.B.; Hotchkiss, J.H. 2001. Physiological responses of fresh-cut apple slices under high CO_2 and low O_2 partial pressures. *Postharvest Biology and Technology*, 22: 197-204.

ISO 4833, 1991. Methods for Microbiological Examination of Food and Animal Feeding Stuffs. Enumeration of Micro-organisms. Colony Count Technique at 30 ºC. International Standards Organisation, Geneva, Switzerland.

ISO 7954, 1988. General guidance for enumeration of yeasts and moulds. Colony count technique at 25 ºC. International Standards Organisation, Geneva, Switzerland.

Kader, A.A.; Saltveit, M.E. 2003. Respiration and Gas Exchange. In: Bartz, J.A.; Brecht, J.K. (Eds.), *Postharvest Physiology and Pathology of Vegetables*. Marcel Dekker, New York, pp. 7-29.

Lamikanra, O.; Watson, M.A. 2000. Cantaloupe melon peroxidase: characterization and effects of food additives on activity. *Nahrung*, 44: 168-172.

Lanciotti, R.; Corbo, M.R.; Gardini, F.; Sinigaglia, M.; Guerzoni, M.E. 1999. Effect of hexanal on the shelf-life of fresh apple slices. *Journal of Agricultural and Food Chemistry*, 47: 4769-4776.

Oms-Oliu, G.; Aguiló-Aguayo, I.; Martín-Belloso, O. 2006. Inhibition of browning on fresh-cut pear wedges by natural compounds. *Journal of Food Science*, 71: 216-224.

Pech, J.C.; Bouzayen, M.; Latché. 2003. Physiological, Biochemical, and Molecular Aspects of Ethylene Biosynthesis and Action. In: Bartz, J.A.; Brecht, J.K. (Eds.), *Postharvest Physiology and Pathology of Vegetables*. Marcel Dekker, New York, pp. 247-285.

Pesis, E. 2005. The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripeneing, enhancement of fruit quality and fruit deterioration. *Postharvest Biology and Technology*, 37: 1-19.

Qi, L.; Wu, T.; Watada, A.E. 1999. Quality changes of fresh-cut honeydew melons during controlled atmosphere storage. *Journal of Food Quality*, 22: 513-521.

Rattanapanone, N.; Lee, Y.; Wu, T.; Watada, A.E. 2001. Quality and microbial changes of fresh-cut mango cubes held in controlled atmosphere. *Hortscience*, 36: 1091-1095.

Rojas-Graü, M.A.; Grasa-Guillem, R.; Martín-Belloso, O. 2007. Quality changes in fresh-cut fuji apple as affected by ripeness stage, antibrowning agents, and stored atmsophere. *Journal of Food Science*, 72: 36-43.

Sapers, G.M.; Miller, R.L. 1998. Browning inhibition in fresh-cut pears. *Journal of Food Science*, 63: 342-346.

Soliva-Fortuny, R.C.; Oms-Oliu, G.; Martín-Belloso, O. 2002a. Effects of ripeness stages on the storage atmosphere, colour, and textural properties of minimally processed apple slices. *Journal of Food Science*, 67: 1958-1963.

Soliva-Fortuny, R.C.; Biosca-Biosca, M.; Grigelmo-Miguel, N.; Martín-Belloso, O. 2002b. Browning, polyphenol oxidase activity and headspace gas composition during storage of minimally processed pears using modified atmosphere packaging. *Journal of the Science of Food and Agriculture*, 82: 1490-1496.

Soliva-Fortuny, R.C.; Martín-Belloso, O. 2003a. Microbiological and biochemical changes in minimally processed fresh-cut 'Conference' pears. *European Food Research and Technology*, 217: 4-9.

Soliva-Fortuny, R.C.; Martín-Belloso, O. 2003b. New advances in extending the shelf-life of fresh-cut fruits: a review. *Trends in Food Science and Technology*, 14: 341-353.

Soliva-Fortuny, R.C.; Alós-Saiz, N.; Espachs-Barroso, A.; Martín-Belloso, O. 2004. Influence of maturity at processing on quality attributes of fresh-cut 'Conference' pears. *Journal of Food Science*, 69: 290-294.

Soliva-Fortuny, R.C.; Ricart-Coll, M.; Martín-Belloso, O. 2005. Sensory quality and internal atmosphere of fresh-cut 'Golden Delicious' apples. *International Journal of Food Science and Technology*, 40: 369-375.

Wills, R.; McGlasson, B.; Graham, D.; Joyce, D. 1998. Physiology and Biochemistry. In *Postharvest an Introduction to the physiology and Handling of Fruit, Vegetables and Ornamentals*. CAB International, New York, pp. 33-59.

Yang, S.F.; Hoffman, N.E. 1984. Ethylene biosynthesis and its regulation in higher plants. *Annual Review of Plant Physiology*, 35: 155-189.

Zwietering, M.H.; Jongenburger, I.; Rombouts, F.M.; Riet, K. van't. 1990. Modelling of the bacterial growth curve. *Applied Environmental Microbiology*, 56: 1875-1881.

Capítulo VII

Inhibition of browning on fresh-cut pear wedges by natural compounds

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Abstract

Mechanical operations as peeling and cutting during minimal processing involve enzymatic browning of fruit tissue. The objective of this work was to evaluate the individual and combined effects of N-acetyl-L-cysteine, reduced glutathione, ascorbic acid and 4-hexylresorcinol to control pear browning. Browning of fresh-cut pears was prevented by a minimum concentration of 0.75% N-acetyl-L-cysteine up to 28 days at 4 ºC. Reduced glutathione treatments were also effective along the storage time although browning was observed after 21 days time storage with a dip of 0.75% reduced glutathione. However, ascorbic acid or 4-hexylresorcinol treatments did not seem to completely prevent browning of pear wedges throughout the storage period. An enhanced antibrowning effect was observed when combining both N-acetyl-L-cysteine and reduced glutathione, considering hue angle as color change index. Thus, hue angle reached maximum levels at 1.5% NAC or 1.5% GSH and 1% NAC with 1% GSH for 28 days. Besides, N-acetyl-L-cysteine, reduced glutathione and 4-hexylresorcinol completely inhibited polyphenol oxidase activity as well as browning inhibitors slightly reduced firmness of fresh-cut pears.

Keywords: fresh-cut pear, browning inhibitors, color, polyphenol oxidase activity, response surface methodology

Introduction

The development of new processing techniques for preserving fresh-cut fruits and vegetables need to overcome some of the hurdles to the commercial distribution of fresh-cut products. Browning is one of the major concerns related to the extension of shelf-life of fresh-cut pear owing to the loss of visual quality of the commodity (Sapers and Miller, 1998; Dong et al., 2000; Gorny et al., 2002).

During minimal processing of fruits and vegetables, tissue integrity is damaged and sensory properties of comodities decrease due to an increase on metabolic activities and decompartimentation of enzymes and substrates (Soliva-Fortuny et al., 2001). Such phenomena are associated to changes on color, flavor and softening besides nutritional properties. The development of enzymatic browning and consequently, production of undesirable brown color is related to polyphenol oxidase (PPO, *o*-diphenol: oxygen oxido-reductase), also known as tyrosinase, phenoloxidase, or cresolase, which catalyze the hydroxylation of monophenols (cresolase activity) and the oxidation of o-diphenol to their corresponding quinones in the presence of oxygen (catecholase activity). The quinones condense and react nonenzymatically with aminoacids and proteins, leading to brown melanin pigments (Dawley and Flurkey 1993).

Reducing agents play a relevant role in the prevention of enzymatic browning either by reducing *o*-quinones to colourless diphenols, or by reacting irreversibly with *o*-quinones to form stable colorless products. Ascorbic acid is commonly used to prevent enzymatic browning of fruit by reducing the *o*-quinones to diphenols (McEvily et al., 1992). Polyphenol oxidase inhibition by ascorbic acid has been attributed to the reduction of enzymatically formed *o*-quinones to their precursor diphenols (Walker 1977). By now, research has been focused on reducing enzymatic browning of fresh-cut pear using ascorbic acid and calcium salt dipping (Gorny et al., 1998; Soliva-Fortuny et al., 2002). However, this treatment is not completely effective to control enzymatic browning of fresh-cut pear, once the ascorbic acid is completely oxidized to dehydroascorbic acid, *o*-quinones are no longer reduced and darkening may occur due to formation of melanines (Nicolas et al., 1994). Some particular post-cutting dips using ascorbic acid in combination with such browning inhibitors as cysteine on 'Bartlett' pears or 4-hexylresorcinol on 'Anjou', 'Bartlett' and 'Bosc' pears packaged under partial vacuum were proven to effectively prevent browning of fresh-cut pears (Dong et al., 2000; Gorny et al., 2002).

The thiol-containing compounds such as N-acetyl-L-cysteine and reduced glutathione are natural chemicals with antioxidant properties, suggested as browning inhibitors to prevent darkening on apple, potato and fresh fruit juices (Rojas-Graü et al., 2005; Molnar-Perl and Friedman, 1990a, 1990b; Friedman et al., 1992). The thiol-containing anti-browning additives react with quinones formed during the initial phase of enzymatic browning reactions to yield the colorless addition products or to reduce *o*-quinones to *o*-diphenols (Richard et al., 1991). Therefore, it can be deduced that thiol-containing anti-browning additives did not inhibit PPO enzymes *per se* although they gave an apparent inhibition activity due to their ability to conjugate with primary oxidation products formed in the reaction (Richard-Forget et al., 1992; Billaud et al., 2004). Besides, it can be deduced that PPO enzymes, according to the vegetal source of enzyme, have differential sensitivities to thiol treatments (Jocelyn, 1958; Saestre and Rabenstein 1978; Sapers and Miller 1998**;** Billaud et al., 2004).

Among the several resorcinol derivatives, 4-hexylresorcinol was proven to have effect on controlling enzymatic browning on shrimp (McEvily et al., 1992*)*, mushroom (Osuga et al., 1994) and apple slices (Monsalve-González et al., 1993). 4-hexylresorcinol is structurally related to phenolic substrates and could have a competitive inhibitory effect on polyphenol oxidase activity (McEvily et al., 1992). 4-hexylresorcinol may specifically interact with polyphenol oxidase, and renders it unable of catalyzing the enzymatic reaction while ascorbic acid reduces quinones generated by polyphenoloxidase (Kahn and Andrawis, 1985).

In a previous work, it has been stated that 4-hexylresorcinol concentration lower than 0.5%, N-acetylcysteine concentration higher than 0.75% and N-acetylcysteine combined with glutathione in concentration higher than 0.60% were the most effective treatments in preventing browning of fresh-cut Fuji apples during 14 days of storage at 4ºC (Rojas-Graü et al., 2005). In such a study, the individual or combined effect of browning inhibitors was assessed by a response surface methodology which consists in a mathematical and statistical procedure to study the relationship between dependent variables and independent variables. Thus, since little research on novel browning inhibitors for fresh-cut pear have been carried out, the main purpose of this work was assessing and comparing the effects of post-cutting dips with ascorbic acid, N-acetyl-L-cysteine, reduced glutathione or 4-hexylresorcinol by a similar experimental design. In addition, several combinations of each browning inhibitors at a wide range concentration were tested in order to

determine the best combination of them to control enzymatic browning throughout the storage period.

Materials and methods

Pears

A winter pear cultivar (*cv. Flor de Invierno*) was purchased at a local distributor. The fruits were stored at 4 ºC for a few days until commercial maturity. Physicochemical characteristics of non-processed pear were determined to characterize pear ripeness (Table 1): soluble solids content (Atajo RX-100 refractometer; Atago Company Ltd., Japan), total acidity (AOAC 2000), pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), antioxidant capacity (De Ancos et al., 2002), color (Minolta CR-400 chroma meter; Konica Minolta Sensing, Inc., Osaka, Japan), Firmness (TA-XT2 Texture Analyzer; Stable Micro Systems Ltd., Surrey, England, UK), gas analysis (Micro-GC CP 2002 gas analyzer; Chrompack Intl., Middelburg, The Netherlands): internal gas composition and respiration quotient (Soliva-Fortuny et al., 2005).

Table 1. Physicochemical characteristics of non-processed pear wedges[a]

Soluble solids (ºBrix)	14.33 ± 0.06
Total acidity (g citric acid /100 g)	0.1237 ± 0.0009
pH	4.15 ± 0.05
Antioxidant capacity (% inhibition $DPPH^{\bullet}$)	19 ± 8
Color parameters	
Skin	
L*	71.5 ± 2.5
a*	-4.9 ± 2.4
b*	42 ± 3
Pulp	
L*	76.6 ± 1.4
a*	-0.20 ± 0.11
b*	10.5± 1.1
Firmness (N)	9.0 ± 1.2
Respiration quotient (RQ)	0.261 ± 0.009
Internal gas composition	
O_2 (%)	20.5 ± 1.1
CO_2 (%)	0.7 ± 0.3
C_2H_4 (ppm)	0.4 ± 0.3
Acetaldehyde (ppm)	-
Ethanol (ppm)	-

[a] mean ± standard deviation

Browning inhibitors

Browning inhibitors were dissolved in distilled water (15 ºC) at different concentration: 0-3% ascorbic acid (AA), 0-3% N-acetyl-L-cysteine (NAC), 0-3% reduced glutathione (GSH) and 0-2% 4-hexylresorcinol (HR) and pH 1.8 to 5.45. Browning inhibitors were applied individually or in combination to observe their antibrowning effectiveness.

Sample preparation

Fruit was cleaned and peeled, the core tissue was completely removed and the remaining tissue was cut into wedges. Pear pieces were dipped for 2 minutes in the test solutions at 15 ºC whereas control samples dipped in distilled water, and the ratio product: solution was 1:2. After drainage of the excess of water, 100 g of pear wedges were packaged in plastic bags. The packages were not completely sealed to test the effectiveness of browning inhibitors when the product was exposed to air. Bags were stored at 4 ± 1ºC in darkness and analyzed throughout the time in duplicate.

Color evaluation

CIE L^* a^* b^* values were directly measured with a Minolta CR-400 chroma meter (Konica Minolta Sensing, Inc., Osaka, Japan). The equipment was set up for D_{65} illuminant and 10º observer angle. Ten replications (five wedges from each two bags) were evaluated from each treatment. Three readings were obtained for each replicate by changing the position of the sample to get uniform color measurements. Color values of CIE L^* (lightness), CIE a^* (red to green) and CIE b^* (yellow to blue) were measured and hue angle h^0 (color itself, 0º: red-purple, 90º: yellow, 180º: bluish-green, and 270º: blue) was calculated by the Eq 1:

$$h^o = \arctan\frac{b^*}{a^*} \qquad \text{(Eq. 1)}$$

The L* color value or hue angle (h^0) were used as indicators of cut surface browning intensity (Sapers and Douglas 1987). A decrease of L* values and lower h^0 angle indicated browning. The color of pear wedges was measured for 28 days at 7-day intervals.

PPO activity measurement

PPO activity of pear wedges was measured using the method described by (Soliva-Fortuny et al., 2002) with some modifications.

The enzyme extracts for determination of PPO activity were obtained by homogenization of 50 g of pear with a McIlvaine buffer solution (Giner et al., 2002) (1:1) at pH = 6.5 and 4 ºC. This buffer contained 1 M NaCl (Riedel-de-Haën AG, Seelze, Germany) and 5% polyvinylpolypyrrolidone (Sigma-Aldrich Chemie, Steinheim, Germany). The homogenate was centrifuged at 12500 rpm for 30 minutes at 4ºC (Centrifuge AVANTI^{TM} J-25, Beckman Instruments Inc., Fullerton, CA, USA). The supernatant was filtered through a Whatman 1 paper and the resulting liquid constituted the enzymatic extract which was immediately used for the PPO activity determination.

Enzyme activity was determined spectrophotometrically. The reaction mixture contained 3 ml of 0.05 M catechol (Sigma-Aldrich, Steinheim, Germany) and 100 µl of extract into a 1 cm path cuvette. The changes in absorbance at 420 nm using a Cecil CE 1010 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK) were recorded every 5 seconds to 2 min from the time the enzyme extract was added. Each experiment was run in triplicate. One unit of PPO activity was defined as a change in absorbance of 0.001 per min and ml of enzymatic extract immediately after extract addition. The initial reaction rate was estimated from the linear portion of the plotted curve. The enzymatic activity of fruit wedges was measured for 28 days at 7-day intervals.

Firmness evaluation

Firmness evaluation was performed using a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, England, UK). Fruit wedges were cut in rectangular shaped samples of 2.0 cm high and were penetrated by 4 mm diameter rod. The downward distance was set at 10 mm at a rate of 5 mm/s and automatic return. Samples were placed so that the rod penetrated their geometric center. Analyses were carried out each 7 days for 28 days and ten samples from two trays of fresh-cut pear were randomly withdrawn from each treatment to obtain representing readings.

Mathematical models and statistical analysis

Statistical analysis was performed using The Statgraphic plus v.5.1 software (Manugistics, Inc., Rockville, MA, USA). An analysis of variance procedure was used to find significant differences

within the range of concentration of each browning inhibitor when being used separately. Significant differences among results were determined by a Duncan multiple range test.

A response surface methodology was performed to observe the effect of combining browning inhibitors on color parameters along the storage period. A central composite design with five factors and faced centered was the proposed experimental design to observe the combined effect of the chemical compounds for 28 days and the storage time on the h^0 value. The browning inhibitors concentration levels were N-acetyl-L-cysteine (0-3%), gluthatione (0-3%), 4-hexylresorcinol (0-2%), ascorbic acid (0-3%) and the storage time (0-28 days). Table 2 shows the uncoded and coded factors (-1, 0, +1), which correspond to each level of independent variables.

Experimental data were fitted to a polynomial response surface. The second-order response function was fitted by the Eq. 2:

$$s = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \qquad \text{(Eq. 2)}$$

where s is the color parameter, β_0 is the centre point of the system; βi, β_{ii} and β_{ij} are the regression coefficients of the linear, quadratic and interactive effects respectively; X_i, X_i^2 and X_iX_j represents the linear, quadratic and interactive effects of the independent variables respectively.

By applying analyses of variance (ANOVAs), the non-significant terms ($p \leq 0.05$) were deleted from the second-order polynomial model, and a new polynomial model was recalculated to obtain the final equation (Reyes-Moreno et al., 2001).

Design Expert 6.0.1 software (Stat Ease Inc., Minneapolis, USA) was used in all analyses and generated plots. A 95% confidence interval was used for all these procedures.

Eventually, the final polynomial equation provided the best combinations of browning inhibitors to maximize the color parameter. Those mixtures of browning inhibitors were tested experimentally to confirm their effectiveness in preventing darkening of fresh-cut pears.

Table 2. Central composite response surface design for hue value (h^0) response on fresh-cut pears treated with browning inhibitors during 28 days of time storage at 4 ºC

	Variables										Response Variable
	CODED					UNCODED					
Assay Number[a]	X_1	X_2	X_3	X_4	X_5	AA (%)	NAC (%)	GSH (%)	HR (%)	t (days)	h^0
1	-1	-1	+1	+1	+1	0	0	3	2	28	99.9
2	+1	+1	+1	-1	-1	3	3	3	0	0	95.3
3	0	0	0	+1	0	1.5	1.5	1.5	2	14	105.2
4	0	0	0	0	0	1.5	1.5	1.5	1	14	106.6
5	0	0	-1	0	0	1.5	1.5	0	1	14	103.6
6	+1	-1	+1	-1	+1	3	0	3	0	28	91.5
7	0	+1	0	0	0	1.5	3	1.5	1	14	103.3
8	0	0	0	-1	0	1.5	1.5	3	1	14	103.1
9	0	0	0	-1	0	1.5	1.5	1.5	0	14	96.2
10	-1	+1	+1	-1	-1	0	3	3	0	0	95.2
11	-1	-1	-1	+1	-1	0	0	0	2	0	91.2
12	+1	+1	-1	-1	-1	3	3	0	0	0	95.2
13	0	0	0	0	-1	1.5	1.5	1.5	1	0	100.6
14	-1	-1	-1	-1	-1	0	0	0	0	0	88.7
15	+1	-1	+1	-1	-1	3	0	3	0	0	95.0
16	-1	+1	+1	+1	-1	0	3	3	2	0	100.1
17	+1	-1	-1	+1	+1	3	0	0	2	28	93.6
18	+1	-1	+1	+1	-1	3	0	3	2	0	98.0
19	-1	+1	+1	-1	+1	0	3	3	0	28	99.0
20	-1	-1	-1	+1	+1	0	0	0	2	28	91.2
21	-1	+1	-1	-1	+1	0	3	0	0	28	97.5
22	+1	+1	-1	+1	+1	3	3	0	2	28	103.1
23	+1	0	0	0	0	3	1.5	1.5	1	14	104.6
24	0	0	0	0	+1	1.5	1.5	1.5	1	28	103.9
25	-1	+1	+1	+1	+1	0	3	3	2	28	101.2
26	+1	+1	+1	-1	+1	3	3	3	0	28	99.7
27	+1	+1	+1	+1	-1	3	3	3	2	0	97.5
28	+1	+1	+1	+1	+1	3	3	3	2	28	100.7
29	+1	+1	-1	-1	+1	3	3	0	0	28	99.7
30	+1	+1	-1	+1	-1	3	3	0	2	0	101.3
31	-1	0	0	0	0	0	1.5	1.5	1	14	102.5
32	-1	-1	-1	-1	+1	0	0	0	0	28	86.5
33	-1	+1	-1	+1	-1	0	3	0	2	0	98.6
34	0	0	0	0	0	1.5	1.5	1.5	1	14	103.8
35	-1	+1	-1	-1	-1	0	3	0	0	0	94.5
36	+1	-1	+1	+1	+1	3	0	3	2	28	101.3
37	+1	-1	-1	+1	-1	3	0	0	2	0	90.8
38	-1	-1	+1	-1	+1	0	0	3	0	28	97.0
39	0	-1	0	0	0	1.5	0	1.5	1	14	87.3
40	+1	-1	-1	-1	-1	3	0	0	0	0	85.9
41	-1	+1	-1	+1	+1	0	3	0	2	28	100.0
42	-1	-1	+1	+1	-1	0	0	3	2	0	98.6
43	-1	-1	+1	-1	-1	0	0	3	0	0	95.7
44	+1	-1	-1	-1	+1	3	0	0	0	28	80.9

[a] this does not correspond to the order of processing

X_1: coded ascorbic acid concentration; X_2: coded cysteine concentration; X_3: coded glutathione concentration; X_4: coded 4-hexylresorcinol; X_5: coded time
AA: ascorbic acid; NAC: N-acetyl-L-cysteine. GSH: glutathione; HR: 4-hexylresorcinol; t: time

Results and Discussion

Effect of browning inhibitors on the color of fresh-cut pears

Individual effect of browning inhibitors

The effect of post-cutting dips on color quality of fresh-cut pears were tested, measuring h^0 and L* parameters along the refrigerated storage. h^0 values did not shifted considerably during the storage time after treatments of 0.75-3% NAC as well as 0.75-3% GSH (Fig. 1). The decrease in h^0 values along time was much greater in control samples than those treated with NAC or GSH. According to Gorny et al., (2002), addition of 0.5% NAC on 'Bartlett' pear induced a red or pink discoloration under the cut skin edge and in core tissue after 4 days. But, a post-cutting dip of 2% ascorbic acid + 1% calcium lactate + 0.5% cysteine adjusted to pH 7 prevented cut surface browning due to nucleophilic attack of quinones by cysteine that may be more effective at a neutral pH since the thiol group of cysteine has a pK_a of 8.33. These results suggest that pH of fruit tissue and in turns, concentration of the inhibitors and other several factors such as cultivar, ripeness, tissue differences and package atmosphere may affect on the response of fresh-cut pear to the browning inhibitor treatment (Sapers and Miller 1998).

The concentration of thiol containing compounds had a significant influence on controlling browning of fresh-cut pear. At the end of storage, h^0 and L* values decreased up to 90º and 68, respectively, on fresh-cut pears treated with 0.75% GSH whereas values declined up to 97 º and 66 when pear wedges were treated with 0.75% NAC (Fig. 1 and 2). In fact, slight significant decreasing on hue values from 21 to 28 days led to browning on pear wedges treated by 0.75% GSH. On the other hand, increased concentrations of GSH or NAC were more effective in preserving browning of fresh-cut pear since higher lightness and hue values were detected for 28 days. Thus, 1.5% NAC or GSH prevented fresh-cut pears from surface darkening, leading to L* values about 71 and 68, respectively, and h^0 around 100º, at the end of storage time. Besides, Fig. 1 shows that h^0 values were significantly lower than 1.5-2.25 % NAC or GSH on wedges pears treated with 3% NAC or 3% GSH.

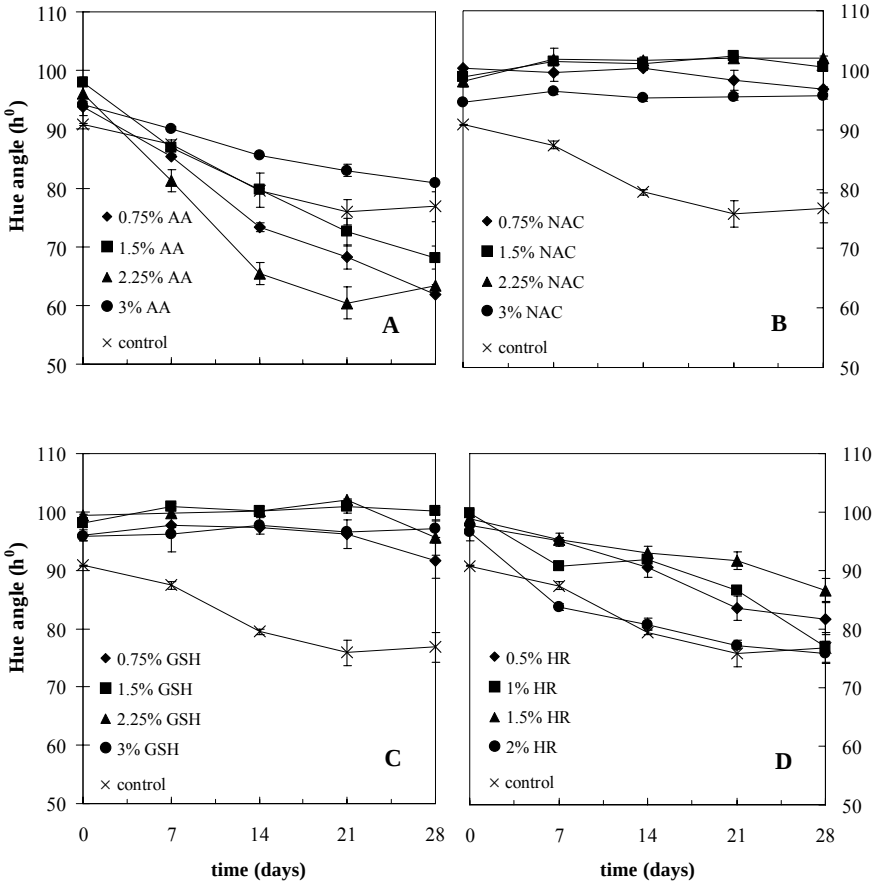

Figure 1. Evolution of hue angle (h^0) response on fresh-cut pears treated with browning inhibitors during the storage at 4 ºC. (A): pear wedges dipped in ascorbic acid (AA) solutions. (B): pear wedges dipped in N-acetyl-L-cysteine (NAC) solutions. (C): pear wedges dipped in glutathione reduced (GSH) solutions. (D): pear wedges dipped in 4-hexylresorcinol (HR) solutions. Data shown are mean ± standard deviation

Fig. 2 proved that L* significantly ($p \leq 0.05$) decreased on pears wedges treated with such high concentration in comparison to pear wedges treated with lower concentration. NAC and GSH as sulphydryl (thiol) compounds may prevent browning by a competitive reaction with polyphenol oxidase, reacting with the intermediates quinones to form stable colorless compounds (Molnar-Perl and Friedman 1990a; Richard-Forget et al., 1992). However, at low concentration, the excess

of *o*-quinones can oxidize cysteine or glutathione-quinone addition compound, leading to phenol regeneration with a deep color formation (Vamos-Vigyazo 1995). Therefore, thiol-containing compounds concentration should be sufficient to transform all the substrate into colorless adduct but not excessive since sulfur-containing compounds at high concentration may produce undesirable off-odor in fruits and vegetables (Son et al., 2001). Hence, apple slices treated with a concentration higher than 0.75% NAC maintained higher hue values than control samples during 14 days of storage at 4ºC, indicating that N-acetyl-L-cysteine is an effective antibrowning agent (Rojas-Graü et al., 2005).

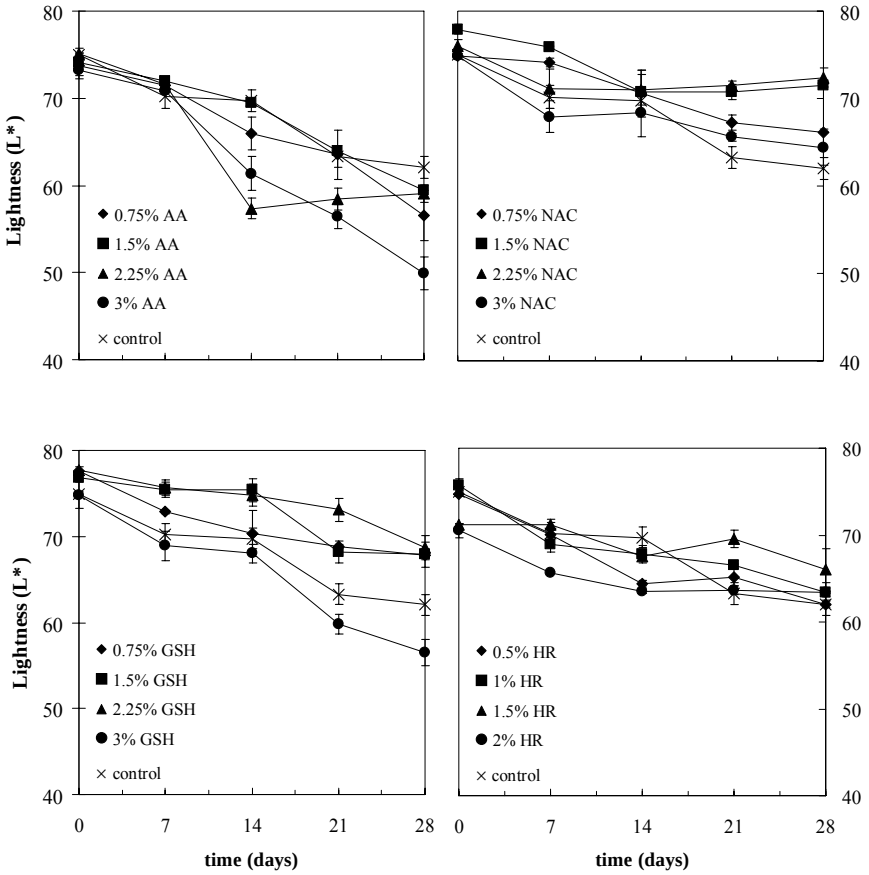

Figure 2. Evolution of lightness (L*) response on fresh-cut pears treated with browning inhibitors during the storage at 4 ºC. (A): pear wedges dipped in ascorbic acid (AA) solutions. (B): pear wedges dipped in N-acetyl-L-cysteine (NAC) solutions. (C): pear wedges dipped in glutathione

reduced (GSH) solutions. (D): pear wedges dipped in 4-hexylresorcinol (HR) solutions. Data shown are mean ± standard deviation

A dip of 0.75% NAC prevented browning up to 28 days whereas 0.75% GSH treatment darkened pear wedges during the fourth week as hue values slightly decreased. pH measurement showed a decrease of pH up to 1.8 of NAC solutions when increasing concentration up to 3% whereas pH of GSH solutions reached a value of 2.57 at 3%. Thus, NAC decreased pH of substrate solution and it appears that the resulting acidification, which depends on browning inhibitor concentration, highly contributes to keep PPO away from its optimum pH of activity.

On the contrary, AA treatment did not prevent fresh-cut pears from darkening since h^0 and L* values depleted continuously along the time (Fig. 1 and 2). Pear wedges treated with 3% AA underwent similar decrease in h^0 values and lower decrease rate in L* values than non treated wedges. Lower AA concentration, 0.75-2.25 %, enhanced browning compared to non treated pear wedges. According to Gorny et al., (2002), 2% AA treatment did not prevent 'Bartlett' pear from surface darkening. A treatment with 1% AA on apple slices revealed that reducing power of AA was weakened and consequently, brown color developed rapidly thereafter (Son et al., 2001). Once added, AA may be completely oxidized to dehydroascorbic acid (DHAA) by its ability to reduce quinones, which can be accumulated and production of brown pigments will occur. According to some researchers, the action of AA is unclear and may even be actively involved in oxidative browning (Kacem et al., 1987).

The HR treatments did not preserve pears wedges from browning and significant decrease in h^0 values was observed since the first week of storage (Fig. 1). Thus, 2% HR led to darkening even more rapidly than control fresh-cut pears. Pear wedges treated with HR underwent similar loss of L* to control samples except to those treated with 1.5% HR, which show higher L* values than non treated pears throughout the time (Fig. 2). Inhibition of enzymatic browning by HR is based on its interaction with polyphenol oxidase, preventing the enzymatic reaction due to structural resemblance to phenolic substrates (Kahn and Andrawis 1985). By now, few studies have been carried out to show the effectiveness of HR itself in controlling enzymatic browning. According to them, neither HR nor AA individually conferred antibrowning protection for a long period (Monsalve-González et al., 1993, 1995). However, combination of 0.2% AA and 200 ppm HR on Red Delicious apple slices was adequate to preserve acceptable color up to 32 days of storage at 25

ºC (Monsalve-González et al., 1995). For 'Anjou', 'Bartlett', and 'Bosc' fresh-cut pears at partial vacuum packaging, the combination treatment of 0.01% HR, 0.5% ascorbic and 1% calcium lactate provided 15 to 30 days shelf-life (Dong et al., 2000).

Combined effect of browning inhibitors

The combined effect of such browning inhibitors as AA, NAC, GSH and HR on browning was studied by a response surface methodology. The experimental design included concentration of inhibitors and storage time as variables and h^0 as response parameter for measuring fresh-cut pear browning, since experimental data did properly fit to the second-order model.

Analysis of variance revealed that the second-order model adequately adjusted to the h^0 experimental measures (Table 3). The regression model explained 84.37% of the total variation ($p \leq 0.05$). The lack-of- fit, which measure the variation of the data around the fitted model, was not significant as the model fit the data properly (Fig. 3).

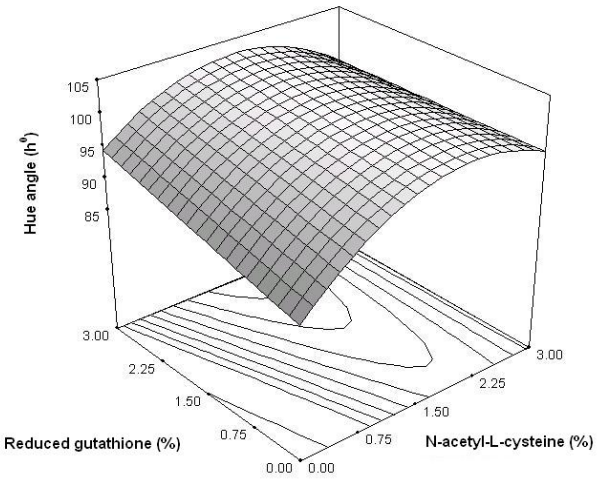

Figure 3. Response surface plots for hue angle (h^0) response as related to N-acetyl-L-cysteine (%) and glutathione reduced (%) after 28 days of refrigerated storage

Browning of fresh-cut pear wedges was affected by NAC, GSH and HR concentration. Table 3 indicated that the linear terms of NAC, GSH and HR, quadratic terms of NAC concentration and NAC-GSH interaction significantly affected browning ($p \leq 0.05$). The range of AA concentration

used for experimental measures did not affected significantly to browning of wedges. Additionally, time storage did not seem to influence on the effect of the browning inhibitors combinations within the studied experimental range.

Table 3. Analyses of variance of regression models for hue angle (h^0) response

SOURCE		SUM OF SQUARES	DF	MEAN SQUARE	F VALUE
Model		1306.08	20	65.3	10.74
Linear	AA	0.51	1	0.51	0.084
	NAC	343.35	1	343.35	56.45 [a]
	GSH	133.28	1	133.28	21.91 [a]
	HR	186.25	1	186.25	30.62 [a]
	t	16.58	1	16.58	2.73
Interactions	AA x NAC	9.96	1	9.96	1.64
	AA x GSH	2.79	1	2.79	0.46
	AA x HR	8.94	1	8.94	1.47
	AA x t	0.023	1	0.023	$3.8.10^{-3}$
	NAC x GSH	147.43	1	147.43	24.24 [a]
	NAC x HR	8.25	1	8.25	1.36
	NAC x t	18.59	1	18.59	3.06
	GSH x HR	4.87	1	4.87	0.80
	GSH x t	2.87	1	2.87	0.47
	HR x t	2.64	1	2.64	0.43
Quadratic	AA^2	2.65	1	2.65	0.44
	NAC^2	127.07	1	127.07	20.89 [a]
	GSH^2	1.75	1	1.75	0.29
	HR^2	7.81	1	7.81	1.28
	t^2	0.12	1	0.12	0.020
Lack of Fit		136.09	22	6.19	1.63
Pure error		3.8	1	3.80	
Cor Total		1445.97	43		

Std. Dev.	2.47	R-Squared	0.90
Mean	97.38	Adj R-Squared	0.82
Coefficient of variation	2.53		

[a] significant at $p \leq 0.05$

AA: ascorbic acid (%); NAC: N-acetyl-L-cysteine (%); GSH: reduced glutathione (%); HR: hexylresorcinol (%); t: time (days)

Once the non-significant terms ($p \leq 0.05$) were deleted from the second-order polynomial model (Table 4), the response was represented by the following polynomial quadratic equation in terms of the uncoded factors (Eq. 3).

$$h^o = +86.1 + 13.2*NAC + 2.7*GSH + 2.3*HR - 3.2*NAC^2 - 0.9*NAC*GSH \quad \text{(Eq. 3)}$$

Table 4. Analyses of variance of regression models for hue angle (h^0) response once the non-significant terms ($p \geq 0.05$) were deleted from the second-order polynomial model

SOURCE		SUM OF SQUARES	DF	MEAN SQUARE	F VALUE
Model		1220.01	5	244.00	41.03 [a]
	NAC	343.35	1	343.35	57.74 [a]
	GSH	133.28	1	133.28	22.41 [a]
	HR	186.25	1	186.25	31.32 [a]
	NAC x GSH	409.70	1	409.70	68.90 [a]
	NAC^2	147.43	1	147.43	24.79 [a]
Lack of Fit		222.16	37	6	1.58
Pure error		3.8	1	3.80	
Cor Total		1445.97	43		

Std. Dev.	2.44	R-Squared	0.84
Mean	97.38	Adj R-Squared	0.82
Coefficient of variation	2.50		

[a] significant at $p \leq 0.05$

AA: ascorbic acid (%); NAC: N-acetyl-L-cysteine (%); GSH: reduced glutathione (%); HR: hexylresorcinol (%)

Maximum h^0 values, about 100º, were achieved when combining 1.2% NAC + 0.75% GSH + 1% HR (Fig. 4A). However, HR dipping provided off-flavor on 'Flor de Invierno' fresh-cut pears after HR treatments from 0.5 to 2%. Monsalve-González et al., (1995) agreed that concentration of 4-hexylresorcinol beyond 0.03% resulted in an increase of the residual content in apple tissue and influenced apple flavour. Therefore, a combination of 1.5% NAC + 1% GSH was suggested to maximize h^0 values without adding HR and thus avoiding the off-flavor (Fig. 4B). A response surface methodology was also used to prove the effect of combining NAC and GSH on controlling browning of apple slices, showing that 0.6% of each browning inhibitor was necessary to maintain a* values near the initial one for 14 days at 4 ºC (Rojas et al., 2005).

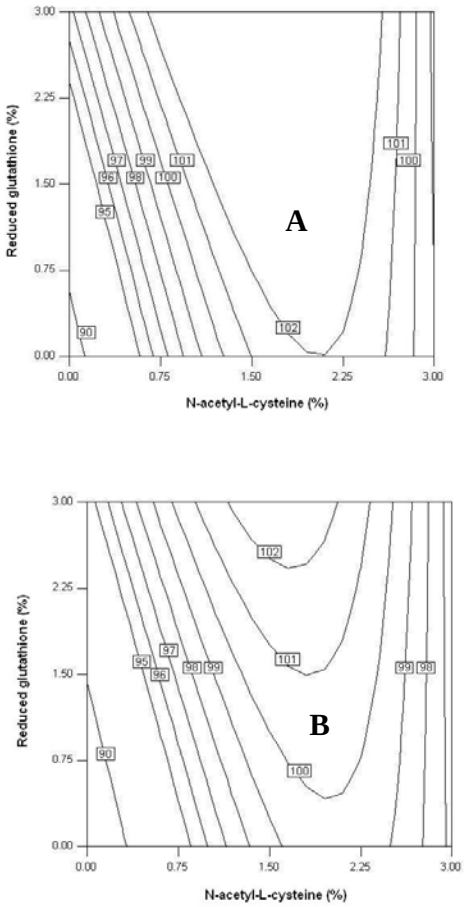

Figure 4. Contour plots for hue angle as related to N-acetyl-L-cysteine (%) and glutathione reduced (%) after 28 days of refrigerated storage. (A): with 1% 4-hexylresorcinol (B): without 4-hexylresorcinol

The negative value of the quadratic term for NAC ($p \leq 0.05$), NAC^2, indicated that h^0 values reached a maximum as NAC concentration rose. In fact, increased concentration beyond 1.5% NAC provided decreasing antioxidant effect than using lower concentration of the browning inhibitor.

The linear term of NAC, GSH and HR were positive ($p \leq 0.05$), which means that the higher the browning inhibitor concentration, the higher h^0 obtained. However, coefficient of NAC in the fitted

model is higher than GSH and HR because its effect seems to be more relevant for the prevention of browning than the two other inhibitors.

In addition, the negative sign of the NAC and GSH interaction indicated the opposite action of such inhibitors. An increase of both NAC and GSH concentration may lead to a decrease in h^0 response. The combined effect of NAC with GSH treatments may decrease when GSH is combined with high concentration of NAC. A dip of 1.5% + 1% GSH has been proved to be effective to prevent browning on fresh-cut pears although has not improved the effect of 1.5% NAC treatment since both dipping preserved h^0 initial values around 100º throughout storage.

Fig. 4B shows suitable combinations of NAC and GSH to preserve color of fresh-cut pears throughout the refrigeration storage. h^0 values above 100º was measured after 28 days when applying dipping solutions of 1.5% NAC with 0.5% or 1% GSH. On the other hand, combinations of 1% NAC with 0.5% or 1% GSH, showed around 96-98º values after 28 days of storage. Table 5 presents experimental measures that confirm the results obtained by the response surface methodology, indicating that a dip of 1.5% NAC as individual treatment or combined with 0.5% or 1% GSH as well as 1% NAC with 1% GSH prevented fresh-cut pear from browning for 28 days. But, 1% NAC + 0.5% GSH just controlled browning up to 21 days since h^0 values slightly decreased during the fourth week (Table 5).

Table 5. Evolution of hue angle (h^0) response on fresh-cut pears treated with N-acetyl-L-cysteine (NAC) in combination with glutathione reduced (GSH) during the storage at 4 ºC.

Time (days)	*Control*	*1.5% NAC + 0.5% GSH*	*1.5% NAC + 1% GSH*	*1% NAC + 0.5 % GSH*	*1% NAC + 1% GSH*
0	90.83±0.12 a	97.7±2.3 a	97.7±1.3 a	96.6±0.9 a	98.31±1.03 a
3	88.8±0.6 ab	99.1±2.2 ab	100.01±1.8 ab	98.3±1.4 a	99.8±0.4 ab
7	87.4±0.7 bc	98.6±2.3 ab	101.6±0.3 b	98.2±1.5 a	100.9±0.8 ab
10	84.9±0.6 c	99.9±0.6 b	102.83±0.15 b	99±3 a	102.0±0.5 b
14	80.3±0.7 d	101.39±0.18 ab	110.0±1.9 b	98±1 a	101±2 b
17	81.6±1.3 d	100.86±0.15 ab	102.0±0.5 b	97.7±2.1 a	100.7±1.7 ab
21	75.9±2.2 e	101.0±0.3 ab	100.5±1.2 b	98.5±0.4 a	100.4±1.7 ab
28	76.8±2.5 e	101.139±0.015 ab	100.63±0.14 b	95.4±2.0 b	100.8±0.9 ab

Different letters in the same column indicate that mean values are significantly different by Duncan's multiple-range test ($p \leq 0.05$)

AA: ascorbic acid (%); NAC: N-acetyl-L-cysteine (%); GSH: reduced glutathione (%); HR: hexylresorcinol (%)

Data shown are mean ± standard deviation

Effect of browning inhibitors on the PPO activity of fresh-cut pears

PPO activity of fresh-cut pear wedges treated with AA was not inhibited throughout the storage time (Fig. 5). Relative PPO activity of pear treated with 3% AA was similar to control samples for 28 days. Additionally, browning of these samples measured by h^0 parameter was alike to non treated pear wedges along the time. On the other hand, darkening of wedges treated with lower AA concentration was more important and in turns, PPO activity depleted up to levels lower than control samples. In general, PPO activity decreased as AA concentration increased due to its reducing properties. However, high AA concentration may diffuse into pear tissue and lead to cell disruption and subsequent decompartmentalisation of enzymes. Thus, activation of soluble tyrosinase forms existing in a latent state, would be otherwise masked (Kahn, 1977). However, browning was similar to non-treated wedges, since such factors as concentrations of both active PPO and phenolic compound contents may be involved in browning reactions of fruit and vegetables (Soliva-Fortuny et al., 2001, 2002).

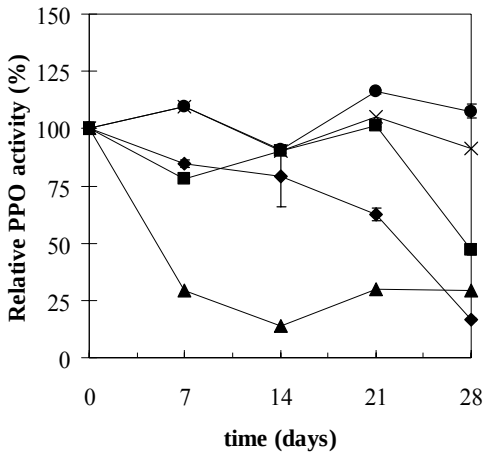

Figure 5. Evolution of relative PPO activity (%) on fresh-cut pears treated with ascorbic acid (AA) solutions during the storage at 4 ºC. Concentration levels: ♦0.75%, ■ 1.5%, ▲ 2.25%, ● 3%, × control. Data shown are mean ± standard deviation

In general, NAC or GSH completely inhibited enzymatic activity of fresh-cut pears. Nevertheless, relative PPO activity detected on fresh-cut pears treated with 0.75% GSG increased during the third week of storage up to 147% in comparison to 100% initial value. Browning was detected on

these samples after 21 days of storage, when an excess of *o*-quinones could have reacted with colorless addition compounds leading to regeneration of phenols and enzymatic formation of colored compounds.

HR inhibited PPO activity although its individual application did not provide browning protection, since the first days greyish and darkish pigments were observed on the surface of the pear wedges. Increased residual content in the tissue may influence on visual appearance and flavor of fresh-cut pear wedges.

Data were not fitted to the proposed polynomial response surface due to NAC, GSH or HR treatments completely inhibited the PPO activity on fresh-cut pears from the beginning of the storage.

Effect of browning inhibitors on the firmness of fresh-cut pears

Firmness values on fresh-cut pears treated with browning inhibitors did not significantly varied throughout storage time. However, the Duncan's multiple-range test proved significant differences ($p \leq 0.05$) on firmness values between browning inhibitors and control treatment (Table 6). Thus, such browning inhibitors dipping as AA, NAC, GSH or HR proved to slightly reduce firmness of pear wedges up to 20% compared to 5% by the control treatment. In fact, browning and softening of fruit tissues detected on pear wedges dipped in AA were also observed by Gil et al., (1998), who pointed out that the use of AA in presence of air did not prevent accelerated softening of fresh-cut apples. Therefore, a response surface design was not performed for firmness as notorious differences were not detected among AA, NAC, GSH and HR concentrations, when they were used separately.

Table 6. Evolution of firmness (N) on fresh-cut pears treated with browning inhibitors during the storage at 4 ºC

Time (days)	*Control*	*0.75% AA*	*1.5% AA*	*2.25% AA*	*3% AA*
0	13.1±0.4 a	11.1±1.1 b	12.6±0.7 ab	12±1 ab	11.6±0.9 b
7	13.3±0.6 a	8.9±0.8 b	10.45±2.4 abc	13.1±1.5 abc	10.44±0.16 c
14	13.0±1.2 a	9.7±1.3 bc	11.03±0.17 c	8.5±0.9 b	9.0±0.4 b
21	12.9±0.5 a	10.1±0.4 b	11.3±1.5 abc	8.3±1.8 c	10±3 abc
28	10.6±1.3 a	9.5±1.5 a abc	9.4±0.8 ac	8.84±0.10 c	7.9±0.7 b

Time (days)	*Control*	*0.75% NAC*	*1.5% NAC*	*2.25% NAC*	*3% NAC*
0	13.1±0.4 a	11.8±0.5 b	12.3±2.4 abc	9.92±0.24 c	9.23±0.14 d
7	13.3±0.6 a	9.6±1.9 b	13.5±0.7 a	7.1±0.4 c	8.2±0.3 d
14	13.0±1.2 a	9.3±0.6 b	11.1±1.1 a	7.0±0.5 c	8.71±0.07 d
21	12.9±0.5 a	9.6±0.4 b	10.1±0.5 b	6.5±2.0 c	8.2±2.2 bc
28	10.6±1.3 a	8.7±0.8 a	9.58±0.07 a	6.78±0.17 b	8.9±0.8 a

Time (days)	*Control*	*0.75% GSH*	*1.5% GSH*	*2.25% GSH*	*3% GSH*
0	13.1±0.4 a	10.9±1.7 bc	13.4±1.4 ab	12.9±0.4 ab	10.1±0.5 c
7	13.3±0.6 a	11±4 ab	12.2±0.3 b	11.6±0.7 b	10.9±1.4 b
14	13.0±1.2 a	7.9±1.9 b	11.3±0.3 c	10.93±0.05 c	10.3±0.4 d
21	12.9±0.5 a	8.014±0.022 b	8.5±1.7 b	10.87±0.16 c	10.55±0.09 d
28	10.6±1.3 a	7±4 abc	7.2±0.9 b	9.9±1.1 a	8.46±0.05 c

Time (days)	*Control*	*0.5% HR*	*1% HR*	*1.5% HR*	*2% HR*
0	13.1±0.4 ab	14±1 a	12.3±0.6 b	12.3±1.8 abc	11.0±0.5 c
7	13.3±0.6 a	12.993±0.012 a	12.5±1.1 a	12.6±0.6 a	10.55±0.14 b
14	13.0±1.2 a	12.00±0.15 a	12.24±0.13 ab	11.3±0.2 bc	11.1±0.6 c
21	12.9±0.5 a	12.4±1.9 a	12.4±0.7 a	11.88±0.04 ab	12.3±0.2 a
28	10.6±1.3 a	12.1±0.9 a	11.74±0.09 a	11.5±0.4 a	12.3±0.5 a

Different letters in the same line indicate that mean values are significantly different by Duncan's multiple-range test ($p \leq 0.05$)
AA: ascorbic acid (%); NAC: N-acetyl-L-cysteine (%); GSH: reduced glutathione (%); HR: hexylresorcinol (%)
Data shown are mean ± standard deviation

Conclusions

As a conclusion, 0.75% NAC dipping was effective to prevent browning of fresh-cut pears up to 28 days at 4 ºC whereas 0.75% GSH avoided darkening up to 21 days at the same temperature. Besides, an enhanced antibrowning effect was observed when applying 1.5% NAC or GSH as well as combining 1% NAC with 1% GSH, observing maximum hue values, approximately 100º, at the

end of 28 days storage. On the contrary, either AA or HR dipping were not adequate to prevent fresh-cut pears from surface darkening. In addition, NAC, GSH and HR inhibited PPO activity whereas browning inhibitors slightly reduced firmness of fresh-cut pears during the refrigerated storage. Hence, both NAC and GSH prevent browning of fresh-cut pear wedges by competitive inhibition of polyphenol oxidase, leading to the formation of stable colorless compounds.

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References

Association of Official Analytical Chemists. 2000. Official Methods of Analysis of AOAC international, edition 17. In: Horwitz, W. (Ed.). AOAC International, Washington.

Billaud, C. ; Brun-Mérimée, S. ; Louarme, L. ; Nicolas, J. 2004. Effect of glutathione and Maillard reaction products prepared from glucose or fructose with glutathione on polyphenoloxidase from apple-I: Enzymatic browning and enzyme activity inhibition. *Food Chemistry*, 84: 223-33.

Dawley, R.M.; Flurkey, W.H. 1993. Differentiation of tyrosinase and laccase using 4-hexylresorcinol, a tyrosinase inhibitor. *Phytochemistry*, 33: 281-4

De Ancos, B.; Sgroppo, S.; Plaza, L.; Cano, M.P. 2002. Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment. *Journal of Science and Food Agriculture*, 82: 790-6.

Dong, X.; Wrolstad, R.E.; Sugar, D. 2000. Extending shelf life of fresh-cut pears. *Journal of Food Science*, 65: 181-5.

Friedman, M.; Molnar-Perl, I.; Knighton, D.R. 1992. Browning prevention in fresh and dehydrated potatoes by SH-containing amino acids. *Food Additives and Contaminants*, 9: 499-503.

Gil, M.I.; Gorny, J.R.; Kader, A.A. 1998. Responses of 'Fuji' apple slices to ascorbic acid treatments and low-oxygen atmospheres. *Hortscience*, 33: 305-309

Giner, J.; Ortega, M.; Resegué, M.; Gimeno, V.; Barbosa-Cánovas, G.V.; Martín-Belloso, O. 2002. Inactivation of peach polyphenoloxidase by exposure to pulsed electric fields. *Journal of Food Science*, 67: 1467-72.

Gorny, J.R.; Gil, M.I.; Kader, A.A. 1998. Postharvest physiology and quality maintenance of fresh-cut pears. *Acta Hortscience*, 464: 231-6.

Gorny, J.R.; Hess-Pierce, B.; Cifuente, R.A.; Kader, A.A. 2002. Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest Biology and Technology*, 24: 271-78.

Jocelyn, P.C. 1958. The importance of thiol compounds in the causation of disease. *Clinica Chimica Acta*, 3(5): 401–418.

Kacem, B.; Cornell, J.A.; Marshall, M.R.; Shiremen, R.B.; Matthews, R.F. 1987. Nonenzymatic Browning in Asceptically Packaged Orange Drinks: Effect of Ascorbic Acid, Aminoacids and Oxygen. *Journal of Food Science*, 52: 1668-72.

Kahn, V. 1977. Latency properties of polyphenol oxidase in two avocado cultivars differing in their rate of browning. *Journal of Science and Food Agriculture*, 28: 233-9.

Kahn, V.; Andrawis, A. 1985. Inhibition of mushroom tyrosinase by tropolone. *Phytochemistry*, 24: 905-8.

McEvily, A.J.; Iyengar, R.; Otwell, W.S. 1992. Inhibition of enzymatic browning in foods and beverages. *Critical Reviews in Food Science and Nutrition*, 32: 253-73.

Molnar-Perl, I.; Friedman, M. 1990a. Inhibition of Browning by Sulfur Amino Acids. 2. Fruit juices and Protein Containing Foods. *Journal of Agriculture and Food Chemistry*, 38: 1648-51.

Molnar-Perl, I.; Friedman, M. 1990b. Inhibition of Browning by Sulfur Amino Acids. 3. Apples and Potatoes. *Journal of Agriculture and Food Chemistry*, 38: 1652-56.

Monsalve-González, A.; Barbosa-Cánovas, G.V.; Cavalieri, R.P.; McEvily, A.J.; Iyengar, R. 1993. Control of Browning during storage of apple slices preserved by combined methods. 4-hexylresorcinol as browning inhibitor. *Journal of Food Science*, 58: 797-826 .

Monsalve-González, A.; Barbosa-Cánovas, G.V.; McEvily, A.J.; Iyengar, R. 1995. Inhibition of enzymatic browning in apple products by 4-hexylresorcinol. *Food Technology*, 49(4): 110-8.

Nicolas, J.J.; Richard-Forget, F.C.; Goupy, P.M.; Amiot, M.J.; Aubert, S.Y. 1994. Enzymatic browning reactions in apple and apple products. *Critical Reviews in Food Science and Nutrition*, 34: 109-57.

Osuga, D.; van der Schaaf A.; Whitaker, J.R. 1994. Control of polyphenoloxidase activity using a catalytic mechanism. In: Yada, R.Y.; Jackman, R.L.; Smith, J.L. (Eds.), *Protein Structure-Function Relationships in foods*. Blackie Academic and Professional, New York, pp. 62-88.

Reyes-Moreno, C.; Parra-Inzunza, M.A.; Milán-Carrillo, J.; Zazueta-Niebla, J.A. 2001. A response surface methodology approach to optimise pretreatments to prevent enzymatic browning in potato (*Solanum tuberosum L*) cubes. *Journal of Science and Food Agriculture*, 82: 69-79.

Richard, F.; Goupy, P.M.; Nicolas, J.J.; Lacombe, J.M.; Pavia, A.A. 1991. Cysteine as an inhibitor of enzymatic browning. 1. Isolation and characterization of addition compounds formed during oxidation of phenolics by apple polyphenol oxidase. *Journal Agricultural and Food Chemistry*, 39: 841-7.

Richard-Forget, F.C.; Goupy, P.M.; Nicolas, J.J. 1992. Cysteine as an inhibitor of enzymatic browning. 2. Kinetic studies. *Journal Agricultural and Food Chemistry*, 40: 2108-13.

Rojas-Graü, M.A.; Sobrino-López, A.; Tapia, M.S.; Martín-Belloso, O. 2005. Browning inhibition in fresh-cut 'Fuji' apple slices by natural antibrowning agent. *Journal of Food Science*, 72: 36-43.

Sapers, G.M.; Douglas, F.W. 1987. Measurement of enzymatic browning at cut surfaces and in juice of raw apple and pear fruits. *Journal of Food Science*, 52: 1258-62, 1285.

Sapers, G.M.; Miller, R.L. 1998. Browning inhibition in fresh-cut pears. *Journal of Food Science*, 63: 342-6.

Saetre, R.; Rabenstein, D.L. 1978. Determination of cysteine and glutathione in fruit by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, 26: 982-3.

Soliva-Fortuny, R.; Grigelmo-Miguel, N.; Odriozola-Serrano, I.; Gorinstein, S.; Martín-Belloso, O. 2001. Browning evaluation of ready-to eat apples as affected by modified atmosphere packaging. *Journal of Agricultural and Food Chemistry*, 49: 3685-90.

Soliva-Fortuny, R.; Biosca-Biosca, M.; Grigelmo-Miguel, N.; Martín-Belloso, O. 2002. Browning, polyphenol oxidase activity and headspace gas composition during storage of fresh-cut pears using modified atmosphere packaging. *Journal of Science and Food Agriculture*, 82: 1490-6.

Soliva-Fortuny, R.; Ricart-Coll, M.; Martín-Belloso, O. 2005. Sensory quality and internal atmosphere of fresh-cut Golden Delicious apples. *International Journal of Food Science and Technology*, 40: 369-75.

Son, S.M.; Moon, K.D.; Lee, C.Y. 2001. Inhibitory effects of various antibrowning agents on apple slices. *Food Chemistry*, 73: 23-30.

Vamos-Vigyazo, L. 1995. Prevention of enzymatic browning in fruits and vegetables. A review of principles and practice. In: Lee, C.Y.; Whitaker, J.R. (Eds.), *Enzymatic Browning Symposium*. American Chemical Society, ACS symposium Series, Washington, 600: 49-62.

Walker, J.R.L. 1977. Enzymatic browning in foods, its chemistry and control. *Food Technology in New Zealand*, 12: 19-25.

Capítulo VIII

Respiratory rate and quality changes in fresh-cut pears as affected by superatmospheric oxygen

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Abstract

Changes in the respiration rate of fresh-cut 'Flor de Invierno' pears stored under superatmospheric oxygen concentrations were studied and compared to those observed under traditional modified atmosphere packaging conditions. Changes in package headspace O_2 and CO_2 concentrations throughout storage were curve-fitted to non-linear equations, calculating respiration rates by combining the derivatives of the equations and the gas permeations throughout storage. Moreover, relationships between respiratory activity and quality parameters of fresh-cut 'Flor de Invierno' pears dipped into and antioxidant solution (0.75% N-acetylcysteine and 0.75% glutathione) were assessed. CO_2 production of fresh-cut 'Flor de Invierno' pears stored under 70 kPa O_2 atmospheres was successfully estimated with the proposed mathematical procedure. This method also proved to well describe CO_2 production rates of fresh-cut pears stored under initial 2.5 kPa O_2 + 7 kPa CO_2 or 21 kPa O_2. In addition, a modification of Michaelis-Menten enzyme kinetics was adequate to describe the changes in estimated CO_2 production due to fermentative processes occurring under low oxygen concentrations. Superatmospheric O_2 concentrations seem to promote oxidative processes, which result into a dramatical modification of some quality attributes of fresh-cut pears.

Keywords: fresh-cut pear, modified atmosphere packaging, superatmospheric O_2 levels, modeling, quality

Introduction

Pears (*Pyrus communis* L cv Flor de Invierno) have great potential to become a high quality fresh-cut commodity. Its pulp is firm, sweet and with a crispy juicy white flesh. This variety could have interesting organoleptical attributes for fresh-cut processing and excellent physiological response to processing operations. However, several factors can affect its shelf-life including stage of ripeness at cutting or storage regime before processing (Sapers and Miller 1998; Gorny et al., 2000). Atmospheres low in O_2 and elevated in CO_2 can extend shelf-life of fresh-cut products by slowing browning reactions at cut surface, reducing the rates of water loss and respiration, and decreasing ethylene biosynthesis and action (Gorny 1997). Day (1996) also found that superatmospheric O_2 levels (more than 60 kPa) were effective for preventing anaerobic fermentation reactions, undesirable moisture and odour losses, and influencing aerobic and anaerobic microbial growth, reducing decay of fresh-cut produce. However, modified atmosphere packaging (MAP) alone does not completely control post-cutting enzymatic browning of fresh-cut pears (Gorny et al., 2002). Therefore, efforts have been made to inhibit or reduce browning of fresh-cut pears during storage under modified atmospheres. The use of dips containing antioxidants such as ascorbic acid, 4-hexylresorcinol, cysteine, glutathione or combinations of them have been shown to delay browning of fresh-cut pears (Sapers and Miller 1998; Dong et al., 2000; Gorny et al., 2002; Oms-Oliu et al., 2006).

Respiration involves the oxidative breakdown of complex substrate molecules normally present in plant cells such as starch, sugars, and organic acids to simpler molecules as CO_2 and H_2O. In general, there is an inverse relationship between respiration rate and postharvest shelf-life of fresh-cut fruits. The beneficial effect of MAP can be attributed to a decrease of the overall metabolic activity of plant tissues. However, respiration induces a reduction in O_2 and an increase in CO_2 partial pressures inside packages, creating gradients that cause O_2 to enter and CO_2 to exit the package at rates proportional to the created gradient. Steady O_2 and CO_2 levels are dependent on interactions of respiration of the product and permeability properties of packaging film (Lakakul et al., 1999). Polymeric films widely used in MAP have some limitations because of their structure and permeation characteristics (Fonseca et al., 2000). Films available for fresh-cut fruits often do not have sufficient O_2 and CO_2 transmission rates to reach steady gas concentrations before reaching too low O_2 levels and/or excessive amounts of CO_2 inside packages, which are detrimental to the produce quality. If O_2 partial pressure in modified atmosphere packages

decreases below the fermentation threshold limit, the tissue will initiate anaerobic respiration, with the corresponding production of off-flavours and off-odours. Thus, the use of superatmospheric O_2 levels (≥ 70 kPa O_2) may be effective to prevent anaerobic conditions, avoiding fermentative metabolites in packages and inhibiting microbial growth (Day 1996, 2000). However, knowledge about the effect of superatmospheric O_2 atmospheres on postharvest physiology and quality of fresh-cut fruits is limited and its basic biological mechanisms are not completely understood.

Most respiratory models reported in the literature to describe the respiration rate of fresh-cut produce are based on Michaelis-Menten type enzyme kinetics. These models can be used under steady-state conditions (Lakakul et al., 1999) or under non-steady conditions, in the early period of storage (Lee et al., 1996; Del Nobile, et al., 2006; Rocculi et al., 2006) but have only been tested for O_2 concentrations ranging from 0 to 21 kPa (Fonseca et al., 2002). Moreover, the respiration models developed to describe CO_2 production that results from fermentative processes (Peppelenbos et al., 1996) have not been validated under superatmospheric O_2 atmospheres.

Therefore, our objective was to describe the changes in respiration rate of fresh-cut 'Flor de Invierno' stored in a permeable system under superatmospheric oxygen conditions and other traditional modified atmosphere packaging. Then, we aimed to investigate relationships between respiratory activity and quality parameters of fresh-cut produce dipped into N-acetylcysteine and glutathione solution throughout storage.

Materials and methods

Sample preparation

Pears (*Pyrus communis* L cv Flor de Invierno) harvested in Lleida (Spain) were stored at 5 ºC prior to processing. The selected ripeness stage of 'Flor de Invierno' pears corresponded to 4.3 ± 0.7 kg, determined by sampling 10 fruits. Pears were sanitized in a 200 ppm NaClO solution for 2 min, rinsed with tap water, and dried prior to cutting operations. Pears were peeled, the core tissue was completely removed and the remaining tissue was cut into wedges. Pear wedges were dipped for 1 min in a solution of 0.75% w/v N-acetylcysteine (Acros Organics, New Jersey, USA) plus 0.75% w/v gluthatione (Acros Organics, New Jersey, USA), according to previous studies (Oms-Oliu et al., 2006). Once excess water was completely drained, 100 g of fruit were packaged in polypropylene trays (173 x 129 x 50 mm) and sealed with a polypropylene plastic film. The O_2 and CO_2 permeance, ratio between oxygen permeability coefficient and film thickness (64 μm), of

the film at storage temperature were 0.8 $cm^3 \cdot m^{-2} \cdot d^{-1} \cdot kPa^{-1}$ and 1.25 $cm^3 \cdot m^{-2} \cdot d^{-1} \cdot kPa^{-1}$ at 5 ºC and 0% RH, respectively (ILPRA Systems España, S.L., Mataró, Spain). The initial in-package O_2 and CO_2 concentrations were 2.5 kPa O_2 + 7 kPa CO_2, air (21 kPa O_2) and 70 kPa O_2 in a product: injected gas ratio of 1:2 (v/v). The trays were thermosealed with a vacuum packing machine ILPRA Food Pack Basic V/6 (ILPRA Systems. CP. Vigevono, Italia) and stored at 5 ± 1ºC in darkness.

Package headspace and internal gas analysis

The gas composition in package headspace was determined with a gas analyzer (Micro-GC CP 2002, Chrompack International, Middelburg, The Netherlands) equipped with a thermal conductivity detector. A little adhesive septum was stuck to the film wrap to reinforce the place where the gas sample is taken, thus avoiding leakage of gas. A sample of 1.7 mL was automatically withdrawn from the headspace atmosphere through a pin-needle connected to the injection system. The determination of the oxygen concentration was carried out by injecting a sample of 0.25 μL to the a CP-Molsieve 5Å column (Chrompack International, Middelburg, The Netherlands) (4m x 0.35 mm, df=10 μm) at 60ºC and 100 kPa whereas a portion of 0.33 μL was injected to a Pora-PLOT Q column (Chrompack International, Middelburg, The Netherlands) (10m x 0.32 mm, df=10 μm) at 75 ºC and 200 kPa for carbon dioxide determination. Initial gas compositions were determined within the next 15-30 min after packaging, thus corresponding to the values at time 0 showed in Fig. 1. A number of 5 test runs were carried out for fresh-cut 'Flor de Invierno' pears. For each run, two trays were taken at each time to perform the analysis and 2 readings were carried out for each package.

The internal ethanol concentrations of pear tissue were determined according to the procedure proposed by Soliva-Fortuny et al., (2007). A sample of 100 g pear was placed in a saturated NaCl solution; the internal gas content was vacuumed and collected in a bell placed at the top of the system. The gas sample was withdrawn from the headspace atmosphere through a rubber septum and injected into a Micro-GC CP 2002 (Chrompack International, Middelburg, The Netherlands) for ethanol quantification. The determination of the ethanol concentration was carried out by injecting a sample of 0.33 μL was injected onto a Pora-PLOT Q column (10m x 0.32 mm, df=10 μm) at 75 ºC and 200 kPa. Two trays were taken at each time to determine the internal gas composition of two replicates.

Mathematical modeling

Models based on the Michaelis-Menten type enzyme kinetics have been widely used to express the dependence of experimental respiration rates of fresh or fresh-cut produce on O_2 concentrations (Peppelenbos et al., 1996; Fonseca et al., 2002). In our study, respiration rates of fresh-cut pears needed to be estimated from the modifications in the package headspace. Gas concentration changes had to be curve-fitted to appropriate nonlinear equations and respiration rates were then calculated by combining the derivatives of the nonlinear equations and the gas permeations through the film. The mathematical procedure used in this study was based on that reported by Lee (1996), who developed a method to calculate the respiration rate of fresh produce in a permeable package and then, estimated the parameters of a respiration model based on enzyme kinetics.

The dependence of gas concentration changes on time storage inside packages of fresh-cut pears has shown to be well described by models not based on physiology, since changes of gas concentrations in permeable systems not only depend on respiration of the commodity but also on the package permeability characteristics, package dimensions and product mass (Fonseca et al.,, 2002). Oxygen and carbon dioxide pressures in package headspace of fresh-cut 'Flor de Invierno' pears decreased and increased throughout storage, respectively. This dependence could well be described by fitting models such as Weibull model Eq. (1) and Logistic model Eq. (2):

$$y_{O_2(t)} = y_{O_2(i)} \times e^{-\left(\frac{t}{\tau}\right)^{\beta}} \quad \text{(Eq. 1)}$$

where $y_{O_2(t)}$ (kPa) are the in-package O_2 partial pressure at time t, $y_{O_2(i)}$ (kPa) are the in-package O_2 partial pressure at time zero, τ (d) is the scale factor and β is a constant, that determines the shape of the rate function (dimensionless).

$$y_{CO_2(t)} = \frac{A_{max}}{1 + e^{\left[k \times \left(t_{1/2} - t\right)\right]}} \quad \text{(Eq. 2)}$$

where $y_{CO_2(t)}$ (kPa) are the in-package CO_2 partial pressure at time t, A_{max} (kPa) is the maximum in-package CO_2 partial pressure, k (d^{-1}) is the accumulative velocity and $t_{1/2}$ (d) is the required time to reach half of the maximum in-package CO_2 partial pressure, the inflection point.

The respiration rates were calculated using a mass balance on the mole of O_2 and CO_2 in the package headspace as follows:

$$R_{O_2} = \left[-\frac{d\left(n_{O_2\,(t)}\right)}{dt} + \left[S \times P_{O_2} \times \left(y^{e}_{O_2} - y_{O_2\,(t)}\right)\right]\right] / m \quad \text{(Eq. 3)}$$

$$R_{CO_2} = \left[\frac{d\left(n_{CO_2\,(t)}\right)}{dt} + \left[S \times P_{CO_2} \times \left(y_{CO_2\,(t)} - y^{e}_{CO_2}\right)\right]\right] / m \quad \text{(Eq. 4)}$$

where R_{O_2} (O_2 mol kg^{-1} d^{-1}) and R_{CO_2} (CO_2 mol kg^{-1} d^{-1}) are the O_2 consumption rate and CO_2 production rate, respectively, $n_{O_2\,(t)}$ and $n_{CO_2\,(t)}$ are the in-package moles of O_2 and CO_2 at time t, respectively, S (m^2) is the area of package surface, the P_{O_2} (O_2 mol m^{-2} d^{-1} kPa^{-1}) and P_{CO_2} (CO_2 mol m^{-2} d^{-1} kPa^{-1}) are O_2 and CO_2 permeance of film, $y_{O_2(t)}$ and $y_{CO_2(t)}$ (kPa) are the O_2 and CO_2 partial pressures in the package headspace at time t, $y^{e}_{O_2}$ and $y^{e}_{CO_2}$ (kPa) are the external O_2 and CO_2 partial pressures and m (kg) is the mass product.

To obtain the accumulative terms of the balances Eq. (3) and (4) concentration data need to be first converted to moles as follows (Rocculi et al., 2006):

$$y_{O_2(t)} = \frac{n_{O_2\,(t)} \cdot R \cdot T}{V_{st}} \quad \text{(Eq. 5)}$$

$$y_{CO_2(t)} = \frac{n_{CO_2\,(t)} \cdot R \cdot T}{V_{st}} \quad \text{(Eq. 6)}$$

where $y_{O_2(t)}$ (kPa) and $y_{CO_2(t)}$ (kPa) are the in-package O_2 and CO_2 partial pressure at time t, $n_{O_2\,(t)}$ and $n_{CO_2\,(t)}$ are the in-package moles of O_2 and CO_2 at time t, R (8.206 L kPa / K mol) is the universal gas constant, T is the temperature expressed in K, V_{st} (0.44 L) is the free volume of the package headspace.

Then, the first derivatives of Weibull model Eq. (1) and Logistic model Eq. (2), which well described changes in mole of oxygen and carbon dioxide throughout storage (Table 1 and 2), were used to determine the accumulative terms of the balances Eq. (3) and (4):

$$\frac{d\left(n_{O_2\,(t)}\right)}{dt} = n_{O_2\,(i)} \times e^{-\left(\frac{t}{\tau}\right)^{\beta}} \times \left[-\frac{\beta}{\tau}\left(\frac{t}{\tau}\right)^{\beta-1} \right] \quad \text{(Eq. 7)}$$

$$\frac{d\left(n_{CO_2\,(t)}\right)}{dt} = \frac{A_{max}}{\left[1 + e^{\left[k \times (t_{1/2} - t)\right]}\right]^2} \times \left[-k \times e^{\left[k \times (t_{1/2} - t)\right]} \right] \quad \text{(Eq. 8)}$$

Finally, the respiration data obtained from Eq. (3) and (4) were used to estimate parameters of a Michaelis-Menten model which assumes that O_2 acts as a competitive inhibitor of fermentative

CO_2 production, as proposed by Peppelenbos et al., (1996). According to these authors, the production rate of CO_2 production has an oxidative and a fermentative term. This latter term will be important at low O_2 concentrations and become zero for high O_2 concentrations:

$$R_{CO_2} = RQ_{ox} \cdot R_{O_2} + \frac{Vm_{CO_2(f)}}{1 + \frac{y_{O_2(t)}}{Kmc_{O_2(f)}}} \quad \text{(Eq. 9)}$$

where RQ_{ox} represents the respiration quotient for oxidative respiration, R_{O_2} (O_2 mol kg^{-1} d^{-1}) and R_{CO_2} (CO_2 mol kg^{-1} d^{-1}) are the O_2 consumption rate and CO_2 production rate, respectively, $Vm_{CO_2(f)}$ (CO_2 mol kg^{-1} d^{-1}) the maximum specific respiration rate for CO_2, $Kmc_{O_2(f)}$ the Michaelis constant for the competitive inhibition of fermentative CO_2 production by O_2 and $y_{O_2(t)}$ (kPa) are the O_2 partial pressures in the package headspace at time t.

Determination of Colour and Firmness

Cut pear surface color values were directly measured with a color meter (Minolta Chroma Meter Model CR-400, Minolta Sensing, Inc., Osaka, Japan). The equipment was set up for a D65 illuminant and 10º observer angle. Five fruit pieces from each of two replicate packages were evaluated for each treatment at each sampling time. Colour changes of fresh-cut pears were measured through hue angle (h^0) [Eq. (10)] parameter. Changes in h^0 parameter have previously been shown to be effective in monitoring enzymatic browning of fresh-cut 'Flor de Invierno' pears (Oms-Oliu et al., 2006).

$$h^o = \arctan\frac{b^*}{a^*} \quad \text{(Eq. 10)}$$

Firmness evaluation was performed using a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, England, UK) by measuring the maximum penetration force. Cylindrical samples of 10 mm high and 15 mm diameter obtained from pear wedges were positioned to be penetrated by a 4 mm diameter rod through their geometric center. The downward distance was set at 5 mm at a rate of 5 mm/s and automatic return. Two trays were taken at each sampling time to perform the analyses, and 5 fruit wedges from each replicate were randomly withdrawn to carry out repetitions.

Determination of Vitamin C, soluble solids and pH

The determination of the vitamin C concentration in fresh-cut pear was performed by HPLC-UV. The extraction procedure and the chromatographic conditions were based on a previous study carried out by Odriozola-Serrano et al., (2007). A portion of 25 g of pear homogenized was added to 25 mL of a solution containing 45 g of metaphosforic acid and 7.2 g of DL-1, 4-dithiothreitol (DTT) per L. The mixture was stirred and centrifuged at 22100 x g for 15 min at 4ºC (Centrifuge AVANTI^{TM} J-25, Beckman Instruments Inc., Fullerton, CA, USA). The supernatant was vaccum-filtered through Whatman No 1 paper. The sample was passed through a Millipore 0.45 μm membrane and injected into the HPLC system.

The HPLC system was equipped with a 600 Controller and a 486 Absorbance Detector (Waters, Milford, MA) working at 245 nm. Duplicates of 20 μL of each extract were injected into a reverse-phase C18 Spherisorb® ODS2 (5μm) stainless steel column (250 mm x 4.6 mm) (Waters, Milford, MA), used as stationary phase. A 0.01% solution of sulphuric acid adjusted to pH 2.6 was used as the mobile phase. The flow rate was fixed at 1 mL min^{-1} at room temperature. Results were expressed as milligrams of vitamin C in 100 g of fresh-cut pear.

The soluble solids content of the juice obtained from a 40 g sample was determined at 20 ºC with a temperature-compensated refractometer ATago RX-1000 (Atago, Japan). The pH was measured using a CRISON 2001 pH-meter (Crison, Barcelona, Spain).

A pair of trays was taken at each sampling time to perform analyses, and 5 fruit wedges from each replicate package were randomly withdrawn to carry out repetitions.

Statistical analysis

Significance of the results and statistical differences were analyzed and experimental data were fitted to the models by non-linear regression procedures using the Statistica 7.0 software (Statsoft, Tulsa, OK, USA). Estimated parameters are given with their respective confidence intervals, product of the standard error of the estimates by the Student-t-adjusted at the degree of freedom. The Duncan's multiple range test was employed to determine differences between means at a 5% significance level.

Fitting accuracy of the models was evaluated through the analysis of R^2 coefficients and the accuracy factor A_f. The higher the R^2 value, the better is the adequacy of the model to describe the data (Neter et al., 1996). The accuracy factor was proposed by Ross (1996) to evaluate the

performance of models. Eq. (11) computes A_f from J experimental observations of O_2 and CO_2 concentrations (n) and their respective J predicted values by the fitted model. The nearer the A_f value to the unit, the better the accuracy.

$$A_f = 10^{\frac{\sum_{j=1}^{j=J}\left|\log\left(\frac{\text{predicted } n_j}{\text{observed } n_j}\right)\right|}{J}} \qquad j = 1, 2, 3,\ldots, J \qquad \text{(Eq. 11)}$$

Results and Discussion

Headspace gas concentrations

Fig. 1 shows the variation of headspace gas partial pressures during storage of fresh-cut pears packaged under different modified atmospheres. As expected, a decrease in the headspace O_2 concentrations during storage together with an increase in the CO_2 concentrations was observed for all initial in-package conditions.

Weibull model Eq. (1) and Logistic function Eq. (2) well fitted experimental O_2 and CO_2 concentrations as a function of storage time, respectively (Fig. 1). The Weibull model was previously used to describe changes in gas concentrations throughout the time of shredded Gale kale (Fonseca et al., 2002). Tables 1 and 2 show the parameters calculated for the fitted models, the determination coefficients (R^2) as well as the accuracy factors (A_f) for each initial modified atmosphere condition when relating O_2 and CO_2 concentrations with time. As can be seen in Table 1, the Weibull model fitted well O_2 experimental data (R^2>0.98) and exhibited good accuracy (A_f =1.082-1.147). Both τ and β computed parameters resulted to be significantly ($p \leq 0.05$) dependent on initial atmosphere conditions. In the present study, τ-values, a time constant, were very high for 70 kPa O_2 atmospheres compared to the values obtained for 21 kPa O_2 and 2.5 kPa + 7 kPa CO_2 atmospheres. The β-value, related to the shape of the curve, was lower than 1 for packages stored under low O_2 atmospheres, slightly lower than 1 under superatmospheric O_2 levels whereas it took values of 1 under air. Values equal to 1 correspond to an exponential function, higher than 1 to a convex curve and lower than 1 to a concave curve.

A logistic model fitted well the CO_2 experimental data (R^2>0.99) and exhibited good accuracy (A_f =1.044-1.082). The A_{max}-values of the model were significantly ($p \leq 0.05$) non-dependent on the MAP conditions (Table 2). On the other hand, k-values were significantly lower under 2.5 kPa O_2 + 7 kPa CO_2 atmospheres than under 70 kPa O_2 atmospheres or 21 kPa O_2. The time required to

reach a fifty percent of the maximum CO_2 production ($t_{1/2}$) was about 12-13 days under 21 kPa O_2 or 70 kPa O_2 levels but 21 days under 2.5 kPa + 7 kPa CO_2 atmospheres.

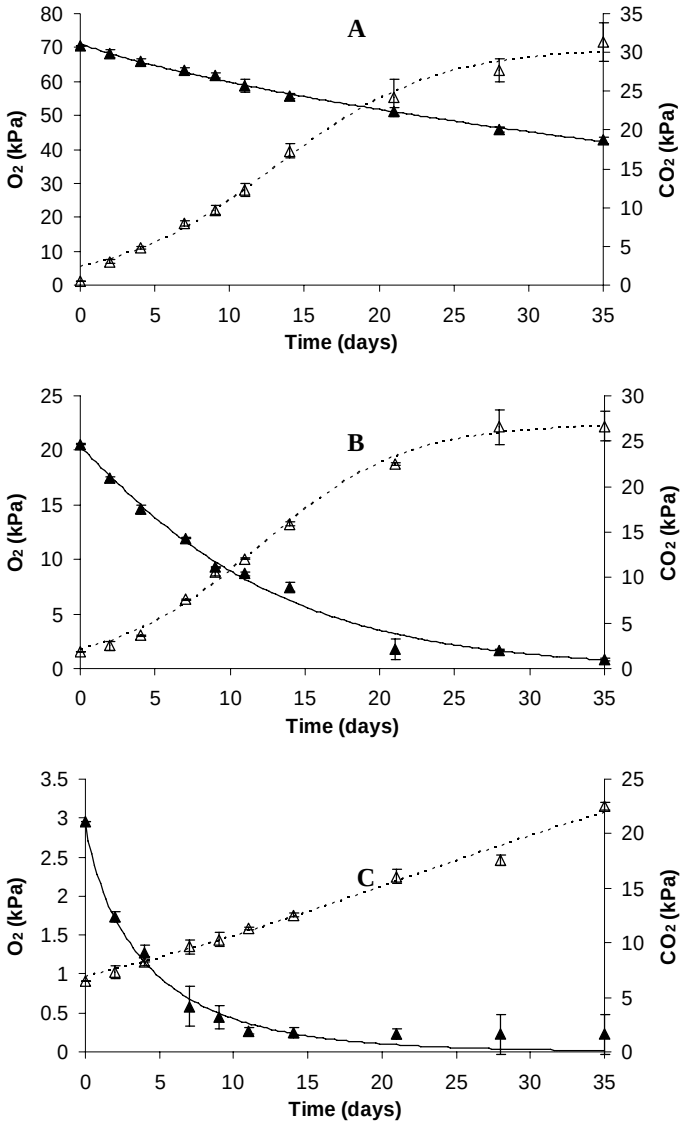

Figure 1. O_2 and CO_2 concentrations in the package headspace of fresh-cut 'Flor de Invierno' pears stored under different modified atmospheres and fit of Weibull model and Logistic model to O_2 and CO_2 experimental data, respectively: 70 kPa O_2 (A), 21 kPa O_2 (B); 2.5 kPa O_2 + 7 kPa

CO_2 (C). O_2 concentrations (bold symbols); CO_2 concentrations (empty symbols). Data shown are mean of 10 determinations ± standard deviation.

Table 1. Kinetic constants of Weibull distribution function (Eq. 1) for the headspace O_2 concentrations of fresh-cut pears stored under modified atmosphere[a].

Modified atmosphere	$n_{O_{2(i)}} \times 10^2 (O_2 \text{ mol})$	τ (d)	β	R^2	A_f
2.5 kPa O_2 + 7 kPa CO_2	0.058±0.002	4.3±0.3	0.79±0.06	0.985	1.147
21 kPa O_2	0.4±0.01	12.0±0.5	1.01±0.08	0.993	1.138
70 kPa O_2	1.37±0.01	72±3	0.91±0.04	0.994	1.082

R^2: determination coefficient; A_f: accuracy factor
[a] Values ± confidence interval at $p \leq 0.05$

Table 2. Kinetic constants of Logistic function (Eq. 2) for the headspace CO_2 concentrations of fresh-cut pears stored under modified atmosphere[a].

Modified atmosphere	$A_{max} \times 10^2$ (CO_2 mol)	k (d^{-1})	$t_{1/2}$ (d)	R^2	A_f
2.5 kPa O_2 + 7 kPa CO_2	0.61±0.07	0.059±0.006	21±4	0.990	1.044
21 kPa O_2	0.52±0.03	0.207±0.012	12.0±0.8	0.981	1.071
70 kPa O_2	0.593±0.05	0.188±0.013	13.1±0.5	0.991	1.082

R^2: determination coefficient; A_f: accuracy factor
[a] Values ± confidence interval at $p \leq 0.05$

Respiratory activity of fresh-cut 'Flor de Invierno' pears

Changes in O_2 consumption and CO_2 production rates throughout storage of fresh-cut 'Flor de Invierno' pears, estimated by fitting Eq. (3) and Eq. (4) to package headspace O_2 and CO_2 concentrations, are shown in Fig. 2. The accumulative terms of these equations are obtained from the first derivative of the adjusted Eq. (1) and Eq. (2). As shown in Fig. 2A, O_2 consumption of just-packaged fresh-cut pears was promoted more significantly ($p < 0.05$) under 2.5 kPa O_2 and 7 kPa CO_2 atmospheres than under initial 21 kPa O_2. This phenomenon may be due to the vacuum created in packages before flushing the gas mixture, thus promoting changes in the pear tissue structure, as well as a dramatic modification of the internal atmosphere. On the contrary, packages of fresh-cut pears stored under 21 kPa O_2 were just sealed and did not suffer such stress due to vacuum. The amount of O_2 available inside the trays stimulated the O_2 consumption rate during the first two weeks, tending to stabilize arround 0.012 mol O_2 d^{-1} kg^{-1} (Fig. 2A and 3A). Under

superatmospheric O_2 levels, O_2 consumption rates decreased continuously throughout time (Fig. 2A). This can be due to the drop in O_2 concentrations at the same time (Fig. 3A). However, O_2 consumption rates of fresh-cut pears stored under a 70 kPa O_2 atmosphere were far higher than those of fresh-cut produce packaged under 2.5 kPa O_2 + 7 kPa CO_2 or 21 kPa O_2 concentrations throughout storage as a consequence of oxidative processes in tissue (Fig. 2A). On the other hand, O_2 consumption rates were almost maintained constant during storage under low O_2 and elevated CO_2 storage atmospheres, since package headspace O_2 concentrations were already low from the beginning of storage. Therefore, our data suggested that superatmospheric O_2 storage atmospheres enhanced oxidative processes in tissue at early period of storage and thus, O_2 consumption rate may not be a good measure of the quality decay rate related to product respiratory activity. Under such storage atmospheres, respiratory activity of fresh-cut produce should be better expressed through CO_2 production rate.

The changes in CO_2 production rates throughout storage are shown in Fig. 2B. At the early stage of storage, the CO_2 production rate of fresh-cut pears packaged under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere was slightly higher than that of pears packaged under other conditions, due to wounding stress promoted under low O_2 and elevated CO_2 concentrations, which may be just above the fermentation threshold (Lakakul et al., 1999). Under these conditions, rates progressively increased throughout storage, reaching the lowest values of 0.02 mol CO_2 d^{-1} kg^{-1} at the end of storage. CO_2 production rate of fresh-cut pears increased similarly under air concentrations and superatmospheric O_2 levels, reaching maximum rates around 0.03 mol CO_2 d^{-1} kg^{-1} at the end of storage. An increase in CO_2 production rates with increased headspace CO_2 concentrations was observed under all tested conditions (Fig. 3B). On the other hand, CO_2 production rates increased with time in spite of O_2 concentrations depletion due to fermentative processes, since ethanol has been shown to accumulate after 10 days. Exposure to O_2 levels ≥ 21 kPa may stimulate, maintain or reduce physiological responses including respiration or production of fermentative metabolites (Poubol and Izumi 2005). Ethanol production detected in fresh-cut pear stored under superatmospheric O_2 levels could be a response to the stress that tissue undergoes under such conditions. The most accepted explanation for oxygen toxicity is the formation of superoxide radicals (O_2^-), which are destructive to cell metabolism (Fridovitch 1975). According to Wszelaki and Mitcham (2000), the volatile content (acetaldehyde, ethanol and ethyl acetate) of strawberry fruit stored under superatmospheric O_2 treatments (≥ 60 kPa O_2) for 14 days

increased greatly compared to that observed in air-stored fruit. Ethanol production in pear tissue was well correlated ($R^2 \geq 0.925$) with CO_2 production rates for all storage atmospheres (Fig. 4). Thus, anaerobic respiration led to an increase in CO_2 production rates of fresh-cut 'Flor de Invierno' pears. Andrich et al., (1994) used exponential functions to describe fermentative CO_2 production. However, Peppelenbos et al., (1996) described CO_2 production as a result from both oxidative and fermentation processes simultaneously (Eq. 9), assuming O_2 as an inhibitor of fermentative CO_2 production. This model fitted well CO_2 production rates ($R^2 \geq 0.984$-0.995) of fresh-cut 'Flor de Invierno' pears packaged under low O_2 and elevated CO_2 atmospheres or air concentrations. The RQ_{ox} respiration model parameter seems to be independent of O_2 concentrations within the range of 0-21 kPa O_2 (Peppelenbos et al., 1996). In our study, RQ_{ox}-values (0.61-0.69) were similar for fresh-cut pears stored under 2.5 kPa O_2 + 7 kPa CO_2 or 21 kPa O_2 atmospheres. The maximum specific respiration rate for CO_2, $Vm_{CO_2(f)}$, was higher under 21 kPa O_2 than 2.5 kPa O_2 + 7 kPa CO_2 atmospheres. Thus, ethanol production would be triggered under air concentrations rather than low O_2 and elevated CO_2 atmospheres. On the other hand, the model (Eq. 9) did not fit CO_2 production rates of fresh-cut pears stored under superatmospheric O_2 levels (Table 3). Other models based on Michaelis Menten-type which consider an inhibition of respiration by CO_2 were also tested to describe CO_2 production of fresh-cut 'Flor de Invierno' pears stored under superatmospheric O_2 concentrations. This approach assumes no CO_2 production at 0 kPa O_2, which accounts for non additional CO_2 production at low O_2 concentrations. However, neither the uncompetitive type of inhibition (Lee et al.,, 1991) -which assumes that the maximum respiration rate is not much influenced under high CO_2 concentrations-, nor the noncompetitive inhibition (Peppelenbos et al.,, 1996) -that leads to an increase of respiration rates at high CO_2 concentrations- have proved to be suitable to describe the changes in respiration rates under superatmospheric O_2 concentrations.

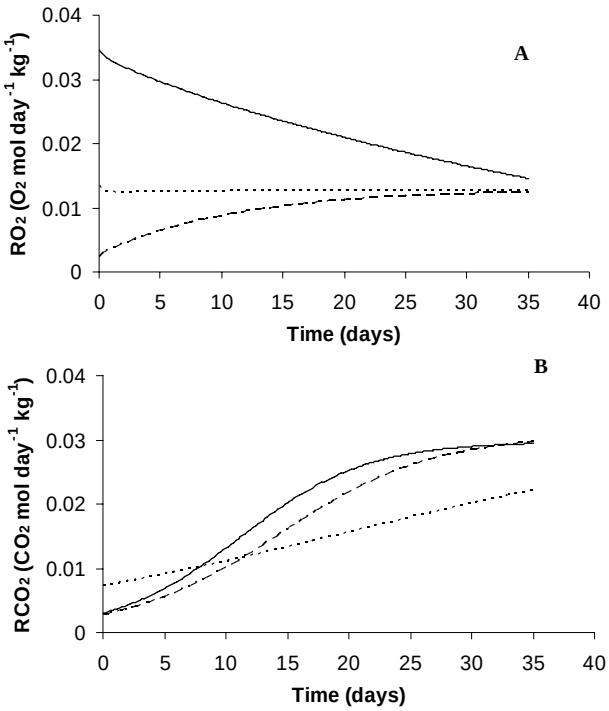

Figure 2. O_2 consumption rate (A) and CO_2 production rate (B) in the package headspace of fresh-cut 'Flor de Invierno' pears stored under different modified atmospheres as predicted by means of Eq. (3) and Eq. (4) using data shown in Figures 1A and B: 2.5 kPa O_2 + 7 kPa CO_2 (·····); 21 kPa O_2 (– – –); 70 kPa O_2 (——).

Table 3. Respiration Model parameters obtained in Eq. (9) for describing CO_2 production rates of fresh-cut 'Flor de Invierno' pears stored under different modified atmospheres, according to Peppelenbos et al. (1996)

Modified atmosphere	RQ_{ox}	$Vm_{CO_2(f)}$ (mol kg^{-1} d^{-1})	$Kmc_{O_2(f)}$	R^2	A_f
2.5 kPa O_2 + 7 kPa CO_2	0.69±0.03	0.0117±0.0003	0.053±0.007	0.984	1.067
21 kPa O_2	0.61±0.07	0.0238±0.0016	1.4±0.5	0.995	1.042
70 kPa O_2	Not fitted to estimated CO_2 production rates				

Values ± confidence interval at $p \leq 0.05$

RQ_{ox}: represents the respiration quotient for oxidative respiration; $Vm_{CO_2(f)}$: the maximum fermentative CO_2 production rate; $Kmc_{O_2(f)}$: the Michaelis constant for the competitive inhibition of fermentative CO_2 production by O_2; R^2: determination coefficient

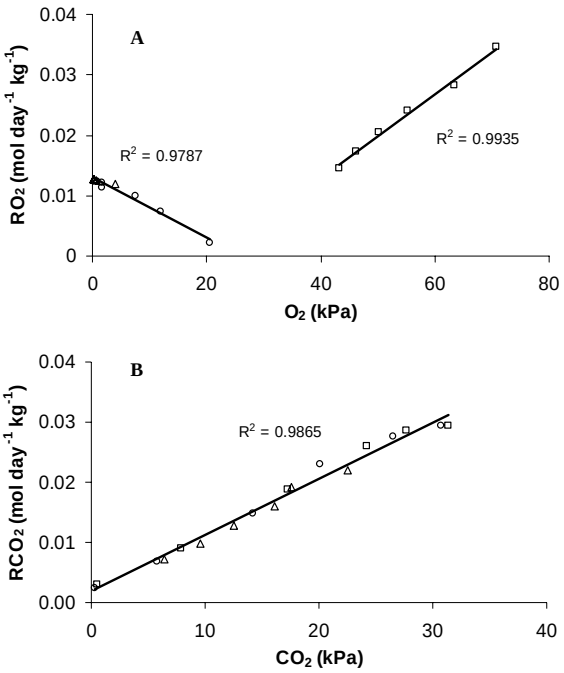

Figure 3. Relationship between O_2 consumption rates and O_2 concentrations (a) and CO_2 production rates and CO_2 concentrations (b) in the package headspace of fresh-cut 'Flor de Invierno' pears stored under 70 kPa O_2 (□), 21 kPa O_2 (○) or 2.5 kPa O_2 + 7 kPa CO_2 atmospheres (Δ).

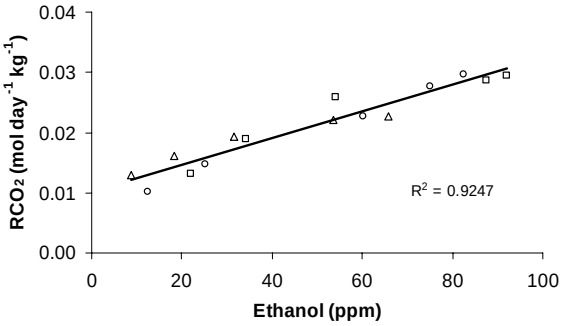

Figure 4. Relationship between descriptive values of CO_2 production rates and average values of ethanol concentrations in 'Flor de Invierno' pear tissue stored under 70 kPa O_2 (□), 21 kPa O_2 (○) or 2.5 kPa O_2 + 7 kPa CO_2 atmospheres (Δ).

Evaluation of physico-chemical parameters of fresh-cut 'Flor de Invierno' pears

Data on some physico-chemical characteristics of fresh-cut pears during 35 days storage as affected by different modified atmosphere conditions are shown in Table 4. Colour changes were determined by h^0 values. A dipping treatment consisting of 0.75% w/v of N-acetylcysteine and 0.75% w/v of glutathione prevented browning of fresh-cut pears packaged under 2.5 kPa O_2 + 7 kPa CO_2 and 21 kPa O_2 atmospheres throughout storage. However, browning was observed on the cut surface of pears packaged under superatmopheric O_2 atmospheres beyond the third week of storage. Oms-Oliu et al., (2006) and Rojas-Graü et al., (2006) reported the effectiveness of N-acetylcysteine and glutathione to prevent browning of fresh-cut 'Flor de Invierno' pear and 'Fuji' apple. The thiol-containing anti-browning additives react with quinones formed during the initial phase of enzymatic browning reactions to yield the colorless addition products or to reduce *o*-quinones to *o*-diphenols (Richard et al., 1991).

Firmness of fresh-cut 'Flor de Invierno' pears was maintained under 2.5 kPa O_2 + 7 kPa CO_2 atmospheres, 21 kPa O_2 or 70 kPa O_2 atmospheres throughout storage. Thus, firmness of fresh-cut pears did not appear to be affected by the respiratory activity of fruit or fermentative metabolite production. Our results contrast with those found by Gorny et al., (2002), who reported that low O_2 (0.25 or 0.5 kPa) elevated CO_2 (air enriched with 5, 10, or 20 kPa CO_2), or superatmospheric O_2 (40, 60, 80 kPa) atmospheres did not effectively prevent softening of fresh-cut 'Bartlett' pear and thus, suggesting that several factors such as cultivar, ripeness and tissue differences may affect the response of fresh-cut pear to modified atmosphere conditions.

Changes in vitamin C content of fresh-cut pears packaged under different initial modified atmosphere conditions are shown in Table 4. As expected, the presence of O_2 inside packages had a negative effect on keeping vitamin C. The vitamin C content of fresh-cut pears stored under superatmospheric O_2 concentrations was rapidly lost and reached the lowest concentrations in comparison with other packaging conditions by the end of storage. On the other hand, 2.5 kPa O_2 + 7 kPa CO_2 atmospheres best maintained vitamin C content although a progressive depletion was also observed throughout storage. According to Lee and Kader (2000), the loss of vitamin C can be reduced by low O_2 and/or moderate CO_2 levels. Although the main factor affecting vitamin C degradation is the initial availability of O_2, high CO_2 production rates appeared to have a negative effect on vitamin C content, especially when hypoxic conditions are reached inside packages (Fig. 5). High CO_2 levels may accelerate vitamin C depletion as it was reported by Agar et al., (1997) in

berry fruits. In fact, high CO_2 levels could accelerate vitamin C loss by oxidation of ascorbic acid catalyzed by ascorbate peroxidase (Tudela et al., 2002).

Table 4. Evaluation of physico-chemical parameters of fresh-cut 'Flor de Invierno' pears packaged under modified atmosphere conditions.

2.5 kPa O_2 + 7 kPa CO_2					
Days	hº [a]	F [a]	TAA [b]	pH [b]	SSC [b]
0	96.3a	9.3a	4.0a	4.6c	11.5a
7	96.9a	9.3a	4.1a	4.5c	11.3a
14	97.0a	8.6a	3.7ab	5.0b	11.2a
21	96.1a	8.7a	2.9bc	5.3b	11.1a
28	97.3a	8.6a	2.2c	5.6a	11.1a
35	98.0a	7.5a	2.1c	5.7a	11.3a
21 kPa O_2					
Days	hº	F	TAA	pH	SSC
0	96.4a	8.8a	3.9a	4.5c	11.2a
7	98.4a	9.1a	2.9b	4.7c	11.1a
14	96.9a	8.2a	2.6b	5.1b	10.9b
21	96.4a	9.3a	2.9b	5.3b	10.7b
28	95.8a	7.8a	2.2c	5.5a	10.6b
35	96.8a	8.3a	2.1c	5.7a	10.1b
70 kPa O_2					
Days	hº	F	TAA	pH	SSC
0	95.9a	9.4a	3.3a	4.8b	11.8a
7	96.4a	9.4a	2.2b	5.1a	11.2ab
14	96.0a	8.3a	1.9b	4.9a	10.6b
21	85.8b	8.4a	0.9c	5.0a	10.7b
28	82.4b	8.2a	0.9c	5.0a	10.7b
35	61.3c	8.7a	0.3d	5.2a	10.2b

Values within a column followed by the same letter indicate that mean values are not significantly different by Duncan's multiple-range test ($p \leq 0.05$);
[a]: mean of ten determinations
[b]: mean of two determinations
hº: hue angle; F: Firmness (N); TAA: vitamin C content (mg / 100 g fw); SSC: soluble solids content (ºBrix)

Values of pH are kept constant in fresh-cut 'Flor de Invierno' pears stored under superatmospheric O_2 levels, but increased under low O_2 or air concentrations, from 4.5 to 5.7 values after 35 days. A positive correlation was detected between the increase in pH values and ethanol production (Fig. 6). Thus, the increase of pH values throughout storage could be related to onset of anaerobic

processes. The pH of fresh-cut 'Conference' pears packaged under 0 kPa O_2 and 2.5 kPa O_2 + 7 kPa CO_2 atmospheres rose slightly but continuously throughout storage (Soliva-Fortuny and Martín-Belloso, 2003). Enhanced physiological activity as well as the proliferation of microorganisms that may be promoted under anaerobic conditions can play an important role in the degradation of organic acids in fresh-cut produce, leading to an increase of pH values. The ability of foodborne pathogens to growth on fresh-cut produce may be promoted by a rise of pH values although it is restricted under refrigeration temperatures (Soliva-Fortuny et al.,, 2003).

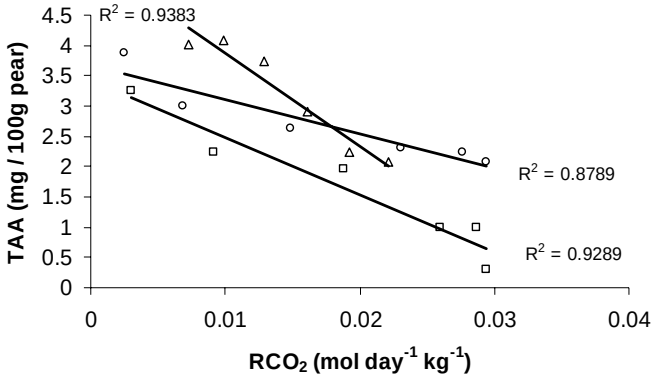

Figure 5. Relationship between descriptive values of CO_2 production rates and average values of vitamin C (TAA) concentrations in 'Flor de Invierno' pear tissue stored under 70 kPa O_2 (□), 21 kPa O_2 (○) or 2.5 kPa O_2 + 7 kPa CO_2 atmospheres (Δ).

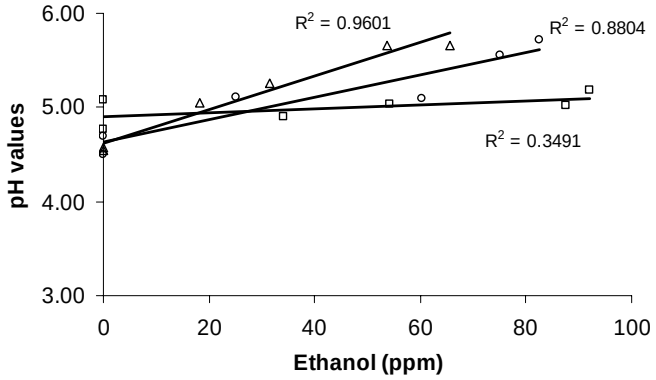

Figure 6. Relationship between average values of internal ethanol production and pH values of fresh-cut 'Flor de Invierno' pears stored under 70 kPa O_2 (□), 21 kPa O_2 (○) or 2.5 kPa O_2 + 7 kPa CO_2 atmospheres (Δ).

Soluble solids content was best maintained in fresh-cut pears stored under low O_2 atmosphere whereas a strong depletion was observed in packages stored under superatmospheric O_2 levels or air concentrations. Such a decrease was due to higher respiration of fresh-cut pears stored under a 70 kPa O_2 or 21 kPa O_2 atmospheres, which could have involved a higher consumption of carbohydrates. Wszelaki and Mitcham (2000) also reported a depletion of soluble solids content in strawberry treated with 90-100 kPa O_2 due to high respiration rates.

Conclusions

The respiratory activity of fresh-cut 'Flor de Invierno' pears was adequately described through CO_2 production rates, rather than through O_2 intake. High rates of O_2 consumption under superatmospheric O_2 concentrations are a consequence not only of the respiratory activity but also of induced oxidative processes, thus difficulting the use of traditionally fitted Michaelis-Menten models. The setting-up of the experiment needs to be improved to better calculate respiration rates of fresh-cut produce under superatmospheric O_2 concentrations. On the other hand, a Michaelis-Menten based model adequately described CO_2 production rates under low O_2 concentrations. Low O_2 and elevated CO_2 atmospheres seem to stimulate CO_2 production rates at early storage although rates increased the lowest during 35 days of storage in comparison with superatmospheric O_2 atmospheres. These latter conditions promoted the highest depletion of vitamin C and soluble solids content. Initial low O_2 concentrations prevented the loss of vitamin C, although CO_2 production may dramatically affect the vitamin C content, especially when hypoxic conditions were reached in the packages.

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References

Agar, I.T.; Streif, J.; Bangerth, F. 1997. Effect of high CO_2 and controlled atmosphere (CA) on the ascorbic and dehydroascorbic acid content of some berry fruits. *Postharvest Biology and Technology*, 11: 47-55.

Andrich, G.; Zinnai, A.; Balzini, S.; Silvestri, S.; Florentini, R. 1994. The kinetic effect of PCO_2 on the respiration rate of Golden Delicious apples. *Acta Horticulturae*, 368: 374-381.

Gorny, J.R. 1997. A summary of CA and MA requirements and recommendations for the storage of fresh-cut (minimally processed) fruits and vegetables. In: Gorny, J.R. (Ed.). *Proceedings of Seventh International Controlled Atmosphere Research Conference*, 13-18 July 1997, University of California, Davis, vol.5, pp. 30-66.

Gorny, J.R.; Cifuentes, R.A.; Hess-Pierce, B.; Kader, A.A. 2000. Quality changes in fresh-cut pear slices as affected by cultivar, ripeness stage, fruit size, and storage regime. *Journal of Food Science*, 65: 541-44.

Gorny, J.R.; Hess-Pierce, B.; Cifuente, R.A.; Kader, A.A. 2002. Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest Biology and Technology*, 24: 271-78.

Day, B. 1996. High oxygen modified atmosphere packaging for fresh prepared produce. *Postharvest News and Information*, 7: 31-4.

Day, B. 2000. Novel MAP for freshly prepared fruit and vegetable products. *Postharvest News and Information*, 11: 27-31.

Del Nobile, M.A.; Baiano, A.; Benedetto, A.; Massignan, L. 2006. Respiration rate of minimally processed lettuce as affected by packaging. *Journal of Food Engineering*, 74(1): 60-9.

Dong, X.; Wrolstad, R.E.; Sugar, D. 2000. Extending Shelf-life of Fresh-cut Pears. *Journal of Food Science*, 65: 181-186.

Fonseca, S.C.; Oliveira, F.A.R.; Lino, I.B.M.; Brecht, J.K.; Chau, K.V. 2000. Modelling O_2 and CO_2 exchange for development of perforation mediated modified atmosphere packaging. *Journal of Food Engineering*, 43: 9-15.

Fonseca, S.C.; Oliveira, F.A.R.; Frias, J.M.; Brecht, J.K.; Chau, K.V. 2002. Modelling respiration rate of shredded Galega kale for development of modified atmosphere packaging. *Journal of Food Engineering*, 54: 299-307

Fridovitch, I. 1975. Superoxide dismutase. *Annual Review of Biochemistry*, 44: 147-59.

Lakakul, R.; Beaudry, R.M.; Hernandez, R.J. 1999. Modeling respiration of apple slices in modified atmosphere packages. *Journal of Food Science*, 64(1): 105-10.

Lee, D.S.; Song, Y.; Yam, K.L. 1996. Application of an enzyme kinetics based respiration model to permeable system experiment of fresh produce. *Journal of Food Engineering*, 27: 297-310.

Lee, S.K.; Kader, A.A. 2000. Preharvest hand postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*, 20: 207-220.

Neter, J.; Kutner, M.H.; Nachtsheim, C.J.; Wasserman, W. 1996. Applied linear regression models. The McGraw-Hill Co., Chicago, USA.

Odriozola-Serrano, I.; Hernández-Jover, T.; Martín-Belloso, O. 2007. Comparative evaluation of UV-HPLC methods and reducing agents to determine vitamin C in fruits. *Food Chemistry*, 105 (3): 1151-1158.

Oms-Oliu, G.; Aguiló-Aguayo, I.; Martín-Belloso, O. 2006. Inhibition of browning on fresh-cut pear wedges by natural compounds. *Journal of Food Science*, 71: 216-24.

Peppelenbos, H.W.; Tijskens, L.M.M.; Van't Leven, J.; Wilkinson, E.C. 1996. Modelling oxidative and fermentative carbon dioxide production of fruits and vegetables. *Postharvest Biology and Technology*, 9: 283-95.

Poubol, J.; Izumi, H. 2005. Physiology and microbiological quality of fresh-cut mango cubes as affected by high-O_2 controlled atmospheres. *Journal of Food Science*, 70: 286-91.

Richard, F.; Goupy, P.M.; Nicolas, J.J.; Lacombe, J.M.; Pavia, A.A. 1991. Cysteine as an inhibitor of enzymatic browning. 1. Isolation and characterization of addition compounds formed during oxidation of phenolics by apple polyphenol oxidase. Journal of Agricultural and Food Chemistry, 39: 841-7.

Rocculi, P.; Del Nobile, M.A.; Romani, S.; Baiano, A.; Dalla Rosa, M. 2006. Use of a simple mathematical model to evaluate dipping and MAP effects on aerobic respiration of minimally processed apples. *Journal of Food Engineering*, 76: 334-40.

Rojas-Graü, M.A.; Sobrino-López, A.; Tapia, M.S.; Martín-Belloso, O. 2006. Browning inhibition in fresh-cut "Fuji" apple slices by natural antibrowning agents. *Journal of Food Science*, 71: 59-65.

Ross, T. 1996. Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Bacteriology*, 81: 501-8.

Sapers, G.M.; Miller, R.L. 1998. Browning Inhibition in Fresh-cut Pears. *Journal of Food Science*, 63(2): 342-6.

Soliva-Fortuny, R.C.; Martín-Belloso, O. 2003. Microbiological and biochemical changes in minimally processed fresh-cut Conference pears. *European Food Research and Technology*, 217: 4-9.

Soliva-Fortuny, R.C.; Ricart-Coll, M.; Elez-Martínez, P.; Martín-Belloso, O. 2007. International atmosphere, quality attributes and sensory evaluation of MAP packaged fresh-cut Conference pears. *International Journal of Food Science Technology*, 42: 208-13.

Tudela, J.A.; Espín, J.C.; Gil, M.I. 2002. Vitamin C retention in fresh-cut potatoes. *Postharvest Biology and Technology*, 26: 75-84.

Wszelaki, A.L.; Mitcham, E.J. 2000. Effects of superatmospheric oxygen on strawberry fruit quality and decay. *Postharvest Biology and Technology*, 20: 125-33.

Capítulo IX

Physiological and microbiological changes in fresh-cut pears stored in high oxygen active packages compared with low oxygen active and passive modified atmosphere packaging

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Abstract

The application of high O_2 atmospheres (HOA) of 70 kPa O_2, balance N_2, for active modified packaging of fresh-cut 'Flor de Invierno' pears was evaluated as an alternative to conventional low O_2 atmosphere (LOA) active modified packaging (2.5 kPa O_2 + 7 kPa CO_2, balance N_2) and to traditional passive atmosphere (PA) packaging. Gas exchange, color, firmness and microbiological stability were assessed throughout 28 d storage at 4 ºC. HOA did not prevent the production of acetaldehyde and ethanol during storage of fresh-cut pears but their accumulation seemed to be promoted under anoxic conditions. Although LOA reduced CO_2 production and inhibited ethylene production, moderate CO_2 concentrations in combination with excessively low O_2 levels inside packages accelerated the accumulation of fermentative metabolites. Both LOA and HOA significantly reduced the growth of microorganisms during storage. In addition, HOA had an inhibitory effect on some spoilage microorganisms such as *Rhodotorula mucilaginosa* isolated from fresh-cut 'Flor de Invierno' pears. Microbiological stability of fresh-cut pears stored under HOA was assured throughout storage but commercial shelf-life may be limited by the browning appearance of cut surface and off-odors beyond 14 d storage.

Keywords: fresh-cut pears, superatmospheric O_2 concentrations, N-acetylcysteine, glutathione, gas exchange, shelf-life

Introduction

Fresh-cut fruit appeared in the market as a response to the consumer trend towards fresh-like high quality products as well as an increase in popularity of ready-to-eat commodities. The greatest hurdle to the commercial marketing of fresh-cut pears is the limited shelf-life due to their susceptibility to enzymatic browning and tissue softening. Good sensorial quality and microbiological stability are also critical factors in maintaining the commercial marketability of fresh-cut produce (Martín-Belloso et al., 2006).

Atmospheres low in O_2 (1-5%) and high in CO_2 (5-10%) have been shown to extend the shelf-life of fresh-cut fruit by reducing respiration, ethylene biosynthesis and product transpiration (Gorny et al., 2002). The proliferation of aerobic microorganisms can be substantially delayed with reduced O_2 levels. The growth of gram-negative aerobes such as *Pseudomonas* is especially inhibited in front of Gram-positive, microaerophilic species such as *Lactobacillus*. CO_2 inhibits most aerobic microorganisms, specifically gram-negative bacteria and moulds (Al-Ati and Hotchkiss, 2002). However, the atmosphere modification during storage may promote excessively low O_2 and high CO_2 concentrations in package headspace that can stimulate the growth of anaerobic psychrotrophic microorganisms and the production of undesirable metabolites (Zagory and Kader, 1988; Soliva-Fortuny et al., 2002a). Hence, the depletion of O_2 and the accumulation of CO_2 in packages of strawberries and raspberries resulted in a deterioration of the sensory quality, odor, taste and firmness (Van der Steen et al., 2002).

Superatmospheric O_2 concentrations ($\geq$ 70 kPa) have been proposed as an alternative to low O_2 modified atmosphere in order to inhibit the growth of typical spoilage microorganisms of fresh-cut produce, prevent undesired anoxic fermentation and maintain fresh sensory quality (Amanatidou et al., 1999; Jacxsens et al., 2001; Van der Steen et al., 2002). Wszelaki and Mitcham (2000) found that 80-100 kPa O_2 inhibited the in-vivo growth of *Botrytis cinerea* on strawberries. Consistently, an initial atmosphere of $\geq$ 70 kPa O_2 retarded the growth of moulds (Van der Steen et al., 2002) and yeasts (Jacxsens et al., 2003) on strawberries and raspberries. High O_2 concentrations can generate reactive oxygen species that damage vital cell components and thereby reduce microbial growth when oxidative stresses overwhelm cellular antioxidant protection systems (Amanatidou et al., 1999). On the other hand, the effects of elevated O_2 concentrations on respiration and ethylene production will depend on the commodity, ripeness stage, O_2 concentration, storage time and temperature, or in-package CO_2 and ethylene concentrations (Kader and Ben-Yehoshua, 2000).

Information on the effects of elevated O_2 concentrations on postharvest physiology, microbiological and sensorial quality of fresh-cut pears is scarce. The exposure of 'Barlett' pears slices to 40, 60 or 80 kPa O_2 decreased their respiration rates over 4 d at 10 ºC (Gorny and Kader, 1998, unpublished, cited by Kader and Ben-Yehoshua, 2000) although it did not effectively prevent cut surface browning and softening (Gorny et al., 2002). Thus, although winter pear varieties such as 'Flor de Invierno' could have interesting characteristics for fresh-cut processing because of their outstanding firmness retention throughout storage, dips in aqueous solutions containing antibrowning compounds need to be used. Thiol-containing compounds such as glutathione, cysteine and their derivatives have a great antioxidant potential towards polyphenol oxidases and have been shown to effectively prevent fresh-cut 'Flor de Invierno' pears browning development (Oms-Oliu et al., 2006).

The objective of our research was to investigate the effectiveness of the application of high O_2 atmospheres (HOA) of 70 kPa O_2, balance N_2, on physiological, physico-chemical and microbiological quality of fresh-cut 'Flor de Invierno' pears compared with low O_2 atmospheres (LOA) consisting of 2.5 kPa O_2 + 7 kPa CO_2, balance N_2, and to traditional passive atmosphere (PA) packaging.

Materials and methods

Quality characteristics of pear flesh

Pears (*Pyrus communis* L cv Flor de Invierno) harvested in Lleida (Spain) were stored at 4 ºC for 1 month prior to processing. A physicochemical characterisation of pear flesh was carried out by sampling 10 fruit (Table 1): soluble solids content (Atago RX-100 refractometer; Atago Company Ltd., Japan), total acidity (AOAC 2000), pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), color (Minolta CR-400 chroma meter; Konica Minolta Sensing, Inc., Osaka, Japan) and firmness (Mechanical Fruit Firmness Tester, QA Supplies, LLC., Norfolk, Virginia, USA).

Sample preparation

'Flor de Invierno' pears at commercial ripeness were sanitized in a 200 $\mu l \cdot l^{-1}$ NaClO solution for 2 min, rinsed with tap water, and dried prior to cutting operations. Pears were peeled, the core tissue was completely removed and the remaining tissue was cut into wedges. Pear wedges were dipped

for 1 min in an aqueous solution of N-acetylcysteine (Sigma-Aldrich Chemic, Steinhein, Germany) at 0.75% w/v and glutathione (Sigma-Aldrich Chemic, Steinhein, Germany) at 0.75% w/v. The concentrations of the antibrowning agents were chosen in accordance to previous studies (Oms-Oliu et al., 2006). Once the excess of water was completely drained (2 min), 100 g of fruit were packaged in polypropylene trays. The active modification of package atmosphere was carried out by flushing a mixture of 2.5 kPa O_2 + 7 kPa CO_2, balance N_2, (LOA) or 70 kPa O_2, balance N_2, (HOA) before sealing the trays using a digitally controlled compensated vacuum ILPRA Food Pack Basic V/6 system (ILPRA Systems. CP., Vigevono, Italy). For passive modification of package atmospheres (PA), the trays were sealed without flushing any gas mixture. The relation between the amount of product and the injected gas mixture was 1:2 v/v. The O_2 and CO_2 permeance of 64-μm thick polypropylene sealing film were $5.2419 \cdot 10^{-13}$ mol $O_2 \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$ and $2.3825 \cdot 10^{-12}$ mol $CO_2 \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$ at 23 ºC and 0% RH, respectively (ILPRA Systems España, S.L. Mataró, Spain). The packages were stored at 4 ± 1ºC in darkness up to random withdrawal for analysis.

Table 1. Physicochemical characteristics of 'Flor de Invierno' pear before processing

Soluble solids (ºBrix)	12.6 ± 0.5[a]
Total acidity (g citric acid / 100 g)	0.33 ± 0.05[a]
pH	4.03 ± 0.09[a]
Flesh colour	
*L**	73.7±2.3[b]
*a**	-1.65±2.07[b]
*b**	10.9±1.5[b]
h^0	99.8±2.9[b]
Firmness (N)	40.4 ± 1.5[b]

[a] Mean of three analyses ± standard deviation
[b] Mean of ten analyses ± standard deviation

Package headspace gas composition

A micro-GC CP 2002 gas analyser (Chrompack International, Middelburg, The Netherlands), equipped with a thermal conductivity detector, was used to analyse the package headspace gas composition. A sample of 1.7 mL was automatically withdrawn from the headspace atmosphere, and an aliquot of 0.25 μl was injected to a CP-Molsieve 5Å column (4m x 0.35 mm, df=10 μm) at 60 ºC and 100 kPa for O_2 quantification. A sample of 0.33 μl was injected to a Pora-PLOT Q

column (10m x 0.32 mm, df=10 μm) at 75 ºC and 200 kPa for CO_2 quantification. Two trays were taken at each time to perform the analysis and 2 readings were carried out for each package.

Color evaluation

The color of fresh-cut pears was determined with a Minolta CR-400 chromameter (Konica Minolta Sensing, Inc., Osaka, Japan), equipped with a D65 light source and the observer at 10º. Two trays were taken at each time to perform the analysis and five fruit pieces were evaluated for each package. Three readings were obtained for each replicate by changing the position of the pear wedges in the optical glass cell to get uniform colour measurements. Colour values of CIE L* (lightness), a* (red-green) and b* (yellow-blue) were measured through reflectance values. Hue angle (h^0) was calculated by Eq. 1.

$$h^o = \arctan\frac{b*}{a*} \quad \text{(Eq. 1)}$$

Texture evaluation

Firmness evaluation was performed using a TA-XT2 Texture Analyser (Stable Micro Systems Ltd., Surrey, England, UK) by measuring the maximum penetration force. Fruit wedges cut in rectangular-shaped 2.0 cm-high pieces were penetrated by a 4 mm diameter rod. The downward distance was set at 10 mm at a rate of 5 mm/s and automatic return. Samples were placed so that the rod penetrated their geometric centre.

Microbiological analyses

Total aerobic psychrophilic microorganisms and yeast and mold populations were evaluated during storage of fresh-cut 'Flor de Invierno' pears. The analyses were carried out twice a week during the first two weeks and then weekly up to 28 d. In sterile conditions, 10 g of sample were homogenized for 2 min with 90 ml of 0.1% sterile peptone water with a Stomacher Lab Blender 400 (Seward medical, London, England). Serial dilutions of fruit homogenates were poured in plate count agar (PCA; Biokar Diagnostics. Beauvais, France) at 7ºC ± 1ºC for 7 d for psychrophilic aerobic bacteria counts (ISO 4833, 1991) and chloramphenicol glucose agar (GCA) at 25ºC ± 1ºC for 5 d for yeast and mold counts (ISO 7954, 1988). Two counts were obtained at each sampling time from two replicate packages at the same experimental condition.

Isolation and identification of spoilage microorganisms on fresh-cut pears

Two types of yeasts were isolated as prevalent spoilage microorganisms from fresh-cut 'Flor de Invierno' pear packages stored under modified atmospheres packaging at 4ºC for their identification. Colonies were isolated and purified on glucose chloramphenicol agar (GCA) (Biokar Diagnostics. Beauvais, France) and incubated for 24 h. The yeast strains purified were kept on extract malt agar slants (EMA) (Biokar Diagnostics. Beauvais,France) at 4 ºC until their identification. Yeasts were identified with an API biochemical kit (api® 20C AUX, bioMérieux, Marcy l'Etoile, France). The two yeasts isolated from fresh-cut 'Flor de Invierno' pear stored under LOA, HOA or PA were *Rhodotorula mucilaginosa* and *Candida parapsilosis*.

Strain, inoculums preparation and fresh-cut pear inoculation

The dominant yeast strains associated with spoilage of fresh-cut 'Flor de Invierno' pears were isolated from samples stored under modified atmospheres at 4 ºC. These microorganisms, *Candida parapsilosis* and *Rhodotorula mucilaginosa*, were used to evaluate the effect of different modified atmosphere packaging conditions on the microbial stability of the commodity. Stock cultures of *Candida parapsilosis* and *Rhodotorula mucilaginosa* were grown in malt extract broth (EMB) at 30ºC for 24h and 80 rpm. One 1:10 dilution for *Candida parapsilosis* stock culture and three consecutive 1:10 dilutions for *Rhodotorula mucilaginosa* stock culture were made before inoculation using saline peptone (0.1% peptone, Biokar Diagnostics. Beauvais, France. + NaCl, Scharlau Chemie, S.A. Barcelona, Spain). Fifty grams of fresh-cut pear processed as described in section 2.2 were inoculated by uniformly spreading 500 µl of *Candida parapsilosis* or *Rhodotorula mucilaginosa* over its entire upper surface using a sterile micropipette in order to obtain an estimated final inoculum level of 10^4 -10^5 CFU ml^{-1}. Trays of inoculated samples were then packaged under LOA, HOA or PA as it is indicated in section 2.2. Non inoculated fresh-cut pear, dipped into N-acetylcysteine (0.75% w/v) and glutathione (0.75% w/v) solution and packaged under the same atmosphere conditions, were used as control samples. Trays containing 50 g of fresh-cut pear were stored at 4ºC for 28 d. A pair of trays of inoculated and non-inoculated fresh-cut pear packaged under each storage atmosphere condition was analyzed at 0, 3, 7, 10, 14, 21 and 28 d.

Statistical analysis

Statistical analysis was performed using the Statgraphics plus v.5.1 software (Manugistics, Inc., Rockville, MA, USA). Data were analyzed by multifactor analysis of variance and a Duncan multiple range test was applied in order to find significant differences among means, with a level of significance of 95%.

Results and Discussion

Effect of modified atmosphere packaging on the package headspace composition of fresh-cut pears

Fig. 1 shows the variation of headspace gas partial pressures during storage of fresh-cut pears packaged under different modified atmospheres. As expected, a decrease in the headspace O_2 concentrations during storage together with an increase in the CO_2 concentrations was observed for all initial in-package conditions. Initial gas package headspace concentrations significantly ($p < 0.05$) affected to changes in O_2 and CO_2 throughout storage. The application of HOA did not significantly reduce respiration rate of fresh-cut 'Flor de Invierno' pears compared with PA or LOA. The amount of O_2 inside packages stored under HOA decreased continuously during storage, while the accumulation of CO_2 under HOA did not differ from packages stored under PA (Fig 1a and b). For fresh-cut pears stored under HOA, the O_2 concentrations inside the packages remained > 50 kPa throughout storage, while CO_2 increased up to levels > 20 kPa at the end of storage. According to Jacxsens et al. (2001), more reactive O_2 species are produced in stressed plant tissue, when exposed to high O_2-levels, and antioxidants are not present in sufficient concentrations to prevent membrane damage, which can result in a wounding response that would in turn, lead to increased respiration rate. In addition, the active modification of package headspace atmosphere involved the creation of vacuum in trays before flushing 70 kPa O_2 concentrations or the gas mixture consisting of 2.5 kPa O_2 + 7 kPa CO_2, which may have promoted changes in the pear tissue structure and stressed the fruit. A rapid depletion of package headspace O_2 concentrations was observed in fresh-cut pears stored under LOA, reaching concentrations below 1 kPa after 1 week (Fig. 1c). Rojas-Graü et al. (2007) also reported a dramatic consumption of the O_2 available inside trays of fresh-cut 'Fuji' apples packaged under 2.5 kPa O_2 + 7 kPa CO_2 compared with progressive O_2 depletion with PA. The package headspace of fresh-cut pears stored under LOA exhibited the lowest CO_2 accumulation compared with other packaging atmospheres. Consistently, CO_2 production of fresh-cut 'Fuji' apples was significantly lower in samples stored

under a LOA than under PA (Rojas-Graü et al., 2007). In fresh-cut pears, the effectiveness of low O_2 and elevated CO_2 atmospheres on reducing respiratory activity has also been proved (Gorny et al., 2002; Soliva-Fortuny et al., 2002b).

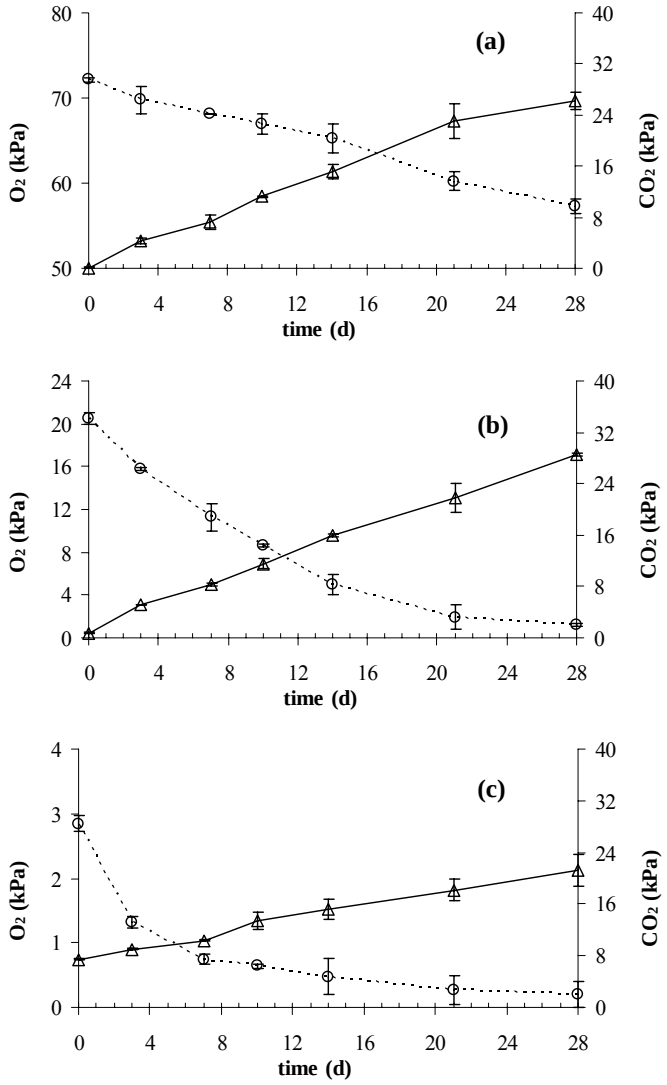

Figure 1. Package headspace O_2 and CO_2 concentrations of fresh-cut 'Flor de Invierno' pears dipped into N-acetylcysteine at 0.75% w/v and glutathione at 0.75% w/v solution and packaged under HOA (a), PA (b) and LOA (c) over 28 d at 4 ºC. Data shown are mean ± standard deviation.

Ethylene concentrations in package headspace were significantly influenced by storage atmosphere ($p < 0.05$). Thus, ethylene production was inhibited in fresh-cut pears stored under LOA, while the amounts of ethylene under HOA or PA rose during the first two weeks, reaching maximum values of 16.9-18.2 $\mu l \cdot l^{-1}$ at 14-d of storage (Fig. 2). It has been demonstrated that low O_2 concentrations combined with elevated amounts of CO_2 may act synergistically to inhibit ethylene production (Solomos, 1997). In addition, thiol-containing compounds could also reduce the ethylene production of fresh-cut pears. Rojas-Graü et al. (2006) found that a dip in a N-acetylcysteine solution reduced significantly the ethylene production of fresh-cut 'Fuji' apples stored under LOA or PA compared with the use of ascorbic acid.

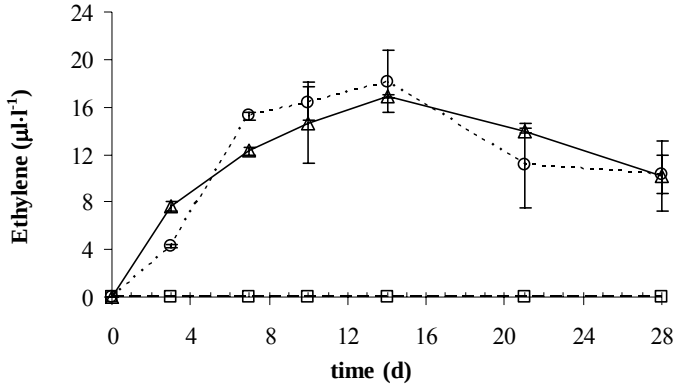

Figure 2. Package headspace ethylene concentrations of fresh-cut 'Flor de Invierno' pears dipped into N-acetylcysteine at 0.75% w/v and glutathione at 0.75% w/v solution and packaged under HOA (○), PA (△) and LOA (□) over 28 d at 4 ºC. Data shown are mean ± standard deviation.

Changes in acetaldehyde and ethanol concentrations inside packages were significantly influenced by storage atmosphere ($p < 0.05$). Acetaldehyde began to accumulate in packages of fresh-cut pears stored under HOA or LOA after 1 week of storage and it continuously increased throughout time. In contrast, acetaldehyde production did not occur for up to 14 d in PA packaged samples but after that, it sharply increased due to excessively low O_2 and high CO_2 concentrations that developed inside packages (Fig. 3). The accumulation of ethanol under HOA was lower than those observed under LOA and PA (Fig. 4). Under these latter conditions, a dramatic accumulation of ethanol was observed after 10-14 d probably due to the onset of fermentative pathways. Under

HOA, accumulated ethanol in package headspace reached concentrations of 65 $\mu l \cdot l^{-1}$ while maximum values of ethanol under LOA and PA were 150 $\mu l \cdot l^{-1}$ approximately, after 28 d of storage. Our results suggest that HOA did not prevent the synthesis of fermentative metabolites in fresh-cut pears but substantially reduced ethanol accumulation compared with atmospheres low in O_2 in combination with high CO_2 concentrations. According to Wszelaki and Mitcham (2000), the volatile content (acetaldehyde, ethanol and ethyl acetate) of strawberry fruit stored under superatmospheric O_2 treatments ($\geq$ 60 kPa O_2) for 14 d increased greatly compared with that observed in air-stored fruit. The most accepted explanation for oxygen toxicity is the formation of superoxide radicals (O_2^-), which are destructive to cell metabolism (Fridovitch, 1975).

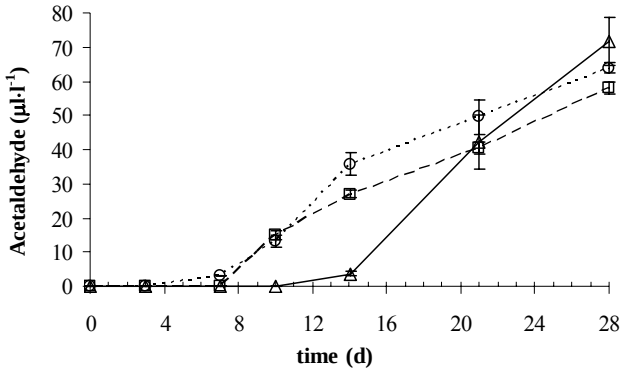

Figure 3. Package headspace acetaldehyde concentrations of fresh-cut 'Flor de Invierno' pears dipped into N-acetylcysteine at 0.75% w/v and glutathione at 0.75% w/v solution and packaged under HOA (○), PA (△) and LOA atmospheres (□) over 28 d at 4 ºC. Data shown are mean ± standard deviation.

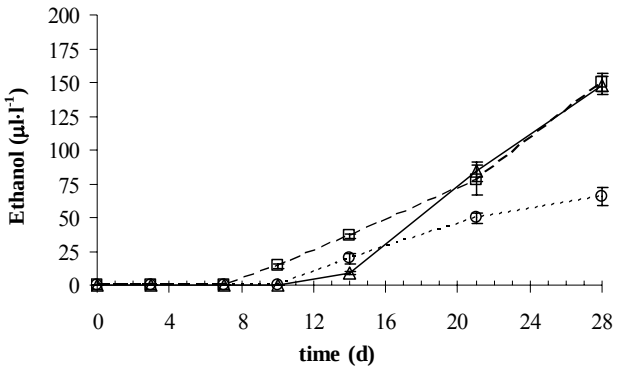

Figure 4. Package headspace ethanol concentrations of fresh-cut 'Flor de Invierno' pears dipped into N-acetylcysteine at 0.75% w/v and glutathione at 0.75% w/v solution and packaged under HOA (○), PA (△) and LOA (□) over 28 d at 4 ºC. Data shown are mean ± standard deviation.

Effect of modified atmosphere packaging on the sensory quality of fresh-cut pears

The combined effect of N-acetylcysteine and glutathione was previously shown to act synergistically to prevent browning of fresh-cut 'Flor de Invierno' pears stored under atmospheric conditions during 28 d at 4 ºC (Oms-Oliu et al., 2006). However, the effect of thiol-containing antibrowning agents to preserve color of fresh-cut 'Flor de Invierno' pears stored under HOA has not been evaluated. Table 2 shows changes in color and firmness of fresh-cut 'Flor de Invierno' pears dipped into 0.75% of N-acetylcysteine and 0.75% of glutathione solution and stored under different modified atmosphere conditions. Initial L* and h^0 values were best maintained under LOA or PA. On the other hand, a significant decrease of L* and h^0 values on cut surface of pears packaged under HOA was observed in comparison with the other packaging conditions. Namely, browning on pear wedges stored under HOA could be visually assessed after two weeks of storage.

Table 2. Changes in lightness, hue angle and firmness of fresh-cut 'Flor de Invierno' pears dipped into N-acetylcysteine at 0.75% w/v and glutathione at 0.75% w/v solution and packaged under HOA, PA and LOA over 28 d at 4 ºC [a]

	HOA			PA			LOA		
d	L^*	h^0	F_{max}	L^*	h^0	F_{max}	L^*	h^0	F_{max}
0	72.8aA	102.1aA	9.3aA	73.7aA	99.9aA	9.4aA	67.1abA	97.8abA	9.4abA
3	72.4aA	100.4aA	9.2aA	74.4aA	99.3abA	9.0abA	69.2abA	99.5aA	9.8aA
7	74.2aA	100.5aA	9.1aA	74.9aA	100.0aA	8.8bA	64.9bB	99.6aA	9.4abA
10	72.6aA	101.0aA	8.6abA	72.2aA	99.0abA	8.7bA	63.0bB	100.1aA	9.1bcA
14	73.6aA	100.2aA	8.3abA	75.2aA	98.4abA	8.8abA	62.9bB	99.9aA	8.6cdA
21	73.5aA	99.9aA	8.1abA	74.1aA	94.9bB	7.8cA	63.2bB	95.2bB	8.5dA
28	73.4aA	99.5aA	7.5bB	74.5aA	95.6abAB	7.8cAB	65.0bB	88.1cB	8.2dA

[a] HOA: 70 kPa O_2, PA: passive atmosphere; LOA: 70 kPa O_2, L^*: lightness; h^0: hue angle; F_{max}: firmness
Values within a column followed by the same small letter indicate that mean values are not significantly different by Duncan's multiple-range test ($p < 0.05$)
Values within a line for the same parameter followed by the same capital letter indicate that mean values are not significantly different by Duncan's multiple-range test ($p < 0.05$)

The changes in firmness values observed during storage of fresh-cut 'Flor de Invierno' pears were significantly influenced by the packaging atmospheres ($p < 0.05$). HOA best maintained initial firmness of fresh-cut pears (Table 2). Consistently, firmness of fresh-cut 'Piel de Sapo' melon stored under HOA was higher than that observed under LOA or PA during 28 d storage (Oms-Oliu et al., 2007). The loss of firmness observed in fresh-cut pears stored under LOA or PA, especially after 10-14 d of storage, may be related to excessively low O_2 and high CO_2, and ethanol accumulation in packages. However, the progressive decrease in firmness values observed in all packaging conditions did not dramatically affect to general appearance of fresh-cut pears.

Effect of modified atmosphere packaging on the growth of aerobic psychrophilic microorganisms on fresh-cut pears

The growth of aerobic psychrotrophic microorganisms on fresh-cut 'Flor de Invierno' pears is shown in Fig. 5. A lag phase preceding the growth of aerobic psychrophilic microorganisms on fresh-cut 'Flor de Invierno' pears was not observed. The growth rate for aerobic psychrophilic microorganisms was higher in pears stored under PA than under both LOA and HOA. Hence, packaging under HOA or LOA prolonged significantly the microbiological shelf-life of fresh-cut pears. Under these conditions, the limit of acceptability for aerobic microorganisms of 7 log (CFU

g^{-1}), according to the Spanish regulation (RD 3484/2000), was not exceeded up to 28 d of storage. On the other hand, fresh-cut pears stored under PA reached a maximum count at the stationary phase of 7.0 (CFU g^{-1}) after 10-14 d. In previous studies, the growth of aerobic psychrophilic microorganisms on fresh-cut melon was also significantly reduced under either HOA or LOA compared with PA during the first week of storage (Oms-Oliu et al., 2007). In addition, our results agree with those reported by Jacxsens et al. (2001), who found no difference in aerobic psychrotrophic growth on chicory endives stored under conventional (3 kPa O_2 and 5 kPa CO_2) and superatmospheric O_2 (95 kPa O_2 and 5 kPa CO_2) atmospheres after 1 week storage. High CO_2 concentrations are generally effective in controlling the growth of most aerobic microorganism, especially bacteria and molds (Al-Ati and Hotchkiss, 2002). Thus, the application of HOA in a low O_2 permeable film allows the accumulation of CO_2 and thus, enhances its inhibitory effect on the growth of microorganisms. High O_2 levels combined with high CO_2 concentrations (10-20%) were more effective in microbial control than the individual gases alone (Amanatidou et al., 1999).

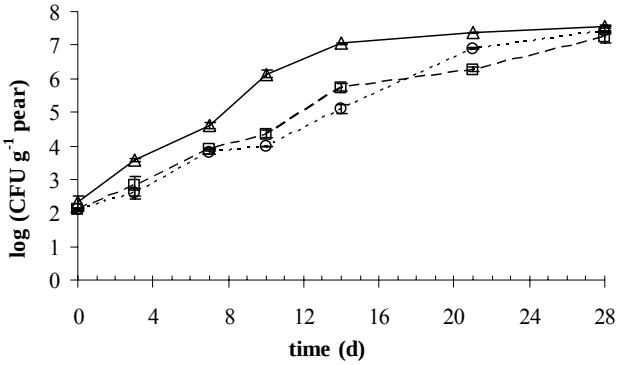

Figure 5. Growth of aerobic psychrotrophic microorganisms on fresh-cut 'Flor de Invierno' pears dipped into N-acetylcysteine at 0.75% w/v and glutathione at 0.75% w/v solution and stored under different modified atmospheres over 28 d at 4 ºC: packaging under LOA (□) packaging under HOA (○), packaging under PA (△). Data shown are mean ± standard deviation

Effect of modified atmosphere packaging on the growth of yeasts and molds on fresh-cut pears

The proliferation of yeasts and molds on fresh-cut 'Flor de Invierno' pears was significantly affected by the packaging atmosphere ($p < 0.05$). Yeast populations were prevalent in fresh-cut

'Flor de Invierno' pears throughout storage compared with molds, regardless storage atmosphere. *Candida parapsilosis* and *Rhodotorula mucilaginosa* were isolated as the most important spoilage microorganisms from fresh-cut 'Flor de Invierno' pears packaged under modified atmosphere conditions. *Candida* and *Rhodotorula* yeast genera have also been identified in other fresh-cut commodities such as mango cubes (Poubol et al., 2005) and melon slices (Oms-Oliu et al., 2007). *Candida parapsilosis* survived on inoculated samples of fresh-cut 'Flor de Invierno' pears and initial levels of inoculation [5 log (CFU g^{-1})] were maintained throughout storage under all tested packaging conditions (Fig 6a). In contrast, the growth of *Rhodotorula mucilaginosa* seems to be greatly affected by the packaging atmosphere composition (Fig. 6b). Thus, different organisms vary greatly in their sensitivity to O_2 partial pressure and some of them could have developed strategies, such as the induction of other enzymes that decompose ROS, to avoid their lethal damage (Kader and Ben-Yehoshua, 2000). The counts of *R. mucilaginosa* in inoculated samples stored under HOA decreased throughout storage up to values of 3.2 log (CFU g^{-1}) by the end of storage (Fig. 6b). *Rhodotorula* yeast genera found in fresh-cut 'Piel de Sapo' melon and 'Nam Dokmai' mango cubes were shown to be also sensitive to superatmospheric O_2 concentrations (Oms-Oliu et al., 2007; Poubol et al., 2005). Initial counts of *R. mucilaginosa* in inoculated samples stored under LOA were maintained throughout storage, while the yeast growth on inoculated samples stored under PA increased greatly after 1 week of storage (Fig. 6b). PA also promoted the highest increase of yeast and mold counts on non inoculated samples, especially after 14 d of storage (Fig. 6a and b). On the other hand, exposure to LOA or HOA during refrigerated storage of fresh-cut 'Flor de Invierno' pears maintained the initial counts of yeasts and molds on non inoculated samples during 28 d of storage. Initial yeast and mold counts of fresh-cut 'Conference' stored under 2.5 kPa O_2 and 7 kPa CO_2 were also maintained during 4 weeks of storage (Soliva-Fortuny et al., 2003). These authors also reported an enhanced effect of low O_2 atmospheres on the inhibition of yeasts and molds in comparison with bacteria.

Spoilage in fresh-cut fruit and vegetables is usually detected by consumers when yeast counts on fresh-cut fruit reach levels above 5 log (CFU g^{-1}) (Jacxsens et al., 1999). According to our results, the application of LOA or HOA maintained the yeast counts below 5 log (CFU g^{-1}) during 28 d of storage, while fresh-cut 'Flor de Invierno' pears packaged under PA exceeded this level after three weeks.

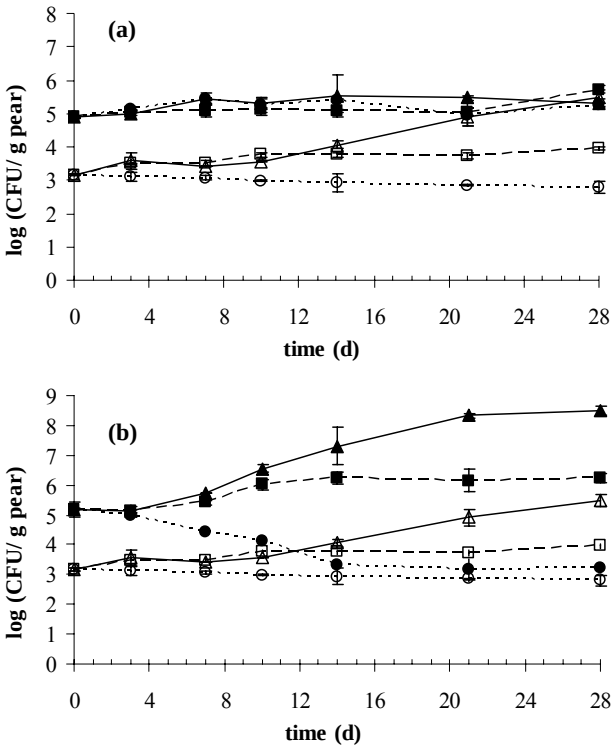

Figure 6. Growth of yeast and molds on fresh-cut 'Flor de Invierno' pears inoculated with *Candida parapsilosis* (a) and *Rhodotorula mucilaginosa* (b), dipped into N-acetylcysteine (0.75% w/v) and glutathione (0.75% w/v) solution and stored under different modified atmospheres over 28 d at 4 ºC: LOA (■)(□), HOA (●)(○), PA (▲) (△); Inoculated samples (full symbols) and control samples (empty symbols). Data shown are mean ± standard deviation.

Conclusions

The application of HOA did not inhibit the synthesis of fermentative metabolites although anoxic conditions created under LOA or PA during storage promoted their accumulation inside packages. Respiration rates and ethylene production of fresh-cut 'Flor de Invierno' under HOA did not seem to differ from those observed under PA. On the other hand, under LOA, ethylene production was completely inhibited and CO_2 accumulation was reduced. Fresh-cut pears stored under HOA were susceptible to undergo enzymatic browning although high O_2 concentrations did not affect to firmness. Microbiological shelf-life of fresh-cut 'Flor de Invierno' pears packaged under both LOA or HOA was substantially extended in comparison with PA. Exposure to superatmospheric

O_2 concentrations seems to clearly reduce the growth of *R. mucilaginosa*, one important spoilage microorganism of fresh-cut 'Flor de Invierno' pears. However, the effect of HOA on foodborne pathogens needs to be also evaluated in further studies.

Acknowledgements

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References

Al-Ati, T.; Hotchkiss, J.H. 2002. Application of Packaging and Modified Atmosphere to Fresh-cut Fruits. In: Lamikanra, O. (Ed.), *Fresh-Cut Fruits and Vegetables: Science, Technology, and Market*. CRC Press, Florida, pp. 305-338.

Amanatidou, A.; Smid, E.J.; Gorris, L.G.M. 1999. Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable-associated microorganisms. *Journal of Applied Microbiology*, 86: 429-438.

Fridovitch, I. 1975. Superoxide dismutase. *Annual Review of Biochemistry*, 44: 147-159.

Gorny, J.R.; Hess-Pierce, B.; Cifuente, R.A.; Kader, A.A. 2002. Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest Biology and Technology*, 24: 271-278.

Jacxsens, L.; Devlieghere, F.; Falcato, P.; Debevere, J. 1999. Behavior of Listeria monocytogenes and Aeromonas spp. of fresh-cut produce packaged under equilibrium modified atmosphere. *Journal of Food Protection*, 62: 1128-1135.

Jacxsens, L.; Devlieghere, F.; Van der Steen, C.; Debevere, J. 2001. Effect of high oxygen atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. *International Journal of Food Microbiology*, 71: 197-210.

Jacxsens, L.; Devlieghere, F.; Van der Steen, C.; Siro, I.; Debevere, J. 2003. Application of ethylene absorbers in combination with high oxygen atmsopheres for the storage of strawberries and raspberries. *Acta Horticulturae*, 600: 311-318.

Kader, A.A.; Ben-Yehoshua, S. 2000. Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. *Postharvest Biology and Technology*, 20: 1-13.

Martín-Belloso, O.; Soliva-Fortuny, R.; Oms-Oliu, G. 2006. Fresh-cut fruits. In: Hui, Y.H. (Ed.), *Handbook of fruits and fruit processing*. Blackwell Publishing, Iowa, pp. 129-144.

Oms-Oliu, G.; Aguiló-Aguayo, I.; Martín-Belloso, O. 2006. Inhibition of browning on fresh-cut pear wedges by natural compounds. *Journal of Food Science*, 71: 216-224.

Oms-Oliu, G.; Raybaudi-Massilia Martínez, R.M.; Soliva-Fortuny, R.; Martín-Belloso, O. 2007. Effect of superatmospheric and low oxygen modified atmospheres on shelf-life extension of fresh-cut melon. *Food Control*, doi: 10.1016/j.foodcont.2007.03.009

Poubol, J.; Izumi, H. 2005. Physiology and Microbiological Quality of Fresh-cut mango cubes as affected by high-O_2 controlled atmospheres. *Journal of Food Science*, 70: 286-291.

Rojas-Graü, M.A.; Sobrino-López, A.; Tapia, M.S.; Martín-Belloso, O. 2006. Browning inhibition in fresh-cut "Fuji" apple slices by natural antibrowning agents. *Journal of Food Science*, 71: 59-65.

Rojas-Graü, M.A.; Grasa-Guillem, R.; Martín-Belloso, O. 2007. Quality changes in fresh-cut Fuji apple as affected by ripeness stage, antibrowning agents, and storage atmosphere. *Journal of Food Science*, 72: 36-43.

Soliva-Fortuny, R.C.; Oms-Oliu, G.; Martín-Belloso, O. 2002a. Effects of ripeness stages on the storage atmosphere, colour, and textural properties of minimally processed apple slices. *Journal of Food Science*, 67: 1958-1963.

Soliva-Fortuny, R.C.; Grigelmo-Miguel, N.; Hernando, I.; Lluch, M.A.; Martín-Belloso, O. 2002b. Effect of minimal processing on the textural and structural properties of fresh-cut pears. Journal of Science and Food Agriculture, 82: 1490-1496.

Soliva-Fortuny, R.C.; Martín-Belloso, O. 2003. Microbiological and biochemical changes in minimally processed fresh-cut Conference pears. European Food Research and Technology, 217: 4-9.

Solomos, T. 1997. Principles underlying modified atmosphere packaging. In: Wiley, R.C. (Ed.), *Minimally Processed Refrigerated Fruits and Vegetables*. Chapman & Hall, New York, pp. 183-225.

Van der Steen, C.; Jacxsens, L.; Devlieghere, F.; Debevere, J. 2002. Combining high oxygen atmospheres with low oxygen modified atmosphere packaging to improve the keeping quality of strawberries and raspberries. *Postharvest Biology and Technology*, 26: 49-58.

Wszelaki, A.L.; Mitcham, E.J. 2000. Effects of superatmospheric oxygen on strawberry fruit quality and decay. *Postharvest Biology and Technology*, 20: 125-133.

Zagory, D.; Kader, A.A. 1988. Modified atmosphere packaging of fresh produce. *Food Technology*, 42: 70-77.

Capítulo X

Antioxidant content of Fresh-cut Pears Stored in High O_2 Active Packages Compared with Conventional Low O_2 Active and Passive Modified Atmosphere Packaging

G. Oms-Oliu, I. Odriozola-Serrano, R. Soliva-Fortuny, O. Martín-Belloso

Journal of Agricultural and Food Chemistry, aceptado

Abstract

The antioxidant content of fresh-cut 'Flor de Invierno' (*Pyrus communis* L.) pears dipped into a 0.75% w/v N-acetylcysteine + 0.75% w/v glutathione solution and packaged under 70 kPa O_2 atmospheres (HOA) was evaluated as an alternative to actively modified low O_2 atmosphere (LOA) and passively modified atmospheres (PA). Changes in color, vitamin C, individual phenolic compounds and antioxidant activity of fresh-cut pears as well as in O_2, CO_2 and ethylene headspace concentrations inside the packages were assessed for 14 days at 4 ºC. The use of antioxidants not only prevented browning and reduced ethylene production of fresh-cut pears but also their application under LOA best maintained vitamin C, chlorogenic acid and antioxidant capacity compared with HOA. Our results show that the use of glutathione and N-acetylcysteine enhanced the formation of phenylpropanoids in fresh-cut pears stored under LOA.

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Keywords: minimal processing; N-acetylcysteine; glutathione; ethylene; color; phenolic compounds; vitamin C; antioxidant capacity

Introduction

Antioxidant constituents of fruits and vegetables have been widely reported to have beneficial effects on the maintenance of health and the prevention of cancer and cardiovascular diseases (Block et al., 1992; Liu et al., 2000). Their biological properties result from their capacity of decreasing oxidative damage and sequestering reactive oxygen species (ROS), which could initiate cascade reactions that result in the production of hydroxyl radicals and other deleterious species such as lipid peroxides (Lurie, 2003). Pears are fruits with a low antioxidant capacity compared to pigmented fruits (Prior et al., 2000). In this fruit, the contribution of phenolic compounds to antioxidant capacity has been reported to be much greater than that of vitamin C (Galvis-Sánchez et al., 2003).

'Flor de Invierno' pear (*Pyrus communis* L.) is a winter pear variety with a big and yellowish-green skin and a firm, sweet, crispy, juicy, and white flesh (Varela et al., 2007). Apart from their interesting organoleptic attributes, it shows an excellent physiological response to minimal processing, especially when processed at optimal ripeness state (Oms-Oliu et al., 2007a).

Browning of cut surfaces is one of the greatest hurdles to the commercial marketing of fresh-cut pears. The destruction of fruit cellular compartmentation allows the oxidation of phenolic compounds by polyphenol oxidase (PPO), thus decreasing the nutritional content of fresh-cut commodities (Gil et al., 2006). In addition, several factors such as physical damage, presence of O_2, extended storage, low relative humidity, high temperatures or chilling injury may promote vitamin C losses with subsequent browning if ascorbic acid falls below a threshold level (Eccher et al., 2002).

Natural thiol-containing compounds with antioxidant properties, such N-acetylcysteine and glutathione, have been reported to prevent browning in 'Flor de Invierno' pears (Oms-Oliu et al., 2006). Thiol-containing antibrowning additives react with *o*-quinones formed during the initial phase of enzymatic browning reactions yielding colorless adducts or reducing them back to *o*-diphenols (Richard et al., 1991). Low O_2 and elevated CO_2 atmospheres can also reduce surface browning. This decrease in the browning phenomena is accompanied by several physiological effects such as a decrease in respiration rates and a delay in the climacteric onset of the rise in ethylene (Solomos, 1997). Day (1996) hypothesized that high O_2 concentrations may cause substrate inhibition of PPO or alternatively, high levels of colorless quinones formed may cause feedback inhibition of the enzyme. However, the results achieved in this field are often

controversial but agree to conclude that modified atmosphere packaging alone cannot effectively prevent fresh-cut fruit browning. Gorny et al. (2002) found that low O_2 (0.25 or 0.5 kPa) elevated CO_2 (air enriched with 5, 10 or 20 kPa CO_2), or high O_2 (40, 60 or 80 kPa) active atmospheres alone did not effectively prevent surface browning of fresh-cut pear slices.

Studies on the influence of modified atmosphere packaging and the use of antioxidant compounds on the shelf-life of fresh-cut pears have mainly focused on sensory quality of the commodity (Sapers and Miller, 1998; Gorny et al., 2002; Soliva-Fortuny et al., 2002; Soliva-Fortuny et al., 2004). Knowledge about the impact of dipping treatments and packaging conditions on antioxidant properties of fresh-cut pears is still incomplete, especially in what regards to high O_2 active packaging. Therefore, the present work aims to assess the combined effect of a N-acetylcysteine + glutathione dip and packaging under high O_2 atmospheres (HOA) on color, vitamin C, phenolic compounds and antioxidant capacity of fresh-cut 'Flor de Invierno' pears, as well as to compare the results with those obtained under conventional low O_2 atmospheres (LOA) and passively modified atmospheres (PA). Ethylene, O_2 and CO_2 headspace concentrations were also evaluated during 14 days of storage at 4 ºC.

Materials and methods

Reagents

N-acetylcysteine, glutathione, metaphosphoric acid and DL-1,4-dithiotreitol (DTT) were purchased from Acros Organics (New Jersey, USA); 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2.2-azino-bis (3-thylbenzthiozoline-6-sulfonic acid) (ABTS), 4-hydrozy-2,5,7,8-tetramethyl-2-carboxylic acid (TROLOX), sulphuric acid, methanol, hydrogen chloride (HCl), potassium persulphate ($K_2S_2O_8$), formic acid (HCOOH) and acetonitrile were obtained from Sharlau Chemie, S.A. (Barcelona, Spain); chlorogenic acid, (-)-epicatechin, ferulic acid, *p*-coumaric acid, sinapic acid and quercetin were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Plant Material

Pears (*Pyrus communis* L cv Flor de Invierno) were harvested at commercial maturity from the same orchard (Lleida, Spain) in mid October. Immediately after harvesting, pears were placed in a refrigerated chamber (4 ºC) for 1 month. The ripeness stage was characterised prior to processing by measuring the maximum force necessary to penetrate the flesh of a whole fruit with an 8-mm

diameter probe (Mechanical Fruit Firmness Tester, QA Supplies, LLC., Norfolk, Virginia, USA). Firmness and other physicochemical characteristics of pear flesh (10 fruits) are shown in Table 1: soluble solids content (Atajo RX-100 refractometer; Atago Company Ltd., Japan), total acidity (AOAC 2000), pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain) and color (Minolta CR-400 chroma meter; Konica Minolta Sensing, Inc., Osaka, Japan).

Table 1. Physicochemical characteristics of 'Flor de Invierno' pear before processing

Soluble solids (ºBrix)	13.92 ± 0.05
Total acidity (g citric acid / 100 g)	0.31 ± 0.07
pH	4.3 ± 0.5
Pulp color	
L*	73.0
a*	-1.4
b*	10.4
Firmness (N)	43.3 ± 0.7

Values are the mean of three independent determinations ± standard deviation

Sample preparation

'Flor de Invierno' pears were sanitized by immersion for 2 min in water containing 200 ppm free-chlorine, rinsed with tap water (10-15 ºC) for 3-5 min and drained. Processing operations were carried out at room temperature (20 ºC). Pears were peeled and the core tissue was completely removed with a pear peeler and coring device. The remaining tissue was cut manually into 6-cm wedges. Pear wedges were dipped for 1 min in aqueous solutions (4 ºC) of 0.75% w/v N-acetylcysteine + 0.75% w/v glutathione. The concentrations of the antibrowning agents were chosen in accordance to previous studies (Oms-Oliu et al., 2006). Sterile distilled water was used as the control treatment. Once the excess of water was completely air drained, 100 g of pear wedges were packaged in polypropylene trays (173 x 129 x 50 mm). The trays were sealed with a 64-μm thick polypropylene film with O_2 and CO_2 permeance of 110 $cm^3 \cdot m^{-2} \cdot d^{-1} \cdot bar^{-1}$ and 500 $cm^3 \cdot m^{-2} \cdot d^{-1} \cdot bar^{-1}$ at 23ºC and 0% RH, respectively (ILPRA Systems España, S.L., Mataró, Spain). In addition, the film water vapor permeance was 3 $g \cdot m^{-2} \cdot d^{-1}$ at 38ºC and 90% RH according to manufacturer information. Active modification of package atmospheres was carried

out before sealing the trays by flushing a mixture of 2.5 kPa O_2 + 7 kPa CO_2 (balance N_2) (LOA) or 70 kPa O_2 (balance N_2) (HOA) using a digitally controlled compensated vacuum ILPRA Food Pack Basic V/6 system (ILPRA Systems. CP., Vigevono, Italy). For passive modification of package atmospheres (PA), the trays were sealed without flushing any gas mixture. The relationship between the amount of product and the injected gas mixture was 1:2 v/v. Eighty four packages were stored at 4 ± 1 ºC in darkness. Initial analyses were determined within the next 4 hours after packaging, thus corresponding to values at time 0.

Headspace gas analysis

The gas composition of the package headspace was analyzed with a gas analyzer (Micro-GC CP 2002, Chrompack International, Middelburg, The Netherlands) equipped with a thermal conductivity detector. An aliquot of 1.7 mL was automatically withdrawn through an adhesive rubber septum with a sampling needle directly connected to the injection system. The determination of the O_2 concentration was carried out by injecting a sample of 0.25 μL to a CP-Molsieve 5Å column (4m x 0.35 mm, df=10 μm) at 60ºC and 100 kPa whereas a sample of 0.33 μL was injected to a Pora-PLOT Q column (10m x 0.32 mm, df=10 μm) at 75 ºC and 200 kPa for CO_2 and ethylene. Two trays were taken at each time to perform the analysis and two readings were carried out for each package.

Vitamin C content

The determination of the vitamin C concentration in fresh-cut pear was performed by HPLC-UV. The extraction procedure and the chromatographic conditions were based on a previous study carried out by Odriozola-Serrano et al. (2007). A portion of 25 g of fruit was added to 25 mL of a solution containing 45 g of metaphosforic acid and 7.2 $g \cdot L^{-1}$ of DL-1, 4-dithiothreitol (DTT). The mixture was stirred and centrifuged at 22100 x g for 15 min at 4ºC (Centrifuge AVANTI^{TM} J-25, Beckman Instruments Inc., Fullerton, CA, USA). The supernatant was vaccum-filtered through Whatman No 1 paper. The sample was passed through a Millipore 0.45 μm membrane and injected into the HPLC system.

The HPLC system was equipped with a 600 Controller and a 486 Absorbance Detector (Waters, Milford, MA) working at 245 nm. Duplicates of 20 μL of each extract were injected into a reverse-

phase C18 Spherisorb® ODS2 (5µm) stainless steel column (250 mm x 4.6 mm) (Waters, Milford, MA), used as stationary phase. A 0.01% solution of sulphuric acid adjusted to pH 2.6 was used as the mobile phase. The flow rate was fixed at 1 mL min^{-1} at room temperature. Results were expressed as milligrams of vitamin C in 100 g of fresh-cut pear. Two trays were taken at each sampling time to perform replicate analyses throughout 14 days of storage.

Phenolic compounds

A high-performance liquid chromatography (HPLC) method was used for the analysis of individual phenolic compounds. The extraction was carried out following the method validated by Hertog et al. (1992). A sample of 0.50 g of freeze-dried pear tissue was carefully mixed with 40 ml of 62.5% aqueous methanol (2g/L TBHQ) and 10 ml of 6 M HCl. After refluxing at 90ºC for 2 h with regular swirling, the extract was allowed to cool and was subsequently made up to 100 ml with methanol and sonicated for 5 min. The extract was then passed through a 0.45µm filter prior to injection. The HPLC system was equipped with a 600 Controller and a diode array detector (Waters, Milford, MA) which was set to scan from 200 to 600 nm. Separations were performed on a reverse-phase C18 Spherisorb® ODS2 (5 µm) stainless steel column (4.6mm x 250mm) operating at room temperature with a flow rate of 1mL/min. A gradient elution was employed with a solvent mixture of 2.5% HCOOH in water (solvent A) and 2.5% HCOOH in acetonitrile (solvent B) as follows: linear gradient from 5 to 13% B, 0-15 min; linear gradient from 13 to 15% B, 15-20 min; linear gradient from 15 to 30% B, 20-25 min; isocratic elution 30% B, 25-28 min; linear gradient from 30 to 45% B, 28-32 min; isocratic elution 45% B, 32-35 min; linear gradient 45 to 90% B, 35-40 min; isocratic elution 90% B, 40-45 min; linear gradient to reach the initial conditions after 5 min; post-time 10 min before the next injection. Individual phenolic components were quantified by comparison with external standards of phenolic compounds such as chlorogenic acid, (-)-epicatechin, ferulic acid, p-coumaric acid, sinapic acid and quercetin. Results were expressed as milligrams of phenolic compounds in 100 g of fresh-cut pear. Two trays were taken at each sampling time to perform replicate analyses throughout 14 days of storage.

Antioxidant capacity

The antioxidant capacity of fresh-cut pear was analyzed using two independent methods.

The determination of free radical-scavenging effect of antioxidants on 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) radical was carried out according to the procedure described by Elez-Martínez and Martín-Belloso (2007). The ABTS assay, based on the ability of the antioxidants to scavenge the blue-green radical cation 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulphonate) (ABTS$^{\bullet+}$), was conducted according to the method described by Re et al. (1999) with some modifications. Pear samples were centrifuged at 22100 x g for 15 min at 4 ºC (Centrifuge AVANTI^{TM} J-25, Beckman Instruments Inc., Fullerton, CA, USA) and filtered through Whatman No 1 paper. Aliquots of 0.01 mL of the supernatant were mixed with 3.9 ml of methanolic DPPH or ABTS$^{\bullet+}$ solutions and 0.09 mL of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. The absorption of the samples was measured with a CECIL CE 2021 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK) at 515 nm for DPPH assay or at 734 nm for ABTS assay.

The percentage of inhibition of the absorbance was calculated and plotted as a function of the concentration of Trolox for the standard reference data. The final DPPH and ABTS values were calculated by using a regression equation between the Trolox concentration and the % inhibition and results were expressed as milligram Trolox equivalent in 100 g of fresh-cut pear. Two trays were taken at each sampling time to perform replicate analyses throughout 14 days of storage.

Color attributes

The color of fresh-cut pears was determined with a Minolta CR-400 chromameter (Konica Minolta Sensing, Inc., Osaka, Japan), equipped with a D65 light source and the observer at 10º. Two trays were taken at each time to perform the analysis and five fruit pieces were evaluated for each package. Three readings were obtained for each replicate by changing the position of the pear wedges in the optical glass cell to get uniform colour measurements. Color values of CIE L* (lightness), a* (red-green) and b* (yellow-blue) were measured through reflectance values. Hue angle (h^0) was calculated by Eq. 1.

$$h^o = \arctan\frac{b^*}{a^*} \qquad \text{(Eq. 1)}$$

Data analysis

Statistical analysis was performed using Statgraphics plus v.5.1 software (Manugistics, Inc., Rockville, MA, USA). Data were analyzed by multifactor analysis of variance and a Duncan multiple range test was applied to determine differences among means, with a level of significance of 0.05. Principal component analysis (PCA) was carried out to obtain an overview of correlations among variables. PCA is a multivariate statistical technique based on the calculation of linear combinations between the variables that explain the most variance of the data. As a result, data can be reduced to a set of new variables called principal components (PCs). The correlation matrix is used to standardize the variables which are not measured on the same scale. The loadings of the PC define the direction of greatest variability and the score values represent the projection of each object onto PC.

Results and Discussion

Changes in headspace gas composition

Fig. 1 shows the variation of headspace gas partial pressures during storage of fresh-cut pears packaged under different modified atmospheres. As expected, a decrease in the O_2 concentrations throughout storage together with an increase in the CO_2 concentrations was observed for all initial in-package conditions. Headspace O_2 and CO_2 concentrations were not significantly influenced by the dipping treatment ($p \geq 0.05$). The amount of O_2 inside packages stored under HOA decreased continuously but remained > 50 kPa for 14 days, while the accumulation of CO_2 under HOA exceeded 15 kPa, which was also observed in packages stored under PA (Fig. 1a and b). Previous works showed that the application of HOA on fresh-cut 'Flor de Invierno' pears did not significantly reduce respiration rates compared with PA or LOA (Oms-Oliu et al., 2007b, c). Superatmospheric O_2 levels may stimulate, have no effect, or reduce respiration rates, depending on the commodity, maturity and ripeness stage, O_2 concentration, time and storage temperature, as well as CO_2 and ethylene concentrations (Kader and Ben-Yehoshua, 2000). A rapid decrease of package headspace O_2 concentrations was observed in fresh-cut pears stored under LOA, reaching concentrations below 1 kPa after 1 week (Fig. 1c). Rojas-Graü et al. (2006) also reported a rapid depletion of headspace O_2 concentrations in fresh-cut 'Fuji' apples packages stored under LOA compared with a progressive decrease under PA, while the use of antibrowning agents, N-acetylcysteine or ascorbic acid did not significantly affect O_2 consumption rates. The package

headspace of fresh-cut pears stored under LOA exhibited the lowest CO_2 accumulation compared with other packaging atmospheres, reaching concentrations below 15 kPa after two weeks of storage (Fig. 1c). Consistently, CO_2 production of fresh-cut 'Fuji' apples was significantly lower in samples stored under a LOA than under PA (Rojas-Graü et al., 2006). Gorny et al. (1999) found that low O_2 (0.25%) and elevated CO_2 (20%) acted synergistically to decrease respiration of fresh-cut peach slices.

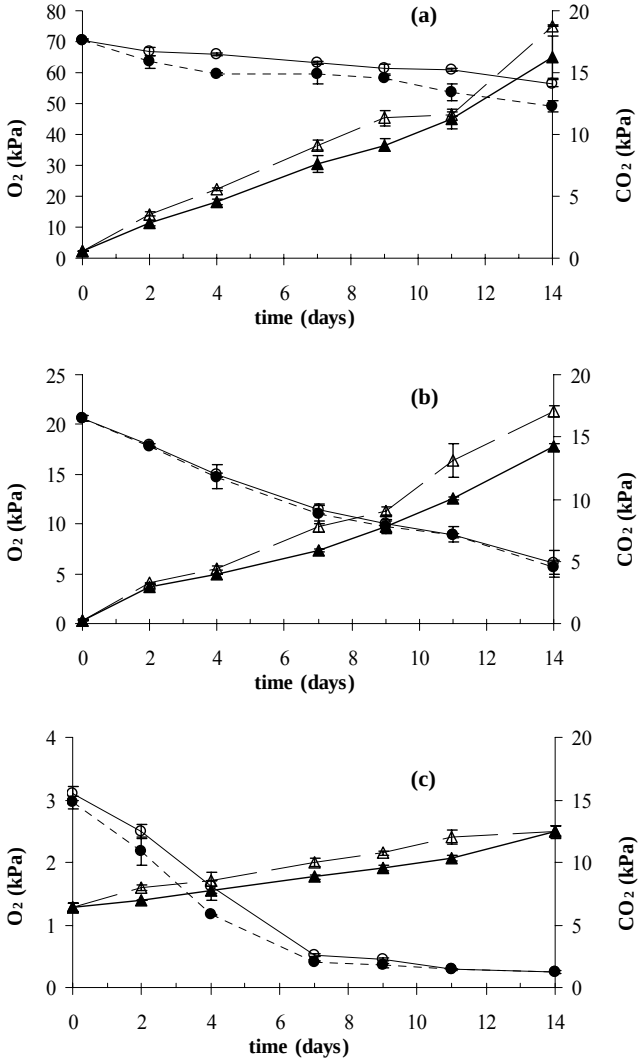

Figure 1. O_2 (●,○) and CO_2 (△,▲) headspace composition in packages of fresh-cut 'Flor de Invierno' pears packaged under HOA (a), PA (b) and LOA (c) during 14 days at 4 ºC. Samples

dipped into N-acetylcysteine (0.75%) and glutathione (0.75%) solution (empty symbols) and dipped into distilled water (full symbols).

Ethylene concentrations in the package headspace atmosphere were significantly influenced ($p \leq 0.05$) by storage atmosphere and dipping treatments. The application of a dip consisting of N-acetylcysteine and glutathione decreased ethylene production of fresh-cut pears (Fig. 2). Rojas-Graü et al. (2006) also found reduced ethylene accumulation in packages of fresh-cut 'Fuji' apples dipped into N-acetylcysteine (1% w/v) solution and stored under LOA or PA compared with the use of ascorbic acid. The action mechanism of thiol-containing compounds on ripening process and ethylene inhibition is not clear. However, Frenkel (1976) observed that the onset of ripening is influenced by a decline of sulphydryl gradient in fruit. These authors demonstrated that SH compounds such as cysteine or dithiothreitol could retard ripening of 'Bartlett' pears.

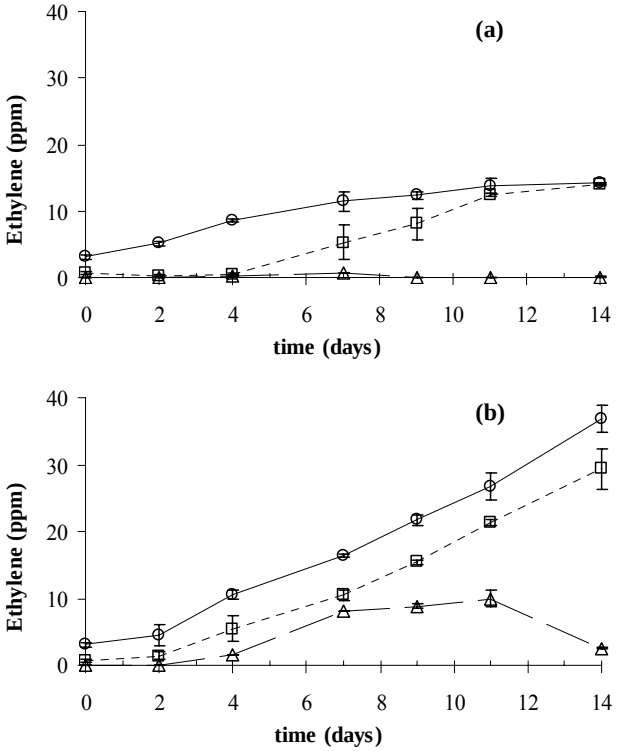

Figure 2. Ethylene headspace concentrations in packages of fresh-cut 'Flor de Invierno' pears dipped into N-acetylcysteine (0.75%) and glutathione (0.75%) solution (a) or dipped into distilled water (b), and packaged under HOA (○), PA (□) and LOA atmospheres (△) during 14 days at 4 ºC.

Ethylene production was reduced or completely inhibited in 'Flor de Invierno' pear wedges stored under LOA, while the headspace concentrations in HOA and PA packages containing non-dipped samples reached maximum values of 30-40 ppm after 14 days (Fig. 2). Rojas-Graü et al (2006) also reported lower ethylene production in fresh-cut 'Fuji' apples under LOA than under PA. Consistently, Soliva-Fortuny et al. (2007) reported an almost complete inhibition of ethylene production in fresh-cut 'Conference' pears stored under absence of O_2 or LOA, given that O_2 is required for ethylene synthesis. According to these authors, low O_2 concentrations combined with elevated amounts of CO_2 may act synergistically to inhibit ethylene production. The inhibition of ethylene under anaerobic or low O_2 concentrations has been reported by many authors, suggesting that O_2 participates in the conversion of 1-amino-cyclorpopane-1-carboxylic acid (ACC) to ethylene (1981). Therefore, high O_2 atmospheres may dramatically promote the biosynthesis of ethylene due to an excessive amount of O_2. ACC oxidase follows an ordered binding mechanism in which it first binds to O_2 and then to ACC. However, when the O_2 concentration was increased from 21 to 100 kPa there was little influence on the apparent K_m for ACC (Yip et al., 1988).

Changes in color attributes

L* and h^0 values are useful as indicators of browning of fresh-cut 'Flor de Invierno' pears (Oms-Oliu et al., 2006). In addition, changes in a* and b* values have previously been used in monitoring pear surface color (Soliva-Fortuny et al., 2002, 2004). Tables 2 and 3 show color quality of fresh-cut 'Flor de Invierno' pears throughout storage. Modified atmospheres alone were not effective to control enzymatic browning of fresh-cut 'Flor de Invierno' pears. These findings agree with those reported by Gorny et al. (2002) on fresh-cut 'Bartlett' pear slices packaged under low O_2 (0.25 or 0.5 kPa), elevated CO_2 (air enriched with 5, 10 or 20 kPa CO_2) or superatmospheric O_2 (40, 60 or 80 kPa). As a consequence of mechanical operations during processing, non-dipped pear wedges developed browning rapidly thereafter. The use of N-acetylcysteine and glutathione prevented cut surface browning under all packaging conditions ($p \leq 0.05$) although their antioxidant effect seems to decrease under HOA. On the other hand, L* and h^0 initial values of dipped pears were maintained along the time without any significant decrease under LOA (Table 2). A dip of N-acetylcysteine and glutathione was also shown to reduce changes in a* and b* values during storage, especially under initially LOA (Table 3). The antibrowning effect of thiol-containing compounds was also proved by Rojas-Graü et al. (2006) in

fresh-cut 'Fuji' apples dipped into 1% N-acetylcysteine and stored under LOA or PA. Although LOA combined with dips of ascorbic acid and calcium chloride have proved to maintain the overall sensory quality of fresh-cut 'Conference' pears for 3 weeks, low quantities of O_2 combined with high CO_2 concentrations were detrimental to flavor perception and caused massive production of fermentative metabolites beyond 3 weeks (Soliva-Fortuny et al., 2007).

Table 2. Changes in L* and h^0 values on fresh-cut 'Flor de Invierno' pears stored under HOA, LOA and PA during 14 days at 4 ºC

L*	LOA		PA		HOA	
Days	D	C	D	C	D	C
0	73.8 a	70.6 a	73.7 a	71.9 a	72.6 a	70.2 a
2	74.1 a	70.4 a	74.2 a	71.3 ab	72.1 ab	70.8 a
4	73.9 a	69.9 a	74.4 a	71.2 ab	71.2 bc	70.6 a
7	74.2 a	68.9 a	74.9 a	70.3 bc	70.9 c	69.3 b
9	74.1 a	67.1b	74.2 a	69.5 c	70.5 c	66.8 c
11	74.4 a	66.3 b	73.2 a	69.4 c	70.5 c	66.1 cd
14	74.1 a	66.7 b	73.9 a	69.7 c	70.5 c	65.6 c
h^0	LOA		PA		HOA	
Days	D	C	D	C	D	C
0	102.4 a	86.1 c	100.6 a	95.3 a	101.2 a	97.9 a
2	101.4 a	86.6 bc	100.0 a	94.5 a	100.4 a	94.1b
4	100.0 a	88.1 a	99.3 a	92.0b	99.5 a	88.6 c
7	100.4 a	87.6 ab	99.8 a	85.9 c	100.6 a	88.2 c
9	103.3 a	88.0 a	98.3 ab	84.4 cd	99.2 a	85.4 d
11	101.8 a	88.2 a	96.3 b	84.2 cd	99.1 a	85.1 d
14	100.9 a	88.0 a	95.8 b	83.5 d	99.8 a	84.8 d

HOA: 70 kPa O_2; PA: passive atmosphere; LOA: 2.5 kPa O_2 + 7 kPa CO_2; L*: Lightness; h^0: hue angle; D: samples dipped into N-acetylcysteine (0.75%) and glutathione (0.75%); C: samples dipped into distilled water

Values within a column followed by the same small letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$)

Table 3. Changes in a* and b* values on fresh-cut 'Flor de Invierno' pears stored under HOA, LOA and PA during 14 days at 4 ºC

a*	LOA		PA		HOA	
Days	D	C	D	C	D	C
0	-2.3 a	0.9 a	-1.9 ab	-1.4 a	-1.9 a	-1.7 a
2	-2.3 a	0.8 a	-1.9 ab	-1.2 a	-1.9 a	-1.0 b
4	-2.1 a	0.5 a	-2.0 a	-0.5 b	-1.7 a	0.4 c
7	-2.1 a	0.6 a	-2.0 a	1.3 c	-1.9 a	0.6 d
9	-2.8 a	0.5 a	-1.8 abc	2.1 d	-1.7 a	1.5 e
11	-2.4 a	0.5 a	-1.4 bc	2.2 d	-1.8 a	1.7 ef
14	-2.2 a	0.6 a	-1.3 c	2.4 d	-1.9 a	1.8 f
b*	LOA		PA		HOA	
Days	D	C	D	C	D	C
0	10.2 a	12.5 a	9.9 a	15.0 a	9.7 a	12.5 a
2	11.4 b	13.1 ab	10.8 ab	15.1 a	10.3 ab	14.3 b
4	11.9 b	14.1 bc	12.4 b	15.8 a	10.5 ab	15.9 c
7	11.4 b	13.9 abc	11.7 bc	18.4 b	10.0 a	19.4 d
9	11.7 b	14.8 cd	12.2 b	21.3 c	10.4 ab	19.3 d
11	11.4 b	16.0 d	12.6 b	21.9 c	11.0 b	19.5 d
14	11.5 b	16.1 d	12.8 b	21.0 c	11.2 b	19.5 d

HOA: 70 kPa O_2; PA: passive atmosphere; LOA: 2.5 kPa O_2 + 7 kPa CO_2; L*: Lightness; h^0: hue angle; D: samples dipped into N-acetylcysteine (0.75%) and glutathione (0.75%); C: samples dipped into distilled water

Values within a column followed by the same small letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$)

Vitamin C

Fig. 3 shows changes in vitamin C concentrations of fresh-cut 'Flor de Invierno' pears throughout storage. As it was reported by Gil et al., (2006) in fruits such as mango, strawberry and watermelon, processing operations do not seem to have a significant effect on the loss of vitamin C in fresh-cut pears. Initial vitamin C reduction in fresh-cut pears after processing may be due to packaging conditions rather than processing operations (Fig. 3). Initial vitamin C concentrations (3.3 - 4.6 mg·100 g^{-1} fw) were shown to be significantly affected by the dipping treatment and packaging atmospheres during storage ($p \leq 0.05$). As expected, ascorbic acid oxidation was greatly favored by the presence of O_2. According to Soliva-Fortuny et al. (2003), vitamin C content of fresh-cut 'Conference' pears were kept almost constant in absence of O_2 in packages throughout storage.

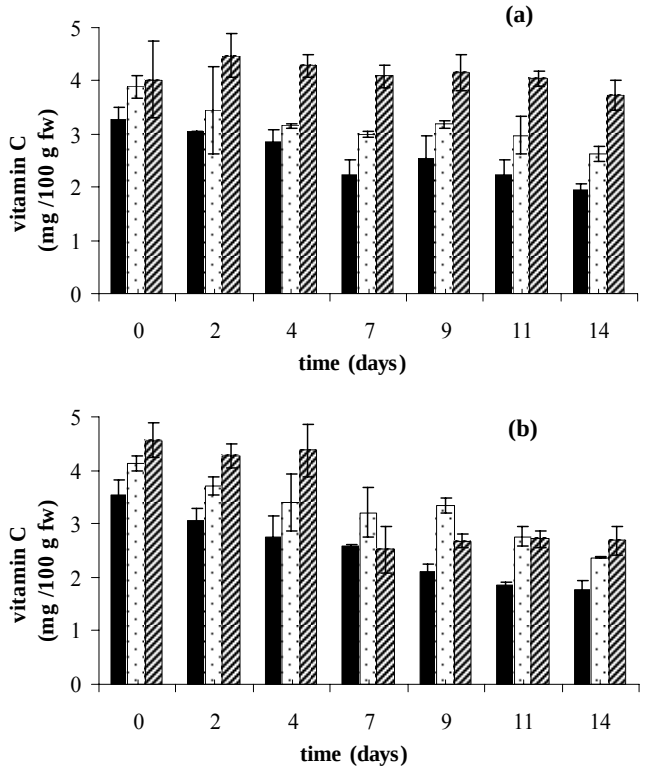

Figure 3. Vitamin C content of fresh-cut 'Flor de Invierno' pears dipped into N-acetylcysteine (0.75%) and glutathione (0.75%) solution (a) or dipped into distilled water (b), and packaged under HOA (■), PA (⊡) and LOA (▨) during 14 days at 4 ºC.

The information about the effects of high O_2 concentrations on antioxidant content of fresh produce is scarce. Day et al. (1998) reported that high O_2 modified atmosphere packaging did not preferentially decrease ascorbic acid content in prepared lettuce. However, our results show that HOA led to the greatest decrease in vitamin C content. Vitamin C concentrations of non-dipped pear wedges stored under HOA could have fallen bellow the threshold of acceptability, corresponding to 50% of initial AA content (< 2 mg kg^{-1}), beyond 9-day storage. Physiological disorders related to browning appeared in 'Conference' pears when ascorbic acid decreased below 2 mg kg^{-1} (Eccher et al., 2002). Although the main factor affecting vitamin C degradation is the initial availability of O_2, non-dipped samples stored under LOA underwent a substantial loss of vitamin C after 1 week of storage, when anoxic conditions could have been reached inside the packages. According to Tudela et al. (Tudela et al., 2002), high CO_2 levels could increase vitamin

C loss by accelerating ascorbate peroxidase-catalyzed oxidation processes. In fact, previous studies showed an important increase in peroxidase activity of fresh-cut 'Piel de Sapo' melon stored under LOA (Oms-Oliu et al., 2008). On the other hand, a dip of N-acetylcysteine + glutathione maintained vitamin C content of fresh-cut pears stored under LOA. In fruit tissue, glutathione and ascorbic acid can form a redox couple which is involved in the regeneration of ascorbic acid (Winkler et al., 1994).

Phenolic compounds

An initial phenolic content of 17-18 mg·100 g^{-1} fw was observed in fresh-cut 'Flor de Invierno' pears (Table 4). Chlorogenic acid, a hydroxycinnamic acid derivative, constituted the main phenolic in fresh-cut 'Flor de Invierno' pears. Chlorogenic acid concentrations were significantly affected by the dipping treatment and storage atmosphere ($p \leq 0.05$). Fresh-cut pears stored under HOA underwent a substantial loss of chlorogenic acid throughout storage. High O_2 atmospheres induced the loss of certain phenolic compounds in fresh-cut prepared lettuce compared with air or low O_2 modified atmosphere (Day, 1998). Cocci et al. (2006) also reported that the O_2 availability in the package headspace of fresh-cut apples stored under air packaging could have led to a stronger degradation of phenolic compounds than under 5 kPa O_2 + 5 kPa CO_2 atmospheres. This phenomenon could be due to the fast oxidation of phenolic compounds on the cut surface, directly in contact with the O_2 in the package headspace. Enzymatic oxidation of chlorogenic acid via polyphenol oxidase (PPO) has been associated with pear browning (Galvis-Sánchez et al., 2003). Our results show that the chlorogenic acid content was higher in fresh-cut pears dipped into N-acetylcysteine and glutathione solution than in non-dipped samples under LOA and PA (Table 4). Thus, the application of antioxidants may reduce chlorogenic acid degradation, which is known to happen much faster than for other fruit phenolics such as catechin and proanthocyanidins (Oleszek et al., 1989).

(-)-Epicatechin and quercetin content increased significantly throughout storage of fresh-cut 'Flor de Invierno' pears (Table 4). (-)-Epicatechin is the flavan-3-ol compound found in fresh-cut 'Flor de Invierno' pears at concentrations of 1.8-2.0 mg /100 g fw at day 0, whereas the initial concentrations of the flavonol compound, quercetin, were about 0.18-0.2 mg /100 g fw. The increase in their content during storage period could be directly associated with a physiological response to stress conditions. Physiological stress may stimulate phenylalanine ammonia lyase

(PAL, E.C. 4.3.1.5) activity with a consequent further production of phenylpropanoids (Salveit et al., 1997). The PAL activation of the phenylpropanoid metabolism could be elicited through induced reactive oxygen species (Reyes et al., 2006). A substantial increase of (-)-epicatechin and quercetin content was observed in non-dipped samples stored under HOA or PA during storage period. However, production of (-)-epicatechin as a wounding response seems to be triggered in dipped pear wedges stored under LOA.

Changes in other phenolic compounds found in fresh-cut 'Flor de Invierno' at minor initial concentrations such as *p*-coumaric acid (0.5-0.7 mg / 100 g fw), ferulic acid (0.8-0.9 mg /100 g fw) and sinapic acid (0.03-0.04 mg / 100 g fw) are shown in Table 4. Fresh-cut 'Flor de Invierno' pears packaged under LOA, PA or HOA underwent a substantial depletion of *p*-coumaric and ferulic acid during storage period, which may be a consequence of their conversion to sinapic acid. The *p*-coumaric acid is formed in plant products via the action of PAL due to the phenylpropanoid metabolism. This compound may be hydroxylated further in positions 3 and 5 and possibly methylated via O-methyl transferase with S-adenosylmethionine as methyl donor, leading to the formation of caffeic, ferulic and eventually sinapic acid (Shahidi, 2004). A dip of N-acetylcysteine and glutathione solution did not significantly affect the phenolic production under PA or HOA. However, dipped pear wedges initially packaged under LOA exhibited the most important accumulation of sinapic acid and decrease of *p*-coumaric acid and ferulic contents ($p \leq 0.05$). The glutathione and N-acetylcysteine, a precursor of glutathione, may be involved in the formation of phenylpropanoids under stress conditions. Ascorbate peroxidase-mediated conjugation of glutathione to *trans*-cinnamic and *p*-coumaric acids has been shown in plants under anoxia conditions (Blokhina et al. 2003).

Table 4. Phenolic content of fresh-cut 'Flor de Invierno' pears stored under HOA, LOA and PA during 14 days at 4 ºC

	chlorogenic acid		(-)-epicatechin		ferulic acid		*p*-coumaric acid		sinapic acid		quercetin		Total phenolics *	
Days	D	C	D	C	D	C	D	C	D	C	D	C	D	C
LOA														
0	17.44 a	16.78 a	2.01 e	1.93 bc	0.95 a	0.83 a	0.65 a	0.72 a	0.04 e	0.03 c	0.20 d	0.18 d	21.29 bc	20.49 bc
2	16.68 b	15.27 b	2.11 e	1.99 bc	0.90 b	0.56 c	0.42 d	0.43 d	0.05 d	0.05 b	0.23 c	0.21 d	20.38 d	18.55 d
4	14.50 d	15.26 b	2.61 d	1.81 bc	0.89 b	0.53 c	0.47 c	0.41 d	0.05 d	0.06 a	0.21 d	0.21 d	18.73 e	18.33 e
7	15.82 c	13.02 c	3.38 bc	1.70 c	0.61 c	0.33 e	0.49 b	0.38 e	0.06 bc	0.06 a	0.19 e	0.35 c	20.56 cd	15.95 cd
9	17.16 ab	12.48 c	3.28 bc	2.12 ab	0.51 d	0.56 c	0.47 c	0.49 c	0.06 c	0.06 a	0.43 a	0.49 a	21.91 ab	16.23 ab
11	16.69 b	12.30 c	3.65 b	1.99 bc	0.45 e	0.43 d	0.41 d	0.44 d	0.07 b	0.06 a	0.42 b	0.48 a	21.69 b	15.73 b
14	17.40 a	11.98 c	4.52 a	2.39 a	0.22 f	0.65 b	0.08 e	0.56 b	0.08 a	0.04 b	0.41 b	0.44 b	22.70 a	16.10 a
PA														
0	17.72 ab	17.99 a	1.88 b	1.88 d	0.90 a	0.87 a	0.59 b	0.62 a	0.04 c	0.03 d	0.21 cd	0.18 d	21.33 a	21.59a
2	17.04 cd	16.31 b	1.87 b	2.25 c	0.64 d	0.32 e	0.45 e	0.48 b	0.06 ab	0.04 c	0.22 c	0.22 c	20.27 c	19.55 b
4	17.65 ab	14.53 c	1.78 b	2.34 c	0.61 d	0.43 d	0.48 d	0.42 d	0.07 a	0.05 ab	0.23 c	0.23 c	20.83 abc	17.99 c
7	18.05 a	13.20 cd	1.86 b	2.35 c	0.64 d	0.49 c	0.44 e	0.37 e	0.06 ab	0.05 ab	0.19 d	0.43 b	21.23 ab	16.94 cd
9	17.24 bc	12.37 de	1.93 b	2.75 b	0.70 bc	0.49 c	0.44 e	0.44 c	0.06 ab	0.05 ab	0.42 ab	0.52 a	20.79 abc	16.64 cd
11	16.97 cd	11.15 ef	2.35 a	2.90 b	0.66 cd	0.44 d	0.54 c	0.47 bc	0.06 ab	0.06 a	0.43 a	0.53 a	21.01 ab	15.56 d
14	16.52 d	10.62 f	2.28 a	3.71 a	0.73 b	0.58 b	0.63 a	0.64 a	0.04 c	0.04 c	0.40 b	0.50 a	20.59 bc	15.91 d
HOA														
0	18.47 a	17.31 a	1.84 b	1.99 c	0.87 a	0.84 a	0.64 a	0.73 a	0.03 d	0.04 bc	0.19 d	0.20 d	22.04 a	21.10 a
2	15.47 bc	14.52 b	1.73 b	3.04 b	0.73 c	0.53 c	0.42 e	0.43 de	0.05 bc	0.06 a	0.23 c	0.26 c	18.62 c	18.85 ab
4	14.28 d	14.42 b	1.83 b	3.04 b	0.80 b	0.57 c	0.42 de	0.44 d	0.05 bc	0.06 a	0.22 cd	0.27 c	17.59 d	18.79 ab
7	14.81 cd	13.67 b	1.96 ab	2.77 b	0.42 e	0.43 d	0.41 e	0.43 de	0.06 ab	0.06 a	0.27 b	0.55 a	17.92 d	17.91 bc
9	16.01 b	12.72 bc	1.75 b	2.93 b	0.49 d	0.47 d	0.45 cd	0.41 e	0.06 ab	0.07 a	0.44 a	0.54 a	19.19 b	17.14 bcd
11	11.05 e	10.13 cd	2.17 a	2.90 b	0.50 d	0.68 b	0.48 b	0.47 c	0.06 ab	0.05 b	0.43 a	0.53 a	14.69 e	14.76 d
14	11.15 e	9.51 d	2.16 a	3.95 a	0.36 e	0.73 b	0.47 bc	0.53 b	0.07 a	0.03 d	0.42 a	0.49 b	14.64 e	15.24 cd

HOA: 70 kPa O_2; PA: passive atmosphere; LOA: 2.5 kPa O_2 + 7 kPa CO_2; D: samples dipped into N-acetylcysteine (0.75%) and glutathione (0.75%); C: samples dipped into distilled water; *: Total phenolics quantified by HPLC. The values are the result of the sum of each component. Values within a column followed by the same small letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$)

Table 5. Changes in antioxidant capacity of fresh-cut 'Flor de Invierno' pears stored under HOA, PA and LOA during 14 days at 4 ºC.

DPPH assay	LOA		PA		HOA	
Days	D*	C*	D	C	D	C
0	21.0 a	17.6 a	16.4 a	14.3 a	20.9 a	15.8 a
2	14.0 bc	10.2 b	15.5 a	11.5 b	15.4 b	9.6 b
4	15.2 b	10.6 b	12.0 b	11.9 b	12.9 c	9.4 bc
7	14.7 bc	9.5 b	12.0 b	9.1 c	11.9 cd	6.6 d
9	13.7 bc	9.6 b	11.2 bc	9.1 c	11.8 cd	5.9 d
11	13.6 bc	10.0 b	11.0 bc	8.9 c	10.4 de	5.7 d
14	11.4 c	9.9 b	10.0 c	9.0 c	9.6 e	7.1 cd
ABTS assay	LOA		PA		HOA	
Days	D	C	D	C	D	C
0	20.3 a	18.0 a	17.7 a	15.7 a	20.0 a	14.8 a
2	15.1 b	11.5 b	16.0 ab	12.1 b	14.6 b	10.4 b
4	16.1 b	10.0 b	14.1 b	10.7 bc	12.7 bc	9.6 bc
7	15.8 b	11.0 b	10.9 c	9.1 cd	10.9 c	7.2 cd
9	14.6 b	10.6 b	10.2 c	8.2 d	11.3 c	7.4 cd
11	15.5 b	10.7 b	10.7 c	7.9 d	11.9 c	5.8 d
14	13.0 b	10.1 b	9.8 c	8.3 cd	10.2 c	5.6 d

* D: samples dipped into N-acetylcysteine (0.75%) and glutathione (0.75%); C: samples dipped into distilled water

Values within a column followed by the same small letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$)

HOA: 70 kPa O_2; PA: passive atmosphere; LOA: 2.5 kPa O_2 + 7 kPa CO_2

Antioxidant capacity of fresh-cut pears packaged under LOA was significantly higher than those observed in pears stored under HOA and PA. Under superatmospheric O_2 levels, non-dipped samples underwent a substantial loss in chlorogenic acid content (≥ 45%) and vitamin C (≥ 50%), which could account for the decrease in antioxidant capacity found in these samples beyond 9 days of storage (≥ 60%). The use of N-acetylcysteine + glutathione significantly enhanced the antioxidant capacity of fresh-cut pears under all storage conditions. The DPPH and ABTS values for dipped pear wedges after 14 days of storage were substantially higher than those for non-dipped samples. According to DPPH and ABTS values, fresh-cut 'Flor de Invierno' pears dipped into N-acetylcysteine + glutathione and stored under LOA could best maintain their initial antioxidant capacity. Other authors have shown a substantial depletion in the antioxidant capacity

of wild rocket leaves stored under atmospheric O_2 levels in comparison to samples stored under 5 kPa O_2 + 5 kPa CO_2 or 5 kPa O_2 + 10 kPa CO_2 atmospheres (Martín-Sánchez et al., 2006). Our findings show that thiol compounds such as N-acetylcysteine + glutathione not only play a relevant role in the prevention of enzymatic browning by reducing *o*-quinones to colorless phenol precursors but can also maintain antioxidant potential of fresh-cut pears.

PCA analysis

A principal components analysis (PCA) was performed on all samples and variables (in-package gas concentrations, antioxidant capacity, total phenolic compounds, vitamin C and color attributes) to obtain relationships among the studied parameters (Fig. 4).

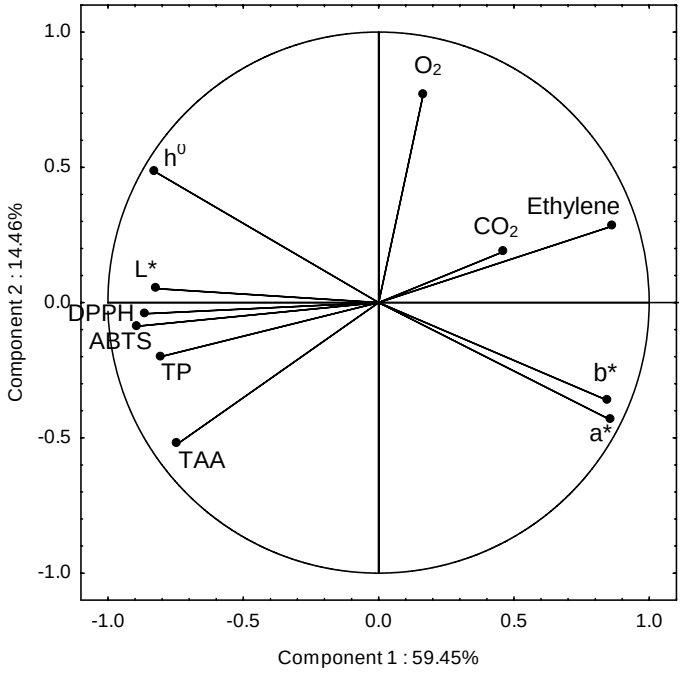

Figure 4. PCA plot of fresh-cut 'Flor de Invierno' pear dipped into N-acetylcysteine (0.75%) and glutathione (0.75%) solution or dipped into distilled water, and packaged under HOA, PA and LOA during 14 days at 4 ºC. DPPH assay; ABTS assay; total vitamin C concentration (TAA); total phenolics (TP)

The factor loadings of the analyzed compounds explained 73.91% of the total variation of the data. The two principal components, PC1 and PC2, explained 59.45% and 14.46% of the total variance,

respectively. As can be seen in Fig. 4, there is a close relationship between DPPH and ABTS values and total phenolic compounds, which in turn, were highly correlated with L* values. Thus, the antioxidant capacity of fresh-cut 'Flor de Invierno' pears could be mainly attributed to total phenolic content rather than to vitamin C concentrations. In a comparative study of six pear cultivars in terms of their phenolic and vitamin C contents, antioxidant capacity correlated well with the content of chlorogenic acid, the most common phenol found in pears (Galvis Sanchez et al., 2003). The scores of PC1 vs. PC2 plotted in Fig. 5 described differences between non-dipped samples and those treated with N-acetylcysteine and glutathione. Thus, it can be observed that a majority of the dipped samples are situated in the left part of the score plot. These samples were related to the highest values of L*, h^0, DPPH, ABTS, vitamin C and total phenolic content. On the other hand, non-dipped samples located in the right part of the plot were related to high a* and b* values. The O_2 and CO_2 in-package concentrations did not seem to affect bioactive compounds and color attributes of fresh-cut 'Flor de Invierno' pears throughout the storage (Fig. 4). Pear wedges stored under HOA scored the highest O_2 content as they were located in the upper part of the plot. Under superatmospheric O_2 levels, the non-dipped samples were highly correlated with ethylene production (Fig. 5).

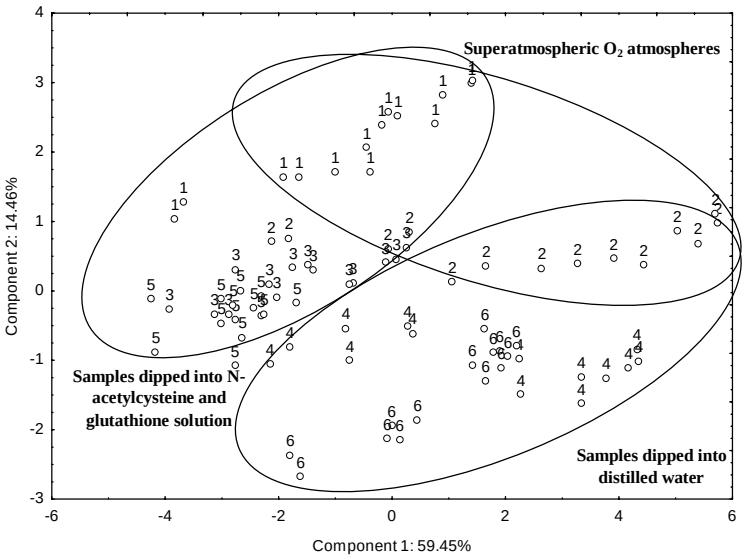

Figure 5. Score plot of PC1 vs. PC2 of all sample labels for fresh-cut 'Flor de Invierno' packaged under active 70 kPa O_2 (HOA) or 2.5 kPa O_2 + 7 kPa CO_2 (LOA) atmospheres and passive

atmospheres (PA) during 14 days at 4 ºC. Dipped into N-acetylcysteine (0.75%) and glutathione (0.75%) solution: 1(HOA), 3(PA), 5 (LOA); dipped into distilled water: 2 (HOA), 4 (PA), 6 (LOA).

Conclusions

A dip into a 0.75% w/v N-acetylcysteine + 0.75% w/v glutathione solution had an important influence on the reduction of enzymatic browning and ethylene production of fresh-cut pears stored under LOA, HOA or PA. However, LOA better maintained antioxidant content of pear wedges compared with PA or HOA by preserving vitamin C, phenolic compounds and antioxidant capacity of the commodity throughout storage. The antioxidant capacity of fresh-cut pears seems to be mainly attributed to phenolic compounds rather than to vitamin C content. In addition, our results suggest that glutathione can be involved in the enhanced formation of phenylpropanoids under LOA.

Abbreviations

HOA, high O_2 atmospheres; LOA, low O_2 atmospheres; PA, passive atmospheres; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid; TROLOX, 4-hydrozy-2,5,7,8-tetramethyl-2-carboxylic acid; h^0, hue angle; TAA, total vitamin C concentration; TP, total phenolic content

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References

Block, G.; Patterson, B.; Subar, A. 1992. Fruits and vegetables and cancer prevention: a review of the epidemiological evidence. *Nutrition and Cancer*, 18: 1-29.

Blokhina, O.; Virolainen, E.; Fagerstedt, K.V. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of Botany*, 91: 179-194

Cocci, E.; Rocculi, P.; Romani, S.; Dalla Rosa, M. 2006. Changes in nutritional properties of minimally processed apples during storage. *Postharvest Biology and Technology*, 39: 265-271.

Day, B.P.F. 1996. High oxygen modified atmosphere packaging for fresh prepared produce. *Postharvest News Information*, 7 (3): 31N-34N.

Eccher Zerbini, P.; Rizzoli, A.; Brambilla, A.; Cambiaghi, P.; Grassi, M. 2002. Loss of ascorbic acid during storage of Conference pears in relation to the appearance of brown heart. *Journal of Science and Food Agriculture*, 82: 1007-1013.

Elez-Martínez, P.; Martín-Belloso, O. 2007. Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Food Chemistry*, 102: 201-209.

Frenkel, C. 1976. Regulation of ripening in Bartlett pears with sulfhydryl reagents. *Botanical Gazzete*, 137: 154-159.

Galvis Sánchez, A.C.; Gil-Izquierdo, A.; Gil, M.I. 2003. Comparative study of six pear cultivars in terms of their phenolic and vitamin C contents and antioxidant capacity. *Journal of Science and Food Agriculture*, 83: 995-1003.

Gil, M.I.; Aguayo, E.; Kader, A.A. 2006. Quality changes and nutrient retention in fresh-cut versus whole fruits during storage. *Journal of Agricultural and Food Chemistry*, 54: 4284-4296.

Gorny, J.R.; Hess-Pierce, B.; Kader, A.A. 1999. Quality changes in fresh-cut peach and nectarine slices as affected by cultivar, storage atmosphere and chemical treatments. *Journal of Food Science*, 64: 429-432.

Gorny, J.R.; Hess-Pierce, B.; Cifuente, R.A.; Kader, A.A. 2002. Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest. Biology and Technology*, 24: 271-278.

Hertog, M.; Hollman, P.; Venema, D. 1992. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agricultural and Food Chemistry*, 40: 1591-1598

Kader, A.A.; Ben-Yehoshua, S. 2000. Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. *Postharvest Biology and Technology*, 20: 1-13

Liu, S.; Manson, J.E.; Lee, I.-M.; Cole, S.R.; Hennekens, C. H.; Willet, W.C.; Buring, J.E. 2000. Fruit and vegetable intake and reisk of cardiovascular disease: the Women's Health Study. *American Journal of Clinical Nutrition*,72: 922-928.

Lurie, S. 2003. Antioxidants. In: Hodges, H. (Ed.), *Postharvest oxidative stress in horticultural crops*. Food Products Press, New York, U.S.A., pp. 131-150

Martínez-Sánchez, A.; Marín, A.; Llorach, R.; Ferreres, F.; Gil, M.I. 2006. Controlled atmosphere preserves quality and phytonutrients in wild rocket (Diplotaxis tenuifolia). *Postharvest Biology and Technology*, 40: 26-33.

Odriozola-Serrano, I.; Hernández-Jover, T.; Martín-Belloso, O. 2007. Comparative evaluation of UV-HPLC methods and reducing agents to determine vitamin C in fruits. *Food Chemistry*, 105: 1151-1158

Oleszek, W.; Chang, Y.L.; Jaworski, A.W.; Price, K.R. 1989. Apple phenolics and their contribution to enzymatic browning reactions. *Acta Socieitatis Botanicorum Poloniae*, 58: 273-283

Oms-Oliu, G.; Aguiló-Aguayo, I.; Martín-Belloso, O. 2006. Inhibition of browning on fresh-cut pear wedges by natural compounds. *Journal of Food Science*, 71(3): 216-224.

Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso, O. 2007. Respiratory rate and quality changes in fresh-cut pears as affected by superatmospheric oxygen. *Journal of Food Science*, 72(8): 456-463

Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso. 2007. Physiological and microbiological changes in fresh-cut pears stored in high oxygen active packages compared with low oxygen active and passive modified atmosphere packaging. *Postharvest Biology and Technology*, doi: 10.1016/j.postharvbio.2007.10.002

Oms-Oliu, G.; Odriozola-Serrano, I.; Soliva-Fortuny, R. 2008. The role of peroxidase on the antioxidant potential of fresh-cut 'Piel de Sapo' melon packaged under different modified atmospheres. *Food Chemistry*, 106: 1085-1092

Prior, R.L.; Cao, G. 2000. Antioxidant phytochemicals in fruits and vegetables: diet and health implications. *HortScience*, 35: 588-592.

Re, R.; Pellegrin, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decoloration assay. *Free Radic Biol Med*, 26: 1231-1237.

Reyes, L.F.; Villarreal, J.E.; Cisneros-Zevallos, L.C. 2006. The increase in antioxidant capacity alter wounding depends on the type of fruit or vegetable tissue. *Food Chemistry*, 101: 1254-1262.

Richard, F.; Goupy, P.M.; Nicolas, J.J.; Lacombe, J.M.; Pavia, A.A. 1991. Cysteine as an inhibitor of enzymatic browning. 1. Isolation and characterization of addition compounds formed during

oxidation of phenolics by apple polyphenol oxidase. *Journal of Agriculture and Food Chemistry*, 39: 841-847.

Rojas-Graü, M.A.; Sobrino-López, A.; Tapia, M.S.; Martín-Belloso, O. 2006. Browning inhibition in fresh-cut "Fuji" apple slices by natural antibrowning agents. *Journal of Food Science*, 71: 59-65.

Saltveit, M.E. 1997. Physical and physiological changes in minimally processed fruits and vegetables. In: Tomás-Barberán, F.A.; Robin, R.D. (Eds). *Phytochemistry of Fruits and Vegetables*. Oxford University Press, Oxford, U.K., pp. 205-220.

Sapers, G.M.; Miller, R.L. 1998. Browning inhibition in fresh-cut pears. *Journal of Food Science*, 63: 342-6.

Shahidi, F. 2004. Biosynthesis, classification, and nomenclature of phenolics in food and Nutraceuticals. In: Shahidi, F and Naczk, M. (Eds.). Phenolics in Food and Nutraceuticals. CRC Press, New York, pp. 1-204.

Soliva-Fortuny, R.C.; Biosca-Biosca, M.; Grigelmo-Miguel, N.; Martín-Belloso, O. 2002. Browning, polyphenol oxidase activity and headspace gas composition during storage of fresh-cut pears using modified atmosphere packaging. *Journal of Science and Food Agriculture*, 82: 1490-1496.

Soliva-Fortuny, R.C.; Martín-Belloso, O. 2003. Microbiological and biochemical changes in minimally processed fresh-cut Conference pears. European Food Research and Technology. 207: 4-9.

Soliva-Fortuny, R.C.; Alós-Saiz, N.; Espachs-Barroso, A.; Martín-Belloso, O. 2004. Influence of maturity at processing on quality attributes of fresh-cut 'Conference' pears. *Journal of Food Science*, 69: 290-294.

Soliva-Fortuny, R.; Ricart-Coll, M.; Elez-Martínez, P.; Martín-Belloso. 2007. Internal atmosphere, quality attributes and sensory evaluation of MAP packaged fresh-cut Conference pears. *International Journal of Food Science and Technology*, 42: 208-213.

Solomos, T. 1997. Principles underlying modified atmosphere packaging. In: Wiley, R.C. (Ed.), *Minimally processed refrigerated fruits & vegetables*. Chapman and Hall, New York, U.S.A., pp. 183-225.

Tudela, J.A.; Espín, J.C.; Gil, M.I. Vitamin C retention in fresh-cut potatoes. 2002. *Postharvest Biolology and Technology*, 26: 75-84.

Varela, P.; Salvador, A.; Hernando, I.; Pérez-Munuera, I.; Quiles, A.; Lluch, M.A.; Fiszman, S. 2007. Eating quality of 'Flor de Invierno' pears: chemical and structural aspects. *International Journal of Food Science and Technology*, 42: 1052-1058.

Winkler, B.S.; Orselli, S.M.; Rex, T.S. 1994. The redox couple between glutathione and ascorbic acid: a chemical and physiological perspective. *Free Radical Biology and Medicine*, 17: 333-349.

Yang, S.F. 1981. Biosynthesis of ethylene and its regulation. In: Friend J, Rhodes M.J.C., (Eds.). *Recent advances in the biochemistry of fruit and vegetables*. London: Academic Press. Pp. 89-106.

Yip, W., Jiao, X., Yang, S.F. 1988. Dependence of in vivo ethylene production rate on 1-aminocyclopropane-1-carboxylic acid content and oxygen concentrations. *Plant Physiology*, 88: 553-558.

Capítulo XI

Edible coatings with antibrowning agents to maintain sensory quality and antioxidant properties of fresh-cut pears

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Postharvest Biology and Technology, enviado

Abstract

The effect of alginate- (2% w/v), pectin- (2% w/v) and gellan-based (0.5% w/v) edible coatings containing N-acetylcysteine at 0.75% w/v and glutathione at 0.75% w/v on gas exchange, antioxidant properties, sensory quality and microbial stability of fresh-cut 'Flor de Invierno' pears, was investigated for 14 days at 4 ºC. The use of polysaccharide-based edible coatings increased the water vapor resistance of coatings and reduced ethylene production of fresh-cut pears. The incorporation of N-acetylcysteine and glutathione into coating formulations not only reduced microbial growth compared with samples not containing antioxidants but also was effective in preventing fresh-cut pears from browning for 2 weeks without affecting firmness of fruit wedges. The increased vitamin C and total phenolic content observed in pear wedges coated with alginate, gellan and pectin including antioxidants contributed to maintain their antioxidant potential. In addition, coatings with alginate or pectin best maintained sensory attributes of pear wedges for 14 days.

Keywords: edible coatings, N-acetylcysteine, glutathione, gas exchange, total phenolic compounds, vitamin C, antioxidant capacity, sensory quality

Introduction

Fresh-cut fruit and vegetables offer to consumers highly nutritious, convenient and healthful commodities while maintaining the freshness of the non-processed products. Diets rich in fruit and vegetables can help in the prevention of diseases, including cancer and cardio- and cerebro-vascular diseases, due to antioxidants they contain (Ames, 1993). However, respiration increases when fruits are wounded, causing accelerated consumption of sugars, lipids, and organic acids, and increasing ethylene production, which induces ripening and causes senescence (Kays, 1991). Minimal processing operations damage the tissue integrity of fruit, which triggers wounding and deteriorative processes including oxidative browning, tissue softening, water loss, and production of undesirable flavors and odors (Martín-Belloso et al., 2007). In addition, the removal of the natural protective epidermal barrier and the increase in moisture and dissolved sugars on the surface provide ideal conditions for the colonization and multiplication of microorganisms (Nguyen-The and Carlin, 1994).

Modified atmosphere packaging (MAP) alone did not effectively prevent cut surface browning or firmness loss of fresh-cut pears (Gorny et al., 2002; Soliva-Fortuny et al., 2002a, 2002b). In addition, polymeric films used in MAP have some limitations because of their structure and permeation characteristics. They may promote water loss, which may result in texture changes, translucency, and/or surface dehydration, or contrarily, they can increase the formation of water condensates that favor microbiological proliferation. The semipermeable barrier provided by edible coatings could be used to improve the shelf-life of fresh-cut fruit packaged under MAP, thus reducing moisture and solutes migration, gas exchange, respiration and oxidative reaction rates, as well as suppressing physiological disorders (Wong et al., 1994a; Baldwin et al., 1996; Park, 1999; Rojas-Graü et al., 2008). Pectin, a soluble component of plant fiber derived from cell walls of plants, alginate, a polysaccharide derived from a marine brown algae (*Phaeophyceae*) and gellan, a microbial polysaccharide secreted by the bacterium *Sphingomonas elodea* (formerly referred to as *Pseudomonas elodea*) are capable to form strong gels or insoluble polymers upon reaction with multivalent metal cations like calcium (King, 1983, Rhim, 2004). In general, polysaccharide-based coatings have been used to extend the shelf-life of fruit and vegetables by reducing respiration and gas exchange due to selective permeabilities to O_2 and CO_2 (Nísperos-Carriedo, 1994; Nussinovitch, 1997). Respiration and ethylene production of cut apple pieces coated with a pectin or gellan-lipid bilayer coating was significantly reduced (Wong et al., 1994b).

Alginate and gellan-based edible coatings effectively prolonged the shelf-life of 'Fuji' apple wedges by 2 weeks of storage compared to uncoated fruit slices (Rojas-Graü et al., 2008).

Color is a critical quality parameter of cut pears since cutting operations, although conducted under controlled conditions, often results into enzymatic browning (Sapers and Miller, 1998; Dong et al., 2000; Gorny et al., 2002). Antioxidant constituents of fruit such as phenolic compounds and ascorbic acid are related to enzymatic browning. Phenolic compounds are oxidized to highly unstable quinones which are later polymerized to brown, red and black pigments (Nicolas et al., 1994; Martínez and Whitaker, 1995). In addition, the decrease in ascorbic acid content below a threshold level has been related to brown heart in pears (Eccher Zerbini et al., 2002). Dips in aqueous solutions containing sulfur-containing amino acids as N-acetylcysteine and glutathione have been proved to preserve browning of uncoated fresh-cut 'Flor de Invierno' pears (Oms-Oliu et al., 2006). The incorporation of antioxidant agents such as N-acetylcysteine and glutathione into alginate- and gellan-based coatings helped to prevent fresh-cut apples and papayas from browning (Tapia et al., 2005; Rojas-Graü et al., 2007a, 2008). Nevertheless, as far as we know, there is no information about the effect of the incorporation of these antioxidant substances into coating formulations on the antioxidant properties of fresh-cut fruit.

In the present work, the objective was to compare the effectiveness of alginate-, gellan- or pectin-based coatings containing thiol antibrowning agents on quality of fresh-cut 'Flor de Invierno' pears. The effects of the coatings on gas exchange, vitamin C, total phenolic compounds, antioxidant capacity, sensory quality and microbiological stability of pear wedges were evaluated for 14 days at 4 ºC.

Materials and methods

Materials

Pears (*Pyrus communis* L cv Flor de Invierno) harvested in Lleida (Spain) were supplied by a local distributor and stored at 4 ºC for one month prior to processing. Food grade sodium alginate (Keltone ® LV, ISP, San Diego, CA, USA), food grade deacylated gellan gum (Kelcogel ®, CPKelco, Chicago, IL, USA) and low-methoxyl pectin (~ 30% esterified) (Sigma-Aldrich Chemic, Steinhein, Germany) were the carbohydrate biopolymers used to prepare the coating formulations. Glycerol (Merck, Whitehouse Station, NJ, USA) was added as plasticizer. Calcium chloride (Sigma-Aldrich Chemic, Steinhein, Germany) was used to induce crosslinking reaction. N-

acetylcysteine and glutathione (Sigma-Aldrich Chemic, Steinhein, Germany) were the added antibrowning agents. A 0.025% (w/v) of sunflower oil (La Española, Spain) with the following composition: 11 g monosaturated, 30 g monounsaturated and polyunsaturated 57.4 g: 35 g *omega*-3 and 55-60 g *omega*-6, was used as the lipid source for emulsion films.

Preparation of the film forming and dipping solutions

The concentrations of coating ingredients used in the formulations were set up according to Rojas-Graü et al. (2007a). Film forming solutions were prepared according to their procedure. Coatings were prepared by dissolving alginate (2 % w/v), gellan (0.5 % w/v) or pectin (2 % w/v) powders in distilled water and heating at 70 ºC while stirring until the solution became clear. Glycerol was added as plasticizer at 1.5 % w/v in alginate or pectin solution and at 0.6 % w/v in gellan solution. Film-forming solutions were emulsified with sunflower oil (0.025 % w/v) which was dispersed using an Ultra Turrax T25 (IKA ® WERKE, Germany) with a S25N-G25G device, for 5 min at 24500 rpm, and degassed under vacuum. For the crosslinking of carbohydrate polymers, a calcium chloride solution (2 % w/v) containing N-acetylcysteine at 0.75% w/v and glutathione at 0.75% w/v was prepared. The concentrations of the antibrowning agents were chosen in accordance to previous studies carried out on fresh-cut 'Flor de Invierno' pears (Oms-Oliu et al., 2006).

Fruit coating and packaging

'Flor de Invierno' pears were sanitized in a 200 $\mu l \cdot l^{-1}$ NaClO solution for 2 min, rinsed with tap water, and dried prior to cutting operations. Pears were peeled, the core tissue was completely removed and the remaining tissue was cut into wedges. The fruit pieces were introduced in a wire basket and dipped into the polysaccharide solutions for 2 min. The excess of coating material was allowed to drip off for 1 min before submerging the basket again for 2 min in the calcium chloride solution containing N-acetylcysteine and glutathione. As controls, fruit pieces were dipped in the calcium chloride not incorporing the antioxidants. Uncoated pieces were dipped in an aqueous solution of N-acetylcysteine at 0.75% (w/v) and glutathione at 0.75% (w/v) or dipped into distilled water for 2 min. Then, 100 g of coated pear wedges were placed in polypropylene trays in a product: air ratio of 1:2 (v/v). Trays were thermosealed using a packaging machine ILPRA Food Pack Basic V/6 (ILPRA Systems. CP. Vigevono, Italy). The O_2 and CO_2 permeance of the sealing film were $5.2419 \cdot 10^{-13}$ mol O_2 m^{-2} s^{-1} Pa^{-1} and $2.3825 \cdot 10^{-12}$ mol CO_2 m^{-2} s^{-1} Pa^{-1} at 23ºC and 0%

RH, respectively (ILPRA Systems España, S.L. Mataró, Spain). The packages were stored at 4 ± 1ºC in darkness up to random withdrawal for analyses.

Water vapor resistance (WVR)

The method for determination of WVR of coatings described by Rojas-Graü et al. (2007a) was used to measure the water vapor resistance of coated fruit pieces. Samples were equilibrated for 24 h in desiccators maintained at 98.9% RH with 0.6 m NaCl solution at room temperature. Then, samples were placed in sealed chambers equilibrated at 33% RH with saturated $MgCl_2 \cdot 6H_2O$ (Panreac Quimica SA, Barcelona, Spain) at 25 ºC. The weight was read at 4-h intervals and the slope of the curve of weight loss vs. time was estimated by linear regression analysis. WVR was calculated using a modified Fick's first law equation:

$$\frac{ds}{dt} = \frac{A\Delta C}{R} \qquad \text{(Eq. 1)}$$

where ds/dt is the rate of gas exchange in $mol \cdot s^{-1}$ (slope); A is the exposed area of the fruit pieces (m^2); R is the resistance of the coating to water diffusion ($s \cdot m^{-1}$); ΔC is the concentration of gas ($mol \cdot m^{-3}$) inside and outside the fruit piece at time *t*. For WPR, ΔC = (Pi-Pa) / RcT, where Rc is the gas constant (8.314 $Pa \cdot m^3 \cdot K^{-1} \cdot mol^{-1}$), T is the temperature in degrees Kelvin and (Pi-Pa) is the difference in water vapor pressure (Pa) inside and outside the fruit tissue, being Pi = a_w of the fruit x P_0 (water vapor pressure of liquid water at 25 ºC) and Pa = partial water vapor pressure in the environment with 33.3% RH at 25 ºC.

Control tests with uncoated pears were performed to determine the resistance factor of the uncoated fruit to water vapor. a_w of the samples was measured with an Acqualab CX-2 (Decagon Devices Inc., Pullman, WA).

Package headspace gas composition

A gas analyser (Micro-GC CP 2002, Chrompack International, Middelburg, The Netherlands), equipped with a thermal conductivity detector, was used to analyse the package headspace gas composition. The gaseous content of each tray was gently mixed prior to sampling and an adhesive septum was stuck to the plastic tray. A sample of 1.7 ml was automatically withdrawn from the headspace atmosphere, and an aliquot of 0.25 µl was injected to a CP-Molsieve 5Å column (4m x 0.35 mm, df=10 µm) at 60 ºC and 100 kPa for O_2 quantification. A portion of 0.33 µl was injected

to a Pora-PLOT Q column (10m x 0.32 mm, df=10 μm) at 75 ºC and 200 kPa for CO_2, ethanol and ethylene determinations. Two trays were taken at each time to perform the analysis and 2 readings were carried out for each package.

Vitamin C content

The determination of the vitamin C concentration in fresh-cut pear was performed by HPLC-UV. The extraction procedure and the chromatographic conditions were based on a previous study carried out by Odriozola-Serrano et al. (2007). A portion of 25 g of fruit was added to 25 ml of a solution containing 45 $g \cdot l^{-1}$ of metaphosforic acid and 7.2 $g \cdot l^{-1}$ of DL-1, 4-dithiothreitol (DTT). The mixture was stirred and centrifuged at 22100 x g for 15 min at 4ºC (Centrifuge AVANTI™ J-25, Beckman Instruments Inc., Fullerton, CA, USA). The supernatant was vaccum-filtered through Whatman No 1 paper. The sample was passed through a Millipore 0.45 μm membrane and injected into the HPLC system.

The HPLC system was equipped with a 600 Controller and a 486 Absorbance Detector (Waters, Milford, MA) working at 245 nm. Duplicates of 20 μl of each extract were injected into a reverse-phase C18 Spherisorb® ODS2 (5μm) stainless steel column (250 mm x 4.6 mm) (Waters, Milford, MA), used as stationary phase. A 0.01% solution of sulphuric acid adjusted to pH 2.6 was used as the mobile phase. The flow rate was fixed at 1 ml/min at room temperature. Results were expressed as milligrams of vitamin C in 100 g of fresh-cut pear.

Total phenolic compounds

The amount of total phenolic compounds in fresh-cut pears was determined according to the Folin-Ciocalteu procedure (Singleton et al., 1999) with some modifications. A sample of 50 g was ground and centrifuged at 22100 x g for 15 min at 4 ºC (Centrifuge AVANTI™ J-25, Beckman Instruments Inc., Fullerton, CA, USA) and then, filtered through a Whatman no 1 filter. An aliquot of 0.5 ml of the supernatant was added to 0.5 ml of Folin-Ciocalteu solution. After 3 min, 10 ml of saturated sodium carbonate solution were added and brought up to 25 ml with distilled water. The absorbance of the blue color that developed was read at 725 nm after 1 hour in darkness conditions. Concentrations were determined by comparing the absorbance of the samples with standards. Results were expressed as milligrams of gallic acid in 100 g of fresh-cut pear. Two trays were taken at each sampling time to perform replicate analyses throughout 14 days of storage.

Antioxidant capacity

The antioxidant capacity of fresh-cut pear was studied through the determination of free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, according to the procedure described by Elez-Martínez and Martín-Belloso (2007). Pear samples were centrifuged at 22100 x g for 15 min at 4 ºC (Centrifuge AVANTI^{TM} J-25, Beckman Instruments Inc., Fullerton, CA, USA) and filtered through a Whatman No 1 paper. Aliquots of 0.01 ml of the supernatant were mixed with 3.9 ml of methanolic DPPH of 0.025 $g \cdot l^{-1}$ and 0.090 ml of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. The absorption of the samples was measured with a CECIL CE 2021 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK) at 515 nm against blank of methanol without DPPH. Results were expressed as a percentage decrease with respect to the absorption value of a reference DPPH solution.

Color and firmness evaluation

Cut pear surface color values were directly measured with color meter (Minolta Chroma Meter Model CR-400, Minolta Sensing, Inc., Osaka, Japan). The equipment was set up for a D65 illuminant and 10º observer angle. Five fruit pieces from each of 2 replicate packages were evaluated for each treatment at each sampling time. Color changes of fresh-cut pears were measured through hue angle (h^0) parameter (Eq. 2). Changes in h^0 parameter have previously been shown to be effective in monitoring enzymatic browning of fresh-cut 'Flor de Invierno' pears (Oms-Oliu et al., 2006).

$$h^\circ = \arctan\frac{b^*}{a^*} \qquad \text{(Eq. 2)}$$

Firmness evaluation was performed using a TA-XT2 Texture Analyser (Stable Micro Systems Ltd., Surrey, England, UK) by measuring the maximum penetration force. Fruit wedges were cut in rectangular shaped 2.0 cm high samples and were penetrated by a 4 mm diameter rod. The downward distance was set at 10 mm at a rate of 5 $mm \cdot s^{-1}$ and automatic return. Samples were placed so that the rod penetrated their geometric centre. Two trays were taken at each sampling time to perform the analyses, and 5 fruit pieces from each replicate were randomly withdrawn to carry out repetitions.

Microbiology analyses

Total aerobic psychrophilic microorganisms and yeast and mold populations were evaluated during storage of fresh-cut 'Flor de Invierno' pears. Two counts were obtained each time from

each of two replicate packages. In sterile conditions, 10 g of sample were homogenized for 2 min with 90 ml of 0.1% sterile peptone water with a Stomacher Lab Blender 400 (Seward medical, London, England). Serial dilutions of fruit homogenates were poured in plate count agar (PCA; Biokar Diagnostics. Beauvais, France) at 7ºC ± 1ºC for 7 days for psychrophilic aerobic bacteria counts (ISO 4833, 1991) and chloramphenicol glucose agar (GCA) at 25ºC ± 1ºC for 5 days for yeast and mold counts (ISO 7954, 1988).

Sensory analyses

Sensory analyses of coated or uncoated pear wedges stored under passive modified atmospheres were carried out at 1 and 14 days of storage to evaluate consumer acceptability. Thirty consumers, aged between 20 and 65 years old, were recruited among students and personnel of the Department of Food Technology, University of Lleida. The panelists evaluated the acceptability of the samples from the point of view of odor, color, taste, and firmness using 10-cm non-structured linear scales, where 0 indicated extreme dislike and 10 indicated extreme like. Results were compared with those obtained for freshly processed samples.

Statistical analysis

Significance of the results and statistical differences were analyzed using The Statgraphics Plus v.5.1 Windows package (Manugistics, Inc., Rockville, MA, USA). Analysis of variance (ANOVA) was performed to compare mean values of different coatings and control samples. The Duncan multiple range test was applied to determinate differences among means at a 5% significance level.

Results and Discussion

WVR of coatings

Fig. 1 shows the water vapour resistance values of alginate-, gellan- or pectin-coated fresh-cut 'Flor de Invierno' pears. WVR of coated pear wedges was significantly higher than that of uncoated fruit pieces (0.117 s/m). Pectin- and alginate-coated samples had an increased WVR of 0.16-0.19 and 0.16-0.18 s/m, respectively, whereas gellan-coated pear pieces had the highest WVR of 0.23-0.24 s/m. The increase in WVR of coated fresh-cut pears is due to the hydrophobic nature of sunflower oil added into formulations. Rojas-Graü et al. (2007a) demonstrated the effect of the

incorporation of sunflower oil at 0.025% concentration into alginate or gellan-based formulations in increasing the WVR of fresh-cut 'Fuji' apples. These authors, in accordance with our results, observed that the increase in WVR was greater when sunflower oil was incorporated in gellan than in alginate formulations. A slight decrease in WVR was observed when N-acetylcysteine and glutathione were incorporated in alginate- or pectin-based coatings (Fig. 1). According to Ayranci and Tunc (2003), the inclusion of antioxidants such as ascorbic acid or citric acid in methylcellulose, in addition to stearic acid, lowers the hydrophobicity of the coatings.

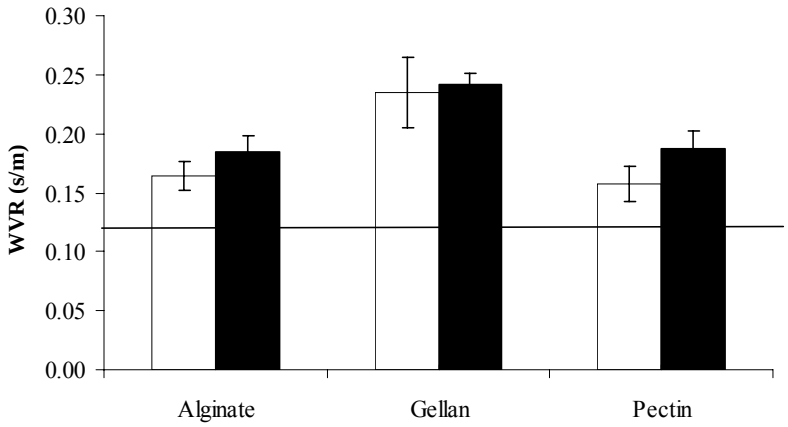

Figure 1. Effect of polysaccharide based coatings on the resistance of fresh-cut 'Flor de Invierno' pears to water vapor. Coating formulations including N-acetylcysteine and glutathione (□) or without antioxidant addition (■). WVR average value of uncoated pear wedges: 0.117 ± 0.004 s/m (——).

Changes in package headspace gas composition

Changes in O_2 and CO_2 package headspace concentrations are shown in Fig. 2. The general trend is that the O_2 concentrations decreased up to 5-8 kPa while CO_2 concentrations increased up to 15-20 kPa. Alginate and gellan-based formulations were also tested by Rojas-Graü et al. (2008). These authors also observed significant variations in O_2 and CO_2 package headspace concentrations of both coated and uncoated fresh-cut 'Fuji' apples along the storage time. Thus, polysaccharide coatings seem not be an effective barrier to O_2 and CO_2 diffusion. On the other hand, N-acetylcysteine and glutathione included in coating formulations may reduce O_2 exchange since gas concentrations were slightly higher in coated pear wedges containing N-acetylcysteine and glutathione than in those coated samples not treated with antibrowning agents (Fig. 2a). Ayranci and Tunc (2003) used limited amounts of ascorbic acid and citric acid in methylcellulose films plasticized with polyethylene glycol, to lowering the O_2 permeability. In addition, CO_2 accumulation was similar in coated and uncoated samples (Fig. 2b). Wong et al. (1994b) also investigated the effect of various bilayer coatings (alginate and pectin included) on respiratory activity of coated apple pieces measuring internal CO_2 and ethylene production in fruit tissue. In contrast with our results, they observed a reduction in the rate of CO_2 evolution and about 90% decrease in ethylene in apple pieces coated with a polysaccharide/lipid compared with uncoated samples. This was explained due to a substantial modification of the internal fruit atmosphere during the first 24 hours, which was attributed to diffusion barrier properties of the polysaccharide/lipid bilayer.

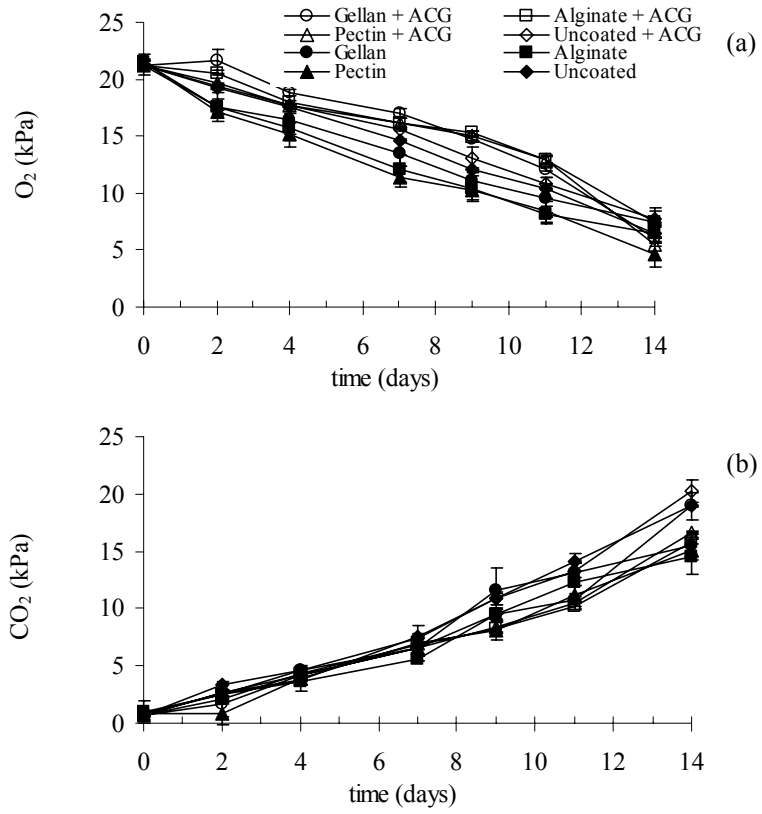

Figure 2. O_2 and CO_2 package headspace concentrations of fresh-cut 'Flor de Invierno' pears coated with gellan, alginate, pectin or uncoated samples during 14 days at 4 ºC. Coating formulations including N-acetylcysteine and glutathione (+ ACG). Data shown are mean ± standard deviation.

Fig. 3 shows the changes in ethylene concentrations in the package headspace of fresh-cut 'Flor de Invierno' pears during storage. The accumulation of ethylene in package headspace was non-significant in pear wedges treated with coatings containing N-acetylcysteine and glutathione during the first week of storage. Gas concentrations increased beyond that period, but achieving low ethylene concentrations (≤ 16 kPa). On the other hand, ethylene accumulation was observed in uncoated and coated pear wedges not including antioxidants from the beginning of storage, reaching maximum ethylene concentrations of 45 $\mu l \cdot l^{-1}$ in packages of uncoated samples after 14 days (Fig. 3). Thus, an inhibitory effect of polysaccharide-based coatings on ethylene synthesis of

fresh-cut pears was observed. Some authors have suggested that the reduction of the initial respiration rate and ethylene production of coated fresh-cut apples may be due to calcium ions contained in the film forming solution rather than the effect of oxygen barrier properties of coatings (Wong et al., 1994b; Lee et al., 2003). Calcium ion (Ca^{2+}) is involved in the regulatory function of many enzyme actions in cellular and physiological processes (Wong et al., 1994b). However our results show that N-acetylcysteine and glutathione could also have reduced the synthesis of ethylene on pear wedges. Rojas-Graü et al. (2007b) found that a dip in an N-acetylcysteine solution reduced significantly the ethylene production of uncoated fresh-cut 'Fuji' apples compared with the use of ascorbic acid. The action mechanism of thiol-containing compounds on ripening process and ethylene inhibition is not clear. Frenkel (1976) observed that the onset of ripening is influenced by a decline of sulphydryl gradient in fruit. These authors demonstrated that SH compounds such as cysteine or dithiothreitol can retard ripening of 'Bartlett' pears.

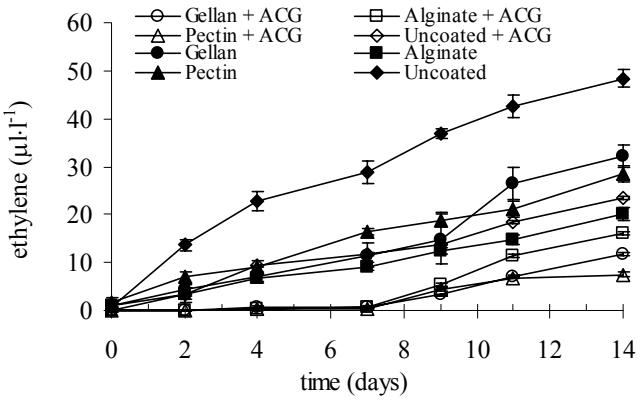

Figure 3. Ethylene package headspace concentrations of fresh-cut 'Flor de Invierno' pears coated with gellan, alginate, pectin or uncoated samples during 14 days at 4 ºC. Coating formulations including N-acetylcysteine and glutathione (+ ACG). Data shown are mean ± standard deviation.

Ethanol accumulation was not observed in both uncoated and coated pear wedges without N-acetylcysteine and glutathione. On the other hand, ethanol began to accumulate in the package headspace of pear wedges coated with antioxidants after 11 days of storage (Fig. 4). Although the amount of ethanol in packages of gellan-coated 'Flor de Invierno' pear wedges was 2-fold higher

than that of alginate- and pectin-coated samples, ethanol concentrations were not high enough to confer off-flavors and to be detrimental to quality. According to Ayranci and Tunc (2003), the incorporation of antioxidants into coatings may reduce the O_2 diffusion through edible coatings. Thus, internal O_2 concentrations may be low enough in pears coated with N-acetylcysteine and glutathione to promote the synthesis of volatiles associated with anaerobic pathways after 1 week. Rojas-Graü et al. (2008) also observed a higher accumulation of acetaldehyde and ethanol in packages of alginate and gellan coated apple wedges containing 1% w/v N-acetylcysteine than uncoated samples.

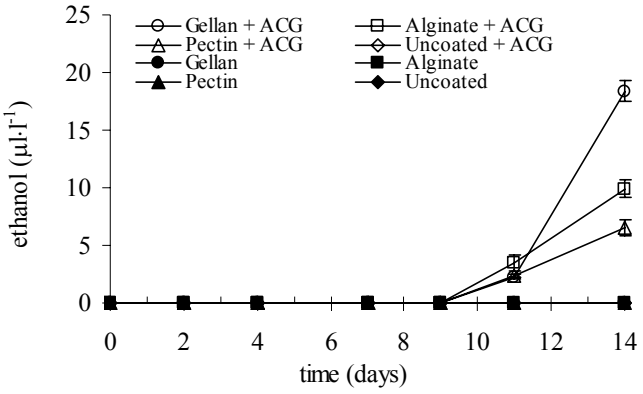

Figure 4. Ethanol package headspace concentrations of fresh-cut 'Flor de Invierno' pears coated with gellan, alginate, pectin or uncoated samples during 14 days at 4 ºC. Coating formulations including N-acetylcysteine and glutathione (+ ACG). Data shown are mean ± standard deviation.

Antioxidant properties

Both forms of ascorbic acid and dehydroascorbic acid have the vitamin property. In this study, the loss of vitamin C does not represent the oxidation of ascorbic acid to dehydroascorbic acid but the conversion of dehydroascorbic acid to diketogulonic acid. Initial vitamin C of both coated and uncoated fresh-cut 'Flor de Invierno' pears was 4.4 / 100 g fw. The use of polysaccharide based edible coatings including antibrowning agents reduced significantly vitamin C loss of fresh-cut pears during more than one week (Fig. 5a). After 11 days, vitamin C content in samples coated with polysaccharide-based edible coatings containing N-acetylcysteine and glutathione was 3.6-3.7 mg / 100 g fw whereas vitamin C in uncoated or coated pieces without incorporation of

antioxidants was 2.3-3.0 mg / 100 g fw. Since vitamin C loss can be greatly favored by the presence of O_2, the incorporation of N-acetylcysteine and glutathione to coating formulations may reduce O_2 diffusion and consequently better preserved vitamin C content of fresh-cut pears. Our results are consistent with others obtained in mushrooms and cauliflower. It was found that methyl cellulose-based edible coating containing ascorbic acid and citric acid reduced the vitamin C loss of both commodities (Ayranci and Tunc, 2003).

Total phenolic content of pear wedges was significantly higher in coated or uncoated samples containing N-acetylcysteine and glutathione (50 mg / 100g fw) than those samples not treated with the antioxidants (40 mg / 100g fw). Concentrations were maintained relatively constant during the whole storage period (Fig. 5b). The addition of thiol-containing compounds as antibrowning agents could be responsible for the overestimation of total phenolic content. In fact, the Folin-ciocalteu assay has been reported to include contribution from L-ascorbic acid, reducing sugars, soluble proteins and other substances (Prior et al., 2005).

Changes during storage in the percentage of DPPH radical inhibited by antioxidants present in fresh-cut pears are shown in Fig. 5c. The use of polysaccharide-based edible coatings by themselves does not seem to substantially contribute to enhance antioxidant capacity of fresh-cut pears. On the other hand, the incorporation of N-acetylcysteine and glutathione best maintain the antioxidant capacity of both uncoated and coated pear wedges. Although the antioxidant capacity of pears has been correlated well with the content of chlorogenic acid, the most common phenol found in pears (Galvis-Sánchez et al., 2003), the compounds formed by the partial oxidation of polyphenols (Nicoli et al., 1999) or the incorporation of antioxidants such as N-acetylcysteine or glutathione may have a higher contribution to radical scavenging activity of fresh-cut fruits.

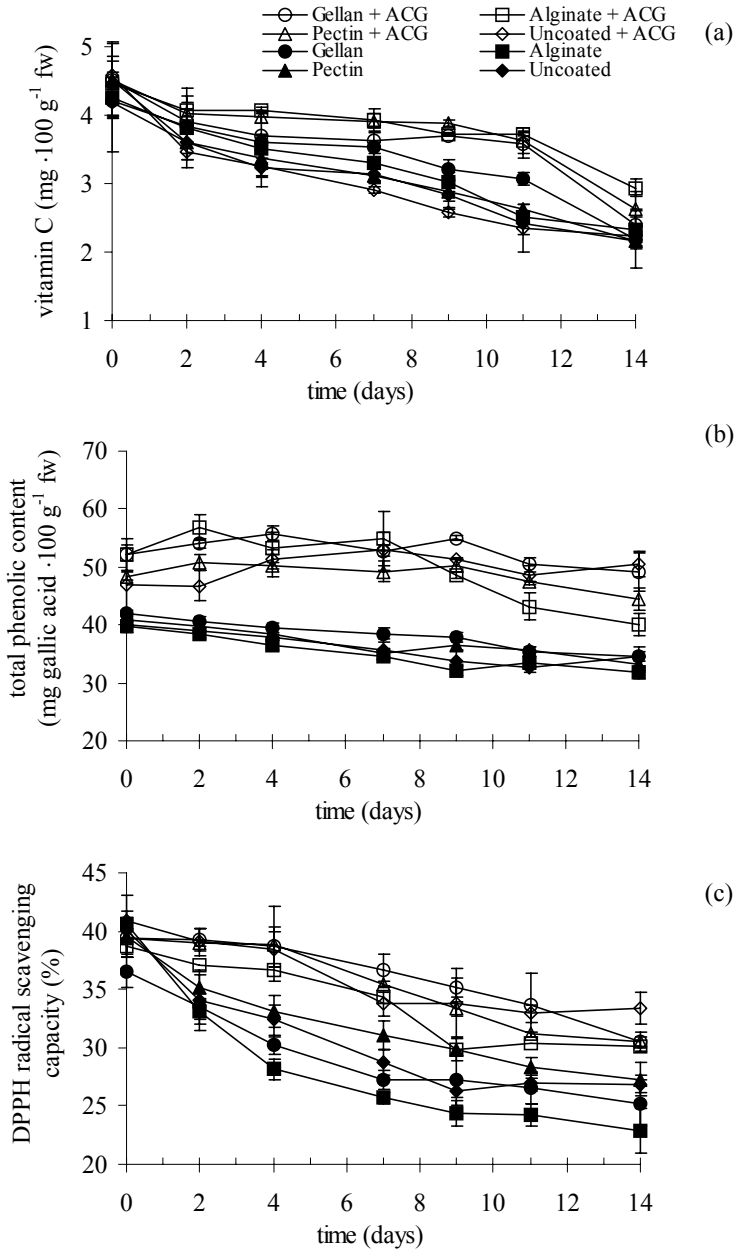

Figure 5. Antioxidant potential of fresh-cut 'Flor de Invierno' pears coated with gellan, alginate, pectin or uncoated samples during 14 days at 4 ºC. Coating formulations including N-acetylcysteine and glutathione (+ ACG). Data shown are mean ± standard deviation.

Color and firmness

Fig. 6a shows a substantial decrease in h^0 values, associated with browning development, on coated or uncoated fresh-cut 'Flor de Invierno' pears not treated with antioxidants. On the other hand, a dipping treatment with N-acetylcysteine (0.75% w/v) and glutathione (0.75% w/v) prevented browning of pear wedges throughout storage. The incorporation of these antioxidant substances into alginate, gellan or pectin coating formulations was also effective to avoid browning. N-acetylcysteine and glutathione incorporated into coating formulations contributed to maintain the initial h^0 values on fresh-cut 'Flor de Invierno' pears during storage period. Rojas et al. (2007b) observed a substantial decrease in h^0 values during the first 48 h after coating apple pieces with alginate and gellan, while the incorporation of N-acetylcysteine or glutathione in concentrations around 1% w/v into coatings effectively prevented browning. Olivas et al. (2003) also reported the positive effect of the incorporation of some additives (ascorbic acid, calcium chloride and sorbic acid) into methylcellulose and methylcellulose-stearic acid coatings on the browning control of fresh-cut 'Anjou' pears.

Losses in texture and a consequent drop in consumer acceptability are the most noticeable changes occurring in 'Flor de Invierno' pears during prolonged storage in a controlled atmosphere (Varela et al., 2007). However, our previous studies have shown that 'Flor de Invierno' pear wedges, at adequate ripeness stage and packaged under traditional passive atmosphere or active modified atmosphere (2.5 kPa O_2 + 7 kPa CO_2), exhibited good firmness retention for 35 days at 4 ºC (Oms-Oliu et al., 2007). In the present study, firmness of both coated and uncoated fresh-cut 'Flor de Invierno' was maintained throughout the storage (Fig. 6b). Several authors have reported the beneficial effect of the incorporation of calcium chloride into coatings on firmness retention of fresh-cut fruit, especially those commodities which exhibit a substantial softening of tissues (Lee et al., 2003; Olivas et al., 2007; Rojas et al., 2008). However, results of this study show that texture of uncoated fresh-cut pears was retained over the storage period and thus, the use of calcium chloride seem not to be necessary to maintain firmness of fruit wedges.

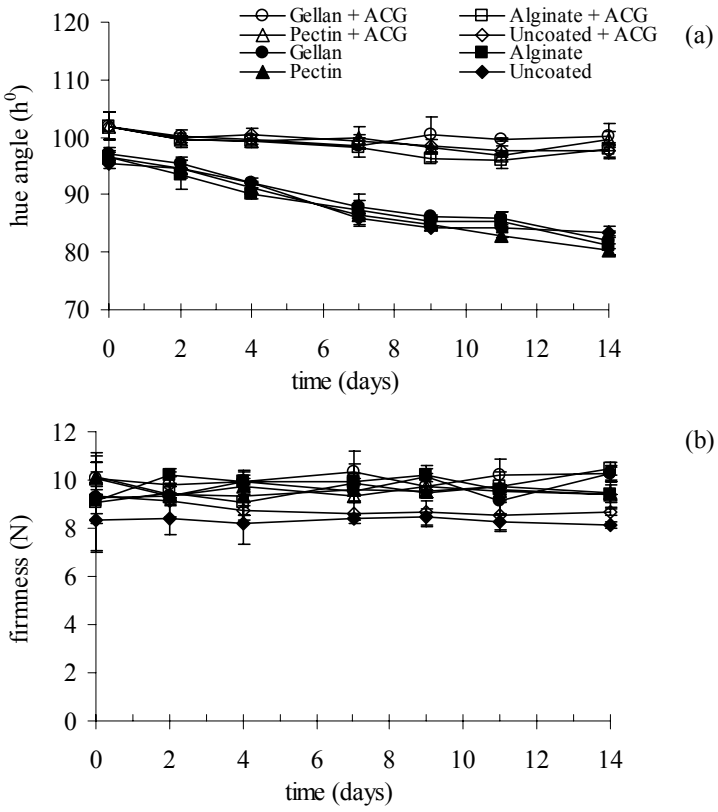

Figure 6. Changes in hue angle (a) and firmness (b) of fresh-cut 'Flor de Invierno' pears coated with gellan, alginate, pectin or uncoated samples during 14 days at 4 ºC. Coating formulations including N-acetylcysteine and glutathione (+ ACG). Data shown are mean ± standard deviation.

Microbial stability

Fig. 7 shows growth of aerobic psychrotrophic and yeast and molds microorganisms on fresh-cut 'Flor de Invierno' pears coated with alginate, gellan, pectin or uncoated fruit wedges. Microbial counts on pear wedges coated with different polysaccharide-based coatings did not differ from uncoated samples. However, the incorporation of N-acetylcysteine and glutathione into coating formulations or a dipping of pear wedges into an antioxidant solution slightly decreased microbial counts. In these samples, aerobic psychrophilic microorganisms did not exceed 5 log CFU g^{-1} after 14 days (Fig. 7a). Lee et al. (2003) reported consistent results for fresh-cut apples coated with

various types of carbohydrate polymers and whey protein concentrate, using ascorbic acid, citric acid and oxalic acid as antibrowning agents. In fresh-cut 'Fuji' apples, Rojas-Graü et al. (2008) observed that alginate and gellan coatings, containing N-acetylcysteine as antibrowning agent, had a marked effect on reducing mesophilic and psychrophilic counts. The incorporation of N-acetylcysteine and glutathione into coating formulations may limit microbial growth. According to Raybaudi-Massilia et al. (2007), microbial loads of uncoated fresh-cut apples dipped into N-acetylcysteine (1% w/v) + glutathione (1% w/v) + calcium lactate (1% w/v) solution were reduced compared with untreated fresh-cut apples.

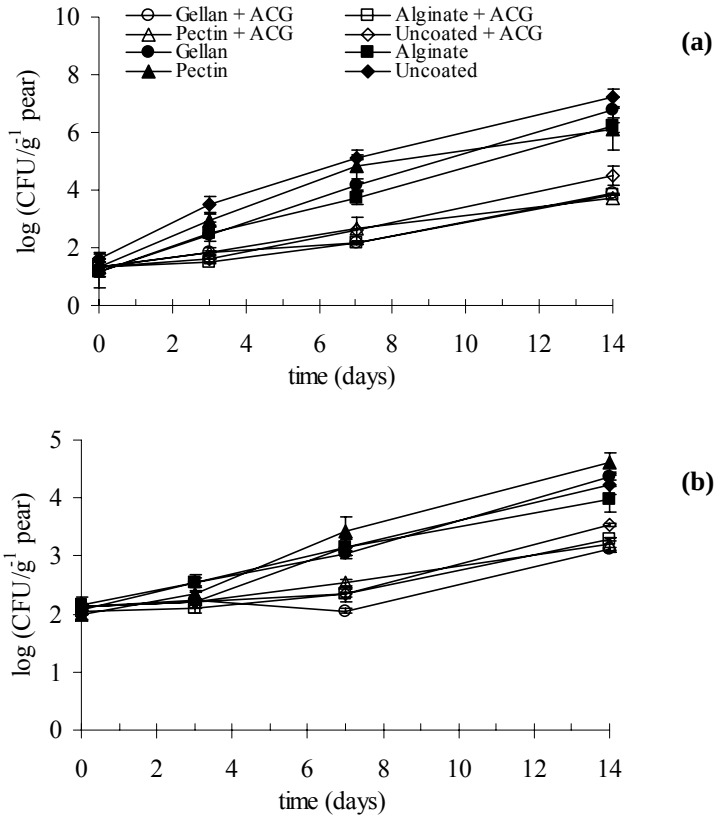

Figure 7. Growth of aerobic psychrotrophic (a) and yeast and molds microorganisms (b) on fresh-cut 'Flor de Invierno' pears coated with gellan, alginate, pectin or uncoated samples during 14 days at 4 ºC. Coating formulations including N-acetylcysteine and glutathione (+ ACG). Data shown are mean ± standard deviation.

Sensory quality

Changes in sensory attributes including odor, color, firmness and taste of coated and uncoated fresh-cut 'Flor de Invierno' pears during storage are shown in Fig. 8.

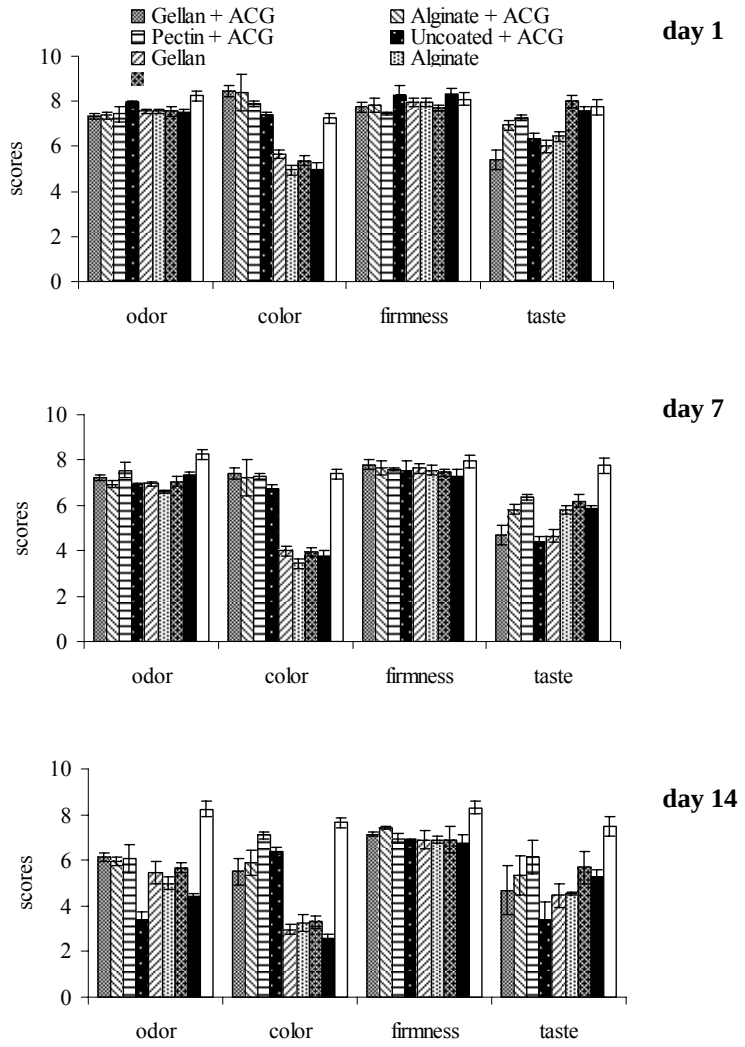

Figure 8. Sensory characteristics of fresh-cut 'Flor de Invierno' pears coated with gellan, alginate, pectin or uncoated pear wedges during 14 at 4 ºC. Coating formulations including N-acetylcysteine and glutathione (+ ACG). Data shown are mean ± standard deviation.

Coated fresh-cut pears scored similar to uncoated samples for odor and firmness attributes at day 1. Softening of fresh-cut pears did not appear to be perceived differently by panelists and storage time did not affect substantially to firmness scores. On the other hand, odor scores for coated pear wedges were higher than for uncoated samples (≤ 5), but substantially lower than those for fresh samples (≥ 8) after 14 days. In addition, odor attributes of coated pear wedges is related to a good retention of volatile compounds. Olivas et al. (2007) suggested that high amount of volatile compounds in apples coated with alginate could indicate that the coatings acted as good barriers, thus reducing loss of volatiles, or affecting the metabolism of volatile production. A dipping treatment consisting into N-acetylcysteine and glutathione solution or their incorporation into coating formulations prevented enzymatic browning reducing color changes for 14 days. Color scores for fruit pieces coated with pectin including the antioxidants or samples dipped into N-acetylcysteine and glutathione solution were higher than 6.

Taste scores of fresh-cut pear wedges significantly decreased during storage compared with fresh pears. However, pectin coating by itself did not promote an initial taste modification of pear wedges compared with uncoated samples. In addition, sulfur-containing compounds incorporated into alginate or pectin coating formulations seem not be detected by panelists. These substances could be more easily detected when fruit pieces were dipped into an antioxidant solution.

Conclusions

The use of polysaccharide-based edible coatings increased substantially the coating water vapor resistance. In addition, the incorporation of N-acetylcysteine and glutathione into gellan, alginate or pectin formulations not only helped to control enzymatic browning but also retarded the microbiological deterioration and resulted in a reduction of ethylene production of fresh-cut pears. The best maintenance of vitamin C and the increased phenolic content observed with the use of coatings containing N-acetylcysteine and glutathione could contribute to maintain higher antioxidant potential of fresh-cut pears compared with uncoated or coated fruits without incorporation of antioxidants. In addition, alginate- or pectin-based coatings containing the antibrowning agents were effective in keeping sensory quality for 2 weeks.

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References

Ames, B.M.; Shigena, M.K.; Hagen, T.M. 1993. Oxidants, antioxidants and the degenerative diseases of aging. *Proceedings of the National Academay of Science*, 90: 7915-7922.

Ayranci, E.; Tunc, S. 2003. A method for the measurement of the oxygen permeability and the development of edible films to reduce the rate of oxidative reactions in fresh foods. *Food Chemistry*, 80: 423-431.

Baldwin, E.A.; Nisperos, M.O.; Chen, X.; Hagenmaier, R.D. 1996. Improving storage life of cut apple and potato with edible coating. *Postharvest Biology and Technology*, 9: 151-163.

Dong, X.; Wrolstad, R.E.; Sugar, D. 2000. Extending shelf life of fresh-cut pears. *Journal of Food Science*, 65: 181-185.

Eccher Zerbini, P.; Rizzoli, A.; Brambilla, A.; Cambiaghi, P.; Grassi, M. 2002. Loss of ascorbic acid during storage of Conference pears in relation to the appearance of brown heart. *Journal of Science and Food Agriculture*, 82: 1007-1013.

Elez-Martínez, P.; Martín-Belloso, O. 2007. Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Food Chemistry*, 102: 201-209.

Frenkel, C. 1976. Regulation of ripening in Bartlett pears with sulfhydryl reagents. *Botanical Gazzete*, 137: 154-159.

Galvis-Sánchez, A.C.; Gil-Izquierdo, A.; Gil, M.I. 2003. Comparative study of six pear cultivars in terms of their phenolic and vitamin C contents and antioxidant capacity. *Journal of Science and Food Agriculture*, 83: 995-1003.

Gorny, J.R.; Hess-Pierce, B.; Cifuente, R.A.; Kader, A.A. 2002. Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest Biology and Technology*, 24: 271-278.

Kays, S.J. (Ed.), 1991. Metabolic processes in harvested products. Postharvest physiology of perishable plant products. Van Nostrand Reinhold, New York, pp. 75-142.

King, A.H. 1983. Brown seed extracts (Alginates). *Food Hydrocolloids*, 2: 115-188.

Lee, J.Y.; Park, H.J.; Lee, C.Y.; Choi, W.Y. 2003. Extending shelf-life of minimally processed apples with edible coatings and antibrowning agents. *LWT-Food Science and Technology*, 36: 323-329.

Martín-Belloso, O.; Soliva-Fortuny, R.; Oms-Oliu, G. 2007. Fresh-cut fruits. In: Hui, Y.H. (Ed.), *Handbook of food products manufacturing. Principles, Bakery, Beverages, Cereals, Cheese, Confectionary, Fats, Fruits, and Functional Foods*. John Wiley & Sons, New Yersey, pp. 879-899.

Martínez, V.M.; Whitaker, J.R. 1995. The biochemistry and control of enzymatic browning. *Trends in Food Science and Technology*, 6: 195-200.

McHugh, T.H.; Senesi, E. 2000. Apple wraps: A novel method to improve the quality and extend the shelf life of fresh-cut apples. *Journal of Food Science*, 65: 480-485.

Nguyen-The, C.; Carlin, F. 1994. The microbiology of minimally processed fresh fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, 34: 371-401.

Nicolas, J.J.; Richard-Forget, F.C.; Goupy, P.M.; Amiot, M.J.; Aubert, S. 1994. Enzymatic browning reactions in apple and apple products. *Critical Reviews in Food Science and Nutrition*, 34: 109-157.

Nicoli, M.C.; Anese, M.; Parpinel, M. 1999. Influence of processing on the antioxidant properties of fruit and vegetable. *Trends in Food Science and Technology*, 10: 94-100.

Nísperos-Carriedo, M.O. 2004. Edible coatings and films based on polysaccharides. In: Krochta, J.M.; Baldwin, E.A.; Nísperos-Carriedo, M.O. (Eds.), *Edible coatings and films to improve food quality*, Technomic Publishing Co., Switzerland, pp. 305-335.

Nussinovitch, A. (Ed.). 1997. Agricultural uses of hydrocolloids. *Hydrocolloid applications: Gum technology in the food and other industries*. Blackie Academic and Professional, London, pp. 169-189.

Odriozola-Serrano, I.; Hernández-Jover, T.; Martín-Belloso, O. 2007. Comparative evaluation of UV-HPLC methods and reducing agents to determine vitamin C in fruits. *Food Chemistry*, 105: 1151-1158.

Olivas, G.I.; Rodriguez, J.J.; Barbosa-Cánovas, G.V. 2003. Edible coatings composed of methylcellulose stearic acid, and additives to preserve quality of pear wedges. *Journal of Food Processing and Preservation*, 27: 299-320.

Olivas, G.I.; Mattinson, D.S.; Barbosa-Cánovas, G.V. 2007. Alginate coatings for reservation of minimally processed 'Gala' apples. *Postharvest Biology and Technology*, 45: 89-96.

Oms-Oliu, G.; Aguiló-Aguayo, I.; Martín-Belloso, O. 2006. Inhibition of browning on fresh-cut pear wedges by natural compounds. *Journal of Food Science*, 71: 216-224.

Oms-Oliu, G.; Aguiló-Aguayo, I.; Soliva-Fortuny, R.; Martín-Belloso, O. 2007. Effect of ripeness at processing on fresh-cut 'Flor de Invierno' pears packaged under modified atmosphere conditions. *International Journal of Food Science and Technology*, doi: 10.1111/j.1365-2621.2007.01635.x

Park, H.J. 1999. Development of advanced edible coatings for fruits. *Trends in Food Science and Technology*, 10: 254-260.

Prior, R. L.; Wu, X. L.; Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53 (10): 4290-4302.

Raybaudi-Massilia, R.M.; Mosqueda-Melgar, J.; Sobrino-López, A.; Soliva-Fortuny, R.; Martín-Belloso, O. 2007. Shelf-life extension of fresh-cut 'Fuji' apples at different ripeness stages using natural substances. *Postharvest Biology and Technology*, 45: 265-275.

Rhim, J.W. 2004. Physical and Mechanical properties of water resistant sodium alginate films. *LWT-Food Science and Technology*, 37: 323-330.

Rojas-Graü, M.A.; Tapia, M.S.; Rodríguez, F.J.; Carmona, A.J.; Martín-Belloso, O. 2007a. Alginate and gellan based edible coatings as support of antibrowning agents applied on fresh-cut Fuji apple. *Food Hydrocolloids*, 21: 118-127.

Rojas-Graü, M.A.; Grasa-Guillem, R.; Martín-Belloso, O. 2007b. Quality changes in fresh-cut Fuji apple as affected by ripeness stage, antibrowning agents, and storage atmosphere. *Journal of Food Science*, 72(1): 36-43

Rojas-Graü, M.A.; Tapia, M.S.; Martín-Belloso, O. 2008. Using polysaccharide-based edible coatings to maintain quality of fresh-cut Fuji apples. *LWT-Food Science and Technology*, 41 (1): 139-147

Sapers, G.M.; Miller, R.L. 1998. Browning inhibition in fresh-cut pears. *Journal of Food Science*, 63: 342-346.

Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299: 152-178.

Soliva-Fortuny, R.C.; Biosca-Biosca, M.; Grigelmo-Miguel, N.; Martín-Belloso, O. 2002a. Browning, polyphenol oxidase activity and headspace gas composition during storage of fresh-cut pears using modified atmosphere packaging. *Journal of Science and Food Agriculture*, 82: 1490-1496.

Soliva-Fortuny, R.C.; Grigelmo-Miguel, N., Hernando, I., Lluch, M.A., Martín-Belloso, O. 2002b. Effect of minimal processing on the textural and structural properties of fresh-cut pears. *Journal of Science and Food Agriculture*, 82: 1490-1496.

Tapia, M.S.; Rodríguez, F.J.; Rojas-Graü, M.A.; Martín-Belloso, O. 2005. Formulation of alginate and gellan based edible coatings with antioxidants for fresh-cut apple and papaya. *IFT Annual Meeting*. New Orleans, U.S.A., Paper 36E-43.

Varela, P.; Salvador, A.; Hernando, I.; Pérez-Munuera, I.; Quiles, A.; Lluch, M.A.A.; Fiszman, S.M. 2007. Eating quality of 'Flor de invierno' pears: chemical and structural aspects. *International Journal of Food Science and Technology*, 42: 1052-1058.

Winkler, B.S.; Orselli, S.M.; Rex, T.S. 1994. The redox couple between glutathione and ascorbic acid: a chemical and physiological perspective. *Free Radical Biology and Medicine*, 17: 333-349.

Wong, D.W.S.; Camirand, M.W.; Pavlath, A.E. 1994a. Development of edible coatings for minimally processed fruits and vegetables. In: Krochta, J.M., Baldwin, E.A., Nisperos-Carriedo, M.O. (Eds.), *Edible coatings and films to improve food quality*. Technomic Publishing Co., Switzerland, pp. 65-88.

Wong, D.; Tillin, S.J.; Hudson, J.S.; Pavlath, A.E. 1994b. Gas exchange in cut apples with bilayer coatings. *Journal of Agriculture and Food Chemistry*, 42, 2278-2285.

4.Discusión General

4. Discusión General

La modificación de la atmósfera de envasado de frutas frescas cortadas pretende limitar la actividad respiratoria y los procesos de anaerobiosis, además de obtener un producto microbiológicamente estable que conserve sus propiedades físico-químicas y organolépticas. En base a este planteamiento, se evaluaron distintas alternativas de envasado en atmósfera modificada para la conservación de melón y pera frescos cortados.

En primer lugar, se determinaron las condiciones óptimas de procesado de la materia prima, así como el efecto individual y combinado de distintos agentes antioxidantes de origen natural para el control del pardeamiento enzimático en pera fresca cortada. Seguidamente, se procedió a la evaluación de los efectos de distintas atmósferas de envasado en la actividad respiratoria, estabilidad microbiológica, atributos de calidad y propiedades antioxidantes de melón y pera frescos cortados. Se compararon sistemas tradicionales de envasado sin modificación de la atmósfera inicial o en concentraciones iniciales de O_2 bajas y CO_2 elevadas con concentraciones de O_2 altas. El estudio se complementó con la aplicación de diferentes recubrimientos comestibles a base de alginato, gelano y pectina en los trozos de melón y pera como una nueva estrategia de conservación de fruta fresca cortada.

4.1. MELÓN PIEL DE SAPO

4.1.2. Efecto del estado de madurez en la calidad final de melón fresco cortado

Se estudió la influencia del estado de madurez de los frutos destinados al procesado en la calidad de melón 'Piel de Sapo' fresco cortado mediante el empleo de distintas atmósferas de envasado e inmersión de la fruta troceada en disoluciones de ácido ascórbico (AA) y cloruro cálcico ($CaCl_2$). Se seleccionaron tres estados de madurez distintos (temprano, intermedio y avanzado) los cuales se caracterizaron en base al contenido de azúcares, acidez total, pH, color y firmeza. El contenido en sólidos solubles varió entre 11,1 a 14,3 ºBrix, dependiendo del estado fisiológico del fruto. Los frutos de madurez avanzada se caracterizaron por una disminución de su firmeza y acidez total, además de un aumento del pH respecto a los frutos de una madurez temprana. Los valores de firmeza disminuyeron de 6.5 a 3.9 N durante la maduración de los frutos. Esta tendencia se asoció a un ablandamiento de los tejidos como consecuencia de una degradación enzimática de los compuestos de las paredes celulares.

4.1.2.1. Evolución de la composición de la atmósfera de envasado

El estado de madurez del fruto ni la inmersión de los trozos de fruta en soluciones de AA y $CaCl_2$ no influyeron en la evolución de las concentraciones de oxígeno (O_2) en el espacio de cabeza de envases de melón fresco cortado durante un período de 35 días. En cambio, el tipo de atmósfera de envasado afectó significativamente a la evolución de las concentraciones de O_2. La conservación sin modificación de la atmósfera inicial conllevó una disminución gradual de las concentraciones de O_2 durante el almacenamiento, mientras que la conservación bajo concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 indujo un descenso brusco de las concentraciones iniciales de O_2, alcanzándose valores por debajo de 1 kPa al cabo de 10 días.

Durante los 15 primeros días de almacenamiento, la acumulación de dióxido de carbono (CO_2) no se vio influida por el estado de madurez del melón. Sin embargo, se observó una acumulación máxima de 45,5 kPa CO_2 al final del almacenamiento en melón de madurez avanzada envasado sin modificación de la atmósfera inicial. Esta mayor acumulación de gas podría ser debida al crecimiento microbiano y a un deterioro general del producto. Además, se demostró la efectividad de la inmersión de la fruta en una solución de $CaCl_2$ y AA para disminuir la producción de CO_2. Esta disminución se ha atribuido al tratamiento con sales de calcio a baja temperatura (Luna-Guzmán et al., 1999; Lamikanra y Watson, 2004).

Ni el estado de madurez ni la inmersión de la fruta en una solución de AA y $CaCl_2$ afectó significativamente a la acumulación de etileno en el espacio de cabeza de los envases. En cambio, la producción de etileno de muestras envasadas sin modificación de la atmósfera inicial fue mayor que la de muestras envasadas bajo concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2. No obstante, los valores máximos de etileno no superaron las 0,8 ppm, detectándose, en general, una actividad fisiológica baja de los frutos cortados. Trabajos de otros autores también muestran la baja actividad fisiológica del melón 'Piel de Sapo' en comparación con otros cultivares como el 'Cantaloupe' (Valdenegro et al., 2004).

Se ha sugerido el empleo de concentraciones mínimas de 1 kPa O_2 y máximas de 15 kPa CO_2 en melón fresco cortado para evitar condiciones de anaerobiosis en el envase y así mantener la calidad del producto (Bai et al., 2003). La producción de etanol es una consecuencia del metabolismo anaeróbico que se ve favorecido por las concentraciones bajas de O_2 y altas de CO_2. En nuestras condiciones de envasado, la acumulación de etanol se evidenció tras una semana de almacenamiento del melón fresco cortado con una atmósfera inicial de 2,5 kPa O_2 + 7 kPa CO_2,

mientras que la acumulación de gas en el envase se retrasó hasta los 15 días en muestras envasadas sin modificación de la atmósfera inicial. No obstante, independientemente de las condiciones de envasado, el melón fresco cortado de una madurez temprana acumuló elevadas concentraciones de etanol en los envases, sobretodo al final del período de almacenamiento. Esto podría ser debido a una mayor susceptibilidad del fruto de madurez temprana al estrés fisiológico bajo concentraciones muy bajas de O_2 y altas de CO_2. De hecho, estas condiciones se han relacionado con desórdenes fisiológicos y daños en la membrana celular (Lester, 2003). En cambio, la acumulación de etanol se redujo considerablemente en melón procesado en un estadio intermedio de maduración.

4.1.2.2. Evolución del color y firmeza

Los cambios de color durante el almacenamiento de melón 'Piel de Sapo' fresco cortado se deben en gran medida al desarrollo de translucidez, que se manifiesta a través de una disminución de la luminosidad (L*) y del índice de blancura (IB):

$$IB = 100 - \left[(100 - L^*)^2 + (a^*)^2 + (b^*)^2 \right]^{\frac{1}{2}} \qquad \text{(Ecuación 1)}$$

El desarrollo de translucidez también es la causa principal de deterioro de melón fresco cortado de las variedades 'Honeydew' y 'Cantaloupe' envasadas en atmósfera modificada (Bai et al., 2001; Bai et al., 2003; Aguayo et al., 2003; Aguayo et al., 2004; O'Connor-Shaw et al., 1994). Nuestros resultados demuestran que la translucidez podría ser un síntoma de senescencia atribuido a estados avanzados de madurez y a la modificación de la atmósfera inicial de envasado. Sin embargo, la inmersión de la fruta en una disolución de AA y $CaCl_2$ fue efectiva en la estabilización del color de melón 'Piel de Sapo' fresco cortado.

Inmersiones en disoluciones de $CaCl_2$ durante 1-5 min han demostrado ser eficaces en el mantenimiento de la firmeza de melón fresco cortado almacenado a 5 ºC (Luna-Guzmán et al., 1999). No obstante, la degradación de la textura de los trozos de fruta se ha relacionado estrechamente con el estado de madurez del fruto en el momento del procesado. Los trozos de melón procesados en una madurez temprana sufrieron un importante ablandamiento debido al metabolismo anaeróbico de sus tejidos que provocó una acumulación importante de etanol. Aunque concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 junto con la inmersión de la fruta en una solución de AA y $CaCl_2$ retrasaron el ablandamiento de melón procesado en una madurez

temprana o intermedia, su efecto no fue significativo en melón procesado en una madurez avanzada. Esto podría relacionarse con los procesos de maduración en los que la acción de las enzimas pécticas y liberación de calcio soluble se traduce en un debilitamiento de las membranas (Soliva-Fortuny, 2003a).

4.1.2.3. Evolución de la estabilidad microbiológica

La influencia del estado de madurez de melón fresco cortado en la evolución de microorganismos aerobios mesófilos, mohos y levaduras se estudió en función de las distintas condiciones de procesado y envasado. Además, los recuentos microbianos durante el almacenamiento se modelizaron mediante la ecuación de Gompertz (Ecuación 2), que define cuatro parámetros fundamentales: carga inicial (k), crecimiento máximo alcanzado en la fase estacionaria (A), velocidad máxima de crecimiento (μ_{max}) y fase de latencia (λ).

$$y = k + A\exp\left\{-\exp\left[\left(\frac{\mu_{max} e}{A}\right)\cdot(\lambda - t)+1\right]\right\} \qquad \text{(Ecuación 2)}$$

Los recuentos de mohos y levaduras en el momento de envasado oscilaron entre 10 y 10^2 ufc g^{-1}, independientemente del estado de madurez de la fruta procesada. Sin embargo, se observaron recuentos superiores de microorganismos aerobios de aproximadamente 10^3 ufc g^{-1} en melón fresco cortado de una madurez temprana e intermedia, y de 10^6 ufc g^{-1} en melón de una madurez avanzada. Esta rápida proliferación de microorganismos aerobios en tejidos de madurez avanzada podría ser consecuencia del debilitamiento de las membranas que podría favorecer la presencia de exudados en las superficies cortadas, y la proliferación de los microorganismos.

En nuestras condiciones de trabajo se observó una rápida sustitución de la flora nativa, constituida por mohos y levaduras, por bacterias como consecuencia del procesado y envasado. La μ_{max} de los microorganismos aeróbicos mesófilos fue superior al de mohos y levaduras excepto para melón procesado en un estadio de madurez avanzado, debido a los elevados recuentos bacterianos iniciales. Bai et al. (2001) también observó en melón 'Cantaloupe' fresco cortado una mayor velocidad del crecimiento bacteriano que de mohos y levaduras.

En general, las concentraciones bajas de O_2 y altas de CO_2 inhiben el crecimiento microbiano. Además, el uso de $CaCl_2$ fue efectivo en el mantenimiento estructural de los tejidos, evitando exudados que favorecen la proliferación microbiana. No obstante, la modificación de la atmósfera y las disoluciones estabilizantes no inhibieron el crecimiento microbiano en los trozos de melón de

madurez avanzada. En cambio, las concentraciones bajas de O_2 y altas de CO_2 junto a la inmersión de la fruta de madurez temprana o intermedia en una solución de AA y $CaCl_2$ redujo significativamente la μ_{max}. También se observó cierto efecto del estado de madurez y las condiciones de envasado en la λ y el A. Los valores de λ para microorganismos aerobios mesófilos y hongos aumentaron cuando la fruta se procesó en una madurez temprana consiguiendo menores recuentos en fase estacionaria, sobretodo bajo concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 e inmersión de la fruta en AA y $CaCl_2$. La adición de calcio podría reforzar las estructuras celulares y evitar una descompartimentación de enzimas y sustratos que conllevaría un mayor intercambio de solutos y fluidos, promoviendo la proliferación microbiana.

4.1.2. Aplicación de distintas alternativas de EAM

4.1.2.1. Evolución de la actividad fisiológica

La respiración aeróbica y anaeróbica de melón 'Piel de Sapo' fresco cortado envasado sin modificación de la atmósfera inicial y bajo diferentes concentraciones iniciales de O_2 y CO_2 (2,5 kPa O_2 + 7 kPa CO_2 y 70 kPa O_2), se calculó a partir del procedimiento matemático descrito en el Capítulo II. En base a los valores predichos se estudió el efecto de diferentes atmósferas modificadas en la actividad respiratoria y en los cambios de calidad del melón fresco cortado durante 35 días a 4 ºC.

La tasa inicial de consumo de O_2 de melón fresco cortado envasado en una atmósfera inicial de 70 kPa O_2 fue tres veces superior a la observada en muestras envasadas sin modificación de la atmósfera o en concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2. La tasa de consumo de O_2 de melón almacenado en condiciones de 70 kPa O_2 fue disminuyendo durante el período de conservación aunque los valores observados fueron superiores a los de otras condiciones de envasado durante 21 días de almacenamiento. Durante los primeros 7-10 días, la tasa de producción de CO_2 fue menor en melón cortado almacenado en concentraciones iniciales de 70 kPa O_2 que en muestras envasadas e concentraciones de 2,5 kPa O_2 + 7 kPa CO_2. Por tanto, el consumo inicial elevado de O_2 en muestras envasadas en concentraciones iniciales de 70 kPa O_2 podría ser debido a reacciones de oxidación más que a la actividad respiratoria. Aunque el uso de niveles de O_2 bajos puede disminuir la respiración debido a una reducción de la actividad metabólica de los frutos (Fonseca et al., 2002), la conservación en concentraciones excesivamente bajas de O_2 podría producir daño fisiológico a los tejidos, lo cual explicaría la estimulación de la

tasa de respiración bajo dichas condiciones al principio del período de conservación. Lakakul et al. (1999) resaltaron la importancia de mantener niveles suficientes de O_2 en los envases para evitar daño en los tejidos y procesos de anaerobiosis.

Los coeficientes de respiración (CR) iniciales de los trozos de melón 'Piel de Sapo' fresco cortado oscilaron entre valores de 0,77 y 1,20 en muestras envasadas sin modificación de la atmósfera inicial o bajo concentraciones de 2,5 kPa O_2 + 7 kPa CO_2. En cambio, el CR presentó valores iniciales muy bajos a concentraciones de 70 kPa O_2, debido a procesos oxidativos en los tejidos que implican un importante consumo de O_2. Los valores normales de CR en la respiración aeróbica de frutos están comprendidos entre 0,7 y 1,3 (Kader et al., 1989). En cambio, en el metabolismo anaeróbico la producción de CO_2 es mucho mayor y los valores de CR son muy superiores a 1,0 (Fonseca et al., 2002). Nuestros resultados muestran valores de CR superiores a 1,3 a los 14 días de almacenamiento en muestras envasadas en 2,5 kPa O_2 + 7 kPa CO_2 o sin modificación de la atmósfera inicial. La producción de etanol en estas condiciones de envasado fue evidente a partir de las dos semanas de conservación. La producción de etanol detectada en atmósferas de envasado de 70 kPa O_2 después de tres semanas podría ser debida al estrés fisiológico sufrido por la célula durante un período largo de almacenamiento en concentraciones altas de O_2.

4.1.2.2. Evolución de la calidad del producto

Nuestros resultados mostraron que la disminución del IB como consecuencia del desarrollo de translucidez en el producto fue favorecida por las condiciones de anaerobiosis creadas en el espacio de cabeza de la fruta envasada en concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 después de 2-3 semanas. La translucidez en melón 'Cantaloupe' se ha relacionado con una acumulación de etanol, acetaldehído y etil acetato en el fruto (Flores et al., 2004). Concentraciones iniciales bajas de O_2 y altas de CO_2 también favorecieron el ablandamiento de los tejidos, mientras que concentraciones altas de O_2 mantuvieron la masticabilidad y la firmeza inicial de melón durante más de dos semanas. Así, concentraciones de 70 kPa O_2 podrían retrasar el metabolismo anaeróbico de los trozos de melón, y el desarrollo de olores y sabores desagradables, además de reducir el estrés por daño fisiológico debido a las concentraciones excesivamente bajas de O_2 y altas de CO_2. Aunque concentraciones altas de O_2 incrementaron el consumo de O_2 de melón fresco cortado respecto a las demás condiciones de envasado, éste no se relacionó con una disminución del contenido de sólidos solubles. Por tanto, el elevado consumo de O_2 se debió

principalmente a procesos de oxidación, más que a un mayor consumo de carbohidratos por la respiración del fruto.

4.1.2.3. Evolución de la estabilidad microbiológica

La producción de especies de O_2 reactivas en el producto bajo concentraciones altas de O_2 puede dañar los componentes celulares de las células y reducir la viabilidad de los microorganismos cuando el sistema de protección celular no puede recuperarse del estrés oxidativo que se ha producido (Escalona et al., 2006; Jacxsens et al., 2001; Kader y Ben-Yehoshua, 2000; Poubol y Izumi, 2005). Las poblaciones de levaduras fueron dominantes durante el almacenamiento de melón fresco cortado frente a las de mohos. La modificación inicial de la atmósfera de envasado mediante la inyección de concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 y 70 kPa O_2 no inhibieron el crecimiento de levaduras pero alargaron la fase de latencia y redujeron la velocidad de crecimiento durante las dos primeras semanas respecto a la conservación sin modificación de la atmósfera inicial. Se ha demostrado que bajo concentraciones altas de O_2, cuanto mayor es la concentración de O_2, mayor es la fase de latencia (Amanatidou et al., 1999). Las concentraciones iniciales de 70 kPa O_2 aumentaron la viabilidad de las poblaciones de levaduras a partir de las dos semanas, superándose recuentos de 10^5 UFC g^{-1} a los 21 días. En cambio, los recuentos de melón fresco cortado envasado bajo 2,5 kPa O_2 + 7 kPa CO_2 no superaron estos niveles durante el almacenamiento de 28 días a 5 ºC. Según Jacxsens et al. (1999), el deterioro de los productos parece ser detectable por los consumidores cuando el recuento de levaduras en frutos cortados supera los niveles de 10^5 UFC g^{-1}.

Aunque los recuentos de aerobios psicrófilos fueron máximos, 10^8 ufc g^{-1}, a partir de los 14 días de almacenamiento independientemente de la atmósfera de envasado, se observó una disminución de la tasa de crecimiento por concentraciones de 70 kPa O_2 y 2,5 kPa O_2 + 7 kPa CO_2 respecto a la conservación sin modificación de la atmósfera inicial durante las dos primeras semanas. El crecimiento de aerobios mesófilos y psicrófilos en lechuga fresca cortada también fue menor bajo 80 kPa O_2 que en atmósfera no modificada durante 8 días de conservación (Allende et al., 2003). Además, el efecto de concentraciones iniciales de 70 kPa O_2 en la inhibición del crecimiento de microorganismos aerobios en melón 'Piel de Sapo' cortado no difirió del de aquel con concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2. Este comportamiento se ha observado también en vegetales frescos cortados como endibias, no encontrándose diferencias en el

crecimiento de microorganismos aerobios psicrófilos entre el envasado convencional (3 kPa O_2 + 5 kPa CO_2) y el realizado bajo concentraciones altas de O_2 (95 kPa O_2 + 5 kPa CO_2) después de una semana de almacenamiento (Jacxsens et al., 2001). Según el cálculo de la vida útil, realizado mediante una modificación de la ecuación de Gompertz (Capítulo III), las atmósferas de envasado con un contenido inicial de 70 kPa O_2 y 2,5 kPa O_2 + 7 kPa CO_2 alargaron la vida útil de melón fresco cortada 10-14 días, mientras que sin modificación de la atmósfera inicial la vida útil fue de 7-10 días.

Los principales microorganismos que se aislaron de trozos de melón 'Piel de Sapo' fresco cortado fueron levaduras: *Rhodotorula mucilaginosa*, *Candida famata* y *Candida ciferii*. Al principio del almacenamiento, se observó una clara predominancia de *R. mucilaginosa* de alrededor un 90% de la población total de levaduras. No obstante, su viabilidad disminuyó durante el almacenamiento, ya que se vio claramente afectada por la conservación en atmósfera modificada. La reducción fue del 40%, 30% y 20% en muestras envasadas bajo concentraciones de 70 kPa O_2 y 2,5 kPa O_2 + 7 kPa CO_2 o sin la modificación de la atmósfera, respectivamente. En cubos de mango almacenados bajo concentraciones altas de O_2 se detectaron géneros similares de levaduras, los cuales demostraron ser también sensibles a las concentraciones altas de O_2 (Poubol y Izumi, 2005). La exposición a niveles de O_2 altos mantuvo los recuentos iniciales de *R. mucilaginosa* durante 10 días, disminuyendo en los días posteriores. Los recuentos finales fueron < 10^2 ufc g^{-1}, es decir por debajo del nivel mínimo de detección del método de siembra usado en el estudio. En cambio, concentraciones bajas de O_2 y altas de CO_2 redujeron la velocidad de crecimiento de *R. mucilaginosa* aunque los recuentos siguieron aumentando ligeramente después de los primeros 7 días. Bajo estas condiciones se detectó un incremento del recuento total de levaduras después de 14 días debido a la proliferación de otras levaduras menos sensibles a las concentraciones altas de O_2. La sensibilidad de los diferentes organismos al O_2 puede variar ya que algunos de ellos pueden desarrollar estrategias como la inducción de enzimas capaces de descomponer las especies de O_2 reactivas para así evitar su daño letal (Kader y Ben-Yehoshua, 2000).

4.1.2.4. Impacto sobre las propiedades antioxidantes

El melón 'Piel de Sapo' contiene una moderada concentración de vitamina C (41,7 - 48,7 mg 100 g^{-1}) y un contenido bajo en compuestos fénolicos (15,4 - 20 mg de ácido gálico 100 g^{-1}). La presencia de O_2 en los envases favoreció la degradación del contenido inicial de vitamina C y

compuestos fenólicos, mientras que los niveles de CO_2 no afectaron significativamente al contenido en compuestos antioxidantes. Además, se observó una relación entre la pérdida de compuestos bioactivos, vitamina C y compuestos fenólicos, y el aumento de la actividad de la enzima peroxidasa (POD), demostrándose así, su afinidad por ambos sustratos.

Los contenidos iniciales de vitamina C de melón fresco cortado envasado bajo concentraciones de 2,5 kPa O_2 + 7 kPa CO_2 fueron sustancialmente más elevadas que las del producto envasado en atmósferas con mayor contenido de O_2 (10 kPa O_2 + 7 kPa CO_2, 21 kPa O_2, 30 kPa O_2, 70 kPa O_2). No obstante, a pesar de la restricción de O_2 en el envase, las atmósferas con un bajo contenido en O_2 no evitaron la pérdida de vitamina C durante el almacenamiento, además de provocar un incremento de más del 40% de la actividad de la enzima POD. Concentraciones excesivamente bajas de O_2 y muy altas de CO_2 podrían provocar un aumento del estrés oxidativo que explicaría la actuación de enzimas como la ascorbato POD que catalizan la oxidación del AA (Pinto et al., 2001; Tudela et al., 2002). El daño oxidativo asociado a la acumulación de especies de O_2 reactivas como el superóxido ($O_2^{\cdot-}$) y peróxido de hidrógeno (H_2O_2), el cual actúa como sustrato de las reacciones catalizadas por la POD, puede causar la peroxidación de lípidos de las membranas celulares con la subsiguiente pérdida de su integridad. Las concentraciones altas de O_2 podrían disminuir el estrés fisiológico de los tejidos de melón fresco cortado y reducir los cambios de deterioro atribuidos a una elevada actividad de la enzima POD en el tejido.

La acumulación de compuestos fenólicos en trozos de melón a los 9 días de almacenamiento podría ser una respuesta al daño fisiológico, favorecido por las concentraciones excesivamente bajas de O_2 y altas de CO_2. Dichas condiciones podrían haber estimulado la actividad de la enzima fenilalanina amonio-liasa (PAL) con la consecuente producción de compuestos fenólicos (Salveit, 1997). Este incremento de la actividad enzimática podría producirse a través de la formación de especies de O_2 reactivas como consecuencia del estrés oxidativo (Reyes et al., 2006). Así, el mayor estrés oxidativo observado en muestras envasadas en concentraciones bajas de O_2 y altas de CO_2 explicaría la mayor producción de compuestos fenólicos. Se ha demostrado que la síntesis y acumulación de fenilpropanoides en diferentes tejidos es una respuesta a distintas situaciones de estrés (Kang y Saltveit, 2002).

El almacenamiento de melón cortado en atmósferas de 2,5 kPa O_2 + 7 kPa CO_2 o 10 kPa O_2 + 7 kPa CO_2 conllevó un mayor incremento de capacidad antioxidante que el envasado en concentraciones iniciales de 21, 30 o 70 kPa O_2. Después de 14 días de almacenamiento, la

capacidad antioxidante fue mayor en el producto envasado bajo concentraciones bajas de O_2, mientras que muestras almacenadas en 70 kPa O_2 mostraron la capacidad antioxidante menor. En nuestro estudio, la capacidad antioxidante se relacionó mejor con el contenido en compuestos fenólicos que con la vitamina C. De hecho, el incremento de la capacidad antioxidante fue consecuencia del incremento en compuestos fenólicos debido a condiciones de estrés. Algunos autores han relacionado la capacidad antioxidante con el contenido en AA de frutas cítricas (Del Caro et al., 2004). No obstante, un gran número de sustancias como flavonoides, ácidos fenólicos, aminoácidos, AA, tocoferoles y pigmentos contribuyen a las propiedades antioxidantes de frutas y vegetales (Piga et al., 2002).

4.1.3 Efecto de la aplicación de RC sobre la calidad de melón fresco cortado

Se estudió el empleo de recubrimientos comestibles (RC) de base polisacárida (alginato, gelano y pectina) como complemento al EAM con el fin de evaluar la vida útil de melón fresco cortado. Se estudiaron los cambios en la actividad respiratoria, color, textura, crecimiento microbiano, características sensoriales y propiedades antioxidantes de trozos de melón envasados en bandejas de plástico de permeancia baja al O_2 (110 cm^3 O_2 m^{-2} bar^{-1} d^{-1}).

Se estudió la resistencia al vapor de agua (RVA) del melón fresco cortado recubierto y sin recubrir. En general, los valores de RVA observados en trozos de melón recubiertos de gelano (23,35 s/cm), alginato (20,6 s/cm) y pectina (19,3 s/cm) fueron en todos los casos superiores a los observados en muestras sin recubrir, donde, en promedio, se observó una RVA de 10,5 s/cm. Este aumento de RVA de los trozos de melón recubiertos se atribuye al aceite de girasol añadido en las formulaciones, ya que se ha demostrado que las matrices formadas a partir de compuestos polisacáridos no proporcionan una protección efectiva frente a la pérdida de agua debido a su naturaleza hidrofílica (Ben-Yehoshua et al., 1985; Kester y Fennema, 1986; Wong et al., 1994).

4.1.3.1. Evolución de la actividad respiratoria

El efecto de la aplicación de RC elaborados a base alginato, gelano o pectina en la actividad respiratoria de trozos de melón se evaluó mediante la evolución de la composición gaseosa en la atmósfera interna y en el espacio de cabeza de los envases durante 15 días de almacenamiento. Nuestros resultados muestran que los RC aplicados no actuaron de barrera al intercambio de O_2 y CO_2 de los trozos de melón puesto que se observó una importante modificación de la atmósfera del

espacio de cabeza en las muestras recubiertas. Estos cambios pueden atribuirse al intercambio gaseoso a través del tejido y a la permeancia baja al O_2 y CO_2 de la película plástica de envasado. En muestras de gelano, la importante modificación de la atmósfera interna puede ser debida al incremento de la actividad respiratoria respecto al resto de muestras.

Como ya se había observado en estudios anteriores, la acumulación de etileno en el espacio de cabeza de los envases fue casi despreciable en muestras recubiertas y sin recubrir. No obstante, se pudo apreciar una acumulación de gas en el interior de los trozos de melón, observándose una menor producción de etileno en las muestras recubiertas que en las no recubiertas. Li y Barth (1998) atribuyeron la síntesis de etileno a la respuesta al stress de los tejidos como consecuencia de los daños sufridos durante el procesado.

4.1.3.2. Impacto sobre las propiedades antioxidantes

La evolución de la composición gaseosa durante el almacenamiento afectó el contenido de vitamina C de melón fresco cortado. Así, las muestras de gelano que sufrieron un mayor descenso de la concentración de O_2 en el espacio de cabeza y en el interior del fruto, mantuvieron contenidos de vitamina C más elevados.

Como ya se ha discutido previamente, la capacidad antioxidante de melón 'Piel de Sapo' fresco cortado aumentó durante su almacenamiento debido a la síntesis de compuestos fenólicos como respuesta al estrés fisiológico de los tejidos. La aplicación de alginato y pectina disminuyó la acumulación de estos compuestos respecto a las muestras sin recubrir. En cambio, en el caso de muestras de gelano, la síntesis se puede ver favorecida por una modificación excesiva de la atmósfera interna de los frutos.

4.1.3.3. Evolución de la estabilidad microbiológica, propiedades físico-químicas y sensoriales

La aplicación de recubrimientos de alginato, gelano o pectina no alargó significativamente la vida útil de trozos de melón 'Piel de Sapo' fresco cortado. Durante la segunda semana los recuentos de mohos y levaduras superaron niveles de 10^5 UFC g^{-1} y el recuento de microorganismos aerobios psicrófilos excedió el límite de 10^7 UFC g^{-1}, pudiendo ser fácilmente detectable el deterioro del producto. De acuerdo con estos resultados y otros anteriores, la vida útil de trozos de melón 'Piel de Sapo' recubiertos o sin recubrir y envasados sin modificación de la atmósfera inicial se

estableció en 7-10 días. Por tanto, en este estudio, se decidió llevar a cabo el análisis sensorial durante 1 semana de almacenamiento.

La aplicación de un recubrimiento de pectina y alginato no modificó de forma apreciable las características sensoriales de los trozos de melón. Los recubrimientos de pectina y alginato mantuvieron el aspecto visual de las muestras durante 1 semana debido probablemente a una menor deshidratación superficial. En cambio, los RC a base de gelano causaron cambios importantes en el color, olor y sabor. Los cambios de color se asociaron a una disminución del IB como consecuencia de translucidez, síntoma de senescencia de los tejidos. Los resultados del análisis sensorial indicaron que la aplicación de RC en trozos de melón contribuyó al mantenimiento de su firmeza inicial. Estos resultados coinciden con los obtenidos instrumentalmente, en los que se observó que los trozos de melón recubiertos mantenían una firmeza mayor que aquellos sin recubrir. El efecto de los RC en el mantenimiento de la firmeza de las muestras se podría asociar al control que ejercen éstos sobre la pérdida de humedad y mantenimiento de la turgencia de los tejidos. No obstante, Olivas et al. (2007), observaron que el ablandamiento de manzana fresca cortada se atribuía más a una degradación de la pared celular que a la disminución de turgencia de los frutos. Por tanto, la presencia de iones calcio en las formulaciones de los RC podría contribuir en la mejora de la firmeza de la fruta cortada.

4.2. PERA FLOR DE INVIERNO

4.2.1. Variación de la calidad de pera fresca cortada con el estado de madurez en el momento del procesado

Los parámetros físico-químicos y fisiológicos de pera 'Flor de Invierno' fresca cortada evolucionaron de forma distinta en los diferentes estados de madurez (temprano, intermedio y avanzado), observándose un mayor contenido en sólidos solubles y una menor acidez al alcanzar la madurez avanzada. El color de piel y pulpa varió en función de la madurez del fruto, siendo remarcable el aumento progresivo de la coordenada a*, debido a la desaparición de la clorofila durante la maduración, quedando al descubierto otros colorantes que antes estaban enmascarados por dicho compuesto. Del mismo modo, durante la maduración, el ablandamiento de los tejidos fue aumentando, siendo la fuerza máxima de penetración cada vez menor. La firmeza de los frutos enteros disminuyó durante el proceso de maduración desde valores de 65,2 N hasta 36,1 N. La firmeza de los frutos de madurez intermedia en el momento del procesado fue de 43,3 N. En

general, los cambios fisiológicos y físico-químicos en la fruta son el resultado de la reestructuración metabólica y química que se desencadena dentro del fruto al madurar, destacándose un incremento de la tasa respiratoria, endulzamiento, ablandamiento y cambios en el aroma, coloración y valor nutritivo (Gorny et al., 2002).

Al aumentar el grado de madurez de la fruta entera, se observó un aumento progresivo de la producción de etileno en la atmósfera interna del tejido, debido probablemente al estado pre-climatérico del fruto, ya que el etileno es el inductor que estimula la mayoría de los mecanismos bioquímicos asociados a la maduración.

4.2.1.1. Evolución de parámetros fisiológicos

Se observó una disminución similar de las concentraciones de O_2 en el espacio de cabeza de los envases de pera procesada en diferentes estados de madurez, observándose, en general, el mismo comportamiento para cada una de las condiciones de envasado aplicadas. Durante las primeras dos semanas, la disponibilidad de O_2 en muestras envasadas sin modificación de la atmósfera fue superior a la de las muestras almacenadas con modificación de la misma (2,5 kPa O_2 + 7 kPa CO_2), aumentando la cantidad de CO_2 acumulada debido a la respiración aeróbica, sobretodo en muestras de madurez avanzada. No obstante, a partir de los 14 días se apreció una estabilización de las concentraciones de O_2, no detectándose diferencias significativas en los niveles de O_2 entre las diferentes condiciones de envasado. En pera 'Conference' fresca cortada envasada en una atmósfera inicial de 2,5 kPa O_2 + 7 kPa CO_2 y permeancia muy baja al O_2 de las películas plásticas (15 $cm^3\ m^{-2}\ d^{-1}\ bar^{-1}$ y 30 $cm^3\ m^{-2}\ d^{-1}\ bar^{-1}$), se observó un descenso brusco de las concentraciones de O_2 durante la primera semana de almacenamiento (Soliva-Fortuny et al., 2002), alcanzándose concentraciones por debajo de los valores definidos por Lakakul et al. (1999) como perjudiciales para la conservación del producto. En el presente estudio, el uso de películas plásticas de permeancia mayor al O_2 (110 $cm^3\ m^{-2}\ d^{-1}\ bar^{-1}$), podría favorecer el mantenimiento de niveles mínimos de O_2 en el interior del envase y evitar así condiciones de anaerobiosis.

Las concentraciones de CO_2 en el interior de los envases de pera fresca cortada aumentaron con el tiempo en todas las condiciones experimentales estudiadas. En general, la producción de CO_2 aumentó al avanzar el estado de madurez de los frutos ($p \leq 0.05$). Estos resultados concuerdan con los de Soliva-Fortuny et al. (2004) en pera 'Conference' fresca cortada. En dicho estudio, la acumulación de CO_2 fue mayor, ya que la permeancia de las películas plásticas a los gases eran

menores, y por lo tanto, la acumulación de concentraciones altas de CO_2 inhibían mucho antes la ruta aerobia (Gorny et al., 2002). A partir de la segunda semana de almacenamiento, los niveles de CO_2 fueron significativamente inferiores en muestras envasadas en concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 que en envases sin la modificación de la atmósfera. Este efecto fue claramente significativo en frutos cortados de madurez intermedia y avanzada mientras que las concentraciones de CO_2 alcanzadas en pera cortada de madurez temprana fueron similares en las dos condiciones de envasado. De acuerdo con nuestros resultados, la composición gaseosa de envases de pera cortada almacenados bajo concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 fue adecuada durante 10-14 días, independientemente del estado de madurez del fruto. Concentraciones elevadas de CO_2 de hasta 30 kPa a los 21 días de almacenamiento en envases almacenados bajo concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 podrían tener efectos perjudiciales en la fisiología de los tejidos cortados. Gorny et al. (2002) observaron un efecto perjudicial de las concentraciones altas de CO_2 en la respuesta fisiológica de pera 'Bartlett' fresca cortada, causada por atmósferas de aire + 20 kPa CO_2 que aceleran el pardeamiento y la necrosis de los tejidos.

La síntesis de etileno se inició rápidamente después del procesado en las muestras envasadas sin modificación inicial de la atmósfera, alcanzándose valores máximos durante los primeros 15 días, sobretodo en las muestras de madurez avanzada. La reducida acumulación de etileno en los envases almacenadas en concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 se relacionó con el efecto inhibidor de concentraciones bajas de O_2 y altas de CO_2 en la producción de etileno (Soliva-Fortuny & Martín-Belloso, 2003b). La inhibición de la síntesis de etileno en ausencia o concentraciones bajas de O_2 ha sido relacionada con la participación del O_2 en la conversión del ácido 1-amino-ciclopropano-1-carboxílico a etileno (Yang, 1981). Además, a pesar que se desconoce el mecanismo de acción de los niveles altos de CO_2, éstos se consideran inhibidores competitivos de la acción del etileno y limitantes de la acción autocatalítica de la 1-aminociclopropano-oxidasa (ACC oxidasa), responsable de la síntesis de etileno (Alzamora et al., 1996).

El etanol es un producto de la respiración anaerobia que normalmente se asocia a olores y sabores desagradables y a una marcada degradación de la calidad del producto. Durante la primera semana, los niveles no fueron lo suficientemente elevados como para detectar alteraciones en el aroma. En cambio, se produjo un incremento considerable de la concentración de etanol a partir de tres

semanas, especialmente en pera de madurez más avanzada, debido probablemente a procesos fermentativos como consecuencia de los niveles bajos de O_2 presentes en el envase. No obstante, las cantidades de etanol acumuladas en los envases no fueron muy elevadas a pesar de las concentraciones altas de CO_2 en las atmósferas de envasado. Esto podría explicarse por el efecto inhibitorio del CO_2 en la acumulación de metabolitos fermentativos. Según Gunes et al. (2001), concentraciones elevadas de CO_2 de 15-30 kPa pueden inhibir en un 50% la producción de acetaldehído, etanol y etil acetato en tejidos de manzana bajo condiciones de anaerobiosis o concentraciones bajas de O_2, comparado con muestras almacenadas en atmósferas en ausencia de CO_2.

El estado de madurez del fruto afectó significativamente a la cantidad de etanol acumulada en los envases. Independientemente de los tratamientos de envasado, los procesos fermentativos se desarrollaron con mayor intensidad en peras procesadas en un estado de madurez avanzado. Concentraciones muy bajas de O_2 y altas de CO_2 favorecieron un incremento drástico de las concentraciones de etanol durante la tercera semana de almacenamiento. Según Pesis (2005), la respiración anaeróbica de los frutos sobremaduros podría verse favorecida por la disminución de la actividad mitocondrial de los tejidos como consecuencia del daño a nivel de la membrana celular. Algunos trabajos realizados en pera 'Conference' fresca cortada mostraron una mayor acumulación de etanol en tejidos de madurez avanzada tanto en condiciones restrictivas de O_2 como sin la modificación de la atmósfera inicial (Soliva-Fortuny et al., 2004). En pera 'Flor de Invierno' fresca cortada de madurez intermedia y envasada bajo concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 no se detectó acumulación de etanol durante los 7 primeros días, manteniéndose concentraciones bajas hasta los 10-14 días.

4.2.1.2. Evolución del color y firmeza

Los cambios de color durante el almacenamiento de pera 'Flor de Invierno' fresca cortada, se determinaron mediante la luminosidad y el tono (h^0), este último calculado a partir de los parámetros colorimétricos a* y b* (Ecuación 3). El tono puede ser un buen indicador del pardeamiento de pera cortada, ya que un descenso de su valor se ha relacionado con un mayor grado de oscurecimiento de la superficie del tejido (Sapers y Douglas, 1987).

$$h^o = \arctan\frac{b^*}{a^*} \qquad \text{(Ecuación 3)}$$

La modificación de la atmósfera de envasado no evitó el pardeamiento enzimático de los tejidos de pera fresca cortada, observándose un oscurecimiento rápido de las superficies cortadas después del procesado mínimo (pelado y cortado), especialmente en pera de madurez avanzada. Según Soliva-Fortuny et al. (2004), un mayor ablandamiento de los tejidos de madurez avanzada podría favorecer la descompartimentación de enzimas y sustratos debido a las operaciones de corte y así acelerar el pardeamiento enzimático.

El estado de madurez de la materia prima influyó decisivamente en la firmeza de pera fresca cortada mientras que las condiciones de envasado no tuvieron una influencia significativa en los fenómenos de degradación de la misma. Se observó valores de firmeza excesivamente elevados de los frutos enteros de madurez temprana (65,2 N) para su consumo en fresco y su procesado. En cambio, la firmeza inicial de los frutos de madurez más avanzada (36,1 N) fue más adecuada para su consumo, aunque se observó una pérdida importante de la firmeza de los trozos de pera al cabo de dos semanas de almacenamiento, lo que podría ser debido al mayor estrés fisiológico que sufrieron estos tejidos. En pera 'Conference' fresca cortada también se observó una progresiva pérdida de firmeza en fruta fresca cortada procesada en un estado avanzado de madurez (Soliva-Fortuny et al., 2004).

4.2.1.3. Evolución de la estabilidad microbiológica

De acuerdo con las constantes cinéticas que describen la evolución de los recuentos microbiológicos según el modelo modificado de Gompertz (Ecuación 2), la velocidad máxima de crecimiento de microorganismos aerobios mesófilos en pera 'Flor de Invierno' fresca cortada fue mayor que la de mohos y levaduras y aumentó en frutos de madurez más avanzada. Durante las dos primeras semanas, los recuentos microbianos fueron superiores sin la modificación de la atmósfera inicial que bajo concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2. El efecto de las concentraciones bajas de O_2 y altas de CO_2 fue mayor sobre el crecimiento de mohos y levaduras que sobre microorganismos aerobios mesófilos. Estos resultados concuerdan con los obtenidos por Soliva-Fortuny et al., (2003c) en pera 'Conference' fresca cortada. Según estos autores, las concentraciones bajas de O_2 y/o concentraciones altas de CO_2 no inhiben el crecimiento de las bacterias aerobias, e incluso en presencia de cantidades pequeñas de O_2, los recuentos bacterianos aumentan rápidamente con el tiempo. No obstante, los recuentos de microorganismos aerobios mesófilos de pera 'Flor de Invierno' fresca cortada se mantuvieron por debajo de 5.10^7 ufc g^{-1}

durante 35 días de almacenamiento, un nivel aceptable de acuerdo con la legislación española que regula este tipo de productos (BOE, 2000). Se ha visto que el efecto del EAM depende del estado de madurez del fruto en el momento del procesado. Aunque concentraciones de 2,5 kPa O_2 + 7 kPa CO_2 inhibieron el crecimiento de mohos y levaduras en pera fresca cortada de madurez de conservación, no fueron efectivas en frutos más maduros. Los recuentos microbianos en la fase estacionaria fueron similares independientemente de las condiciones de envasado para frutos procesados en estados de madurez comercial y de consumo.

Por tanto, se concluyó que el envasado en concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 es recomendable para mantener la estabilidad microbiológica y firmeza de pera 'Flor de Invierno' fresca cortada procesada en un estado intermedio de madurez durante 10-14 días, aproximadamente. No obstante, estas condiciones de procesado y envasado no son suficientes para la inhibición del pardeamiento enzimático. Por tanto, una vez establecido el estado de madurez más adecuado para el procesado se procedió a evaluar el efecto de diferentes agentes antioxidantes de origen natural en el control del pardeamiento enzimático de este producto.

4.2.2. Empleo de antioxidantes en el mantenimiento de la calidad de pera fresca cortada

Como consecuencia de las operaciones mecánicas de pelado y cortado, las superficies cortadas de pera 'Flor de Invierno' sufrieron un rápido pardeamiento. Como se comprobó anteriormente, los sistemas tradicionales de envasado en atmósfera modificada, tales como la modificación pasiva de la atmósfera o modificación activa mediante la aplicación de concentraciones bajas de O_2 y altas de CO_2, no inhibieron el pardeamiento enzimático de la pera cortada. Por tanto, es necesaria la aplicación de agentes antioxidantes para mantener la calidad visual del producto. Se estudió el efecto individual de agentes antioxidantes de origen natural, ácido ascórbico (AA), 4-hexilresorcinol (4-HR), glutatión reducido (GSH) y N-acetilcisteína (NAC). Además, el efecto combinado de los distintos antioxidantes y del tiempo de almacenamiento en el color se evaluó mediante un diseño de superficie de respuesta (Capítulo VII). Este estudio permitió optimizar la concentración de antioxidantes necesaria para obtener los cambios mínimos en el parámetro colorimétrico h^0.

4.2.2.1. Efecto individual de los antioxidantes

La disminución de los valores de h^0 a través del tiempo fue más pronunciada en las muestras control que en aquellas tratadas con NAC o GSH. Además, la concentración de dichos agentes influyó significativamente en la evolución de h^0. Se comprobó que la disminución de los valores de h^0 fue mayor en pera fresca cortada tratada con la menor concentración de NAC o GSH (0,75 %) aunque observaciones visuales mostraron que estos tratamientos evitaban el pardeamiento durante al menos 21 días de almacenamiento. Estos agentes antioxidantes previenen del oscurecimiento por competencia con la enzima polifenoloxidasa (PPO), reaccionando con quinonas intermedias para formar compuestos estables incoloros (Molnar-Perl et al., 1990a; Richard-Forget et al., 1992). Según Richard et al. (1991), las quinonas forman compuestos incoloros con los compuestos sulfhídricos como la cisteína o glutatión a un pH superior a 4. Se comprobó en los tejidos de pera 'Flor de Invierno' que el pH de aquellos tejidos tratados con NAC o GSH a una concentración de 0,75% era de aproximadamente 4,8. En este sentido, el pH podría tener una influencia clara en la acción de compuestos como NAC o GSH. La aparición de coloraciones rojizas y rosáceas en pera 'Bartlett' fresca cortada tratada con cisteína se evitó mediante el ajuste del pH a 7 de una solución de 2% ácido ascórbico + 1% lactato cálcico + 0,5% cisteína. Esto podría ser debido a que la acción de la cisteína sobre las quinonas se ve favorecida por un pH neutro ya que el grupo tiol de la cisteína tiene un pKa de 8,33 (Gorny et al., 2002). Estos resultados sugieren que el pH del tejido vegetal y por tanto, la concentración de antioxidantes así como la variedad, el estado de madurez, y la atmósfera de envasado podría afectar significativamente a la respuesta de la pera fresca cortada al tratamiento antioxidante (Sapers y Miller, 1998).

En cambio, los valores de h^0 disminuyeron continuamente a lo largo del período de conservación en muestras tratadas con AA y HR y por tanto, estos tratamientos no controlaron el pardeamiento enzimático de pera fresca cortada. El oscurecimiento del tejido tratado con AA (0,75-2,25%) o HR (2%) era incluso mayor que el de las muestras control. En estudios anteriores, se había observado ya que el AA y HR no inhibían eficazmente el pardeamiento enzimático de manzana por si solos (Monsalve-González et al., 1993, 1995) e incluso se había sugerido que el AA podía favorecer los procesos oxidativos (Kacem et al., 1987).

4.2.2.2. Efecto combinado de los antioxidantes

El efecto combinado de los antioxidantes AA, NAC, GSH y HR, a concentraciones de 0-3%, en el control del pardeamiento enzimático de pera fresca cortada se estudió mediante el diseño de una superficie de respuesta (Capítulo VII). El diseño experimental incluyó la concentración de los antioxidantes y el tiempo de conservación como variables independientes y el h^0 como variable respuesta.

Un modelo de segundo orden se ajustó adecuadamente a la evolución del parámetro h^0. El modelo de regresión, una vez eliminados los términos no significativos, explicó el 97,38 % del total de variación ($p \leq 0.05$). La falta de ajuste de los datos experimentales al modelo de regresión no fue significativa.

La concentración de NAC, GSH y HR afectó significativamente al pardeamiento de pera fresca cortada. En cambio, el rango de concentraciones de AA aplicado y el tiempo de almacenamiento del producto no afectaron significativamente al pardeamiento del tejido vegetal. Al aumentar la concentración de NAC, GSH o HR se observó un incremento del efecto inhibitorio del pardeamiento de estos antioxidantes. El incremento de la concentración de los antioxidantes podría provocar una disminución del pH y a la vez, un aumento del efecto inhibitorio de la actividad polifenoloxidásica (Billaud et al., 2004). Además, se observó que h^0 alcanzaba valores máximos al aumentar la concentración de NAC hasta 1,5%. A concentraciones superiores a 1,5% de NAC, el incremento de las concentraciones de NAC o GSH no mejoraba el efecto inhibidor de la mezcla antioxidante.

Ya que los tratamientos con HR (0,5-2%) proporcionaron sabores desagradables al producto, combinaciones de NAC con GSH podrían ser las más adecuadas para el mantenimiento de los valores de h^0 durante la conservación de pera 'Flor de Invierno' fresca cortada. Aplicando soluciones de 1,5% NAC con 0,5% o 1% GSH se detectaron valores de h^0 superiores a 99º en muestras almacenadas en condiciones atmosféricas después de 28 días. Por otro lado, se observaron valores cercanos a 96-97º en mezclas de 1% NAC con 0,5% o 1% GSH, al final del período de conservación. Dichas mezclas se aplicaron en fruta fresca cortada para confirmar los resultados obtenidos en la superficie de respuesta. A partir de observaciones visuales y medidas colorimétricas, se demostró que una solución de 1,5% NAC con 0,5% o 1% GSH así como la combinación de 1% NAC con 1% GSH controlaban eficazmente el pardeamiento de pera fresca cortada, manteniendo valores máximos de h^0 durante los 28 días de almacenamiento. Además, un

tratamiento de 1% NAC con 0,5% GSH evitó el oscurecimiento durante al menos los primeros 21 días.

4.2.2.3. Efecto de los antioxidantes sobre la actividad PPO

La aplicación de antioxidantes en pera fresca cortada fue efectiva en la inhibición de la actividad PPO, a excepción de un tratamiento con AA. La actividad PPO en pera cortada tratada con un 3% de AA fue similar a la del control durante 28 días de almacenamiento. Además, en estas muestras, el pardeamiento fue similar al de muestras no tratadas, como indicó la disminución del parámetro h^0 durante el período de almacenamiento. En cambio, el pardeamiento observado en los trozos de fruta tratados con concentraciones más bajas de AA fue más pronunciado y a la vez, la actividad PPO relativa disminuyó hasta niveles inferiores al control. En general, la actividad PPO disminuye al aumentar la concentración de AA probablemente debido a sus propiedades reductoras. Sin embargo, concentraciones elevadas de AA podrían tener un efecto pro-oxidante en los tejidos vegetales.

En general, NAC o GSH inhibieron completamente la actividad PPO de la fruta fresca cortada. No obstante, en pera cortada tratada con 0,75% de GSH se detectó actividad enzimática, aumentando durante las dos últimas semanas de almacenamiento hasta valores superiores que los de las peras no tratadas. Estas muestras no sufrieron pardeamiento hasta los 21-28 días de almacenamiento, cuando un exceso de quinonas podría haber reaccionado con compuestos incoloros dando lugar a regeneración de fenoles y formación de substancias coloreadas (Vámos-Vigyázó et al., 1995). En trozos de pera tratados con GSH, un aumento del pH durante el período de conservación podría haber favorecido la actividad PPO, cuya actividad es óptima a pH neutros o cercanos a la neutralidad.

El HR inhibió la actividad PPO aunque su aplicación individual no evitó el pardeamiento, ya que en los primeros días de conservación se observó pigmentación grisácea en las superficies cortadas. Un contenido residual elevado en el tejido podría influir en la apariencia visual, sabor y olor del producto.

Una vez fijadas las condiciones más adecuadas para el procesado de la pera 'Flor de Invierno' fresca cortada, se procedió al estudio de distintas alternativas de EAM tales como el empleo de concentraciones altas de O_2, la modificación pasiva de la atmósfera o al envasado activo en concentraciones bajas de O_2 y altas de CO_2.

4.2.3. Efecto de la aplicación de distintas alternativas de EAM en combinación con el empleo de antioxidantes en la conservación de pera fresca cortada

4.2.3.1. Evolución de la actividad fisiológica

Se estudió el efecto de distintas alternativas de EAM en la respiración aeróbica y anaeróbica de pera 'Flor de Invierno' fresca cortada tratada con antioxidantes (0,75% NAC + 0,75% GSH). A partir de la evolución de la composición gaseosa de los envases durante el almacenamiento se calcularon las tasas de respiración a través del procedimiento matemático descrito en el Capítulo VIII. En base a los valores predichos, se estudió el efecto de las diferentes condiciones de envasado en los cambios de actividad respiratoria y calidad de la pera fresca cortada durante 35 días a 4ºC.

La tasa inicial de consumo de O_2 y producción de CO_2 de pera fresca cortada fue menor sin la modificación inicial de la atmósfera que bajo concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2. Esto podría ser debido al vacío que se crea en el envase, previo al llenado de gases, el cual podría favorecer condiciones de estrés fisiológico del tejido y una modificación de la atmósfera interna de la fruta. Sin la modificación inicial de la atmósfera, el consumo de O_2 aumentó durante las dos primeras semanas hasta 0,012 mol O_2 d^{-1} kg^{-1} debido a la cantidad de O_2 disponible en el envase que estimuló la respiración aeróbica de los trozos de pera incrementando el consumo de O_2 y la producción de CO_2. Bajo estas condiciones, se alcanzó una tasa de consumo de O_2 similar a la observada bajo concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 después de 14 días. Aunque la producción de CO_2 en pera envasada en concentraciones iniciales bajas de O_2 y altas de CO_2 fue aumentado durante el almacenamiento, alcanzando valores de 0,02 mol CO_2 d^{-1} kg^{-1} al final del mismo, la velocidad de producción fue menor que la observada en muestras envasadas sin modificación de la atmósfera. La producción de CO_2 en manzana 'Fuji' fresca cortada envasada en concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 fue también menor que la observada sin modificación de la atmósfera (Rojas-Graü et al., 2007a).

La tasa de consumo de O_2 en pera 'Flor de Invierno' fresca cortada envasada en concentraciones iniciales de 70 kPa O_2 aumentó de manera importante en comparación con otras condiciones de envasado debido probablemente a procesos de oxidación en los tejidos. Por tanto, la actividad respiratoria de frutos almacenados bajo concentraciones altas de O_2 se debería describir a partir de la producción de CO_2 en lugar de O_2. La tasa de producción de CO_2 de fruta almacenada bajo concentraciones iniciales de 70 kPa O_2 aumentó de manera similar a la de fruta almacenada sin

modificación de la atmósfera, alcanzando valores de 0,03 mol CO_2 d^{-1} kg^{-1} después de 35 días. Por tanto, no se observó una inhibición de la actividad respiratoria de pera 'Flor de Invierno' fresca cortada bajo concentraciones altas de O_2, comparado con el envasado sin modificación de la atmósfera inicial o en concentraciones bajas de O_2 y altas de CO_2.

Las concentraciones de etileno de pera 'Flor de Invierno' fresca cortada se vieron influidas por la atmósfera de envasado. Las concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 inhibieron la producción de etileno, mientras que muestras envasadas en concentraciones iniciales de 70 kPa O_2 o sin modificación de la atmósfera acumularon etileno hasta valores de 16,9-18,2 µl l^{-1} a los 14 días. Se ha demostrado que las concentraciones bajas de O_2 y altas de CO_2 actúan sinérgicamente en la inhibición de la síntesis de etileno (Soliva-Fortuny et al., 2003b; Solomos, 1997). No obstante, la adición de compuestos antioxidantes como NAC y GSH se ha visto que contribuye a la total inhibición de etileno en pera cortada almacenada en concentraciones bajas de O_2 y altas de CO_2, además de la reducción de la síntesis de etileno bajo concentraciones altas de O_2 o en envases sin modificación de la atmósfera inicial. Otros autores observaron una reducción significativa de la producción de etileno en manzana 'Fuji' fresca cortada tratada en una solución antioxidante de NAC en comparación al tratamiento con AA bajo concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 (Rojas-Graü et al., 2007a).

Nuestros resultados sugieren que las concentraciones altas de O_2 no previenen la síntesis de metabolitos fermentativos (acetaldehído y etanol) en pera 'Flor de Invierno' fresca cortada pero reducen su acumulación en los envases, más que las concentraciones bajas de O_2 y altas de CO_2. La producción de etanol observada en pera fresca cortada envasada en concentraciones iniciales de 70 kPa O_2 podría ser una respuesta al estrés que sufren los tejidos bajo concentraciones altas de O_2. La explicación más aceptada sobre la toxicidad del O_2 podría ser la formación de radicales superóxido (O_2^-) que destruyen el metabolismo celular (Fridovitch, 1975). De acuerdo con Wszelaki y Mitcham (2000), el contenido de compuestos volátiles (acetaldehído, etanol o etil acetato) de fresas almacenadas en concentraciones altas de O_2 (≥ 60 kPa) durante 14 días incrementó enormemente comparado con el de fresas almacenadas en condiciones atmosféricas. La respiración anaeróbica de los frutos favoreció el la producción de CO_2, correlacionándose bien la producción de etanol con la tasa de producción de CO_2 ($R^2 \geq 0,925$) para todas las condiciones de envasado. Peppelenbos et al. (1996) describieron la tasa de producción de CO_2, como consecuencia de la respiración aeróbica y anaeróbica, asumiendo el O_2 como un inhibidor de la

síntesis de CO_2 en los procesos fermentativos (Capítulo VIII). Las tasas de producción de CO_2 en pera 'Flor de Invierno' fresca cortada se ajustaron adecuadamente al modelo propuesto por estos autores ($R^2 \geq$ 0,984-0.995), excepto en aquellas muestras envasadas en concentraciones altas de O_2. Según el modelo, los coeficientes respiratorios durante la respiración aerobia (0,61-0,69) fueron similares en fruta envasada sin modificación de la atmósfera y en concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2. En cambio, la tasa máxima de producción de CO_2 fue mayor sin modificación de la atmósfera que en concentraciones bajas de O_2 y altas de CO_2. Los modelos de respiración basados en las cinéticas de Michaelis-Menten asumen una inhibición de la respiración debida al CO_2. No obstante, los mecanismos de acción de las concentraciones altas de O_2 en la actividad respiratoria de los tejidos no se conocen en profundidad, dificultando el desarrollo de modelos adecuados que describan la actividad respiratoria de los productos frescos cortados.

4.2.3.2. Evolución de los parámetros de calidad

El tratamiento antioxidante de 0,75% NAC + 0,75% GSH inhibió eficazmente el pardeamiento enzimático de pera 'Flor de Invierno' fresca cortada envasada sin modificación de la atmósfera inicial o en concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2. En cambio, se observó oscurecimiento de las superficies cortadas almacenadas en 70 kPa O_2 a partir de los 14-21 días de almacenamiento a pesar del tratamiento antioxidante.

Se observó una buena resistencia de la variedad 'Flor de Invierno' al procesado y envasado. Los cambios de firmeza de la pera fresca cortada durante el almacenamiento bajo concentraciones altas de O_2 no afectaron de manera visible la apariencia general del producto. En cambio, se observaron pérdidas significativas de firmeza a partir de los 10-14 días bajo concentraciones excesivamente bajas de O_2 y altas de CO_2. No obstante, la respuesta de los tejidos vegetales a las condiciones de envasado puede depender de muchos factores como la variedad o estado de madurez. El ablandamiento de pera 'Bartlett' fresca cortada no se inhibió bajo concentraciones bajas de O_2 (0,25 o 0,5), altas de CO_2 (aire + 5, 10 o 20 kPa CO_2) o concentraciones altas de O_2 (40, 60, 80 kPa) (Gorny et al., 2002).

Las pérdidas de vitamina C en pera fresca cortada se vieron favorecidas por la presencia de O_2 en los envases. El contenido de vitamina C en fruta envasada en concentraciones iniciales de 70 kPa O_2 disminuyó rápidamente en comparación al envasado sin modificación de la atmósfera o concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2. Sin embargo, se observó una pérdida de

vitamina C en fruta cortada envasada en concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 durante el almacenamiento, a pesar de los niveles bajos de O_2. Aunque el factor principal que afecta la degradación de vitamina C es la presencia de O_2, tasas de producción de CO_2 elevadas podrían haber afectado negativamente al contenido de vitamina C cuando se alcanzan condiciones de anoxia en los envases. Según Agar et al. (1997), la pérdida de vitamina C en frutos rojos se acelera debido a las concentraciones altas de CO_2. Éstas podrían acelerar la pérdida de vitamina C mediante la oxidación de AA, catalizada por la ascorbato peroxidasa (Tudela et al., 2002).

Los valores de pH se mantuvieron constantes en pera 'Flor de Invierno' fresca cortada envasada bajo concentraciones altas de O_2. En cambio, se observó un aumentó de pH de 4,5 a 5,7 en fruta envasada sin la modificación de la atmósfera o en concentraciones de 2,5 kPa O_2 + 7 kPa CO_2. Este aumentó de pH se podría relacionar con la producción de etanol y los procesos de anaerobiosis. El pH de pera 'Conference' fresca cortada envasada en ausencia de O_2 o en concentraciones de 2,5 kPa O_2 + 7 kPa CO_2 también experimentó un ligero aumento continuado a lo largo del almacenamiento (Soliva-Fortuny y Martín-Belloso, 2003c). En condiciones de anaerobiosis, el aumento de la actividad fisiológica y la proliferación de microorganismos podrían jugar un papel importante en la degradación de ácidos orgánicos, aumentando así los valores de pH.

El contenido en sólidos solubles se mantuvo bajo concentraciones bajas de O_2 y altas de CO_2, mientras que se observó una importante disminución de azúcares en muestras almacenadas en concentraciones altas de O_2 o sin la modificación de la atmósfera inicial. Un mayor consumo de carbohidratos debido a la mayor actividad respiratoria, observada en trozos de pera envasados sin la modificación de la atmósfera o en concentraciones altas de O_2, explicaría la disminución en sólidos solubles. Wszelaki y Mitcham (2000) también observaron una disminución del contenido en sólidos solubles en fresas almacenadas en 90-100 kPa O_2 debido a la elevada actividad respiratoria.

4.2.3.3. Evolución de la estabilidad microbiológica

Los recuentos de microorganismos aerobios psicrófilos en pera 'Flor de Invierno' fresca cortada, tratada con una solución de NAC (0,75% p/v) y GSH (0,75% p/v), experimentaron un crecimiento rápido. La velocidad de crecimiento bacteriano fue mayor en fruta cortada envasada sin modificación de la atmósfera inicial que en concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 o

70 kPa O_2. Así, la modificación de la atmósfera inicial alargó significativamente la estabilidad microbiológica del producto. En estas condiciones de envasado, se observaron recuentos totales no superiores a 10^7 ufc g^{-1} durante los 28 días de almacenamiento. En cambio, la pera fresca cortada envasada sin la modificación inicial de la atmósfera alcanzó recuentos máximos en la fase estacionaria de 10^7 ufc g^{-1} después de 10-14 días. Como se ha comentado anteriormente, los recuentos de microorganismos aerobios psicrófilos en melón 'Piel de Sapo' fresco cortado envasado sin modificación de la atmósfera también fueron mayores que en concentraciones de 2,5 kPa O_2 + 7 kPa CO_2 o de 70 kPa O_2 durante la primera semana de almacenamiento. Las concentraciones altas de CO_2 son efectivas en el control del crecimiento de la mayoría de microorganismos aerobios, especialmente bacterias y mohos (Al-Ati y Hotchkiss, 2002). El uso en este estudio de plásticos de permeancia baja al CO_2 para el envasado de fruta en concentraciones altas de O_2 podría haber favorecido la acumulación de este gas y así, aumentar su efecto inhibitorio en el crecimiento microbiano. Se ha demostrado que cantidades de O_2 elevadas combinadas con concentraciones altas de CO_2 (10-20%), son más efectivas en el control microbiano que el uso individual de los mismos gases (Amanatidou et al., 1999).

La presencia de levaduras en pera 'Flor de Invierno' fresca cortada fue mayoritaria en comparación a la de mohos, independientemente de la atmósfera de envasado. Las levaduras más importantes que se aislaron de la fruta fresca cortada fueron *Candida parapsilosis* y *Rhodotorula mucilaginosa*. En otras frutas frescas cortadas como cubos de mango (Poubol y Izumi, 2005) o trozos de melón 'Piel de Sapo' también se han aislado levaduras del mismo género. En muestras inoculadas de pera fresca cortada, niveles de 10^5 ufc g^{-1} de *C. parapsilosis* se mantuvieron durante todo el almacenamiento en todas las condiciones de envasado. En cambio, la composición inicial de la atmósfera de envasado afectó de manera significativa al crecimiento de *R. mucilaginosa*. Por tanto, la sensibilidad al O_2 de los distintos microorganismos puede variar ya que algunos de ellos desarrollan estrategias para evitar daño letal, tales como la inducción de enzimas capaces de descomponer las especies reactivas de O_2 (Kader y Ben-Yehoshua, 2000). Los recuentos de *R. mucilaginosa* en muestras inoculadas almacenadas en concentraciones altas de O_2 disminuyeron durante el almacenamiento hasta alcanzar niveles de 10^3 ufc g^{-1} después de 28 días. En estudios anteriores se demostró la sensibilidad a las concentraciones altas de O_2 de levaduras del género *Rhodotorula*, aisladas de melón 'Piel de Sapo' y mango 'Nam Dokmai' frescos cortados (Poubol y Izumi, 2005). Los recuentos iniciales de *R. mucilaginosa* se mantuvieron constantes en muestras

inoculadas almacenadas en concentraciones bajas de O_2 y altas de CO_2, mientras que los recuentos en las muestras envasadas sin modificación de la atmósfera aumentaron sustancialmente después de la primera semana de almacenamiento. En muestras no inoculadas, los recuentos totales máximos de mohos y levaduras también se obtuvieron en fruta cortada envasada sin modificación de la atmósfera inicial, mientras que los recuentos microbianos se mantuvieron constantes en pera 'Flor de Invierno' fresca cortada en concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 o 70 kPa O_2. Estos resultados concuerdan con los obtenidos por Soliva-Fortuny y Martín-Belloso (2003c) en pera 'Conference' fresca cortada. Estos autores observaron recuentos constantes de mohos y levaduras en fruta envasada en concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 durante 4 semanas de almacenamiento, detectándose también un mayor efecto inhibitorio de las concentraciones bajas de O_2 en el crecimiento de mohos y levaduras que en el de bacterias.

En pera 'Flor de Invierno' fresca cortada tratada con NAC (0,75% p/v) + GSH (0,75% p/v) y almacenada en concentraciones iniciales de 2,5 kPa O_2 + 7 kPa CO_2 o 70 kPa O_2 se mantuvieron los recuentos de levaduras por debajo de 10^5 ufc g^{-1} durante 28 días. En cambio, en muestras tratadas con antioxidantes y envasadas sin la modificación de la atmósfera inicial se excedieron los 10^5 ufc g^{-1} después de tres semanas.

4.2.3.4. Impacto sobre las propiedades antioxidantes

La oxidación del AA se vio favorecida por la presencia de O_2 en los envases. Se observó una pérdida de vitamina C importante en pera fresca cortada almacenada bajo concentraciones altas de O_2, mayor que sin modificación de la atmósfera inicial. En concentraciones iniciales bajas de O_2 y altas de CO_2, el contenido en vitamina C de trozos de pera tratados con NAC (0,75% p/v) y GSH (0,75% p/v) se mantuvo durante 14 días de almacenamiento. En los frutos, el glutatión y ácido ascórbico pueden verse involucrados en la regeneración del AA (Winkler et al., 1994).

El principal compuesto fenólico encontrado en pera 'Flor de Invierno' fresca cortada fue el ácido clorogénico en concentraciones iniciales de 17-18 mg 100 g^{-1}. Su contenido se vio afectado por la atmósfera de envasado y por el tratamiento antioxidante de NAC y GSH. Se observó una pérdida importante de ácido clorogénico en pera fresca cortada almacenada bajo concentraciones altas de O_2. Además, la aplicación de antioxidantes parece que reduce la oxidación del ácido clorogénico de muestras almacenadas bajo concentraciones iniciales bajas de O_2 y altas de CO_2 o en envases sin modificación de la atmósfera inicial.

El incremento del contenido de otros compuestos fenólicos minoritarios como (-)-epicatequina y quercetina (2,0 y 0,18 mg 100 g^{-1} respectivamente) durante el almacenamiento de pera fresca cortada podría asociarse a una respuesta fisiológica del tejido. En condiciones de estrés, la formación de especies de O_2 reactivas podría activar la PAL y en consecuencia, se podría producir un aumento de la síntesis de compuestos fenólicos (Reyes et al., 2006). Aunque se observó un aumento de la síntesis de (-)-epicatequina y quercetina en muestras no tratadas con antioxidantes y almacenadas bajo concentraciones altas de O_2, su producción se vio estimulada en trozos de pera tratados y almacenados en concentraciones bajas de O_2.

La transformación de los ácidos *p*-cumárico y ferúlico en ácido sináptico, compuestos que se encuentran en concentraciones iniciales de 0,03-0,9 mg 100 g^{-1} en pera, podría explicar el incremento del contenido en ácido sináptico y la disminución de ácido *p*-cumárico y ferúlico que se observó durante el almacenamiento de pera fresca cortada. El metabolismo general fenilpropanoide consisten en una serie de reacciones que llevan a la síntesis de ácido *p*-cumárico a partir de fenilalanina, catalizado por la PAL. A partir de la hidroxilación y metilación del ácido *p*-cumárico, se pueden obtener otros compuestos fenólicos como el ácido cafeico, ferúlico y sináptico (Shahidi, 2004). La inmersión de fruta troceada en una solución de NAC y GSH favoreció la síntesis de compuestos fenólicos en muestras almacenadas en concentraciones bajas de O_2 y altas de CO_2. Se ha visto que el glutatión podría participar en la formación de compuestos fenólicos como el ácido *trans*-cinámico y *p*-cumárico bajo condiciones de anoxia (Blokhina et al., 2003).

El contenido fenólico de la pera fresca cortada se relacionó con la capacidad antioxidante del producto. Aunque las concentraciones altas de O_2 disminuyeron la capacidad antioxidante de trozos de pera en comparación a las muestras almacenadas en concentraciones iniciales bajas de O_2 y altas de CO_2 o en envases sin modificación de la atmósfera inicial, el tratamiento antioxidante contribuyó al mantenimiento de la capacidad antioxidante del producto. Los mayores valores de capacidad antioxidante se observaron en pera cortada tratada con la solución antioxidante y almacenada en concentraciones bajas de O_2. Nuestros resultados muestran que el tratamiento con NAC + GSH no solo juega un papel relevante en el control del pardeamiento enzimático sino que también contribuye al mantenimiento del potencial antioxidante de la pera fresca cortada.

4.2.4. Efecto de la aplicación de RC con incorporación de antioxidantes sobre la calidad de pera fresca cortada

Se estudió el efecto de la aplicación de RC, previamente ensayados, con la incorporación de antioxidantes (0,75% NAC p/v + 0,75% GSH p/v) sobre la calidad del producto. Se estudiaron los cambios en la actividad fisiológica, calidad sensorial, propiedades antioxidantes y estabilidad microbiológica de los trozos de pera envasados en películas plásticas de permeancia al O_2 baja (110 cm^3 O_2 m^{-2} bar^{-1} d^{-1}).

Se determinó la RVA de los trozos de pera recubiertos con RC a base de alginato, gelano y pectina. Se observaron valores iniciales de RVA de 23-24 s/cm en trozos de fruta recubiertos con gelano y valores de 16-19 s/cm en los recubiertos con alginato y pectina. En cambio, la RVA de muestras sin recubrir fue de 11,7 s/cm. Como se comentó anteriormente, el aumento de la RVA y consiguiente disminución de pérdida de agua en trozos de pera recubiertos es debida a la naturaleza hidrofílica del aceite de girasol añadido en las formulaciones. Rojas-Graü et al. (2007b) demostró el efecto de la incorporación de una concentración de 0,025% de aceite de girasol en RC a base de alginato y gelano en el aumento de la RVA de manzana 'Fuji' fresca cortada. Además, la incorporación de NAC y GSH en los recubrimientos a base de alginato y pectina provocó una ligera disminución de RVA, atribuible a la disminución de la hidrofibicidad de los recubrimientos por la incorporación de los antioxidantes.

4.2.4.1. Evolución de la actividad fisiológica

La aplicación de RC elaborados a base de alginato, gelano y pectina no evitó el intercambio gaseoso entre los trozos de pera y el espacio de cabeza de los envases. Nuestros resultados concuerdan con los de Rojas-Grau et al. (2007c) obtenidos en manzana 'Fuji' fresca cortada. Estos autores tampoco observaron diferencias significativas entre las composiciones gaseosas de envases de manzana fresca cortada recubierta y sin recubrir. No obstante, en nuestro estudio se observó que la incorporación de 0,75% NAC p/v y 0,75% GSH p/v en los recubrimientos disminuyó ligeramente la permeabilidad al O_2 respecto a las muestras recubiertas sin incorporación de antioxidantes. Wong et al. (1994) investigaron el efecto de varios recubrimientos bicapa (incluidos alginato y pectina) en la actividad respiratoria de trozos de manzana recubiertos, evaluando la producción interna de CO_2 y etileno. Contrariamente a lo observado en este estudio, estos autores detectaron una reducción de la producción de CO_2 y una inhibición casi total de la síntesis de

etileno en manzana cortada recubierta comparado con muestras sin recubrir. Las propiedades de barrera a la difusión gaseosa de los recubrimientos podrían explicar la modificación de la atmósfera interna del fruto durante las primeras 24 horas y la consiguiente reducción de la actividad respiratoria de la fruta cortada recubierta.

Durante la primera semana de almacenamiento, se observó una inhibición de la síntesis de etileno en trozos de pera recubiertos con incorporación de la mezcla de antioxidantes. La acumulación de gas en estas muestras aumentó a partir de los 7 días, aunque alcanzándose concentraciones de etileno al cabo de 14 días no superiores a las de muestras sin recubrir o con recubrimientos sin antioxidantes (20-50 ppm). El efecto inhibitorio de la síntesis de etileno de los RC de alginato y gelano, conteniendo NAC como agente antioxidante, se había demostrado previamente en manzana fresca cortada (Rojas-Graü et al., 2007c). Algunos autores han sugerido que la reducción de la actividad respiratoria y producción de etileno de manzana fresca cortada recubierta podría ser debida principalmente a los iones de calcio necesarios para la formación de la película (Wong et al., 1994; Lee et al., 2003). El calcio participa en la regulación de la actividad de muchos enzimas involucrados en procesos fisiológicos y celulares (Wong et al., 1994). Nuestros resultados muestran que la NAC y el GSH podrían haber contribuido en la reducción la síntesis de etileno de trozos de pera cortada. En fruta no recubierta, se observó una menor acumulación de etileno en muestras tratadas con antioxidantes que en las no tratadas. Compuestos como la cisteína o el dithiothreitol han demostrado retardar la maduración y senescencia de peras 'Bartlett' (Frenkel, 1976) y en consecuencia, podrían tener un efecto importante en la inhibición de la síntesis de etileno.

No se observó acumulación de etanol en el espacio de cabeza de los envases conteniendo trozos de pera no recubiertos o muestras recubiertas sin antioxidantes. En cambio, el etanol se acumuló en los envases de trozos de pera recubiertos con incorporación de NAC + GSH después de 11 días de almacenamiento refrigerado. De acuerdo con Ayranci y Tunc (2003), la difusión del O_2 a través de los recubrimientos podría disminuir con la incorporación de antioxidantes, provocando un importante descenso de las concentraciones de O_2 en el interior de los tejidos. A pesar de la acumulación de etanol, las concentraciones no fueron suficientes como para detectar deterioro de la calidad del producto durante los 14 días de almacenamiento. Rojas-Graü et al. (2007c) también observaron una acumulación mayor de acetaldehído y etanol en envases de manzana cortada recubierta por alginato y pectina conteniendo 1% NAC p/v que en los de fruta sin recubrir.

4.2.4.2. Cambios en las propiedades físico-químicas y sensoriales

Los panelistas no detectaron ablandamiento de los tejidos ni en trozos de pera 'Flor de Invierno' recubiertos por alginato, gelano o pectina ni en muestras sin recubrir. En cambio, se detectó una disminución substancial del olor de pera fresca cortada no recubierta (≤ 5), en comparación a trozos de fruta recubiertos (≥ 5) o muestras frescas (≥ 8) después de 14 días. El olor de las muestras recubiertas está relacionado con la retención de compuestos volátiles. Según Olivas et al. (2007), las propiedades barreras del alginato podrían facilitan la retención de los compuestos volátiles o afectar a las rutas de síntesis de estos compuestos. La incorporación de agentes antioxidantes en los RC o mediante inmersión acuosa aumentó significativamente la calidad visual de los trozos de pera fresca cortada. Además, nuestros resultados sugieren que la incorporación de los antioxidantes en la formulación de los RC de alginato o pectina mejora substancialmente el sabor del producto, en comparación a su adición en los trozos de fruta mediante solución acuosa.

Las medidas instrumentales de color y firmeza corroboraron los cambios en los atributos sensoriales observados en pera fresca cortada. La incorporación de NAC (0,75% p/v) y GSH (0,75% p/v) mediante inmersión acuosa o en las formulaciones de los RC evitó eficazmente el pardeamiento de los trozos de pera. En trozos de pera recubiertos y sin recubrir, el h^0 inicial de muestras no tratadas con NAC y GSH disminuyó rápidamente, mientras que el de las muestras tratadas con antioxidantes se mantuvo durante el almacenamiento. Rojas et al. (2007b) observaron una disminución importante del h^0 de trozos de manzana recubiertos con alginato y gelano durante las 48 primeras horas, mientras que la incorporación de NAC y GSH en concentraciones de 1% p/v a los RC controló de manera eficaz el pardeamiento de las superficies cortadas. Olivas et al. (2003) también observó el efecto positivo de la adición de algunos aditivos (AA, $CaCl_2$ y ácido sórbico) en RC de metilcelulosa y ácido metilcelulosa-esteárico en el control del pardeamiento de pera 'Anjou' fresca cortada.

Entre los principales cambios de pera 'Flor de Invierno' durante el almacenamiento prolongado en atmósfera controlada está la pérdida de textura con la consiguiente disminución de la aceptabilidad del consumidor (Varela et al., 2007). No obstante, en estudios anteriores, se ha observado un mantenimiento de la firmeza inicial de pera 'Flor de Invierno' fresca cortada procesada en un estado de madurez intermedio y envasada sin modificación de la atmósfera inicial o en concentraciones de 2,5 kPa O_2 + 7 kPa CO_2 o 70 kPa O_2, durante 35 días de almacenamiento refrigerado. En el presente estudio, los valores de firmeza de los trozos de pera se mantuvieron

constantes durante el período de almacenamiento, no detectándose diferencias significativas entre las muestras sin recubrir y las recubiertas con gelano, alginato o pectina. Muchos autores han demostrado la efectividad del $CaCl_2$, necesario para la formación de las películas, en la retención de la firmeza de productos frescos cortados susceptibles al ablandamiento (Lee et al., 2003; Rojas et al., 2007b, c; Olivas et al., 2003). No obstante, los resultados del presente estudio demuestran que nuestro producto sin recubrir no experimenta un ablandamiento apreciable durante su almacenamiento, no siendo necesario el uso de calcio para el mantenimiento de su firmeza.

4.2.4.3. Evolución de la estabilidad microbiológica

En pera 'Flor de Invierno' fresca cortada recubierta y sin recubrir, los recuentos microbianos de la fruta tratada con 0,75% NAC p/v y 0,75% GSH p/v fueron menores que los de trozos de fruta no tratados con antioxidantes durante 14 días de almacenamiento. Además, los recuentos microbianos de pera tratada con los distintos RC de alginato, gelano y pectina no difirieron significativamente de sus respectivos controles sin recubrir. Sin adición de antioxidantes, los recuentos de microorganismos aerobios psicrófilos superaron los 10^6 ufc g^{-1} en muestras recubiertas y sin recubrir, mientras que los recuentos en trozos de pera tratados con antioxidantes fueron menores de 10^5 ufc g^{-1} después de 14 días. Estos resultados concuerdan con otros obtenidos en manzana fresca cortada recubierta con diferentes polímeros de carbohidratos y concentrado de proteína de suero, y el empleo de AA, ácido cítrico y ácido oxálico como inhibidores del pardeamiento (Lee et al., 2003). Rojas-Graü et al. (2007c) observaron que un RC de alginato o gelano, conteniendo NAC como antioxidante, tenía una marcado efecto en la reducción de los recuentos de microorganismos aerobios mesófilos y psicrófilos en manzana 'Fuji' cortada. Estos autores sugirieron un cierto efecto antimicrobiano del agente antioxidante. En trozos de manzana sin recubrir, se observaron recuentos microbianos menores en fruta cortada tratada con NAC (1% p/v) + GSH (1% p/v) + lactato de calcio (1% w/v) que en muestras sin tratar (Raybaudi-Massilia et al., 2007).

4.2.4.4. Impacto sobre las propiedades antioxidantes

La incorporación de 0,75% NAC p/v y 0,75% p/v GSH a los RC a base de alginato, gelano o pectina disminuyó significativamente la pérdida de vitamina C de pera 'Flor de Invierno' fresca cortada en comparación con los trozos de pera recubiertos sin antioxidantes y con los controles sin

recubrir. Según Ayranci y Tunc (2003), esto podría ser atribuible a la menor permeabilidad al O_2 de los RC con antioxidantes. Se observó un descenso importante de vitamina C en muestras sin recubrir, las superficies de las cuales se encuentran en contacto directo con el O_2. No obstante, la oxidación del AA no se evitó en trozos de pera recubiertos ya que los RC presentaron una probre barrera al O_2.

El contenido fenólico se mantuvo relativamente constante durante el almacenamiento refrigerado de pera fresca cortada. No obstante, tras la incorporación de NAC (0,75% p/v) y GSH (0,75% p/v) a los RC o su adición mediante inmersión acuosa, el contenido inicial de compuestos fenólicos de los trozos de pera 'Flor de Invierno' fue significativamente superior al observado en muestras recubiertas o sin recubrir no tratadas con antioxidantes. La incorporación de NAC y GSH como agentes antioxidantes en los RC pudo ser responsable de una sobreestimación del contenido fenólico. Según Prior et al. (2005), el metódo de análisis de Folin-Cicalteau podría incluir la contribución del ácido ascórbico, azúcares reducidos, proteínas solubles o otras sustancias.

El uso de RC no contribuyó al mantenimiento de la capacidad antioxidante de la pera 'Flor de Invierno' fresca cortada. No obstante, la incorporación de NAC y GSH mantuvo la capacidad antioxidante tanto de los trozos recubiertos como de los no recubiertos. Aunque la capacidad antioxidante de pera se ha correlacionado bien con el contenido de ácido clorogénico, el compuesto fenólico más común en peras (Galvis-Sánchez et al., 2003), otras sustancias formadas a partir de la oxidación parcial de los polifenoles o la incorporación de antioxidantes como la NAC o GSH podrían tener una contribución mayor a la actividad antioxidante de los frutos cortados (Nicoli et al., 1999).

Bibliografía

Agar, I.T.; Streif, J.; Bangerth, F. 1997. Effect of high CO_2 and controlled atmosphere (CA) on the ascorbic and dehydroascorbic acid content of some berry fruits. *Postharvest Biology and Technology*, 11: 47-55

Aguayo, E.; Allende, A.; Artés, F. 2003. Keeping quality and safety of minimally fresh processed melon. *European Food Research Technology*, 216: 494-499.

Aguayo, E.; Escalona, V.H., Artés, F. 2004. Metabolic behaviour and quality changes of whole and fresh processed melon. *Journal of Food Science*, 69: 148-155.

Al-Alti, T.; Hotchkiss, H. 2002. Application of Packaging and Modified Atmosphere to Fresh-cut Fruits and Vegetables. In: Lamikanra, O. (Ed.), Fresh-cut fruits and vegetables science, technology, and Market. CRC Press, Boca Raton, FL, USA, pp. 305-338.

Allende, A.; Del Carmen Barba, M.; Artés, F. 2003. Effect of High O_2 and CO_2 atmosphere on microbial and sensorial quality of fresh processed 'Lollo Rosso' Letucce. *Acta Horticulturae*, 599: 97-101

Alzamora, S.M.; Tapia, M.S.; Argaiz, A.; Welti, J. 1996. Combination of preservation factors applied to minimal processing for food. *Food Science and Nutrition*, 36(6): 626-659.

Amanatidou, A.; Smid, E.J.; Gorris, L.G.M. 1999. Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable-associated microorganisms. *Journal of Applied Microbiology*, 86: 429-438.

Ayranci, E.; Tunc, S. 2003. A method for the measurement of the oxygen permeability and the development of edible films to reduce the rate of oxidative reactions in fresh foods. *Food Chemistry*, 80: 423-431.

Bai, J.H.; Saftner, R.A.; Watada, A.E; Lee, Y.S. 2001. Modified atmosphere maintains quality of fresh-cut cantaloupe (Cucumis melo L.). *Journal of Food Science*, 66: 1207-1211.

Bai, J.; Saftner, R.A.; Watada, A.E. 2003. Characteristics of fresh-cut honeydew (*Cucumis xmelo L.*) available to processors in winter and summer and its quality maintenance by modified atmosphere packaging. *Postharvest Biology Technology*, 28: 349-359.

Ben-Yehoshua, S.; Burg, S.P.; Young, R. 1985. Resistance of citrus fruit to mass transport of water vapour and other gases. *Plant Physiology*, 79, 1948.

Billaud, C. ; Brun-Mérimée, S. ; Louarme, L. ; Nicolas, J. 2004. Effect of glutathione and Maillard reaction products prepared from glucose or fructose with glutathione on polyphenoloxidase from apple-I: Enzymatic browning and enzyme activity inhibition. *Food Chemistry*, 84:223-33.

Blokhina, O.; Virolainen, E.; Fagerstedt, K.V. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Annals of Botany, 91: 179-194

BOE. (2000). Normas de higiene para la elaboración, distribución y comercio de comidas preparadas. Real Decreto 3484/2000, pp. 1435-1441.

Del Caro, A.; Piga, A.; Vacca, V.; Agabbio, M. 2004. Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage. *Food Chemistry*, 84: 99-105.

Escalona, V.H.; Verlinden, B.E.; Geysen, S.; Nicolaï, B.M. 2006. Changes in respiration of fresh-cut butterhead lettuce under controlled atmospheres using low and superatmospheric oxygen conditions with different carbon dioxide levels. *Postharvest Biology and Technology*, 39: 48-55.

Flores, F.B.; Martínez-Madrid, M.C.; Amor, M.B.; Pech, J.C., Latché, A.; Romojaro, F. 2004. Modified atmosphere packaging confers additional chilling tolerance on ethylene-inhibited cantaloupe Charentais melon fruit. *European Food Research and Technology*, 219: 614-619.

Fonseca, S.C.; Oliveira, F.A.R.; Brecht, J.K. 2002. Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. *Journal of Food Engineering*, 52: 99-119.

Frenkel, C. 1976. Regulation of ripening in Bartlett pears with sulfhydryl reagents. *Botanical Gazette*, 137 (2): 154-159.

Fridovitch, I. 1975. Superoxide dismutase. *Annual Review of Biochemistry*, 44: 147-159.

Galvis-Sánchez, A.C.; Gil-Izquierdo, A.; Gil, M.I. 2003. Comparative study of six pear cultivars in terms of their phenolic and vitamin C contents and antioxidant capacity. *Journal of Science and Food Agriculture*, 83: 995-1003.

Gil, M.I.; Gorny, J.R.; Kader, A.A. 1998. Responses of 'Fuji' apple slices to ascorbic acid treatments and low-oxygen atmospheres. *HortScience*, 33: 305-309.

Gorny, J.R.; Hess-Pierce, B.; Cifuentes, R.A.; Kader, A.A. 2002. Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest Biology and Technology*, 24: 271-278.

Gunes, G.; Watkins, C.B.; Hotchkiss, J.H. 2001. Physiological responses of fresh-cut apple slices under high CO_2 and low O_2 partial pressures. *Postharvest Biology and Technology*, 22: 197-204.

Kester, J.J.; Fennema, O. 1986. Edible films and coatings: A review. *Food Technology*, 40 (12): 47-59.

Jacxsens, L.; Devlieghere, F.; Falcato, P.; Debevere, J. 1999. Behavior of *Listeria monocytogenes* and *Aeromonas* spp. of fresh-cut produce packaged under equilibrium modified atmosphere. *Journal of Food Protection*. 62: 1128-1135.

Jacxsens, L.; Devlieghere, F.; Van der Steen, C.; Debevere, J. 2001. Effect of high oxygen atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. *International Journal of Food Microbiology*, 71, 197-210.

Kacem, B.; Cornell, J.A.; Marshall, M.R.; Shiremen, R.B.; Matthews, R.F. 1987. Nonenzymatic Browning in Asceptically Packaged Orange Drinks: Effect of Ascorbic Acid, Aminoacids and Oxygen. *Journal of Food Science*, 52: 1668-1672.

Kader, A.A.; Ben-Yehoshua, S. 2000. Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. *Postharvest Biology and Technology*, 20, 1-13.

Kader, A.A.; Zagory, D.; Kerbel, E.L. 1989. Modified atmosphere packaging of fruits and vegetables. *CRC Critical Reviews in Food Science and Nutrition*, 28 (1): 1-30.

Kang, H.M.; Saltveit, M.E. 2002. Antioxidant capacity of lettuce leaf tissue increases after wounding. *Journal of Agricultural and Food Chemistry*, 50: 7536-7541.

Lakakul, R.; Beaudry, R.M.; Hernandez, R.J. 1999. Modeling respiration of apple slices in modified atmosphere packages. *Journal of Food Science*, 64(1): 105-110.

Lamikanra, O.; Watson, M.A. 2004. Effect of Calcium Treatment Temperature on Fresh-cut Cantaloupe Melon during Storage. *Journal of Food Science*, 69: 468-472.

Lee, J.Y.; Park, H.J.; Lee, C.Y.; Choi, W.Y. 2003. Extending shelf-life of minimally processed apples with edible coatings and antibrowning agents. *LWT-Food Science and Technology*, 36, 323-329.

Lester, G.E. 2003. Oxidative stress affecting fruit senescence. In: Hodges DM (Ed.), Postharvest oxidative stress in horticultural crops. Food Products Press: New York, USA, pp. 113-129

Li, P.; Barth, M.M. 1998. Impact of edible coatings on nutritional and physiological changes in lightly-processed carrots. *Postharvest Biology and Technology*, 14 (1): 51-60.

Luna-Guzmán, I.; Cantwell, M.; Barreto, D. 1999. Fresh-cut cantaloupe: effects of $CaCl_2$ dips and heat treatments on firmness and metabolic activity. *Postharvest Biology and Technology*, 17: 201-213.

Molnar-Perl, I.; Friedman, M. 1990a. Inhibition of Browning by Sulfur Amino Acids. 2. Fruit juices and Protein Containing Foods. *Journal of Agricultural and Food Chemistry*, 38: 1648-1651.

Monsalve-González A.; Barbosa-Cánovas, G.V.; Cavalieri, R.P.; McEvily, A.J.; Iyengar, R. 1993. Control of Browning during storage of apple slices preserved by combined methods. 4-hexylresorcinol as browning inhibitor. *Journal of Food Science*, 58: 797-826.

Monsalve-González, A.; Barbosa-Cánovas, G.V.; McEvily, A.J.; Iyengar, R. 1995. Inhibition of enzymatic browning in apple products by 4-hexylresorcinol. *Food Technology*, 49(4): 110-118.

Nicoli, M.C.; Anese, M.; Parpinel, M. 1999. Influence of processing on the antioxidant properties of fruit and vegetable. *Trends in Food Science and Technology*, 10: 94-100.

O'Connor-Shaw, R.E.; Roberts, R.; Ford, A.L.; Nottingham, S.M. 1994. Shelf-life of minimally processed Honeydew, kiwifruit, papaya, pineapple, and Cantaloupe. *Journal of Food Science*, 59: 1202-1206.

Olivas, G.I.; Rodríguez, J.J.; Barbosa-Cánovas, G.V. 2003. Edible coatings composed of methylcellulose, stearic acid, and additives to preserve quality of pear wedges. *Journal of Food Processing and Preservation*, 27: 299-320.

Olivas, G.I.; Mattinson, D.S.; Barbosa-Cánovas, G.V. 2007. Alginate coatings for preservation of minimally processed 'Gala' apples. *Postharvest Biology and Technology*, 45(1): 89-96.

Peppelenbos, H.W.; Tijskens, L.M.M.; van't Leven, J.; Wilkinson, E.C. 1996. Modelling oxidative and fermentative carbon dioxide production of fruits and vegetables. *Biology and Technology*, 9: 283-95.

Pesis, E. 2005. The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripeneing, enhancement of fruit quality and fruit deterioration. *Postharvest Biology and Technology*, 37: 1-19.

Piga, A.; Agabbio, M.; Gambella, F.; Nicoli, M.C. 2002. Retention of Antioxidant activity in minimally processed mandarin and satsuma fruits. *LWT-Food Science and Technology*, 35: 344-347.

Pinto, E.; Lentheric, I.; Vendrell, M.; Larrigaudiere, C. 2001. Role of fermentative and antioxidant metabolisms in the induction of core browning in controlled-atmosphere stored pears. *Journal of the Science of Food and Agriculture*, 81: 364-370.

Poubol, J.; Izumi, H. 2005. Physiology and Microbiological Quality of Fresh-cut mango cubes as affected by high-O_2 controlled atmospheres. *Journal of Food Science*, 70 (6): 286-291.

Prior, R. L.; Wu, X. L.; Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53 (10): 4290-4302.

Raybaudi-Massilia, R.M.; Mosqueda-Melgar, J.; Sobrino-López, A.; Soliva-Fortuny, R.; Martín-Belloso, O. 2007. Shelf-life extension of fresh-cut Fuji apples at different ripeness stages using natural substances. *Postharvest Biology and Technology*, 45(2): 265-275.

Reyes, L.F.; Villarreal, J.E.; Cisneros-Zevallos, L.C. 2006. The increase in antioxidant capacity alter wounding depends on the type of fruit or vegetable tissue. *Food Chemistry*, 101: 1254-1262.

Richard, F.; Goupy, P.M.; Nicolas, J.J.; Lacombe, J.M.; Pavia, A.A. 1991. Cysteine as an inhibitor of enzymatic browning. 1. Isolation and characterization of addition compounds formed during oxidation of phenolics by apple polyphenol oxidase. *Journal of Agriculture and Food Chemistry*, 39: 841-847.

Richard-Forget, F.C.; Goupy, P.M.; Nicolas, J.J. 1992. Cysteine as an inhibitor of enzymatic browning. 2. Kinetic studies. *Journal of Agricultural and Food Chemistry*, 40: 2108-2113.

Rojas-Graü, M.A.; Grasa-Guillem, R.; Martín-Belloso, O. 2007a. Quality changes in fresh-cut fuji apple as affected by ripeness stage, antibrowning agents, and stored atmosphere. Journal *of Food Science*, 72: 36-43.

Rojas-Graü, M.A.; Tapia, M.S.; Rodríguez, F.J.; Carmona, A.J.; Martín-Belloso, O. 2007b. Alginate and gellan based edible coatings as support of antibrowning agents applied on fresh-cut Fuji apple. *Food Hydrocolloids*, 21, 118-127

Rojas-Graü, M.A.; Tapia, M.S.; Martín-Belloso, O. 2007b. Using polysaccharide-based edible coatings to maintain quality of fresh-cut Fuji apples. *LWT-Food Science and Technology*, doi: 10.1016/j.lwt.2007.01.009

Saltveit, M.E. 1997. Physical and physiological changes in minimally processed fruits and vegetables. In: Tomás-Barberán, F.A.; Robin, R.D. (Eds.), Phytochemistry of Fruits and Vegetables. Oxford University Press, Oxford, pp. 205-220.

Sapers, G.M.; Douglas, F.W. 1987. Measurement of enzymatic browning at cut surfaces and in juice of raw apple and pear fruits. *Journal of Food Science*, 52:1258-62, 1285

Sapers, G.M.; Miller, R.L. 1998. Browning inhibition in fresh-cut pears. *Journal of Food Science*, 63:342-346.

Shahidi, F. 2004. Biosynthesis, classification, and nomenclature of phenolics in food and Nutraceuticals. In: Shahidi, F and Naczk, M. (Eds.). Phenolics in Food and Nutraceuticals. CRC Press, New York, pp. 1-204.

Soliva-Fortuny, R.; Biosca-Biosca, M.; Grigelmo-Miguel, N.; Martín-Belloso, O. 2002. Browning, polyphenol oxidase activity and headspace gas composition during storage of minimally

processed pears using modified atmosphere packaging. *Journal of the Science of Food and Agriculture*, 82: 1490-1496.

Soliva-Fortuny, R. 2003a. Influencia de las condiciones de envasado y conservación sobre la calidad y vida útil de manzana y pera mínimamente procesadas. Tesis Doctoral. Universidad de Lleida (España).

Soliva-Fortuny, R.; Martín-Belloso, O. 2003b. New advances in extending the shelf-life of fresh-cut fruits: a review. *Trends in Food Science and Technology*, 14: 341-353.

Soliva-Fortuny, R. C.; Martín-Belloso, O. 2003c. Microbiological and biochemical changes in minimally processed fresh-cut 'Conference' pears. *European Food Research and Technology*, 217: 4-9.

Soliva-Fortuny, R.; Alós-Saiz, N.; Espachs-Barroso, A.; Martín-Belloso, O. 2004. Influence of maturity at processing on quality attributes of fresh-cut 'Conference' pears. *Journal of Food Science*, 69: 290-294.

Solomos, T. 1997. Principles underlying modified atmosphere packaging. In: Wiley, R.C. (Ed.), Minimally Processed Refrigerated Fruits and Vegetables. Chapman & Hall, New York, pp. 183-225.

Tudela, J.A.; Espín, J.C.; Gil, M.I. 2002. Vitamin C retention in fresh-cut potatoes. *Postharvest Biology and Technology*, 26: 75-84.

Valdenegro, M.; Ramírez, M.; Flores, F.B.; Romojaro, F. 2004. Influencia de 1-MCP y etileno sobre los daños por frío en melón español durante el almacenamiento. In: Instituto Nacional de Investigação Agrária (Ed.), Maturação e Pós-colheita 2004 – Frutos e Hortícolas, 6-9 Octubro 2004. Instituto Nacional de Investigação Agrária, Oeiras, Portugal, pp. 519-521.

Vámos-Vigyázó, L. 1995. Prevention of enzymatic browning in fruits and vegetables: a review of principles and practice, Enzymatic Browning and its Prevention, American Chemical Society, Washington, DC, 300:49-62

Varela, P.; Salvador, A.; Hernando, I.; Pérez-Munuera, I.; Quiles, A.; Lluch, M.A.; Fiszman, S. 2007a. Eating quality of 'Flor de Invierno' pears: chemical and structural aspects. *International Journal of Food Science and Technology*, 42: 1052-1058.

Winkler, B.S.; Orselli, S.M.; Rex, T.S. 1994. The redox couple between glutathione and ascorbic acid: a chemical and physiological perspective. *Free Radical Biology and Medical*, 17: 333-349.

Wszelaki, A.L.; Mitcham, E.J. 2000. Effects of superatmospheric oxygen on strawberry fruit quality and decay. *Postharvest Biology and Technology*, 20: 125-133.

Wong, D.; Tillin, S.J.; Hudson, J.S.; Pavlath, A.E. 1994. Gas exchange in cut apples with bilayer coatings. *Journal of Agricultural and Food Chemistry*, 42: 2278-2285.

Yang, S.F. 1981. Biosíntesis of ethylene and its regulation. In: Friend, J.; Rhodes, M.J.C. (Eds.). Recent advances in the biochemistry of fruit and vegetables. Academic Press, London, pp 89-106.

5.Conclusiones

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En base a los resultados obtenidos y de su interpretación se pueden deducir las siguientes conclusiones:

→ El estado de madurez del fruto es un factor limitante de la vida útil de melón y pera frescos cortados. La inmersión de los trozos de melón en cloruro cálcico (0,5% p/v) previa al envasado fue necesaria para el mantenimiento de la firmeza. Por otro lado, no se observó ablandamiento de los tejidos de pera durante su almacenamiento pero éstos sufrieron un rápido pardeamiento enzimático. El uso combinado de agentes antioxidantes tales como N-acetilcisteína (0,75% p/v) y glutatión (0,75% p/v) controló eficazmente el pardeamiento de los trozos de pera durante 28 días, inhibiendo completamente la actividad de la enzima polifenoloxidasa.

→ Atmósferas de envasado ricas en O_2 fueron efectivas en la disminución de la velocidad de crecimiento microbiano, observándose efectos similares con un envasado en concentraciones iniciales bajas de O_2 y altas de CO_2. Se demostró la sensibilidad a las concentraciones altas de O_2 de levaduras del género *Rhodotorula*, predominantes en melón 'Piel de Sapo' y pera 'Flor de Invierno' frescos cortados.

→ El uso de concentraciones altas de O_2 indujo un aumento de la actividad respiratoria de melón y pera frescos cortados, en comparación con el envasado en concentraciones iniciales bajas de O_2 y altas de CO_2. No obstante, concentraciones altas de O_2 retrasaron la producción de metabolitos fermentativos relacionados con la iniciación del metabolismo anaeróbico del fruto. Además, la inmersión de la pera fresca cortada en una solución de N-acetilcisteína (0,75% p/v) y glutatión (0,75% p/v) controló el pardeamiento y tuvo un efecto importante en la reducción de la producción de etileno bajo concentraciones altas de O_2.

→ El factor principal que afecta a la degradación de vitamina C es la presencia de O_2 aunque concentraciones muy bajas de O_2 y excesivamente elevadas de CO_2 aumentaron la actividad de la enzima peroxidasa, directamente relacionada con la pérdida de vitamina C durante el almacenamiento de melón 'Piel de Sapo' fresco cortado, además de favorecer el desarrollo de translucidez y ablandamiento en el producto.

→ Se observó formación de compuestos fenólicos durante el almacenamiento de melón y pera frescos cortados envasados en atmósfera modificada, especialmente bajo concentraciones muy bajas de O_2 y excesivamente elevadas de CO_2. Aunque las concentraciones bajas de O_2 redujeron la oxidación de compuestos fenólicos tales como el ácido clorogénico en pera fresca cortada, otros compuestos relacionados con el metabolismo fenilpropanoide tales como (-)-epicatequina o ácido sináptico aumentaron, especialmente en pera tratada con N-acetilcisteína (0,75% p/v) y glutatión (0,75% p/v) bajo concentraciones bajas de O_2 y elevadas de CO_2.

→ La capacidad antioxidante de melón y pera frescos cortados se atribuyó principalmente al contenido de compuestos fenólicos y en menor medida al contenido de vitamina C.

→ Los recubrimientos comestibles a base de alginato y pectina no solo aumentaron la resistencia al vapor de agua y redujeron el ablandamiento de melón fresco cortado, sino que también disminuyeron el estrés oxidativo asociado a la acumulación de compuestos fenólicos en fruta cortada. La incorporación de N-acetilcisteina (0,75% p/v) y glutatión (0,75% p/v) a los recubrimientos comestibles controló eficazmente el pardeamiento enzimático, redujo la producción de etileno, contribuyó al mantenimiento del potencial antioxidante, además de alargar la estabilidad microbiológica de pera fresca cortada.