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# Internal quality profile and influence of packaging conditions on fresh-cut pineapple

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# INTERNAL QUALITY PROFILE AND INFLUENCE OF PACKAGING CONDITIONS ON FRESH-CUT PINEAPPLE

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To my husband, Luis,

To my son and daughters,

Fer, Adri and Meli

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# ABSTRACT

The flesh quality profile of Gold cultivar pineapple and the influence of packaging conditions on fruit pieces were studied as tools to prop up homogeneous, reproducible, and endurable quality of fresh-cut pineapple. Physicochemical, mechanical and antioxidant attributes, as well as the aroma profile of the natural occurring volatiles were determined for three cross-sections cut along the pineapple. The influence of packaging conditions using passive modified atmosphere (AIR) with and without an alginate coating and two active modified atmospheres (low oxygen, LO: 12 % $O_2$, 1% $CO_2$ and high oxygen, HO: 38% $O_2$) at 5 °C on the quality of the fresh-cut fruit was also assessed. Soluble solids content (SSC), titratable acidity (TA), water content, vitamin C and phenolic compounds content, as well as POD activity in the bottom third of the fruit were significantly higher ($p<0.05$) than in other sections, while L* and b* color parameters were smaller. In general, the mechanical response of pineapple flesh to penetration, cut, compression and/or extrusion forces did not significantly vary among pieces from different sections of the fruit, except for the shear test, which showed the largest resistance in pineapple pieces cut from the bottom third of the fruit. In addition, twenty volatile compounds were identified and quantified from the fresh pineapple aroma profile. The most abundant volatile compounds were methyl butanoate, methyl 2-methyl butanoate and methyl hexanoate, whereas the most odor active volatiles of pineapple aroma were methyl 2-methyl butanoate, ethyl 2-methyl butanoate, ethyl hexanoate and 2,5-dimethyl-4-methoxy-3(2H)-furanone. The same aroma profile constituents were found in the three cross-sections of the fruit, but the total volatiles content increased from the top to the bottom third of the fruit (7560 to 10910 µg/kg). The concentration of the main odor active volatiles as well as their relative content varied along the fruit. On the other hand, AIR, LO and HO atmospheres allowed the preservation of SSC, TA, pH and color of fresh-cut pineapple for two weeks without differences among packaging atmospheres. Vitamin C content and antioxidant capacity were smaller in fruit pieces packed under HO atmosphere than in LO or AIR, but no changes were observed along storage. Total phenols content and juice leakage differed among packaging conditions and along storage. Alginate coating helped to reduce juice leaked from pineapple pieces, while high $CO_2$ concentrations were likely to promote it. Shear test hardness and work were bigger for pineapple pieces cut from the bottom third than other parts of the fruit; however, mechanical characteristics of the fruit were not modified during storage. Moreover, volatile compounds content reached a maximum during the second week of storage, and depleted thereafter. The use of passive modified atmosphere and alginate coatings could favor longer withhold of odor active volatile compounds and antioxidant attributes in the fresh-cut fruit, with reduced juice leakage along storage at 5 °C. Adequate mixing procedures during fresh-cut pineapple preparation, accounting for quality attributes differences along the fruit, are needed for homogeneous and reproducible quality of fresh-cut pineapple.

# RESUMEN

Las diferencias en los atributos de calidad de la pulpa de piña del cultivar 'Gold' a lo largo de la fruta y la influencia de las condiciones de envasado fueron estudiadas como instrumentos orientados a la obtención de trozos de piña fresca cortada con una calidad homogénea, reproducible y duradera. Se determinaron las propiedades físico-químicas, mecánicas y antioxidantes, así como y el perfil de los compuestos aromáticos de la pulpa de piña fresca cortada de tres secciones transversales a lo largo de su eje central (superior, medio e inferior). Se evaluó la influencia del uso de atmósferas modificadas pasivas (envasadas en aire, AIR: 20.9% de $O_2$, y los trozos de piña con ó sin una película comestible de alginato) y activas (en atmósferas de bajo oxígeno, LO: 12% de $O_2$, 1% de $CO_2$, y alto oxígeno, HO: 38% de $O_2$) a 5 °C sobre la calidad de la fruta cortada. Se encontró que el contenido de sólidos solubles (SSC), acidez titulable (AT), contenido de agua, vitamina C, compuestos fenólicos y actividad enzimática de la peroxidasa (POD) fue significativamente mayor en los trozos de fruta procedentes del tercio inferior de la fruta ($p < 0,05$), en comparación con los de otras secciones de la fruta, mientras que los parámetros de color L* y b* resultaron menores. En general, la respuesta mecánica de la pulpa de piña a las fuerzas de penetración, corte, compresión y / o extrusión no varió significativamente entre los trozos cortados de diferentes secciones de la fruta; sin embargo, en la prueba de corte, los trozos cortados del tercio inferior, cerca de la base de la fruta, mostraron una mayor fuerza de resistencia. Se identificaron y cuantificaron veinte compuestos volátiles del perfil aromático de la piña. De ellos, los más abundantes fueron butanoato de metilo, metil-2-metil butanoato y hexanoato de metilo, mientras que los de mayor impacto en el olor de esta fruta fueron metil-2-metil butanoato, etil 2-metil butanoato, hexanoato de etilo y 2,5-dimetil-4 -metoxi-3 (2H)-furanona. En las tres secciones transversales de la fruta, se identificaron los mismos componentes del perfil aromático, aunque el contenido total de los mismos fue mayor en los trozos cortados del tercio inferior (10910 mg / kg) con respecto a aquellos cortados del tercio superior, cerca de la corona de la piña (7560 mg / kg). El contenido de los principales compuestos aromáticos de la piña varió a lo largo de la fruta y también su composición relativa. Por otro lado, no se observaron diferencias significativas en SSC, TA, pH y el color de la piña fresca cortada entre ninguna de las atmósferas evaluadas ni durante dos semanas de almacenamiento a 5 °C. El contenido de vitamina C y la capacidad antioxidante fueron 15 y 8% menores, respectivamente, en los trozos de fruta envasados en las atmósferas HO que en LO o AIR, aunque no se observaron cambios a lo largo del almacenamiento. El contenido total de fenoles y la cantidad de líquido drenado de los trozos de piña variaron para las distintas condiciones de envasado y tiempos de almacenamientos. El uso de una película comestible de alginato en los trozos de piña contribuyó a reducir la cantidad de líquido drenado en contraste con las concentraciones altas de $CO_2$ que parecen favorecerlo. La dureza y el trabajo asociado al corte fueron mayores para los trozos de piña cortados del tercio inferior de la fruta (base), pero no mostraron variaciones

a lo largo del tiempo. Adicionalmente, el contenido de compuestos volátiles alcanzó un valor máximo durante la segunda semana de almacenamiento, reduciéndose posteriormente. Dada la variación de los parámetros de calidad a lo largo de la piña, es necesario el uso de procesos de mezclado que permitan la obtención de lotes de fruta fresca cortada con atributos de calidad homogéneos y reproducibles. El uso de atmosferas modificadas pasivas favoreció la retención de de los compuestos volátiles con mayor impacto en el aroma de la piña y de sus propiedades antioxidantes. Su combinación con el uso de una película comestible de alginato, podría favorecer una mayor reducción de la pérdida de líquido durante el almacenamiento a 5 °C.

# RESUM

Les diferències en els atributs de qualitat de la polpa de pinya del cultivar 'Gold' entre diferents parts del fruit i la influència de les condicions d'envasament van ser estudiades com a instruments orientats a l'obtenció de trossos de pinya fresca tallada amb una qualitat homogènia, reproduïble i duradora. Es van determinar les propietats físico-químiques, mecàniques i antioxidants, així com i el perfil dels composts aromàtics de la polpa de pinya fresca tallada de tres seccions transversals al llarg del seu eix central (superior, mig i inferior). Es va avaluar la influència de l'ús d'atmosferes modificades passives (envasades en aire, AIR, combinades amb l'aplicació d'una pel·lícula comestible a base d'alginat), i actives (en atmosferes de baix oxigen, LO: 12% de $O_2$, 1% de $CO_2$, i alt oxigen, HO: 38% de $O_2$) sobre la qualitat de la fruita tallada. Es va trobar que el contingut de sòlids solubles (SSC), acidesa titulable (AT), contingut d'aigua, vitamina C, compostos fenòlics i activitat enzimàtica de la peroxidasa (POD) van ser significativament major en els trossos procedents del terç inferior del fruit ($p < 0,05$), en comparació amb els d'altres seccions de la fruita, mentre que els paràmetres de color L* i b* van resultar més baixos. En general, la resposta mecànica de la polpa de pinya a les forces de penetració, tall, compressió i / o extrusió no varià significativament entre els trossos tallats de diferents seccions de la fruita; tanmateix, en assajos de tall, els trossos procedents del terç inferior de les pinyes mostraren una major resistència mecànica. Es van identificar i quantificar vint composts volàtils del perfil aromàtic de la pinya. D'ells, els més abundants van ser butanoat de metil, metil-2-metil butanoat i hexanoat de metil, mentre que els de major impacte en l'olor d'aquesta fruita van ser metil-2-metil butanoat, etil-2-metil butanoat, hexanoat d'etil i 2,5-dimetil-4-metoxi-3(2H)-furanona. A les tres seccions transversals de la fruita, es van identificar els mateixos components del perfil aromàtic, encara que el contingut total dels mateixos fou major en els trossos tallats del terç inferior (10910 mg / kg) respecte a aquells obtinguts del terç superior, a prop de la corona de la pinya (7560 mg / kg). El contingut dels principals compostos aromàtics de la pinya va variar en les diverses zones del fruit i també la seva composició relativa. D'altra banda, no es van observar diferències significatives en SSC, TA, pH i color de la pinya fresca tallada entre cap de les atmosferes avaluades durant dues setmanes d'emmagatzemament a 5 °C. El contingut de vitamina C i la capacitat antioxidant van ser un 15 i un 8% menors, respectivament, en els trossos de fruita envasats en les atmosferes HO que en LO o AIR, encara que no es van observar canvis durant l'emmagatzemament. El contingut total de fenols i la quantitat de líquid drenat dels trossos de pinya van variar per a les diferents condicions d'envasament i temps d'emmagatzemament. L'ús d'un recobriment comestible d'alginat sobre els trossos de pinya va contribuir a reduir la quantitat de líquid drenat en contrast amb les concentracions altes de $CO_2$, que semblen afavorir-lo. La duresa i el treball associat al tall van ser majors per als trossos de pinya obtinguts a partir del terç inferior dels fruits (base), però no van mostrar variacions durant l'emmagatzemament. Addicionalment, el contingut de compostos volàtils va

assolir un valor màxim durant la segona setmana d'emmagatzemament, reduint-se posteriorment. Donada la variació dels paràmetres de qualitat entre diferents parts de la pinya, és fa necessari l'ús de processos de barrejat que permetin l'obtenció de lots de fruita fresca tallada amb atributs de qualitat homogenis i reproduïbles. L'ús d'atmosferes modificades passives va afavorir la retenció dels compostos volàtils amb un major impacte en l'aroma de la pinya i de les seves propietats antioxidants. La seva combinació amb l'ús d'un recobriment comestible a base d'alginat podria afavorir una major reducció de les pèrdues de líquid durant l'emmagatzemament a 5°C.

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# INTRODUCTION

# TENDENCIAS EN EL PROCESADO MÍNIMO DE FRUTAS Y HORTALIZAS FRESCAS

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## 1. INTRODUCCIÓN

El mercado de frutas y hortalizas mínimamente procesadas está creciendo sostenidamente desde los años 80 y 90, marcado por una continua innovación en los productos y por la mejora de los canales de distribución. Se inició con una pequeña gama de productos dirigida mayoritariamente a los servicios de alimentación, particularmente a la expedición de comida rápida, convirtiéndose en una gran industria con una amplia variedad de productos frescos cortados, los cuales actualmente se comercializan en el sector institucional (hostelería y restauración) y especialmente para su venta directa en supermercados y grandes superficies.

Los productos mínimamente procesados confieren valor añadido a las frutas y hortalizas frescas enteras, ofreciendo al consumidor, por un lado conveniencia en cuanto al espacio y tiempo de preparación, y por otro, un producto con atributos similares a los del producto fresco. En este sentido, el consumidor reconoce la importancia de la incorporación de las frutas y hortalizas frescas en la dieta diaria, por su alto contenido de vitaminas, antioxidantes, minerales, fibra, hidratos de carbono y agua, así como de sustancias fitoquímicas que pueden ayudar a prevenir el riesgo de contraer cáncer y enfermedades del corazón. En la actualidad, el consumidor es más consciente de la importancia de una buena alimentación y busca nuevas alternativas en comidas saludables, según se ve reflejado en la gran cantidad

de nuevos productos enriquecidos con vitaminas y otros nutrientes, que se encuentran actualmente en el mercado. Su estilo de vida también ha cambiado, y cada vez cuenta con menos tiempo para preparar y comer los alimentos, por lo que busca productos alternativos nutritivos, sabrosos, variados y fáciles de preparar.

En este sentido, los vegetales mínimamente procesados, también conocidos como productos frescos cortados, de cuarta gama ó listos para consumir, están dirigidos a satisfacer la demanda actual del consumidor. Estos productos son sometidos a diversas operaciones de procesado, tales como pelado, cortado, reducción de tamaño, lavado y envasado, que persiguen la conservación mediante una combinación de tratamientos parciales minimizando el impacto de dichas operaciones (Wiley, 1997). Estos productos no son sometidos a ningún tratamiento térmico para la destrucción de microorganismos, sino que sus tejidos mantienen sus funciones metabólicas activas hasta que llegan al consumidor final. La conveniencia que ofrecen estos productos, en términos de calidad, disponibilidad, facilidad de preparación, valor nutritivo, sabor y seguridad, responde a las necesidades y preferencias del consumidor. Son alimentos que mantienen las características de los productos frescos recién cortados.

El consumo de frutas y hortalizas frescas cortadas ha crecido vertiginosamente en EE.UU y muchos países europeos; en el año 2005, el consumo per cápita en España estuvo entre 1.5 y 2.0 kg, que se puede considerar bajo comparado con los 30 kg en EE.UU. y 6 kg en Francia. Dentro de este sector, las hortalizas frescas cortadas dominan el mercado, principalmente las lechugas cortadas y las mezclas de ensaladas, seguidos por las espinacas y las acelgas. La introducción de las frutas cortadas ha sido más lenta, por tratarse de productos más perecederos que las hortalizas; sin embargo, ya se pueden encontrar una gran variedad de frutas en el mercado incluyendo trozos de pera, manzana, melocotón, sandía, kiwi, mango, mandarina, uva y piña. En EE.UU. la participación del mercado de frutas frescas cortadas ha crecido en los últimos años, siendo los productos cortados de sandía, melón cantaloupe, mezclas de frutas y piña las más importantes.

El sector de la hostelería y la restauración consumen alrededor del 22% de los productos mínimamente procesados comercializados en España y el crecimiento continúa con la introducción de nuevos productos, materiales de envasado y mejoras en la higiene de los procesos, según la Asociación Española de Frutas y Hortalizas Lavadas y Listas para su empleo (AFHORLA), lo cual resalta la importancia de la venta al detalle. También señalan que el mayor crecimiento a nivel español se da en las

grandes urbes, concentrándose un 45% del consumo total en las áreas metropolitanas de Madrid, Barcelona y Valencia (Agroinformación, 2009).

La calidad de los productos frescos cortados depende principalmente de las variedades que se utilicen, las prácticas antes y después de la cosecha, factores climáticos, índices y método de cosecha, el tiempo que transcurre entre la cosecha y el procesado, y la forma y los equipos con que éstos son preparados (Kader, 2002; Rojas-Graü y Martin-Belloso, 2005; Lamikanra, 2005; Varoquaux y Mazollier, 2002; Hodges y Toivonen, 2008). Para su elaboración, solamente se deben utilizar productos frescos enteros de buena calidad, sin daños fisiológicos ni patológicos, golpes, ni residuos de pesticidas u otros daños que incidirán directamente sobre la calidad y vida útil del producto; por tanto no se podrán aprovechar partes de productos parcialmente deteriorados.

Los tejidos de las frutas y hortalizas frescas cortadas están vivos y por ello, responden a los cortes realizados durante su preparación con un aumento en su actividad fisiológica y una mayor susceptibilidad al deterioro, pues al quitar la piel y disminuir su tamaño se rompen tejidos y se expone una mayor área a las condiciones ambientales externas, favoreciendo la pérdida de humedad, el ablandamiento de los tejidos, la pérdida de aromas, los cambios de color y la entrada de microorganismos indeseables.

Algunos tratamientos estabilizantes ayudan a conservar la calidad de estos productos, tales como la inmersión en soluciones de sales de calcio para conservar la firmeza del producto, agentes antioxidantes para controlar los cambios de color, el uso de sustancias antimicrobianas para controlar el crecimiento de microorganismos indeseables, y otros tratamientos coadyuvantes dirigidos a retardar su deterioro y prolongar su vida comercial, sin afectar sus atributos sensoriales (Rojas-Graü y Martín-Belloso, 2005; Garcia y Barret, 2002).

Por otro lado, es necesario el uso de un envase apropiado con el fin de proteger al producto contra daños físicos a la vez de ofrecer una barrera a la entrada de microorganismos indeseables y la salida de compuestos volátiles aromáticos. Actualmente, existe en el mercado una gran variedad de materiales poliméricos con distintas características de permeabilidad al oxígeno y al dióxido de carbono, con los cuales puede alcanzarse una correcta modificación de la composición de los gases dentro del envase (Al-Ati y Hotchkiss, 2002).

La modificación de la atmósfera puede hacerse pasiva o activamente. En el primer caso, los envases se llenan y se cierran, atrapando el aire, de modo que la

composición inicial en el interior de los envases es similar a la del aire, y ésta cambia durante el almacenamiento como resultado de la respiración del producto envasado y el intercambio de gases a través de la superficie del envase. En el segundo caso, se sustituye el aire por una mezcla de gases antes de sellar los envases. El uso de atmósferas modificadas ayuda a retardar la aparición de síntomas de deterioro como la pérdida de firmeza, cambios en el color y apariencia del producto y reducción en la tasa respiratoria, con lo cual la vida útil puede prolongarse significativamente. Sin embargo, el efecto difiere según el tipo de producto, la composición de los gases y las características de los envases. Atmósferas con bajo contenido de oxígeno (1 a 5%) y alto contenido de dióxido de carbono (5-10%) pueden reducir significativamente la actividad metabólica de frutos como manzana y pera (Oms-Oliu et al., 2008) y hasta pueden retardar el crecimiento de microorganismos indeseables. Sin embargo, cuando las concentraciones de oxígeno son inferiores al 2% pueden ocurrir problemas de crecimiento anaeróbico de patógenos indeseables y reacciones de deterioro que afecten el sabor, aroma y otros atributos de calidad de los productos frescos cortados. Similarmente, el uso de una atmósfera modificada con un alto contenido de oxígeno (mayor de 70%) ayuda a conservar la firmeza de algunas frutas cortadas tales como pera (cultivar Flor de Invierno), aunque no inhibe las reacciones de pardeamiento. Sin embargo su uso en trozos de melón (piel de sapo), permite mantener mejor el color y la firmeza que cuando se emplean atmósferas con una concentración reducida de oxígeno (Oms-Oliu et al., 2007 y 2008). En el caso del envasado de trozos de piña cortada, concentraciones entre 10 y 40% de oxígeno resultaron beneficiosas (Montero-Calderón et al., 2008); sin embargo, es necesario vigilar que la concentración de oxígeno no baje del 2% para evitar reacciones indeseables.

Así pues, la respuesta de los productos frescos cortados al uso de atmósferas modificadas, dependerá del tipo de producto, grado de madurez y prácticas antes y después de la cosecha, pero en todos los casos, deben ir acompañadas por un buen control de la temperatura durante toda la cadena de producción y comercialización del producto, siendo la temperatura ideal de 5 °C. Temperaturas mayores aceleran el deterioro y minimizan el efecto de beneficioso de cualquier tratamiento estabilizante.

La innovación y las mejoras tecnológicas han acompañado el avance de estos productos en los mercados internacionales. Se ha logrado mejorar los procesos para reducir los daños físicos durante la preparación y manipulación de las frutas y hortalizas frescas cortadas, mejorar las condiciones de higiene y las buenas prácticas

de manufactura, reduciendo así el riesgo de contaminación. También se han desarrollado materiales de envase que contribuyen a conservar la calidad del producto por un mayor tiempo.

Actualmente, el uso de recubrimientos comestibles es quizás la técnica más novedosa y prometedora para alargar la vida útil de este tipo de productos, por los beneficios que aporta como barrera a los gases y al vapor de agua, además de la posibilidad de utilizarlo como vehículo de sustancias activas en el alimento, permitiendo conservar la calidad de los trozos de frutas y hortalizas frescas cortadas. Estos recubrimientos comestibles proveen una barrera protectora entre el producto y el ambiente que lo rodea, moderando a su vez el intercambio de gases ($O_2$, $CO_2$, etileno, compuestos aromáticos). Además, dan soporte estructural al alimento, ayudando a conservar su textura, limitando la pérdida de humedad y salida de fluidos del producto fresco cortado (Figura 1).

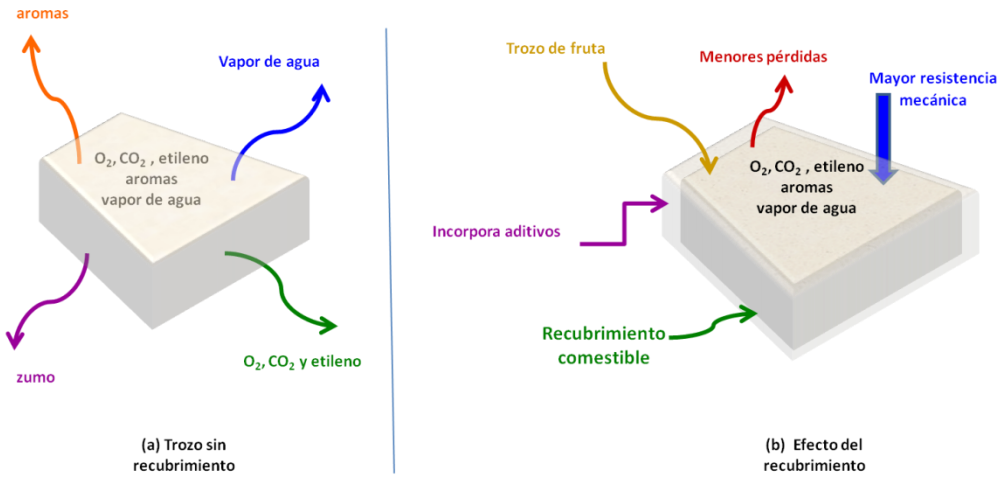


**Figura 1.** Principales propiedades de los recubrimientos comestibles en productos frescos cortados

Generalmente, el recubrimiento comestible se forma directamente sobre la superficie de los trozos de frutas y hortalizas, como una capa uniforme muy fina. Estos recubrimientos pueden ser de origen proteico (caseína, proteínas de suero, colágeno, zeína de maíz y proteína de soja) o de origen polisacáridos (como celulosa, quitosano, pectinas, almidón, alginato, gelano, carragenato, carboximetilcelulosa y

algunos que se preparan con base de purés de frutas como manzana, melocotón, pera y plátano).

Los recubrimientos de proteínas y polisacáridos se complementan con ingredientes lipídicos para aumentar la barrera al vapor de agua y agentes plastificantes como el glicerol, que contribuye a mejorar las características elásticas y de permeabilidad de esa delgada capa sobre la superficie externa de los trozos de frutas u hortalizas frescas cortadas. Según Olivas y Barbosa-Cánovas (2005) y Rojas-Graü et al. (2007a) los recubrimientos comestibles deben prepararse con sustancias seguras (GRAS: generalmente reconocidas como seguras), ser estables en condiciones de humedad relativa alta, buenas barreras al vapor de agua, al oxígeno y al dióxido de carbono, presentar buenas propiedades mecánicas y de adhesión al producto, resultar aceptables sensorialmente y poseer un costo razonable.

Otros aditivos incorporados en los recubrimientos comestibles son las sales de calcio que actúan como agentes texturizantes, aumentando la resistencia mecánica, agentes antioxidantes para prevenir el oscurecimiento en productos susceptibles de pardeamiento (ácido cítrico, ácido ascórbico, cisteína, glutatión, etc.), agentes antimicrobianos (ácidos orgánicos, aceites esenciales, etc.) y otros compuestos que pueden mejorar las propiedades sensoriales o nutricionales de los trozos de frutas y vegetales cortados, como saborizantes, colorantes, nutracéuticos y agentes probióticos.

Entre los campos en los que se ha investigado en los últimos años destaca la combinación de tratamientos estabilizantes empleando sustancias naturales para la conservación de la calidad de las frutas frescas cortadas durante un tiempo más largo. Para cada producto, se debe plantear una estrategia para retardar la aparición de los síntomas de deterioro; así por ejemplo, para productos como manzana y pera, los cambios de color pueden ser controlados con tratamientos antioxidantes y utilizando atmósferas modificadas, y la pérdida de firmeza mediante tratamientos con sales de calcio (Oms-Oliu et al., 2007; Rojas-Graü et al., 2007a); el control del crecimiento de microorganismos indeseables puede hacerse parcialmente con el uso de atmósferas de alto contenido de oxígeno y agentes antimicrobianos naturales (aceites esenciales, ácidos orgánicos) que ayudan a conservar la apariencia y vida comercial de los productos frescos cortados.

Por otro lado, los recubrimientos comestibles complementan los efectos de algunos de estos tratamientos estabilizantes y pueden ser utilizados como vehículo para la aplicación de algunos compuestos que beneficien al producto y ayuden a conservar

su calidad. Se ha encontrado que los recubrimientos comestibles pueden ayudar a conservar la firmeza, color y apariencia de los trozos de manzana, pera, melón y papaya (Figura 2). Además, mediante su uso, se puede reducir la pérdida de fluidos en trozos de piña fresca cortada (Figura 3). La incorporación de agentes antioxidantes en recubrimientos comestibles ha dado buenos resultados en manzana, pera y melón frescos cortados. Otra aplicación novedosa ha sido la incorporación de microorganismos probióticos en recubrimientos de alginato y gelano sobre manzana y papaya (Tapia et al., 2007). Finalmente, se han obtenido muy buenos resultados con la incorporación de aceites esenciales dentro de los recubrimientos comestibles, como tratamiento antimicrobiano, aplicados en trozos de manzana y melón fresco cortado (Raybaudi-Massilia et al., 2007 y 2008; Rojas-Graü, et al., 2007b).

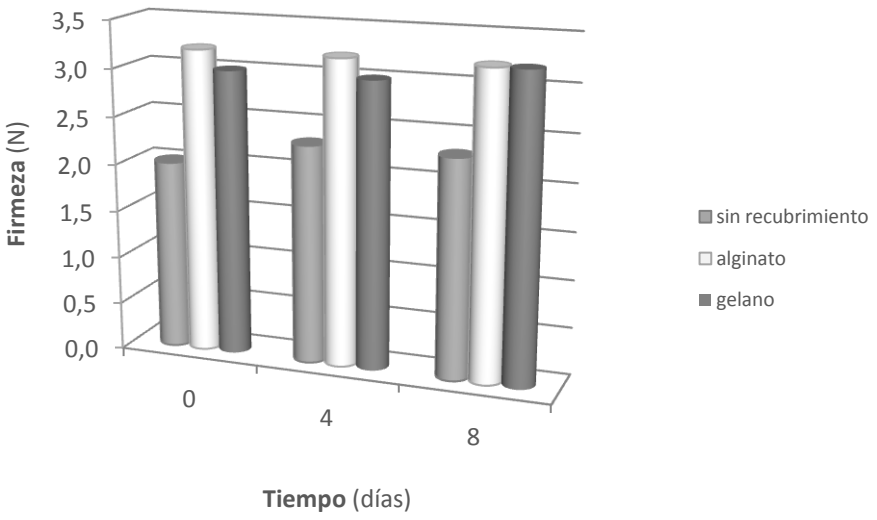


**Figura 2.** Efecto de la incorporación de cloruro de calcio (2% p/v) en recubrimientos de alginato o gelano en la firmeza de trozos de papaya almacenados bajo refrigeración (Adaptado de Tapia et al., 2008)

## CONSIDERACIONES FINALES

Las frutas y hortalizas frescas cortadas ofrecen al consumidor un producto atractivo, con atributos sensoriales y nutritivos que se ajustan a sus necesidades y preferencias. Por ello, la demanda de este tipo de alimentos saludables, seguros y convenientes crece continuamente.

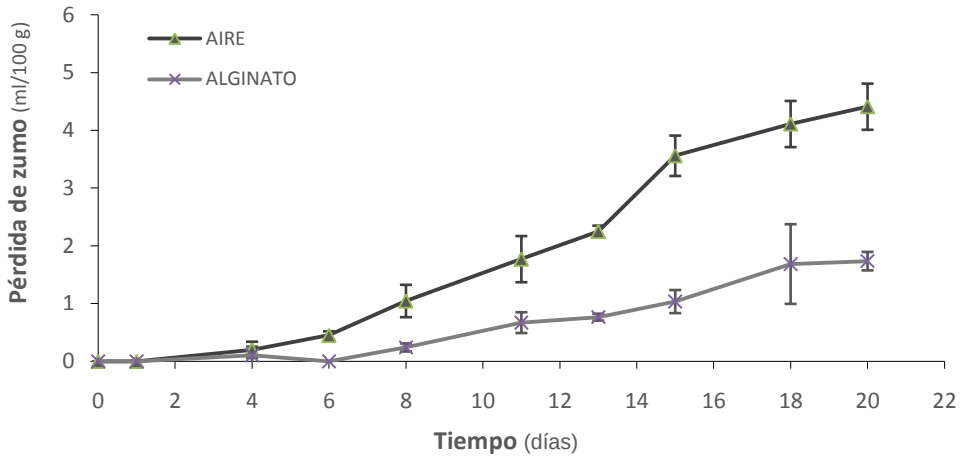


**Figura 3.** Efecto de la aplicación de un recubrimiento de alginato en la pérdida de zumo de trozos de piña fresca cortada almacenadas en envases de polipropileno sin modificación inicial de la atmosfera (Adaptado de Montero-Calderón, et al. 2008)

La calidad final de las frutas y hortalizas frescas cortadas es el resultado de una combinación inteligente de técnicas aplicadas. Así, la refrigeración durante los procesos de elaboración y distribución a una temperatura cercana a 5 °C, se complementa con una buena selección de la materia prima, unas prácticas higiénicas correctas durante la elaboración y manipulación de los productos frescos cortados y la selección adecuada de los envases y de la atmósfera interna que beneficie más a cada producto. Estas pautas básicas se complementan con la identificación y selección de tratamientos estabilizantes que permitan conservar los atributos de calidad del producto fresco recién cortado, como la incorporación de agentes antioxidantes, preferiblemente de origen natural, para conservar su color y apariencia, sales de calcio para mantener la firmeza sin afectar al sabor y otros parámetros de calidad, agentes antimicrobianos para minimizar el crecimiento microbiano, además de recubrimientos comestibles que por sí mismos pueden contribuir a mantener los atributos de textura, sabor, apariencia y reducir las pérdidas de fluidos y de humedad de los trozos de producto fresco cortado, pero que

también pueden utilizarse como medio de transporte para incorporar sustancias que supongan un valor añadido a los vegetales frescos cortados.

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# FRUITS AND VEGETABLES FOR THE FRESH-CUT PROCESSING INDUSTRY

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*IN: ADVANCES IN FRESH-CUT FRUITS AND VEGETABLES PROCESSING (FORTHCOMING)*

## 1. INTRODUCTION

Fruits and vegetables are a gift of nature with the charming to delight people from all ages. They provide a wide range of flavors and textures, loaded with most of the nutrients required by the organism for good health and wellness.

Their consumption as fresh produce is largely recommended all over the world; however, nowadays, consumers have limited time for food preparation. This has lead fresh-cut fruits and vegetables market to grow, as they offer good and convenient produces, ready-to-eat, with the fresh-like attributes.

Fresh-cut fruits and vegetables are composed of living cells, which naturally spoil and deteriorate over time and are affected by all preparation operations and surrounding conditions during pre- and post-harvest handling, processing operations and storage. Respiration and ethylene production rates, color, aroma, and texture change as a response to physical damages produced during cutting operations, as well as the activity of undesirable microorganisms (Wiley 1994, Artés-Hernández et al. 2007; Aguayo et al. 2004, Silveira et al. 2007a).

Quality attributes of the fruit and vegetables used as raw materials for processing have a great influence on final quality and shelf-life of fresh-cut products and hence, it is very important to identify and understand relevant changes for specific product and how they are affected by handling, processing and storage.

Produce to be used for the fresh-cut industry should resist processing and maintain their attributes with minimum variations for as long as possible. This chapter focuses on the fruits and vegetables requirements, handling and conditioning for fresh-cut produce processing.

## 2. QUALITY OF INTACT FRESH FRUITS AND VEGETABLES

Fresh fruits and vegetables are expected to preserve quality during handling, storage, processing and distribution. Even though quality criteria vary among products, it is generally associated to intrinsic properties of the food such as visual appearance, texture, flavor, its nutritive value and safety issues during field production, handling and processing. Appearance has a great influence on product selection for processing; shape, size, color, gloss, uniformity and lack of wilting, browning, and decay symptoms give clues about stage of maturity, freshness, and expected process yield.

Texture attributes gather structural properties of the product and related sensorial attributes perceived as it is bitten. Structures of fresh fruits and vegetables cells and tissues are complex in shape, chemical composition, adhesive and cohesive forces between cells and how they are affected by turgidity, maturity stage and other variables, resulting in a wide range of responses to force stresses during handling and processing (Schouten et al. 2004). Texture can be described by a series of parameters for specific characteristics such as firmness or hardness, fracturability, adhesiveness, gumminess, crispiness, fibrousness, juiciness, flexibility and others, being their relative importance dependent on the product and its final use. Mechanical response of intact produce is affected when fresh-cut products are prepared, because of injured cells and tissues, size reduction, elimination of protective skin, increased water losses and promptness to wilt and decay.

Flavor embraces taste and aroma attributes, with a very wide range of combinations of sweetness, sourness, bitterness, astringency along with the characteristic aromas of each product, and the absence of undesirable off-flavors and off-odors. Kader (2008) highlights today's importance of nutritive and better-flavored fruits and vegetables, as key factor in selecting cultivars, as a key to increase sales and consumption.

Nutrients content and biochemical composition vary with the products as they might come from different parts of the plant. Storage organs such as roots and tubers have high starch content, while stems are rich in fibers and skeleton type tissues with high lignin and cellulose content, and fruits are rich in sugars, organic acids, mineral salts, pectic substances and enzymes (Maestrelli and Chourot 2002). Soluble solids content, total or titratable acidity, pH, water content, density and the ratio of soluble solids content to acidity are commonly used as quality attributes. Fruits and vegetables are very good sources vitamins A and C, minerals, carbohydrates, dietetic

fiber, proteins and antioxidant compounds such us carotenoids, flavonoids and other phenolic compounds (Kader and Barret, 2004); their composition and concentration vary among cultivars and are affected by pre- and postharvest practices.

Safety requirements related with fresh-cut produce include good agricultural (GAP) and good processing practices (GMF), freedom of plagues, micotoxines, pesticide residues and any other chemical or physical contamination which might risk consumer health.

Finally, it is important to consider that fruits and vegetables quality attributes required for the fresh-cut industry may differ from those for intact fruit market, because no alterations are done to the products in the later case. Processors need intact fruits and vegetables that can withstand processing and maintain quality attributes of the fresh-cut product as long as possible, with high production yields, with very good and consistent quality, free form defects with the right maturation stage, and thus, the use of grocery stores surplus or low quality and unmarketable products for processing should be avoided. The right intact fruits and vegetables used as raw matter must allow the preparation of high quality fresh-cut products, with uniform and consistent quality, suitable post-cutting shelf-life and consumer satisfaction.

## 3. DYNAMIC BEHAVIOR OF FRESH FRUITS AND VEGETABLES

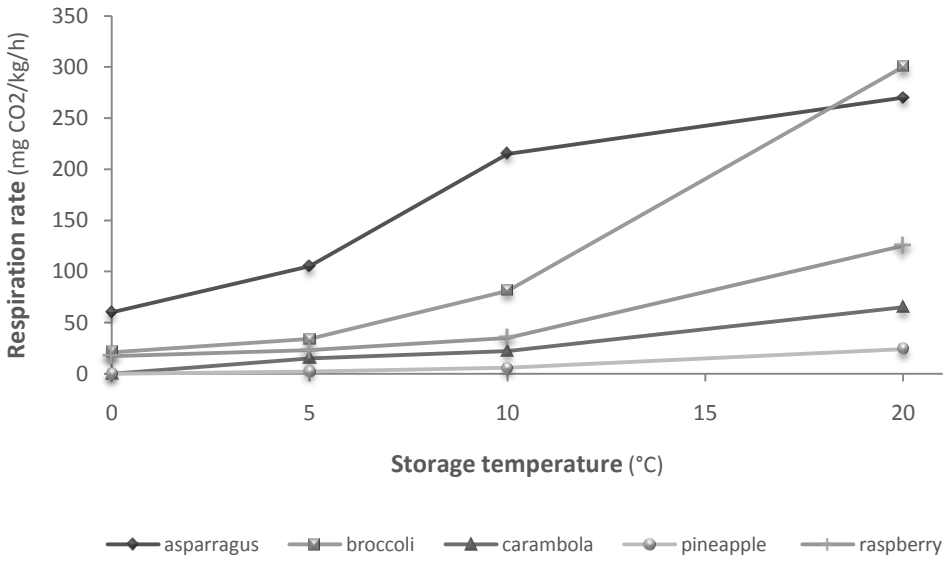
Fruits and vegetables are composed of living tissues and have to withstand two major hurdles: harvest and processing. When they are harvested, water and nutrient supply from the mother plant ends and tissues are injured at the incision point. Processing causes further physical damages as the products are cut, and generally takes away the product skin, which is a natural protection, but the product respiration and other metabolic activities continue all the way up to the consumer table.

For such dynamic system, changes are continually occurring, even though they may not be obvious for the first hours or days after harvesting or cutting. Appearance and texture alterations are the first to be noticed, although many more physical, physiological and biochemical changes are ongoing at the same time at different rates, and influenced by internal and external factors.

The main intrinsic changes of fruits and vegetables are described ahead, but they are directly related to external factors, such as, temperature, relative humidity, handling, microbiological and other stresses occurring during pre- and postharvest operations.

### *Respiration rate*

The rate of respiration is sensible to internal factors such as product type and maturity stage but also to external factors like temperature (Figure 1), ethylene concentration, stress caused during harvest, post-harvest and processing operations, pathogens and physical injuries (Kader et al. 2002a; Varoquaux 2002; Fonseca et al. 2002); the faster the respiration rate, the shorter shelf-life of the intact and fresh-cut products. Respiration rate is a good indicator of ongoing processes inside a product and how fast they are happening; products harvested during active growth (vegetables and immature fruits) usually have high respiration rates, while mature fruits and storage organs have relatively low rates (Saltveit, 2004a).




Sources: Barth et al. 2004; Beaulieu and Gorny 2004; Gross 2004

**Figure 1.** Effect of storage temperature on respiration rate of several fruits and vegetables.

### *Ethylene production*

Ethylene has an active participation in growth, development, maturation, healing and senescence of fresh produce (Kader 2002; Saltveit 2004). Its production rate varies among products, it increases with temperature, diseases incidence, physical injuries, water stress and maturity stage at harvest, and can be partially controlled by low temperature storage, reduced oxygen composition or elevated carbon dioxide levels surrounding the commodity. Very small concentrations of ethylene can damage sensible products (Table 1), and hence they should be handled separated from those which produce it.

### *Water loss*

Fruits and vegetables water content is very high, and can be easily lost through the skin lenticels, stomata, cuticule, and other structures when the surrounding atmosphere has low relative humidity. The skin of fruits and vegetables acts as a natural barrier which helps to partially control water loss; but its effectiveness depends on product morphology, surface characteristics, size, ratio of product surface area to weight or/and volume, maturity stage, physical injuries, as well as environmental temperature, relative humidity and air movement around the product. Some products are more susceptible to lose water, such as lettuce and other leafy vegetables, which wilt and shrivel and generally deteriorate rapidly; others are more resistant to water loss, like apples and pears. The water loss for other products increase for those with rough, uneven or extended surface area or with high stomata or lenticels density, like rambutan, with hair like structures which favor water loss and desiccation, with the consequent external darkening occurring in a few days at 25 °C and 60% relative humidity (Yingsanga et al. 2006), with losses of 7-11% weight during the first storage day.

On the other hand, fresh-cut products can lose water even more rapidly than the whole products, because of their increased surface area to volume ratio, the skin removal and the damaged tissues resulting from cutting operations, which favor cellular content leakage. For such products, the use of sharp knives and proper packaging materials are key elements to reduce water migration.

**Table 1.** Optimum storage temperature and relative humidity for selected fruits and vegetables

| Product | Storage temperature (°C) | Relative humidity (%) | Sensitivity to chilling [1] | Freezing temperature (°C) | Sensitivity to ethylene [2] |
|---|---|---|---|---|---|
| apple (summer) | 0-2 | 85-95 | | | |
| Fuji, Gala | 0 | 90-95 | o | -1.5 | H |
| Golden, McIntosh | 4 | 90-95 | √ | -1.5 | H |
| asparragus | 2.5 | 95-100 | √ | -0.6 | M |
| avocado (ripe) | 0-2 | 85-95 | o | -1.6 | H |
| avocado (unripe cv Fuerte, Hass) | 3-7 | 85-90 | √ | -1.6 | H |
| banana | 13-18 | 85-90 | √ | -0.8 | H |
| beets (topped) | 0-2 | 98-100 | o | -0.9 | L |
| blackberry | -0.5-0.0 | 90-95 | | -1.3 | L |
| broccoli | 0 | 95-100 | o | -0.6 | H |
| brussel sprouts | 0 | 95-100 | o | -0.8 | H |
| cabbage | 0 | 98-100 | o | -0.9 | H |
| cantaloupe | 2-5 | 95 | √ | -1,2 | M |
| carambola | 7-10 | 85-90 | √ | -1.2 | |
| carrots | 0 | 98-100 | o | -1.4 | H |
| cassava | 13-18 | 85-90 | √ | | L |
| cauliflower | 0 | 95-98 | o | -0.8 | H |
| celery | 0 | 98-100 | o | -0.5 | M |
| chayote | 7 | 85-90 | √ | | |
| cherimoya (custard apple) | 13-18 | 85-90 | √ | -2.2 | H |
| coconut | 0-2 | 85-95 | o | -0.9 | |
| cucumber | 10-12 | 85-90 | √ | -0.5 | H |
| eggplant | 10-12 | 90-95 | √ | -0.8 | |
| garlic | 0 | 65-70 | o | -0.8 | L |
| granadilla | 7-10 | 85-90 | √ | | |
| guaba | 7-10 | 85-90 | √ | M | |
| honeydew melon | 13-18 | 85-90 | √ | -1.1 | H |
| kiwifruit (ripe) | 0 | 90-95 | o | -0.9 | H |
| lemon | 10-13 | 85-90 | √ | -1.4 | |
| lettuce head | | | | | |
| butterhead | 0 | 98-100 | o | -0.2 | H |
| iceberg | 0 | 98-100 | o | -0.2 | H |
| lime | 9-10 | 85-90 | √ | -1.6 | |
| mango | 13 | 85-90 | √ | -1.4 | M |
| mushrooms | 0 | 90 | o | -0.9 | M |
| nectarine | -0.5-0.0 | 90-95 | o | -0.9 | M |
| onion (mature bulbs, dryed) | 0 | 65-70 | o | -0.8 | L |
| orange | 7-10 | 85-90 | √ | -0.8 | M |
| oregano | 0-5 | 90-95 | o | | M |
| papaya (ripe) | 13-18 | 85-90 | √ | -0.9 | M |
| passion fruit | 7-10 | 85-90 | √ | | M |
| peach (ripe) | -0.5-0.0 | 90-95 | o | -0.9 | M |
| pear | -1.5-0.5 | 90-95 | o | -1.7 | H |
| pepper (Bell) | 7-10 | 95-98 | √ | -0.7 | L |
| pineapple | 7-13 | 85-90 | √ | -1.1 | L |
| plantain | 13-15 | 90-95 | √ | -0.8 | H |
| pomegranate (arils) | 5-7 | 90-95 | √ | -3.0 | L |
| potato (cured) | 13-18 | 85-90 | √ | -0.8 | M |
| rambutan | 12 | 90-95 | √ | | H |
| raspberry | -0.5-0.0 | 90-95 | o | -0.9 | L |
| rhubarb | 0 | 95-100 | o | -0.9 | L |
| soursop | 13 | 85-90 | √ | | |
| spinach | 0 | 95-100 | o | -0.3 | |
| summer squash | 7-10 | 95 | √ | -0.5 | M |
| sweet potato | 13-18 | 85-90 | √ | -1.3 | L |
| strawberry | 0 | 90-95 | o | -0.8 | L |
| tomato (mature green) | 10-13 | 90-95 | √ | -0.5 | H |
| tomato (firm ripe) | 8-10 | 85-90 | √ | -0.5 | L |
| watermelon | 10-15 | 90 | √ | -0.4 | H |

1 o: no; √: yes; 2 L: low; M: medium; H: high

Sources: Beaulieu and Gorny, 2004; Tompson and Kader, 2004; Cantwell 2002

### *Internal and external color*

Color is one of the main quality attributes of fresh commodities commonly used as selection criterion by both, the consumers and the fresh-cut industry, as well as an indicator of the overall quality and maturity stage of the product. Color varies among products, cultivars, stages of maturity or development; it can be affected by pre-harvest factors such as plant nutrition, seasonality, climate conditions, production lot, temperature, relative humidity, storage time, and postharvest handling conditions. Color changes can result of the degradation or formation of pigment compounds such as chlorophylls (green color), anthocyanins (red, blue and purple colors), and carotenoids and flavonoids (yellow and orange colors) (Kader and Barrett 2005; Maestrelli and Chourot 2002). They can occur as part of the ripening process, but they also can be caused by mechanical stresses on cell wall, membranes and tissues during produce handling and fresh-cut processing. Such damages favor fluids leakage, tissue softening and enzymatic browning reactions, as enzymes and their substrates become in contact. Enzymatic activity of PPO (polyphenol oxidase) and POD (peroxidase) is also associated with color changes in fruits and vegetables. Mangoes, avocados, peaches, apples, pears, bananas, olives, potatoes, mushrooms, lettuce, grapes and other fruits and vegetables are very sensible to enzymatic browning due to PPO activity.

Color changes can be accelerated by external factors such as high temperature, low relative humidity environment, physical damages and other stress conditions. Odriozola-Serrano et al (2009) found that the degradation of antocyanins responsible for the good appealing bright red color of strawberries, was significantly larger as the temperature rises and storage time increases.

Color requirements for fresh intact products consumption varies with those for fresh-cut processing. For the whole produce market, external color is one of the main quality attributes, while for the industry, both, external and internal colors are important, since both or the latter become exposed in the final fresh-cut product. They should be bright, even, and have the color characteristics expected by the consumer. Care must be taken with new cultivars with some improved characteristic for processing but with major changes in color, because they could be rejected by the consumer.

### *Texture*

Texture attributes vary during pre- and postharvest handling as they are affected by stage of maturity, plant nutrition, water stress, storage temperature and relative humidity, rough handling, and ripening processes. Product softening, loss of

turgidity, increased elasticity or toughness are some of the changes occurring during product handling, which may reduce its value and utility for the fresh-cut industry. Changes can be due to water losses, as mentioned earlier, or to the activity of several enzymes or pathological breakdown in combination with handling conditions. Enzymes such as β-galactosidase, polygalacturonase, pectin methyl esterase, cellulose, phenilalanineammonia lyase, peroxidase, and cellulase, participate in cell wall modification, degradation of pectin compounds in tomatoes, melons, avocados and peaches, pectin solubilization in strawberries, ripening initiation processes, tissue weakening and softening in raspberries, avocado, blackberries, mangoes, cherimoya, and tomatoes, and toughness development in asparagus (Bhowmik and Dris 2004).

Physical damages of fresh fruits and vegetables should be minimized throughout harvest, transportation and postharvest and processing operations, because they have a negative effect on quality attributes and shelf-life. Symptoms could show immediately, during processing or after several days or weeks of storage; they are generally described as tissue darkening, loss of firmness, bruises, cracks, cuts and perforations which lead to faster deterioration reactions than the intact products. Damaged tissues increase respiration rates, water loss and other metabolic reactions, allow better contact between enzymes and their substrates, and favor microbial spoilage and other undesirable reactions.

Maestrelli and Chourot (2002) classified fruits as very fragile, fragile, resistant or very resistant to handling. They found cultivar, stage of maturity, and handling practices influenced the response to mechanical injuries of peaches, pears, prunes and apricots. Damages are caused by impact, compression, penetration, vibration and shear forces against the product, and hence, they can be controlled by protecting and immobilizing the product, which can be achieved by careful handling, reducing unnecessary movements, drop heights, and any cut edges or rough surfaces which could threaten product integrity.

Bruising response differences have been reported for different apple and peach cultivars after cutting, but their susceptibility can also be influenced by pre-harvest practices, weather conditions, maturity stage and other factors which might affect phenolic compounds content and enzymatic activity (Varoquaux, 2002).

Impact bruising damages susceptibility is larger for sweet cherries handled bellow 10°C as compared with temperatures up to 20 °C, while mechanical damages due to vibration are not affected by temperature (Crisosto et al. 1993). On the other hand,

physiological disorders such as chilling and freezing injuries also contribute to tissue softening, water soaked areas, ripening problems, surface and flesh discoloration, off-flavors and off-odors production and increased susceptibility to microbial spoilage and breakdown (Kader 2002 a, Kader and Barret 2008).

***Compositional changes***

Internal composition of fruits and vegetables keeps changing during growth and development and after harvest. Changes could be desirable or not, depending on the product and how and when it is going to be processed. Soluble solids content and acidity are two important parameters for fruits, frequently related to stage of maturity, flavor and consumer preferences and used as quality parameters for product selection for processing. Little changes occur in non-climacteric fruits after harvest (pineapple, citrus fruits), while climacteric fruits suffer important changes as they continue to ripen.

Aroma compounds losses or the production of off-flavors and off-odors directly affect fruits and vegetables flavor. They can be associated to maturity stage, unfavorable storage conditions or enzymatic activity of peroxidases and lypoxygenases in chili peppers, broccoli, asparagus, carrots and green beans.

Antioxidant and other nutritional attributes can be lost during handling and processing. Kader and Barret (2004) pointed out the high sensibility of ascorbic acid to high temperatures, light, low humidity environments, physical damages and chilling injuries.

***Growth and development***

Some fresh produce continue to grow and develop after harvest. Rooting can occur on root crops and onions, seed germination on tomatoes and peppers, and elongation in asparagus (Kader 2002a). Proper selection of the harvesting index, handling and storage conditions and special treatments are necessary to diminish these undesirable changes. Most of these changes are undesirable for fresh-cut processing, since metabolic activity increases and product appearance may rapidly deteriorate.

***Microbial spoilage***

Fruits and vegetables meet many of microbial requirements for life, they offer the nutrients and water they need, at environmental conditions at which they can grow. Some can be harmful for consumer health and other cause produce spoilage,

deteriorating their quality attributes. Microorganisms get into the product at different stages of production and postharvest handling or processing.

- Phytopathological decay includes microbial spoilage caused by bacteria, viruses, molds and yeast which affect product quality, but do not represent a risk for consumer health. Some get into the product at earlier stages of development while other during postharvest handling, through incision cuts or by side to side contamination between individual fruits or contaminated surfaces.

- Harmful microorganisms constitute food safety risks, because they can cause illness and even death to consumers, even when little or no deterioration symptoms are observed in fruits and vegetables. Major transmission sources for these pathogens are directly related to deficiencies in both, workers hygiene and agricultural practices.

## 4. TECHNOLOGICAL TOOLS TO PRESERVE FRESH PRODUCE FOR PROCESSING

Quality is a key factor for processed foods, but it is particularly true and important for fresh-cut fruits and vegetables, which must preserve the quality attributes of the intact produce after cutting stresses, without being subjected to any strong temperature stabilizing treatment. Hodges and Toivonen (2008) highlighted the importance of recognizing that all processes applied to a fruit or vegetable cause stress-induced changes in the tissues physiology and metabolism. They also pointed out the need to understand how these changes occur to set effective strategies to preserve the product quality and extend its shelf-life.

The fresh-cut processing industry requires high grade fruits and vegetables as raw materials; they should have good appearance, texture, taste, odor, nutritive attributes and must be safe for the consumer. They should be free from mechanical injuries, decay, insects and other damages, and also, they must resist process operations and further handling and storage procedures.

In addition, effective technological tools must be used in every one of the slabs of the chain, from the production fields to the processing industry, to assure proper quality produce supply and minimize product losses.

The success of a fresh-cut product will depend on the fulfillment of the target consumer needs and expectations, the use of the right fruits and vegetables at their

optimum maturity stage for processing, and the application of the right operation procedures and packages, as well as the utilization of appropriate market tactics.

Strategies for better intact fruits and vegetables for processing involve pre-harvest, harvest and postharvest handling are discussed ahead.

## 4.1 Cultivar evaluation and selection

a. For each type of produce, there could be a few or many cultivars in the market, each of them with their particular benefits and limitations for processing. Studies comparing cultivars usually show considerable variation in respiration rates, color, texture, bruising susceptibility, size, shape, appearance, nutritional value, sensory and other characteristics of many fruits and vegetables (Crisosto et al. 1993, 2002, Deepa et al. 2007, Gorny et al 2000, Maestrelli et al 2002, Schouten et al 2004).

b. Cultivar selection for the fresh-cut industry looks for intact fresh fruits or vegetables that can meet the desired quality attributes pre-established for their fresh-cut product, resist transportation and handling before processing, tolerate processing operations with minor quality alterations and have a prolonged after-cutting shelf-life. These four elements are interrelated because of the dynamic behavior of intact fresh fruits and vegetables, since their characteristics and quality are continually changing and influenced by environmental conditions, production technology during pre-harvest and postharvest handling, fresh-cut processing and packaging, and distribution to the final market.

c. Industry needs reliable agricultural products with high quality throughout the year, when possible, in order to produce uniform fresh-cut products without interruption. Cultivar selection is the first step and it has to be followed by proper pre-harvest practices, harvesting indicators and postharvest handling operations and storage previous to processing and food safety programs to avoid consumer risks (Chiesa et al. 2003).

d. Ideal fruits and vegetables for fresh-cut processing are those with the best and homogeneous quality attributes, right stage of development or maturity, high field production and processing yields, available all year round, free from physical, physiological or pathological disorders, easy to handle, highly resistant to handling and all processing operations and stabilizing treatments, little susceptible to external conditions, with a prolonged shelf-

life after processing to maintain quality attributes all the way to the consumer and safe. It should also meet consumer likes and preferences and market requirements.

e. For specific products, particular requirements are needed. Processors need to clearly define the attributes of their final products to evaluate cultivar response to handling, processing and after-cutting handling and to determine limiting factors, such as juice leaking, discoloration, browning, wilting, microbial spoilage or others which might restrict fresh-cut product shelf-life. Harvesting indicators, handling and preparation to processing practices effect on the final product quality should also be evaluated.

f. Some examples of studies conducted to select the best cultivars for fresh-cut processing include one of Gorny et al. (1999), who found out that the critical factors which impact fresh-cut peach and nectarine slices quality were ruled by product response to cutting, which was better when the flesh firmness was between 13 and 27 N, and the fruits were stored at 0 °C and 90-95% relative humidity. In another study, the same authors (2000) compared the suitability of Anjou, Barlett, Bosc and Red Anjou pear cultivars for fresh-cut slices production, and found significant differences in respiration and ethylene production rates, flesh firmness, color and susceptibility to cut-surface browning, which was very intense for Anjou and Red Anjou cultivars. Apple sensibility to browning of five apple cultivars was also studied by Milani and Amedi (2005), who found differences in browning rate; the Red Delicious cultivar exhibited the highest browning rate, followed by Golden Delicious with a medium rate and Granny Smith and Golden Smoty, which had a weak browning rate. Sweet cherry cultivars were evaluated for fresh-cut processing by Toivonen (2006) who concluded that most of them were adequate for fresh-cut processing, as they maintain firmness, even though they showed differences in post-cutting bleed, weight loss and decay throughout storage.

g. Some hints for fruits and vegetables cultivar selection for fresh-cut processed products:

h. Define desirable quality parameters and tolerance ranges for the fresh-cut product (color, shape, size, flavor, soluble solids content, acidity, texture, juiciness, nutritional content, other).

i. Identify available cultivars with consistent and reliable quality which can meet product concept.

j. Look for limiting factors to fresh-cut product quality and shelf-life, based on visual and eating quality and microbial safety; consider deteriorative changes that reduce their marketability, such as browning, water soaked tissues, translucency, softening, composition changes, microbial growth, decay or others).

k. Evaluate cultivar aptitude for processing by studying product response to handling and processing, susceptibility to deteriorative changes before, during and after cutting.

l. Determine required harvest maturity stage and ripening treatments when needed.

m. Evaluate processing yields (usable product per kg of intact fruit or vegetable, processing time per kg of prepared fresh-cut product, etc.)

n. For those cases, where a cultivar is preferred though it may have some limitations, evaluate alternative treatments to control undesired browning, softening or other changes on the fresh-cut product.

o. Determine expected post-cutting shelf-life of the final product at handling temperatures.

p. Study product compatibility among products for mixed fresh-cut fruits or vegetables.

q. Seek for possible suppliers, production sites, agricultural practices, traceability possibility, product quality and availability throughout the year.

r. When possible and convenient, evaluate product response to mechanization to reduce processing time, contamination risks and improve yields.

## 4.2 Pre-harvest practices to improve intact produce quality

Genetic material, sowing, growing conditions, light intensity along production period, pruning, product thinning, harvest maturity, nutrients and water supply, soil quality, fertilization, weeds control and pest management affect product quality together with climate conditions (Hodges and Toivonen 2008, Kader 2008). Thus, careful production plans must be implemented looking forward to strengthening up the

preferred characteristic of a particular crop and consequently, the final quality and shelf-life of fresh-cut products. The effect of some pre-harvest practices is given ahead.

Crop rotation has shown to have a positive effect on product quality, because decay inoculum of soil borne fungi, bacteria and nematodes builds up with repeated cropping of the same vegetable in the production fields (Crisosto and Mitchell 2002). Fruit size and yield is affected by fruitlet thinning, position inside the tree, pruning and other cultural practices according to the same authors.

Irrigation is very important for all crops since plants tissues need water to live. Low water supply might stress product and increase its sensibility to sun burns, alter maturation processes in pears, provoke a leather like texture on peaches, while moderate water stress can reduce fruit size, increase soluble solids content, acidity and ascorbic acid content (Kader 2002 a). Gelly et al. (2003) also reported that deficit irrigation on peaches (*Prunus pérsica* L.) increased soluble solids content and also helped to maintain fruit color longer. On the other hand, excess water stress could lead to cracking failures in cherries, apricots, tomatoes and other products, reduce firmness and soluble solids content and cause a larger susceptibility to mechanical injuries due to an excess of turgidity (Kader 2002 b). Plants can also be stressed because of salt presence in irrigation water. Kim et al. (2008b) evaluated stress due to water salinity on romaine lettuce (*Lactuca sativa* cultivar Clemente). Sodium chloride concentrations above 100 mM resulted in 1.5 to 3 fold reduction of lettuce height and weight, as compared with control treatment without salt, and color losses increased with sodium chloride concentrations.

Carotenoids and phenolic compounds contents are also affected by irrigation with salt water. The number of days before harvest at which irrigation is stopped also influenced product quality in Iceberg lettuce, as observed by Fonseca (2006), who found out that when irrigation was stopped 4 days before harvesting instead of 16 days, the product weight and diameter were larger, but so did the aerobic bacteria counts resulting in faster quality deterioration of the product. For intact tomatoes, Kim et al. (2008a) found a 30% increase in lycopene and vitamin C content when they were irrigated with salt water, though phenolic compounds content was not affected. Type of substrate also affects quality attributes.

Fertilization affects both quality at harvest and postharvest shelf-life of fruits and vegetables. Nutrients should be balanced, since deficiencies or excesses can favor physiological disorders and reduce products quality and shelf-life. High nitrogen

fertilization is used to increase product size but it can reduce volatile compounds production and promote changes in product flavor; other elements also show opposite response, such as high levels of potassium, which can reduce color disorders while high levels of magnesium can increase them (Crisosto and Mitchell 2002). Plant nutrition differences can affect product size, firmness and weight loss susceptibility. Calcium has been associated with a reduction of respiration rate and ethylene production, firmness increase, and ripening and deteriorative reactions slowdown (Kader 2002b).

Hotdges and Toivonen 2008) compared quality attributes of tomato slices grown in hairy vetch and black polyethylene mulch, and found out that those grown in the hairy vetch mulch were firmer, had less water soaked areas and less increase in electrical conductivity, stresses associated to chilling injuries and membrane damages, respectively.

Calcium chloride sprays have been successfully used to reduce browning core, cork spots, superficial scalds disorders and external and internal rots on “Anjou” pears, with an overall enhancement of fruit appearance and an improvement of fruit juiciness and fruit color (Raese and Drake 2000).

Climatic conditions (temperature, rain, wind, light) affect internal quality attributes of the products as well as their susceptibility to handling and processing. The effect of climatic conditions can be partially controlled in the growing areas by shades, drainage, and wind stopper; however, nowadays, they can be precisely controlled in greenhouse plantations. Lin and Jolliffe (1996) found an important reduction on skin chlorophyll content and shelf-life for low light intensity on greenhouse-grown English cucumbers; such reduction can be avoided by the use of supplemental light during growing which increases product yields, external and internal quality of many vegetables, including dry matter content and skin chlorophyll content on cucumber, higher ascorbic acid and sugar content in tomato and better head firmness on lettuce (Hovi-Pekkanen and Tahvonen 2008).

Nowadays, there is a trend in Europe and Latin America to start moving from traditional growing to protected areas cultivation for a better control during produce growing, they use a wide range of simple and complex technologies to control temperature, relative humidity, irrigation control, and more recently, with the incorporation of floating trays with nutrients solution supply, where small leaves grow with a significant reduction in nitrates accumulation and microbial load, two

characteristics very well appreciated for fresh-cut processing (Rodríguez-Hidalgo et al. 2006).

### 4.3 Harvest and maturity indices

Stage of maturity at harvest is very critical not only to assure product quality and shelf-life of intact fruits and vegetables for the fresh market but also for the fresh-cut industry; it affects product composition, postharvest tolerance to handling and processing operations, and their post-cutting life (Kader 2002b, 2008, Kader and Barret 2004, Martín-Belloso and Rojas-Graü 2005, Toivonen and DeEll 2002).

Maturity at harvest influences fruits and vegetables response to processing and deterioration reactions. Toivonen (2008) studied the effect of maturity at harvest on the susceptibility of anti-browning treated apple slices to cut-edge browning. He found out that cutting surface of slices from 'Granny Smith' apples picked prior to proper harvest maturity are more susceptible to browning even after commercial anti-browning treatments. Fruit ripeness of pear slices at cutting affected their shelf-life (Gorny et al. 2000); it varied from 2 days at 0 °C for ripe fruit to more than 8 days for partially ripe and mature-green pears; and it also affected surface darkening at 0°C which was significantly reduced for partially ripe and mature-green fruit. However, the eating quality of mature-green pear slices exhibited lack of juiciness and aroma.

Harvesting criteria vary among products, how they are to be consumed or processed, distance to market places, intended storage time and temperature, industry requirements, consumer preferences and many other parameters. Some produce may be consumed in several stages of maturity such as mangoes, papayas and plantains, which have different uses for green-mature and fully ripe products; vegetables are obtained from different parts of the plant, such as leaves, flowers, sprouts, roots and tubers which reach their best quality attributes at various stages of growing and developing of the plant so there is a wide range of possibilities for harvesting, depending on the final destination of the produce, the desired quality attributes and their resistance or tolerance to withstand handling and processing.

Maturation or harvesting indices have been set to describe through one or few indicators, the right time to harvest for better quality and shelf-life. Harvest indices must be simple, easy to understand and apply, reliable, product of an objective measurement and whenever possible, nondestructive (Reid 2002). Table 2 shows maturity indices commonly used for fruits and vegetables harvesting. Both,

production yields and quality parameters of the product, are taken into consideration, as well as market prices and buyers requirements. Early harvesting results in low production yields and underdeveloped quality attributes, whereas late harvesting leads to overmature products and excessive postharvest losses.

To develop a maturity index, Reid (2002) suggested the following steps:

1. Determine changes in the commodity throughout its development
2. Look for features whose changes correlate well with the stages of the commodity's development
3. Carry out storage trials and taste panels to determine maturity indices that fulfill minimum acceptability and required shelf-life
4. Select maturity indices and assigned minimally acceptable values
5. Test harvesting indices over several years in several growing locations to ensure that it reflects the quality of the harvested product

This suggestion should be adjusted for fruits and vegetables to be used for fresh-cut processing, after evaluating their response to process, handling and storage operations.

Non-fruit vegetables generally include diameter, length, shape, color, firmness and/or compactness and other appearance parameters as the main harvesting indices; some examples are asparagus, celery, rhubarb, and okra length, bud size of artichokes, compactness of broccoli, cauliflower, Brussels sprouts, cabbage and some lettuce cultivars, and color in lima beans, broccoli, collards, and pea.

Color, size, firmness, appearance and internal quality attributes are used for fruit vegetables harvest, along with observations about natural incision of the fruit to the plant. For instance, cantaloupes and other melons stem appearance and how it naturally breaks is a good indicator for harvesting, together with soluble solids content, aroma, fruit and rind color changes or even days from bloom. Tomato harvesting indices selection will depend on the use given to the product, they can be harvested mature-green, which are very firm tomatoes, with color changing from green to light green; ripe with full red color and soft, but still firm, or in the middle, known as breaker tomatoes, which are firmer than ripe tomatoes but softer than mature-green and exhibit a pink to red color on the blossom end. Color and firmness are very important parameters for cucumber, eggplant, bell pepper, water melon

and other fruit vegetables. Asghary et al. (2005) found “Semsory” muskmelon (*Cucumis melo* L. var. reticulates) harvested at the first stages of yellow color development had higher sugar content, better color, taste, aroma and market value than those harvested at mature green stage.

Beaulieu et al. (2004) highlighted tissue softening as a serious problem and limiting factor for fresh-cut products, and listed softening enzymes, decreased turgidity due to water loss and stage of maturity as the main causes of texture changes. They studied the effect of product firmness on the post-cutting sensory attributes of fresh-cut cantaloupe stored at 4 °C, prepared from melons harvested at four distinct maturity stages (one-quarter to full-slip), and found that those from three-quarters mature cantaloupes exhibit less firmness loss than those from full-slip maturity fruits. Antioxidant characteristics also vary with cultivars and maturity stage; total and individual phenolic content, antioxidant capacity, carotenoids, ascorbic acid and capsaicin content varied among sweet pepper genotypes and maturity stage (Deepa et al. 2007, Marin et al. 2004). Kader (2008) pointed out that non-fruit vegetables have better quality taste when they are harvested immature, while fruit vegetables and fruits get better when they are harvested fully ripe.

Harvesting criteria for fruits also include shape, size and appearance parameters, but flavor and aroma take an important role. They have a great influence on product quality, since aroma related volatile and non-volatile compound synthesis increases as the product matures and ripens (Kader 2008). Optimum levels of such compounds do not always match the harvesting criteria, because other parameters have to be considered, such as the type of product, resistance to handling and processing, time required to reach the final market, produce prices, how it is processed or consumed, and others. For apples, harvest date is determined by several parameters, including days from the full bloom as a rough idea of fruit maturity, background color, ease of separation of the fruit from the spur, soluble solids content, starch conversion into sugars, flesh firmness and internal ethylene concentration (Gast 1994, Toivonen 2008).

For climacteric fruits, proper selection of harvesting indicators is very important, because if fruits are picked prior to physiological maturation, in an early period of pre-climacteric stage, fruits quality attributes would not reach desired levels. Robles et al. (2006) studied changes in Ataulfo mangoes as the fruit ripened and found an important increase in total soluble solids and ethylene production rate, accompanied with a gradual reduction of the respiration rate, acidity and firmness. Maradol

papaya, Keitt mangoes and Red Spanish pineapple give better results for fresh-cut processing, when processed before full ripeness stage (Hernández et al. 2007), explained by less firmness and color alterations during post-cutting storage.

In summary, maturity at harvest affects quality attributes and after-cutting shelf-life of intact and fresh-cut produce and harvesting indices should be adjusted to produce response to handling and processing. Under or over mature produce results in deficient quality attributes and low yields, while over-mature products diminish post-cutting shelf-life of fresh-cut products, increase susceptibility to deterioration, mechanical damages, microbial spoilage and other damages.

**Table 2.** Maturity indices commonly used for fruits and vegetables

| Index | Examples |
|---|---|
| Elapsed days from full bloom to harvest | Apples, pears |
| Mean heat units during development | Peas, apples, sweet corn |
| Development of abscission layer | some melons, apples, feijoas |
| Surface morphology and structure | Cuticle formation on grapes, tomatoes. Netting of some melons. Gloss of some fruits (development of wax) |
| Size | All fruits and many vegetables |
| Specific gravity | Cherries, watermelons, potatoes |
| Shape | Angularity of banana fingers. Full cheeks of mangoes. Compactnes of broccoli and cauliflower |
| Solidity | Lettuce, cabbage, Brussel sprouts |
| Textural properties: | |
| Firmness | Apples, pears, stone fruits |
| Tenderness | Peas, apples, sweet corn |
| External color | All fruits and most vegetables |
| Internal color and structure | Formation of jellylike material in tomato fruits. Flesh color of some fruits |
| Compositional factors: | |
| Starch content | Apples, pears |
| Sugar content | Apples, pears, stone fruits, grapes |
| Acid content, sugar/acid ratio | Pommegranates, citrus, papaya, melons, kiwifruit |
| Juice content | Citrus fruits |
| Oil content | Avocados |
| Astringency (tannin content) | Persimmons, dates |
| Internal ethylene concentration | Apples, pears |

Source: REID 2002.

## 4.4 Postharvest strategies to reduce undesirable changes

***Use optimum storage temperatures to reduce metabolic activity:***
Temperature is the most important external factor to control during postharvest handling and storage previous to processing, because it rules most of the changes occurring inside an intact or fresh-cut fruit or vegetable. As the temperature drops, most reactions slow down, and hence, quality attributes can withstand for longer periods. Optimum storage temperature should always be chosen for intact and fresh-cut fruit and vegetables (table 1). As a general rule, fresh-cut products should be stored at 5 °C or below, but the optimum temperature for the intact products could be higher for chilling sensitive fruits and vegetables, and it must be considered for produce storage before processing. Some produce can withstand temperatures near freezing (0 °C and below), some need temperatures near 0 °C, and those sensible to chilling injury disorders cannot be stored at temperatures below 7 to 13 °C, depending on the product. Storage at lower temperatures than those tolerated by the intact produce will result in uneven ripening, flavor, color and aroma losses, texture changes and other undesirable changes.

Every 10 °C rise on the produce handling temperature in the range from 0 to 30 °C, the rates of respiration and deterioration increase two to three times for non chilling sensitive commodities (Kader 2002a, Saltveit 2004). Crisosto et al (1993) observed that sweet cherry respiration rates of four cultivars rapidly increased from nearly 10 mg $CO_2$/kg/h at 0 °C to 45 to 50 mg $CO_2$/kg/h at 20 °C, though response to temperature varied among cultivars exhibiting differences in fruit sensibility to temperature changes (Crisosto et al. 1993).

Exposure to high temperatures is also detrimental though some product tissues can tolerate them for short periods; it causes phytotoxic symptoms which lead to accelerated deterioration (Saltveit, 2004). Prolonged exposure to sun in the fields, transportation trucks or during storage should be avoided to reduce quality losses.

Once the produces are processed, temperature must be hold at 5 °C, to minimize changes on the quality attributes of the fresh-cut products as well as microbial spoilage.

***Relative humidity and water loss control:***
Following temperature, relative humidity is the second factor in importance for quality maintenance. Shelf-life and value of fruits and vegetables decreases with water loss because it causes appearance deterioration, tissue softening, wilting,

shriveling and weight loss. Such changes also affect product suitability for the fresh market and the fresh-cut industry, since commodities resistance and yields during processing and handling deteriorate and shelf-life of the product is sensible shorter.

Fresh produce are not solid-pack but porous materials filled with their own internal atmosphere, which has a high relative humidity. They lose water through the skin and/or abscission cuts, because of relative humidity differences between the internal atmosphere and that surrounding the product, and because of these, fresh produce should be stored under high relative humidity environments, as a complement to optimum storage temperature. However, storage requirements vary because water losses also depend on skin and other product characteristics, which make some products more susceptible to losses than others (Table 2). Díaz-Pérez et al. (2007) found out water loss relations with intrinsic characteristics of bell pepper such as fruit size, maturity stage, cuticle thickness, natural wax over the product surface, and reported larger water loss through the calyx or stem scar than from the product skin, as previously reported for eggplants and tomato.

Water loss cannot be completely stopped, but it can be reduced by careful handling and proper storage temperature and relative humidity conditions. Temperature should be as low as the product can tolerate without chilling injury symptoms (Table 1) and relative humidity should be higher than 80% for most products, and up to 95-100% for very sensible to water loss products, such as leaves vegetables and strawberries. Packaging materials, produce waxing and reduced exposure to air movement could also help to reduce water losses.

***Air movement***

Cold air is normally used for produce cooling and storage; it removes heat from the produce and delivers it to the evaporator of the refrigeration system. The faster the air passes through the produce, the quicker the product cools down. However, once the product is cold, excess air movement favors water losses, and thus, it should be kept as low as possible, to allow proper ventilation, without major losses. Adequate packages sizes and ventilations and proper product layout in the storage rooms can help to control excessive exposure to air.

***Light***

Potatoes exposure to light favors greening during storage because of the production of solanine and chlorophyll. Such changes are undesirable and can be avoided by storage in darkness. Prolonged storage of green vegetables without light could also discolor them. The light effect starts at the fields or greenhouses, light intensity also

affects flavonoids, thiamine, riboflavin, carotenoids, ascorbic acid and other compounds that are found in fruits and vegetables during growing, thus affecting their composition and nutritional quality (Kader 2002b).

***Atmosphere composition***

Cells and tissues require oxygen and produce carbon dioxide during respiration. Low oxygen and high carbon dioxide concentration in the atmosphere surrounding the fruit or vegetable can be used to delay deterioration and extend shelf life. Table 1 show recommended atmosphere composition for several commodities; however, product benefits and shelf-life extension can significantly vary among products and cultivars.

***Ethylene***

Ethylene is a plant regulator that affects growth, development, ripening and senescence processes and postharvest quality (Watkins (2006). Very low concentrations of ethylene in the atmosphere surrounding the product can trigger ripening processes of climacteric fruits and undesirable reactions on some fruits and vegetables such as color loss and senescence reactions. Sensibility to ethylene varies among products and changes can be desirable or not, but as a general rule, very low concentrations of ethylene are needed to affect product quality. Controlled application of ethylene can be used for uniform maturation and degreening, but should be avoided for long term storage. As a general rule, ethylene producers must be always separated from ethylene sensitive products.

***Handling and processing***

Mechanical damages on fruits and vegetables are caused by impact, compression, shear, and puncture forces applied to the product while harvesting and handling it all the way to the consumer or processing industry. Such damages accelerate metabolic processes and favor microbiological spoilage. Some of these damages symptoms can be detected only after several days of storage, during processing or the subsequent storage, but they greatly affect product quality, stability and shelf-life. Stress caused by physical efforts during handling should be minimized in order to supply raw materials suitable to resist further stress processes during fresh-cut processing.

Varoquaux (2002) suggested that peeling and cutting damage product cells and cause an increase of the membrane permeability and probably, a reduction of phospholipids biosynthesis. These events trigger the reactions of restoration of cellular microstructures and membrane integrity and entail the production of

aldehydes of long carbonated chains, increase respiration rate and lead to rapid consumption of cellular metabolites and the consequent deterioration. However, produce response to stress also depends on the type of fruit or vegetable, its stage of development or maturity and environmental conditions, and thus, it can largely vary for a single product, as kiwi, for which ethylene production rise due to cutting stress can very rapid, or it might take several hours (Varoquaux et al. 2002).

***Field and storage packages***

The main function of packaging is to protect fresh fruits and vegetables against mechanical injuries, contamination or any other damages throughout postharvest handling. Packages should have smooth surfaces and edges, be resistant to staking, have a suitable size and shape for the product, be easy to handle, allow proper ventilation for cooling and be readily available. Some packages should have water loss barriers or some other special requirement. Packages for fresh fruits and vegetables used for fresh-cut processing are temporary, they are only used to protect the product as it is carried from the fields to the processing plant, and for short term storage prior to processing.

## 4.5 Conditioning and storage before processing

Fresh-cut products quality starts with fresh fruits and vegetables, properly handled during pre-harvest, harvest and postharvest handling. The fresher the prime matter, the better the final product.

Temporary storage between harvesting and processing also affects the quality and shelf-life of fresh-cut products; in general, the longer the delay before processing, the shorter the shelf-life is going to be. However, since fresh-cut products are very perishable, lasting between one and two weeks, temporary storage of intact fruits and vegetables will be convenient. Tropical or template fruits, roots or tubers or other vegetables brought from distant markets could be processed at the final market, though some quality attributes could be partially compromised.

As for the cultivar selection, the effect of storage prior to processing should be studied for specific intact fruits and vegetables and their fresh-cut products.

Products to be stored prior to processing should be conditioned before storage or transportation to the market where they are going to be prepared, as a mean to preserve their quality. Some common preparation operations include product selection (separation of culls), washing, classification based on quality criteria,

stabilizing treatments such as application of growth regulators, antifungal treatments, curing, packaging and cooling.

Induced fruit ripening could be useful to obtain uniform product characteristics prior to processing. It is generally carried out under controlled conditions of temperature, humidity and air circulation. Ethylene generator devices yield very good results with banana, tomato, avocado, plantain and other fruits.

Application of 1-methylcyclopropene (1-MCP) has been widely used to reduce the action of ethylene in fruits and vegetables. Several authors have reported that color changes, tissue softening and other changes occurring during ripening are substantially delayed (Schouten and van Kooten, 2002. 1-MCP (1-methylcyclopropene) inhibits ethylene action and ripening reactions. It is applied at 20-25 °C, in low concentrations (2.5 nL/L to 1 µL/L) for 12 to 24 hours, but results depend on cultivar, development stage, time from harvest to treatment and multiple applications. Effects vary among fruits and vegetables including delay in respiration rate, ethylene production, volatile production, color changes, chlorophyll degradation, membrane changes, softening, acidity and sugars variation and the development of disorders and diseases. It protects products from endogenous and exogenous sources of ethylene. Chlorophyll degradation and color changes are prevented or delayed in oranges, broccoli, tomato, avocado and other green vegetables, while volatile development is inhibited in several apple cultivars, apricots, melons, bananas and other fruits. Product softening is also delayed in fruits such as avocado, custard apple, mango, papaya, apple, apricots, pears, mature plums, peaches, nectarines and tomato (Watkins (2006), Blankenship and Dole (2003), Manganaris et al. 2008),) As products ripening and senescence processes are delayed or inhibited, their shelf-life is increased.

McArtney et al. (2008) studied pre-harvest applications of 1-MCP in Golden Delicious apples, and they were able to reduce the rate of softening of the fruit during storage.

Storage of intact fruits and vegetables should be carried out at their optimal temperature and relative humidity levels.

## 5. CONCLUSIONS

Intact and fresh-cut fruits and vegetable characteristics have an intrinsic dynamic behavior because they are composed of living tissues that keep changing over time and are influenced by environmental conditions and handling practices.

Initial quality of the intact fruits and vegetables used for fresh-cut processing will fix the maximum attainable quality and after-cutting shelf-life of the processed product.

Temperature control and reduction of mechanical injuries of the intact produce before processing are key factors to maintain their quality and suitability for processing.

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# FLAVORS FOR FRUIT COMMODITIES: PINEAPPLE (*Ananas comosus*)

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*IN: HANDBOOK OF FRUIT FLAVORS (FORTHCOMING)*

## 1. INTRODUCTION

Pineapple is one of the most popular tropical fruits. It is recognized as a very aromatic fruit, which can be found just about any market around the world. It was first spread as juices or canned pineapple and as the transportation resources, rapid distribution and postharvest technology developed, it also became available as fresh fruit (Flath 1980; Umano 1992). Studies on pineapple aroma have been made since many years ago using both fresh fruits from different cultivars (not always specified) and processed foods.

Near 370 volatile constituents have been recognized in pineapple flavor up to 2005, including alcohols, aldehydes, esters, ketones, lactones, terpenes and terpenoids, hydrocarbons, lactones and others (Paull 1993; Tokitomo and others 2005; Umano and others 1992); however only some of them have been identified as pineapple flavor contributors. Aroma constituents may vary with season, cultivar, maturity, processing conditions, ethylene control, temperature, chemical treatments, modified atmosphere and pre-harvest factors, such us carbon supply, water stress, light, temperature and biotic stresses.

In this chapter, a review of the state of knowledge of pineapple aroma is presented, taking in consideration flavor changes due to stages of maturity, cultivars as well as processing conditions and finally a sensory characterization of pineapple flavor.

## 2. FLAVOR COMPONENTS OF PINEAPPLE

Flavor consists mainly of lipophilic volatile compounds but low and non-volatile materials also play an important part of the overall sensation. As many other fruits and foods, pineapple flavor is a combination of volatile components perceived by the human olfactory system and non-volatile components (sugars, acids) recognized by tongue sensors (Flath 1980). In fact, flavor is a combination of both taste and odor.

The flavor of pineapple is a blend of a number of volatile and non-volatile compounds which are present in small amounts and in complex mixtures, being the non-volatile compounds the more difficult to be analyzed (Pickenhagen 1999). Many of these compounds have been identified and reported by several authors from fresh fruit, processed pineapple products and pineapple essences (Badilla-Porras 2005; Berger and others 1985; Brat and others 2004; Elss and others 2005; Haagen-Smit and others 1945a, Umano and others 1992; Wu and others 1991). However, comparison among reported results is difficult, since different pineapple cultivars and pineapple products have been used, results are given in different units and bases, and separation techniques and analysis varies among works. A summary of pineapple constituents identified by several researchers from 1945 to 2005 are shown in Table 1.

One of the most important flavor compounds in fruits is 2,5-dimethyl-4-hydroxy-3(2H)-furanone, which is a relatively hydrophilic and not very stable molecule (Figure 1). It has been found to be part of the aroma of pineapple where it was identified for the first time (Rodin and others 1965). This compound is generally known under its trade name Furaneol®, HDF, or pineapple furanone. Furaneol has been also identified in strawberries (Re and others 1973), raspberries (Honkanen and others 1980), mangoes (Pickenhagen and others 1981), tomatoes (Buttery and others 1995), and many other fruits. Its content increases as the fruit ripens and it gives the characteristic caramel-like, sweet, floral and fruity aroma (Miller and others 1973; Perez and others 1996; Tonsbeek and others 1968). Also, it is extensively used as food flavoring due to its low odor thresholds and flavor-enhancing properties (Dahlen and others 2001).

Flavor, however, also depends on the presence of small quantities of other volatiles with low threshold values. The characteristic pineapple aroma has been attributed to ethyl 3-(methylthio) propanoate and methyl 3-(methylthio) propanoate (Umano and others 1992). Aliphatic esters, which often have fruity notes such as apple, banana, plum or apricot, have also been reported (Flath 1980). Berger (1991) reported that esters such as 2-methylbutanoates and hexanoates give fruity notes to fresh

pineapple as well as other fruits. Takeoka and others (1991) also identified many sulfur-containing esters among pineapple volatiles, but their concentrations were lower than their odor thresholds. In addition, two minor hydrocarbon compounds, 1-(E,Z)-3,5-undecatriene and 1-(E,Z,Z)-3,5,8-undecatetraene, have been identified as important contributors to fresh-cut pineapple aroma due to their low odor threshold values (Berger and others 1985).

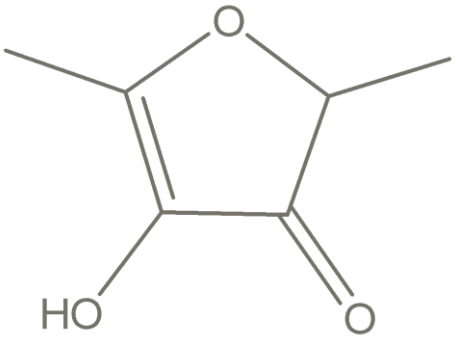



**Figure 1.** Furaneol (2,5-dimethyl-4-hydroxy-3(2H)-furanone).

Other compounds have also been identified and considered important for pineapple aroma. Lactones, can contribute to the pleasant coconut character in some cultivars. In fact, the coconut-like aroma often found in pineapple has been attributed to lactones, namely, γ-octalactone, δ-octalactone and γ-nonalactone (Flath 1980).

First studies on pineapple aroma by Haagen-Smit and others (1945a, b) were done before gas-liquid chromatography techniques were available. These authors studied volatile flavor and odor constituents of fresh pineapple (Smooth cayenne cultivar). They analyzed volatile components of summer and winter fruit grown in Hawaii in order to establish a correlation between flavor and these substances. They found differences among summer and winter fruit in both volatile extraction yield and composition. Summer fruit had much greater volatile oil content than winter fruit (190 and 15.6 mg/kg, respectively). They reported ethanol and ethyl acetate as major components of summer fruit, with smaller quantities of acetaldehyde, ethyl acrylate, ethyl 3-methylbutyrate, ethyl hexanoate, methyl and ethyl esters of $C_5$ unsaturated acid, methyl 3-(methylthio) propanoate and acetic acid. For winter fruit they found ethyl acetate as the major component, followed by acetaldehyde, methyl 3-methylbutyrate, methyl pentanoate, methyl 4-methylpentanoate, methyl octanoate and methyl 3-(methylthio) propanoate. Furthermore, they observed that summer fruit seemed to contain mostly ethyl esters, while winter fruit contained mostly methyl esters, even though this observation has not been confirmed yet in others reported results.

Table 1. Summary of volatile compounds identified in pineapple fruits and its processed products from 1945 to 2005.

| | Esters | | Esters | | Esters | | Esters |
|---|---|---|---|---|---|---|---|
| 1 | 1-butyl formate (5) | 49[a] | ethyl 3-acetoxyhexanoate (4, 5, 9, 10) | 97 | ethyl propenoate (10) | 145 | methyl 4-acetoxyhexanoate (1, 6, 9, 10) |
| 2 | 1-pentyl hexanoate (5) | 50 | ethyl 3-acetoxyoctanoate (9, 10) | 98 | ethyl S-(+)-2-methylbutanoate (7) | 146 | methyl 4-acetoxyoctanoate (6, 9, 10) |
| 3 | 1-propyl acetate (5) | 51 | ethyl 3-acetoxypentanoate (9) | 99[a] | ethyl tetradecanoate (3) | 147 | methyl 4-hydroxybutanoate (3) |
| 4 | 1-propyl formate (5) | 52 | ethyl 3-hydroxy-2-methylbutanoate (9) | 100 | ethyl (E)3-hexenoate (5) | 148 | methyl 4-hydroxyhexanoate (9) |
| 5 | 2,3-butanediol diacetate (9) | 53 | ethyl 3-hydroxybutanoate (9) | 101 | ethyl (E)-3-octenoate (5) | 149 | methyl 4-hydroxyoctanoate (9) |
| 6[a] | 2-methyl-1-butyl acetate (4, 5, 6) | 54[a] | ethyl 3-hydroxyhexanoate (4, 5, 6, 9, 10) | 102 | ethyl (Z)-3-octenoate (4) | 150 | methyl 4-methylpentanoate (5, 10) |
| 7 | 2-methyl-1-propyl acetate (5, 9) | 55 | ethyl 3-hydroxyoctanoate (4, 6, 9) | 103 | geranyl acetate (10) | 151 | methyl 5-acetoxyheptanoate (6, 9) |
| 8 | 2-metyl-1-propyl formate (5) | 56 | ethyl 3-hydroxypentanoate (9) | 104 | hexyl acetate (4) | 152[a] | methyl 5-acetoxyhexanoate (3, 4, 5, 6, 9, 10) |
| 9 | 2-phenylethyl acetate (4) | 57 | ethyl 3-methylbutyrate (5, 6) | 105 | methyl (Z, Z, Z)-octadecatrienoate (2) | 153[a] | methyl 5-acetoxyoctanoate (3, 4, 5, 6, 9, 10) |
| 10 | 2-propenyl n-hexanoate (2) | 58 | ethyl 3-methylbutanoate (4) | 106 | methyl (E)-2-butanoate (7) | 154 | methyl 5-hexenoate (7, 9) |
| 11 | 2-propyl 2-methylpropionate (5) | 59 | ethyl 4-(methylthio) butanoate (7) | 107 | methyl (E)-2-hexenoate (2, 9) | 155 | methyl 5-hydroxy hexanoate (4, 6, 9) |
| 12 | 2-propyl acetate (5) | 60 | ethyl 4-acetoxybutanoate (9) | 108 | methyl (*E*)-3-hexenoate (9) | 156 | methyl 5-hydroxyoctanoate (6, 9) |
| 13 | 3-(methylthio) propyl acetate (4, 9) | 61 | ethyl 4-acetoxyhexanoate (6, 9, 10) | 109 | methyl (E)-4-hexanoate (7) | 157 | methyl acetate (5, 6, 9) |
| 14 | 3-methyl-2-butenyl acetate (4, 9) | 62 | ethyl 4-acetoxyoctanoate (6, 9, 10) | 110 | methyl (*E,E*)-2,4-hexadienoate (9) | 158 | methyl acrylate (5) |
| 15 | 3-methylbut-3enyl acetate (7) | 63 | ethyl 4-acetoxypentanoate (9) | 111 | methyl (methylthio) acetate (4, 5, 9) | 159 | methyl benzoate (4, 6, 7) |
| 16 | 3-methylbutyl acetate (5, 9) | 64 | ethyl 4-hydroxyhexanoate (6, 9) | 112 | methyl (*Z*)-3-hexenoate (9) | 160[a] | methyl butanoate (3, 4, 6, 9, 10) |
| 17 | allyl isothiocyanate (9) | 65 | ethyl 4-hydroxyoctanoate (6, 9) | 113 | methyl (Z)-9-octadecenoate (2) | 161 | methyl butyrate (5) |
| 18 | butyl acetate (3, 4, 6) | 66[a] | ethyl 5-acetoxyhexanoate (4, 5, 6, 9, 10) | 114 | methyl (Z, Z)-9,12-octadecadienoate (2) | 162 | methyl cinnamate (7) |
| 19 | dibutyl phthalate (9) | 67 | ethyl 5-acetoxyoctanoate 4, 5, 6, 7, 9) | 115 | methyl 2,4-hexadienate (6) | 163 | methyl (Z)-4-decenoate (5) |
| 20 | diethyl carbonate (4, 5) | 68 | ethyl 5-hexanoate (7) | 116 | methyl 2-acetoxybutanoate (6) | 164 | methyl (Z)-4-octenoate (5) |
| 21 | diethyl malonate (2) | 69 | ethyl 5-hydroxyhexanoate (9) | 117[a] | methyl 2-hidroxypropanoate (4) | 165 | methyl decanoate (5) |
| 22 | diethyl succinate (9) | 70 | ethyl 5-hydroxyoctanoate (2, 9) | 118 | methyl 2-hydroxy-2-methyl butanoate (3, 4, 6, 9) | 166 | methyl (E)-3-hexenoate (4, 6, 10) |
| 23 | diisobutyl phthalate (9) | 71 | ethyl 5-oxohexanoate (5) | 119 | methyl 2-hydroxy-3-methylbutanoate (4) | 167 | methyl (E)-3-octenoate (4) |
| 24[a] | dimethyl malonate (3, 4, 5, 6, 9, 10) | 72 | ethyl acetate (5, 6, 8, 9, 10) | 120 | methyl 2-hydroxy-hexanoate (6, 9) | 168 | methyl heptanoate (4, 5) |
| 25 | dimethyl succinate (4) | 73 | ethyl acrylate (5) | 121[a] | methyl 2-methyl-3-oxobutanoate (3) | 169[a] | methyl hexanoate (3, 4, 5, 6, 9) |
| 26 | *erythro*- butane-2,3-diol diacetate (9) | 74 | ethyl benzoate (5) | 122[a] | methyl 2-methylbutanoate (3, 4, 6, 8, 9, 10) | 170 | methyl lactate (9) |
| 27 | ethy trans-3-octenoate (5) | 75[a] | ethyl butanoate (3, 4, 6, 8, 9) | 123 | methyl 2-methylbutyrate (5) | 171 | methyl n-dodecanoate (2) |
| 28 | ethyl (E)-2-butanoate (7) | 76 | ethyl butyrate (5) | 124 | methyl 2-methylpropanoate (5, 8) | 172 | methyl n-hexadecanoate (2) |
| 29 | ethyl (E)-2-hexenoate (2) | 77 | ethyl cinnamate (4, 7) | 125 | methyl 2-octenoate (4) | 173 | methyl nicotinate (4) |
| 30 | ethyl (E)-3-hexenoate (2, 4, 9) | 78 | ethyl cis-4-decenoate (5) | 126[a] | methyl 3-(methylthio)-propanoate (1, 3, 4, 5, 9) | 174 | methyl n-octadecanoate (2) |
| 31 | ethyl (methylthio)acetate (4, 5) | 79 | ethyl decanoate (4, 5, 9) | 127 | methyl 3-(methylthio)-(E)-2-propenoate (7) | 175 | methyl nonanoate (5) |
| 32 | ethyl (Z)-3-hexenoate (2, 9) | 80 | ethyl formate (5) | 128 | methyl 3-(methylthio)-(Z)-2-propenoate (7) | 176 | methyl octadienoate (2) |
| 33[a] | ethyl 2-(methylthio) acetate (2) | 81 | ethyl heptanoate (4, 5) | 129 | methyl 3-acetoxy butanoate (6) | 177 | methyl octanoate (3, 4, 5, 6, 9, 10) |
| 34 | ethyl 2-butenoate (4) | 82 | ethyl hexadecanoate (9) | 130 | methyl 3-acetoxy-2-methyl butanoate (6, 9) | 178 | methyl pentanoate (3, 4, 5, 6, 9, 10) |
| 35 | ethyl 2-hydroxy-2-methylbutanoate (4, 6, 9) | 83[a] | ethyl hexanoate (3, 4, 5, 6, 8, 9, 10) | 131[a] | methyl 3-acetoxybutanoate (4, 9) | 179 | methyl phenylacetate (9) |
| 36 | ethyl 2-hydroxy-3-methylbutanoate (4, 9) | 84 | ethyl lactate (5, 9) | 132[a] | methyl 3-acetoxyhexanoate (3, 4, 5, 6, 9, 10) | 180 | methyl propanoate (5) |
| 37 | ethyl 2-hydroxyhexanoate (4, 9) | 85 | ethyl methylmalonate (9) | 133 | methyl 3-acetoxyoctanoate (5, 9, 10) | 181 | methyl (E)-3-hexenoate (5) |
| 38 | ethyl 2-hydroxypropanoate (3, 4) | 86 | ethyl methylsuccinate (9) | 134 | methyl 3-hexenoate (5, 10) | 182 | methyl (E)-3-octenoate (5) |
| 39[a] | ethyl 2-methyl butanoate (3, 4, 6, 8, 9) | 87 | ethyl methylpropanoate (4) | 135 | methyl 3-hydroxy-2-methylbutanoate (6, 9) | 183 | methyl (Z)-3-hexenoate (4, 6) |
| 40 | ethyl 2-methylbutyrate (5) | 88 | ethyl n-dodecanoate (2) | 136 | methyl 3-hydroxy-3-methylbutanoate (3, 4, 6, 9) | 184 | methyl (Z)-3-octenoate (4) |
| 41 | ethyl 2-methylpropanoate (5, 8) | 89 | ethyl n-hexadecanoate (2) | 137 | methyl 3-hydroxybutanoate (3, 4, 6, 9) | 185 | methyl (Z)-4-decenoate (4) |
| 42 | ethyl 2-propenoate (4) | 90 | ethyl n-hexanoate (2) | 138 | methyl 3-hydroxybutyrate (5) | 186 | methyl (Z)-4-hexenoate (6) |
| 43 | ethyl 3 methylbutyrate (5) | 91 | ethyl n-octadecanoate (2) | 139[a] | methyl 3-hydroxyhexanoate (3, 4, 5, 6, 9, 10) | 187 | methyl (Z)-4-octenoate (6) |
| 44[a] | ethyl 3-(methylthio) propanoate (2, 3, 4, 5, 6, 9) | 92 | ethyl nonanoate (5) | 140 | methyl 3-hydroxyoctanoate (4, 5, 9, 10) | 188 | methyldecanoate (4) |
| 45 | ethyl 3-(methylthio)-(E)-2-propenoate (7) | 93 | ethyl octanoate (2, 3, 4, 5, 9) | 141 | methyl 3-hydroxypentanoate (9) | 189[a] | propyl acetate (4, 9, 10) |
| 46 | ethyl 3-(methylthio)-(Z)-2-propenoate (7) | 94 | ethyl pentanoate (4, 5, 9) | 142 | methyl 3-methylbutanoate (4, 6, 8) | 190 | *threo* -butane-2,3-diol diacetate (9) |
| 47 | ethyl 3-acetoxy-2-methylbutanoate (6, 9) | 95 | ethyl phenylacetate (4, 9) | 143 | methyl 3-methylbutyrate (5) | 191 | δ-heptanoate (10) |
| 48 | ethyl 3-acetoxybutanoate (4, 6, 7, 9) | 96 | ethyl propanoate (4, 5, 9) | 144 | methyl 4-(methylthio)butanoate (7) | | |

Table 1. *(Continued).*

| | Alcohols and phenols | | Alcohols and phenols | | Alcohols and phenols | | Alcohols and phenols |
|---|---|---|---|---|---|---|---|
| 201 | (3-hydroxyphenyl) ethyl alcohol (10) | 218 | 2-hexanol (5) | 235 | 3-methylpentan-3-ol (5) | 252 | menthol (9) |
| 202 | (*Z*)-3-hexenol (9) | 219 | 2-methyl-1-propanol (4, 5) | 236 | 3-methylphenol (9) | 253 | methanol (5) |
| 203 | 1-butanol (4) | 220 | 2-methyl-2-butanol (9) | 237 | 3-pentanol (4) | 254 | methyl-3-buten-2-ol (5) |
| 204 | 1-decanol (4) | 221 | 2-methyl-3-buten-2-ol (3, 4, 5, 9) | 238 | 4-ethylphenol (9) | 255 | methoxy furaneol (6) |
| 205 | 1-dodecanol (2) | 222 | 2-methylbutan-1-ol (5) | 239 | 4-allyl-2,6-dimethoxyphenol (10) | 256 | nonanol (7, 9) |
| 206 | 1-hexanol (4, 10) | 223 | 2-methylpentan-2-ol (5) | 240[a] | 4-vinylguaiacol (4, 6) | 257 | *p*-allylphenol (chavicol) (5) |
| 207 | 1-menthen-4-ol (5) | 224[a] | 2-methylpropan-1-ol (5, 9) | 241[a] | 4-vinylphenol (4, 6) | 258 | *p*-cymen-8-ol (4, 9) |
| 208 | 1-octen-3-ol (7, 9) | 225 | 2-pentanol (4, 9, 10) | 242 | benzyl alcohol (6, 9) | 259 | pentyl alcohol (9) |
| 209 | 1-pentanol (4, 5) | 226 | 2-phenylethanol (4, 9) | 243 | butanol (9) | 260 | phenethyl alcohol (9) |
| 210 | 1-penten-3-ol (4) | 227[a] | 3- (methylthio)-1-propanol (4, 9) | 244 | coniferilic alcohol (6) | 261 | phenol (9, 10) |
| 211 | 1-propanol (5) | 228 | 3-hexanol (5) | 245 | (E)-2-hexen-1-ol (4) | 262 | solerol (4) |
| 212[a] | 2,3-butanediol (4, 6) | 229 | 3-methyl pentan-2-ol (9) | 246 | *erytro*-3-acetoxy-2-butanol (9) | 263 | tert-butanol (possible trace) (5) |
| 213 | 2,3-dimethyl-2-butanol (5) | 230 | 3-methyl-2-butan-1-ol (4, 7, 9) | 247 | *erytro*-3-hydroxy-2-butanol (9) | 264 | *threo*-3-acetoxy-2-butanol (9) |
| 214 | 2-/3-methyl-1-butanol (4) | 231 | 3-methyl-2-butenol (9) | 248 | ethanol (5) | 265 | (Z)-3-hexen-1-ol (4) |
| 215 | 2-allylphenol (5, 6) | 232 | 3-methyl-3-butan-1-ol (7) | 249 | furfuryl alcohol (4) | 266 | α-terpineol (4, 5, 9) |
| 216 | 2-butoxy-ethanol (6, 10) | 233 | 3-methyl-3-buten-2-ol (9) | 250 | heptanol (7, 9) | | |
| 217 | 2-ethyl-1-hexanol (9) | 234 | 3-methylbutan-1-ol (5, 9, 10) | 251 | hexanol (9) | | |
| | **Aldehydes** | | **Aldehydes** | | **Aldehydes** | | **Aldehydes** |
| 301 | 1-nonanal (2) | 306 | acetaldehyde (5) | 311[a] | furfural (4, 5, 6, 9) | 316 | *p*-hydroxybenzaldehyde (10) |
| 302 | 2-butyl-2-octenal (9) | 307 | benzaldehyde (4, 5) | 312 | hexanal (3, 4, 5, 9, 10) | 317 | propanal (5) |
| 303 | 3-(methylthio)-propanal (4) | 308 | decanal (3, 4) | 313 | nonanal (3, 4, 9) | 318 | syringaldehyde (10) |
| 304 | 5-(hydroxymethyl) furfural (4, 5, 9) | 309 | (E)-2-hexenal (4) | 314 | octanal (8) | 319 | vanillin (8, 9, 10) |
| 305 | 5-methylfurfural (4) | 310 | formaldehyde (5) | 315[a] | phenylacetaldehyde (4) | | |
| | **Ketones** | | **Ketones** | | **Ketones** | | **Ketones** |
| 401 | (Z)-1,5-octadien-3-one (8) | 406 | 2-hexanone (5) | 411[a] | 3-hydroxy-2-butanone (4, 9) | 416 | acetoxyacetone (5) |
| 402 | 2,3-butanedione (4, 5, 8) | 407 | 2-pentanone (4, 5, 10) | 412 | 3-methyl-2-butanone (4) | 417 | hydroxyacetone (9) |
| 403 | 2-acetylfuran (2-furylmethylketone) (4, 9) | 408 | 3-acetoxy-2-butanone (9) | 413 | 3-pentanone (5) | 418 | methyl amyl ketone (6) |
| 404 | 2-butanone (5) | 409 | 3-hexanone (5) | 414 | 4-hydroxy-4-methyl-2-pentanone (4, 6, 8) | 419 | β-damascenone (8) |
| 405 | 2-heptanone (4) | 410[a] | 3-hydroxy-(2H)-pyran-2-one (4) | 415 | acetone (5, 6, 9) | | |
| | **Lactones** | | **Lactones** | | **Lactones** | | **Lactones** |
| 501[a] | 2,5-dimethyl-3(2H)-furanone (4, 5) | 508 | 3-hydroxy-2-methyl-(4H)-pyran-4-one (maltol) (4) | 515 | γ-decalactone (4, 5, 6, 8, 9, 10) | 522 | γ-valerolactone (9) |
| 502 | 2,5-dimethyl-4-hydroxy-2,3-hydro-3-furanone (5) | 509 | 3-hydroxy-4,5-dimethyyl-2(5H)-furanone (8) | 516 | γ-dodecalactone (4, 5, 8, 9) | 523 | δ-decalactone (4, 6, 8, 9) |
| 503[a] | 2,5-dimethyl-4-hydroxy-3(2H) furanone (1, 3, 4, 6, 8, 9, 10) | 510 | 6-methyl-5-hepten-2-one (4) | 517 | γ-heptalactone (4, 6, 9) | 524 | δ-dodecalactone (4) |
| 504[a] | 2,5-dimethyl-4-methoxy-3(2H)-furanone (3, 4, 8, 9) | 511 | methyl tetrahydrofuran-3-one (9) | 518[a] | γ-hexalactone (3, 4, 5, 6, 9, 10) | 525 | δ-heptalactone (4, 6) |
| 505 | 2-methyl-2(3H)-furanone (4) | 512 | pantolactone (4) | 519 | γ-nonalactone (4, 5, 8, 9, 10) | 526[a] | δ-hexalactone (3, 4, 6, 9, 10) |
| 506 | 2-methyltetrahydrofuran-3-one (9) | 513[a] | solerone (4) | 520[a] | γ-octalactone (3, 4, 5, 6, 8, 9, 10) | 527 | δ-nonalactone (1) |
| 507 | 3,5-dimethyl-4-hydroxy-2,3-dihydroxyfuran-3-one (5) | 514[a] | γ-butyrolactone (3, 4, 5, 6, 9) | 521 | γ-palmitolactone (5) | 528[a] | δ-octalactone (3, 4, 5, 6, 8, 9, 10) |

Table 1. *(Continued)*.

| | Terpenes and terpenoids | | Terpenes and terpenoids | | Terpenes and terpenoids | | Terpenes and terpenoids |
|---|---|---|---|---|---|---|---|
| 601 | (E)-β-cariophyllene (3) | 607 | linalool (4, 5, 9) | 613[a] | α-pinene (3) | 619 | γ- eudesmol (2, 5) |
| 602 | 4-terpinenol (4, 9) | 608 | sabinene (3) | 614 | α-zingiberene (3) | 620 | γ- gurjunene (2) |
| 603 | camphor (5, 9) | 609 | Z-ocimene (4) | 615[a] | β-myrcene (3, 4) | 621 | δ-cadinene (2, 3) |
| 604 | geraniol (4, 7) | 610[a] | α-copaene (3, 5) | 616[a] | β-phellandrene (3) | 622 | 1,4-cineol (5) |
| 605 | germacrene D (2) | 611 | α-muurolene (2) | 617 | β-pinene (3) | 623 | 1,8-cineol (5) |
| 606 | limonene (3, 4, 9) | 612 | α-patchoulene (2) | 618[a] | β-ylangene (2) | | |
| | **Miscellaneous** | | **Miscellaneous** | | **Miscellaneous** | | **Mixcellaneous** |
| 701[a] | (E)-β-ocimene (3,7-dimethyl-1,3,6-octatriene) (3) | 710 | 3-methylbutyric acid (9) | 719 | ethyl ester, C5 unsaturated acid (5) | 728 | methyl mercaptan (methanethiol) (5) |
| 702 | 1-(E,E)-3,5-undecatriene (2) | 711[a] | acetic acid (4, 5, 6, 9) | 720 | eugenol (10) | 729 | N,N-dimethylformamide (9) |
| 703 | 1-(E,E,Z)-3,5,8-undecatraene (2) | 712 | benzene (5) | 721 | hexadecanoic acid (palmitic acid) (6) | 730[a] | octanoic acid (3, 6, 9) |
| 704 | 1-(E,Z)-3,5-undecatriene (2, 8) | 713 | betapinene (3) | 722[a] | hexanoic acid (3, 6, 9, 10) | 731 | *p*-cymene (3, 4, 9) |
| 705 | 1-(E,Z,Z)-3,5,8-undecatraene (2) | 714 | butanoic acid (4, 8) | 723 | linalool oxide (*Z*-furanoid) (4, 7, 9) | 732 | phenylacetic acid (8) |
| 706 | 1,1-diethoxyethane (5) | 715 | cinamic acid (10) | 724 | linalool oxide (E-furanoid) (4, 5, 7, 9) | 733 | propanoic acid (9) |
| 707 | 1,3,5,8-undecatetraene (8) | 716 | decanoic acid (9) | 725 | methyl ester, C5 hydroxy acid (5) | | |
| 708 | 2-methylbutyric acid (9) | 717 | dimethyl disulfide (5) | 726 | methyl ester, C5 keto acid (5) | | |
| 709 | 3-hydroxy-2-methyl-4H-pyran-4-one (maltol) (4) | 718 | dimethyl trisulfide (7) | 727 | methyl ester, C5 unsaturated acid (5) | | |

1-Badilla-Porras 2005, 2-Berger and others 1983, 1985, 3-Brat and others 2004, 4-Elss and others 2005, 5-Flath 1980, 6-Sinuco and others 2004,
7-Takeoka and others 1991, 8-Tokitomo and others 2005, 9-Umano and other 1992, 10-Wu and others 1991.

[a]: volatile compounds composition shown in Figure 1, 2 or 3.

Studies from the 60´s and 70´s were summarized by Flath (1980) which included some researches with canned Malayan pineapple juice with paper chromatography made in 1964 by Mori in Hawaii and continued by Connell with fresh Australian pineapple who were the first researches to use gas chromatography techniques for pineapple aroma studies, and were able to identify 16 new volatile components. In 1965, two researchers studied Smooth cayenne fruit harvested during the winter season from Hawaii; they were able to identify 2,5-dimethyl-4-hydroxy-2,3-dihydrofuran-3-one (furanone) from the juice extracted from the fruit, using magnetic resonance, infrared, ultraviolet and mass spectra (Rodin and other 1965; Silverstein and others 1965). Later on, they worked with pineapple juice concentrate and identified *p*-allylphenol (chavicol) and γ-hexalactone and confirmed the presence of methyl and ethyl 3-(methylthio) propanoates which were previously reported by Haagen-Smit and others since 1945. Flath and Forrey (1970) studied essence extracted from Smooth Cayenne pineapple concentrated juice from Hawaii using tubular gas chromatography-mass spectrometry technique, which simplified the identification of gas chromatography compatible components; they identified 44 volatile compounds, half of them previously identified as well as some other which could not been identified.

Berger and others (1983) pointed out that volatile constituents of pineapple included aliphatic, hydroxyl, acetoxy and carboxylic esters, γ-lactones, sulfur compounds, linalool oxide, 2,5dimethyl-4-hydroxy-3(2H)-furanone, monoterpene alcohols and sesquiterpenoid structures. They isolated pineapple volatiles under enzyme inhibition with methanol, to reduce the formation of secondary aroma compounds. These researchers also identified more than 20 sesquiterpenes with either bi- or tricyclic skeletons, terpenoids, fatty acid/ amino acid derivatives, phenylproponids (furanol) and benzenoids (benzaldehyde) as well as N- and S-containing compounds. Other 19 volatile constituents were found in a later study by Berger and others (1985) with fresh whole ripe pineapple from the Ivory Coast (cultivar was not reported); the newly identified compounds included four nonterpenoid hydrocarbons, carboxylic esters and others; however, the authors found that mechanical damage during sample preparation or processing of the fruit tissue can cause a rapid decrease of all undecaenes concentration, which can be avoided by preventing enzymatic and oxidative degradation.

As reported by Wu and others (1991) aromatic components of fruits are present either in a free form or bound to sugar as glycosides. They prepared pineapple juice from fresh pineapple fruit from Costa Rica (non specified cultivar) and found free and glycosidically bound volatile compound in pineapple. Methyl 3-acetoxyhexanoate,

2,5-dimethyl-4-hydroxy-3(2H)-furanone and methyl 5-acetoxyhexanoate were the most abundant. But they also identified 2-pentanol, 2-butoxyethanol, hexanoic acid, phenol, *p*-hydroxybenzaldehyde, vanillin and syringaldehyde that were not reported before. They found out that free volatile fraction had fruity and pineapple-like aroma, while the glycosidically bound fraction had no odor, until it was released with enzymatic hydrolysis. Lactones and hydroxyl compounds were the main glycosidically bound volatiles; 2,5-dimethyl-4-hydroxy-3(2H)-furanone was the most abundant compound followed by δ-octalactone and ethyl 3-hydroxyhexanoate. Some lactones were only found as glycosidically bound while others free, or, in both forms. In addition, Sinuco and others (2004) identified 17 glycosidically bound aroma compounds (aglycones) in fresh perola cultivar pineapple. Phenolic compound, carboxylic acids and furanic compounds were the main aglycones identified, and coniferlic alcohol, hexadecanoic acid, furaneol and 4-vinylguaiacol were the most important. Table 2 summarizes the free and bound volatile compounds reported by Sinuco and others (2004) and Wu and others (1991).

In their work, Takeoka and others (1991) identified and studied sulfur-containing constituents from pineapple essence and their contribution to pineapple odor. Volatiles were extracted with pentane. They identified for the first time 26 pineapple constituents and reported methyl and ethyl 3-(methylthio)-(Z)-2-propenoate as the major volatile constituents found in pineapple with concentrations in the range of 1 to 6 µg/kg.

## 3. PINEAPPLE FLAVOR PROFILE CHANGES

Volatile compounds play an important role in flavor perception (Kays 1997), however their content can be altered by cultural practices before harvest, postharvest handling practices, maturity stage and processing procedures, which might include refrigeration, minimal processing, juice extraction, filtration, heat processing and others.

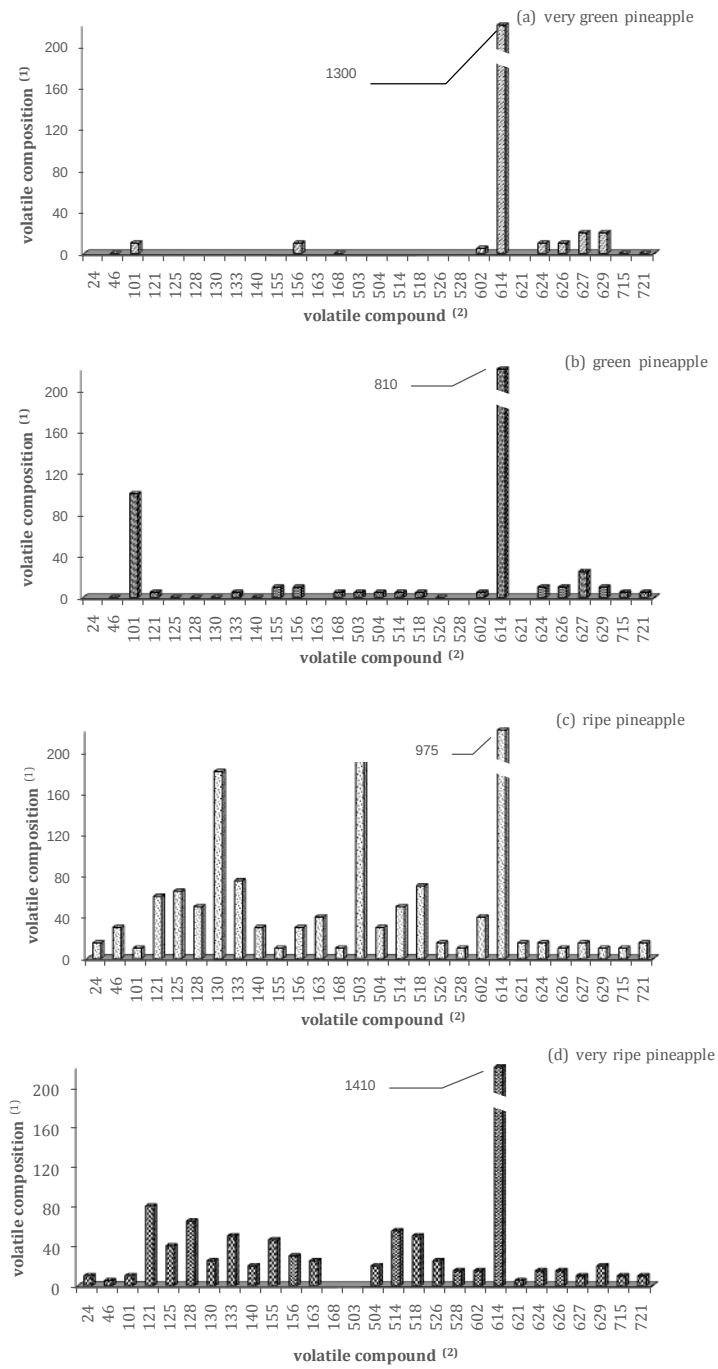
### 3.1 Influence of cultivars and maturity stages on the flavor of pineapple fruit

Many volatile compounds of pineapple have been identified from fresh fruit. However, in many cases their concentrations depend on the cultivar as well as the degree of ripeness.

Umano and other (1992) worked with green and ripened pineapples from the Philippines (cultivar was not reported) and found differences in volatile constituents composition. They identified 157 volatile compound; 144 were found in green fruits and 127 in ripened fruit. Ethyl acetate (24.5%), ethyl 3-(methylthio) propanoate (10.4%) and ethyl 3-acetoxyhexanoate (8.7%) were the major volatile components in green pineapple, compared with ethyl acetate (33.5%), *threo*-butane-2,3-diol diacetate (13.0%) and 3-hydroxy-2-butanone (8.7%) in ripened pineapples.

Brat and others (2004) studied volatile compounds for a new pineapple hybrid (Flhoran 41) for different stages of maturity and compared them with Smooth cayenne cultivar. These authors found that major components were aliphatic, hydroxyl and acetoxy esters and terpenes. Figure 3 show four graphs for main pineapple volatile compounds for Flhoran 41 cultivar. Limonene was the most abundant constituent and it decreased significantly as the fruit ripens (1300, 810, 975 and 1410 µg/100 g, for very green, green, ripe and very ripe fruit, respectively). Volatile compounds composition changes throughout the different stages of maturity; ripen pineapple had larger content of most of the volatile compounds as compared with green and very green fruits.

Additionally, they found some differences in volatile components profile and concentrations for ripen pineapple of Flhoran 41 and Smooth cayenne cultivars (Figure 4). Ripe pineapple from both varieties showed similar volatile composition but some components were only found in Flhoran 41 fruits (n-butyl acetate, ethyl 2-hydroxypropanoate and (E)-β-caryophyllene); some had higher concentrations in Flhoran 41 pineapples ((E)-β-ocimene, γ-butyrolactone, 2,5-dimethyl-4-methoxy-3(2H)furanone, 2,5-dimethyl-4-hydroxy-3(2H)furanone and some esters (methyl 2-methylbutanoate, methyl 2-hydroxy-2-methyl butanoate, methyl 2-methyl 3-oxobutanoate and ethyl 3-(methylthio) propanoate). Other volatile constituents were present in higher concentrations in Smooth cayenne cultivar, such us methyl 5-acetoxyhexanoate, methyl 3-acetoxyhexanoate, dimethyl malonate and methyl hexanoate.




[1] µg of 2-heptanol equivalent per 100g fresh weight; [2] compound numbers correspond to those in Table 1.

**Figure 3.** Changes in major pineapple volatile compounds composition during maturation of Flhoran41 cultivar from French West Indies (a, b, c and d show results for very green, green, ripe, and very ripe pineapple, respectively). Adapted from Brat and others (2004).

## 3.2 Effects of processing on the flavor of pineapple fruit

The pineapple fruit flavor can be easily modified during fruit processing. A representative example is the pineapple juice, which is usually a byproduct (out-flowing juice, the juice from the peel and the pineapple core) obtained during the production process of canned pineapples, even though the majority of commercial pineapple juice is made from concentrate (Askar and Treptow 2001). In both cases, thermal treatments are employed resulting in the loss or transformation of some volatile compounds.

Elss and others (2005) studied the flavor profile of juice made from fresh-cut pineapples fruits, juice concentrates, commercial juices and juice made from concentrate (Figure 2). Their results showed considerable differences among flavor profile of fresh pineapple juice and other processed samples, since in most cases, the characteristic methyl esters and hydroxy or acetoxy esters were lacking completely or had only minor amounts in processed products. Esters content of juice prepared from fresh-cut pineapple was much larger than that of concentrate and juices (Fig 2.a). Differences can be due to losses during processing once the fruit is peeled, cut and processed. Thermal processes used to prepare pineapple concentrates facilitate volatile compound losses and might also contribute to the production of new chemical compounds. They observed that methyl 2-methylbutanoate, methyl 3-(methylthio) propanoate and methyl hexanoate were the most abundant esters in pineapple juice prepared from fresh-cut fruit, followed by methyl 5-acetoxyhexanoate, ethyl hexanoate, methylbutanoate, ethyl 3-(methylthio) propanoate and methyl 3-acetoxyhexanoate.

Commercial juices had similar aroma constituents but in much lower concentrations, while concentrates and juices prepared from concentrates had even lower concentrations. In addition, 2,5-dimethyl-4-hydroxy-3(2H) furanone, 2,5-dimethyl-4-methoxy-3(2H) furanone and γ-hexalactone were the most important not ester volatile components in juice prepared from fresh fruit. The first of them was important for all pineapple products studied, while the second and third were present in much lower concentrations in commercial juices, concentrates and juices from concentrates. Furfural, 4-vinylguaiacol, 4-vinylphenol, furfural, 2,5-dimethyl-3(2H) furanone, 2,5-dimethyl-4-hydroxy-3(2H) furanone and acetic acid were present in higher concentrations in pineapple concentrates, than the juice from fresh fruits and other pineapple products. Differences confirm changes in volatiles composition occurring during processing.

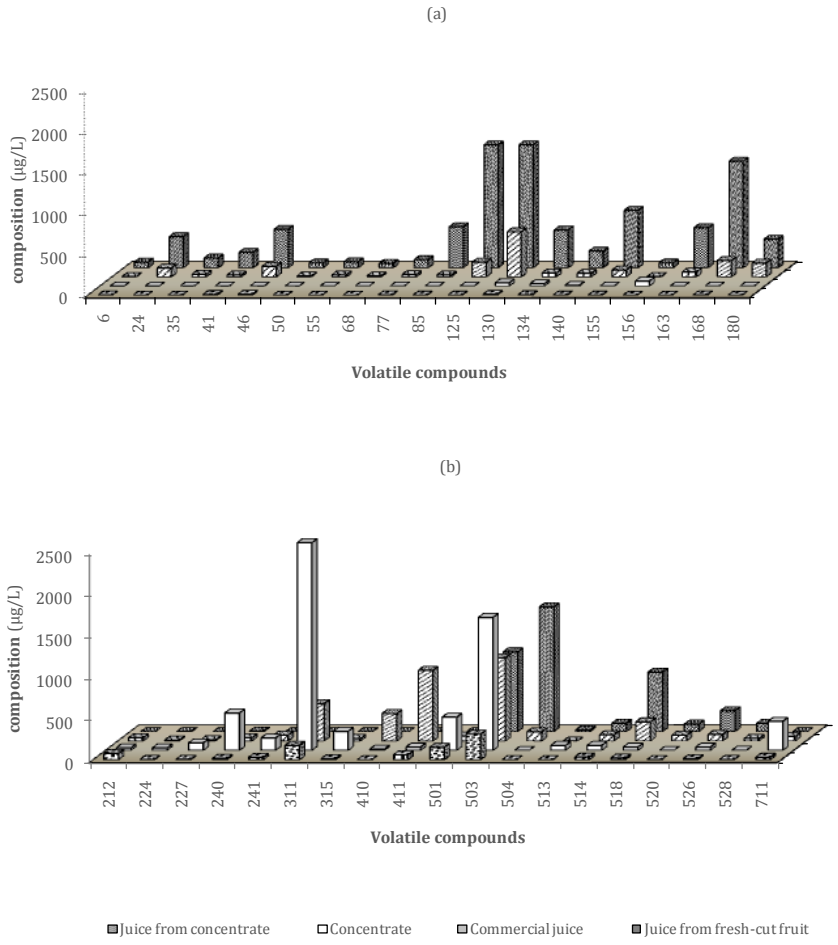



**Figure 2.** Esters (a) and alcohols, aldehydes, ketones, lactones and other compounds (b) average composition (µg/l) in pineapple products. Volatile compound numbers correspond to those in Table 1 (adapted from Elss and others 2005).

Some other volatile compounds found in juices from concentrates include small amounts of terpenes associated with contamination during processing since they were not found in juices made from fresh-cut fruit (*p*-cimene, β-myrcene, α-terpineol or linalool).

Because of the convenience offered to consumers, consumption of fresh-cut fruit including pineapples has increased considerably in the last years. However, the shelf-

life of cut fruits is considerably lower than that of the intact fruit. Once a fruit is cut it becomes a different product from what it was in its entire form. Therefore, producer must ensure the fruit's original flavor characteristics, quality and safety.

Spanier and others (1998) studied the effect of storage (4 °C for 3, 7 and 10 days) on the flavor volatile profile of fresh-cut pineapples. They observed that pineapple-like flavors increased very slightly during storage (acetic acid 1-methylethyl ester, acetic acid propyl ester and 1-butanol 3-methyl acetate). While unpleasant odors and volatiles such as fermented, cheesy, sour dough, alcohol, oily, etc., showed dramatic increases and masked the more desirable pineapple flavor leading to a diminution of the overall flavor quality of the product. The large increase in the level of low boiling alcohols in stored pineapple suggested that fermentative events occurred during storage. They confirmed that yeast was the source of the fermentation derived alcohols.

More recently, Lamikanra and Richard (2004) indicated that the stress adaptation process of fruit to exposure of tissue resulting from fresh-cut processing involves the reduction of volatile aroma compounds, particularly esters, and synthesis of sesquiterpene compounds with phytoalexin properties. In fact, they evaluated the effect of storage and ultraviolet-induced stress on the volatile aroma compounds of fresh-cut pineapple. According to their results, storage at 4 °C for 24h, and exposure of cut fruit to UV radiation for 15 min caused a considerable decrease in the concentration of esters and an increase in the relative amount of copaene, sesquiterpene which inhibit microbial growth in fruits when it is added to fresh-cut fruit (Lamikanra and Richard 2002; Lamikanra and others 2003). Furthermore, they identified other sesquiterpene considered as a potent antimicrobial agent, ocimene, which was present in the fruit but their production was not photo-induced by UV irradiation. The loss of esters and changes in volatile aroma composition during storage, including production of terpene phytoalexins, will potentially affect the fruit flavor during storage. However, sesquiterpene phytoalexins could contribute to the defense mechanism in wounded pineapple tissue.

## 4. SENSORY CHARACTERIZATION OF PINEAPPLE FLAVOR

Total aroma of the fruit is a result of a specific blend of individual component aromas with specific quantity of each of them. For this reason, it is necessary to achieve proper separation and identification of odor contributing constituents in combination with sensory evaluation of the fruit and its individual components. Most of pineapple aroma studies have been done on identification and quantification of

volatile constituents and only a few have been done with sensory analysis (Flath 1980, Tokitomo and others 2005).

Several researchers have studied the contribution of different volatile compounds to overall aroma of pineapple fruit (Flath 1980; Takeoka and others 1991; Tokitomo and others 2005; Wu and others 1991). Relative composition and proper characteristics of each volatile constituent are two factors involved in their contribution to fruit aroma; however, it is not necessarily related to the component concentration. Therefore, determine the contribution of different volatile compounds to overall aroma perception of fresh and processed pineapple is very important. For this reason is essential the use of sensory analysis, in combination with separation techniques and proper analytical analysis to measure volatile components threshold and composition. Table 3 summarizes some sensory data and odor description for pineapple volatile compounds reported in the last years.

Flath (1980) cited studies by Pitter and others (1970) and Rodin and others (1971) who reported odor and taste thresholds of furanone in water as 0.1 to 0.2 ppm and 0.3 ppm, respectively, and while furanone concentration in pineapple flesh reported as 1.2 ppm on winter Smooth cayenne fruit by Silverstein and others (1965). In this work, furanone odor was described as caramel, sweet and fruity. A coconut note has been also reported in the aroma of fully-ripe pineapple, probably caused by odorous lactones like γ-octalactones and δ-octalactones (Flath 1980).

Contribution of volatile constituents to pineapple aroma have been studied by comparing their concentrations and odor detection thresholds (Berger and others 1985, Takeoka and others 1991); as the ratio of average concentration to odor detection threshold increases, the contribution of the volatile compound become larger. Berger and others (1983) considered that α-patchoulene contributed to the strong fruit-spice odor of pineapple. In 1985, the same authors used a capillary gas chromatographic sniffing technique to compare analytical data with sensory judgments. They were able to identify several volatile constituents not reported before from whole ripe pineapple fruits from the Ivory Coast. They reported that even though 1-(E,Z)-3,5-undecatriene and 1-(E,Z,Z)-3,5,8-undecatraene had low average concentrations, their odor detection thresholds was also low, and thus they concluded that these compounds probably have an important contribution to the overall impression of pineapple flavor. Their isomers 1-(E,E)-3,5-undecatriene and 1-(E,E,Z)-3,5,8 undecatraene were found to have much less odor. Ethyl hexanoate and ethyl 3-(methylthio) propanoate were also reported by the same authors as

important contributors to pineapple aroma, both of them were present in larger concentrations, and their odor detection threshold were also higher.

Takeoka and others (1991) reported ethyl-2-methyl-butanoate (S-(+) enantiomer) as a potent odorant with an odor threshold of 0.006 µg/kg, they considered it as the second largest odor contributor to pineapple aroma after pineapple furanone.

Sinuco and others (2004) used high resolution gas chromatography with olfactometry (HRGC/O) to separate and describe the odor of each pineapple aroma components with the help of a group of aroma experts. They studied volatile constituents of fresh pineapple fruits (perolera cultivar), using fruits grown in Colombia. They reported methyl esters of 2-methyl-butanoic and hexanoic acids as responsible for fresh pineapple odor, while reported esters such as ethyl butanoate, ethyl-2-methylbutanoate, butyl acetate, 2-methyl acetate, methyl-3-hexenoate, methyl 2-hydroxy-2-methybutanoate, methyl 3-hydroxybutanoate and ethyl 3-acetoxy-2-methylbutanoate as low impact volatile compounds for pineapple aroma. Furanone, γ-butyrolactone, γ-hexalactone, γ-octalactone, γ-decalactone and δ-octa-lactone were reported as important for perolera cultivar pineapple aroma.

Tokitomo and others (2005) uses the aroma extract dilution analysis approach (AEDA) to identify the most odor-active compounds. They studied Super Sweet (F-2000) pineapple cultivar purchased in Germany and Japan, and found 29 odor-active volatile components and estimated an odor activity value (OAV) to compare among volatile constituents taking into consideration the odor threshold and concentration. They reported furanone (2,5-dimethyl-4-hydroxy-3(2H)-furanone), ethyl 2-methyl propanoate and ethyl 2-methylbutanoate as the three most odor-active compounds, followed by methyl 2-methylbutanoate, 1-(E,Z)-3,5-undecatriene and β–damascenone. They corroborated that fresh pineapple-like aroma was due to 1-(E,Z)-3,5-undecatriene, as reported before. Sensory evaluations were performed by the authors to corroborate the above results. They use the main 12 odorants of pineapple and prepare models using the same compound concentrations as found in pineapple and seven odor descriptors: sweet, citrus-like, fresh, fruity, green or grassy, woody and pineapple-like. When furanone or ethyl 2-methylbutanoate was excluded from the models, panelists noticed aroma changes. Absence of furanone resulted in lack of sweet, pineapple-like aroma, while absence of ethyl 2-methyl butanoate was reflected as a lack of fresh pineapple flavor.

Recently, Schulbach and others (2007) evaluated overall acceptability of fresh pineapple from five different countries and six different producers. They used a

descriptive sensory analysis with eight descriptive terms: sweetness, sourness, pineapple flavor intensity, firmness, juiciness, off-flavor, banana character and coconut character, along with a rating for overall acceptability. Their results showed that the attributes sweetness, pineapple flavor intensity and off-flavor were the most important factors in determining acceptability. Pineapple flavor rating was more significant than sweetness in determining pineapple sensory quality as long as the sugar content of the fruit was adequate. In addition, this experiment provides strong evidence that increasing the aroma volatiles in pineapple will not only result in a pineapple with higher flavor intensity, but also with more apparent sweetness and better overall acceptability.

## 5. OTHER FLAVOR COMPONENTS

Many other constituents which stimulate the sense of taste have also been identified. The sugars, for instance, produce sensations of sweetness, while organic acids are responsible for sour tastes. Both, acidity and sweetness contributes with pineapple aroma. Acids content varies as the fruit develops and ripens. Citric and malic acids are the major nonvolatile acids in pineapple. Malic acid content can vary from 18 to 30% of total acids, while citric acid content is about 28 to 66% (Paull 1993).

Sugar content is an important characteristic which directly affects flavor. It is used as a quality parameter to indicate both maturity stage and quality. Major sugars in ripen fruit includes sucrose, glucose and fructose (Paull 1993). Content of inverted sugars is much larger during the early stages of the fruit development, and decreases as the fruit ripens. Total sugars increase as the fruit ripens up to 12 to 18%, depending on the cultivar, weather conditions and others (Flath 1980).

## 6. FINAL REMARKS

Near 380 volatile and non-volatile constituents have been recognized up to 2005 in fresh and processed pineapple. However, factors as cultivar, stage of maturity, processing conditions as well as pre- and post-harvest practices can directly affect pineapple aroma profile. In fact, original flavor characteristics, quality and safety of pineapple can be seen affected during their processing. In addition, even though MD2 cultivar (Del Monte Gold) has substitute a large portion of pineapple world market, no information is available about its impact aroma compounds and how do they change during processing. Studies in this subject are still very limited, and more

efforts should be made, not only to identify impact components but to study changes due to processing and storage, including sensory analyses of pineapple volatile compounds.

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# OBJECTIVES

## General Objective

The general objective of this work was to study the flesh quality of Gold cultivar pineapple along the fruit and the influence of packaging conditions on fruit pieces throughout storage, as tools to prop up homogeneous, reproducible, and endurable quality of fresh-cut pineapple.

## Specific Objectives

- To determine the quality profile of Gold cultivar pineapple flesh along the fruit.
- To identify changes and limiting factors for the shelf-life of fresh-cut pineapple.
- To evaluate the effect of modified atmospheres packaging and an edible coating on the mechanical, antioxidant and physicochemical properties of fresh-cut pineapple throughout storage at 5 °C.
- To compare the effect of packaging conditions on the aroma profile and odor activity of volatile compounds in fresh-cut pineapple stored at 5 °C.

# MATERIALS AND METHODS

# MATERIALS AND METHODS

## 1. MATERIALS

Fresh 'Gold' cultivar pineapples (*Ananas comosus* L. Merrill) imported from Costa Rica, were bought at a local supermarket in Lleida, Spain and stored at 11 ± 1 °C overnight prior to processing for each of the four studies included in this work. Fruits were free from mechanical injuries, insects, pathogens or other defects. Pineapples had uniform stage of maturity, given by its shell color, which showed several to most of their eyes partially filled with yellow color, all of them surrounded by green (De la Cruz-Medina and García, 2007).

An ascorbic acid (1%) and citric acid (1%) solution was used to keep a low pH level on the fresh-cut pineapple surface. Food grade sodium alginate (Keltone® LV, ISP, San Diego, CA, USA) was used as the carbohydrate biopolymer for coating formulation. Glycerol (Merck, Whitehouse Station, NJ, USA) and sunflower oil (La Española, Spain) were added as plasticizer and emulsifier, respectively. Calcium chloride (Sigma–Aldrich Chemic, Steinhein, Germany) was used to induce cross-linking reactions.

Reference volatile compounds were used as internal and external standards for fresh-cut pineapple aroma analysis are included in Table 1 and 2. They were chosen from previous studies with pineapple flesh. Regents were purchased from Sigma-Aldrich Química SA, Madrid, Spain.

## 2. FRESH-CUT PROCESSING AND SAMPLE PREPARATION

Working area, cutting boards, knives, containers and other utensils and surfaces in contact with the fruit during processing were washed and sanitized with 200 µL/L sodium hypochlorite solution at pH 7 to have a maximum sanitizing effect before processing. Pineapple crown leaves were removed and the fruit was washed twice in two 200 µL/L sodium hypochlorite solutions for 5 min each, letting excess water drain for 3–5 min after each dip. Fruit were peeled and cut into 1.0 to 1.2 cm-thick slices using an electric slicing machine (Food Slicer-6128: Toastmaster Corp, Elgin, USA). Slices from the top, middle and bottom sections of the fruit were separated into different containers for studies 1 and 2. Slices were cored and cut into wedges (6–7 g, each) with sharp knives, and thoroughly mixed in studies 3 and 4 to minimize the effect of flesh quality differences along the fruit. Fresh-cut pineapple pieces were

washed in 20 μL/L sodium hypochlorite solutions for 2 minutes, drained and packed in 500 mL trays and stored at 5 °C for less than two hours before their analysis, for studies 1 and 2.

For storage evaluation (studies 3 and 4), fresh-cut pineapple pieces were immersed in 1% citric acid and 1% ascorbic acid solution for 2 min as anti-browning agents and to keep the surface pH low enough to reduce microbial growth. Excess water was drained for 2 min and 100g pineapple pieces were packaged as described ahead.

When alginate edible coating was used as a protective barrier, ascorbic and citric acid were incorporated directly into the calcium chloride solution to reduce excessive handling of fresh-cut produce.

## 3. FRESH-CUT FRUIT COATING

Alginate coating was prepared as described by Rojas-Graü et al. (2008). Alginate powder (1%, w/v) was dissolved in distilled water under controlled heating (80 °C) and stirred until the mixtures became clear. Glycerol was added as plasticizer (1.5%, w/v). The solution was emulsified with 0.025% (w/v) sunflower oil, using an Ultra Turrax T25 (IKA® WERKE, Germany) with a S25N-G25G device for 5 min at 24,500 rpm and degassed under vacuum. Fresh-cut pineapple pieces were submerged for 2min in the coating solution, drained for 2min and submerged for another 2min in a 2% (w/v) calcium chloride bath for carbohydrate polymer cross linking. Ascorbic acid (1%) and 1% citric acid were also added to the latter solution.

## 4. PACKAGING CONDITIONS AND STORAGE

Portions of 50 or 100g of fresh-cut pineapples were placed into PP trays (500 $cm^3$, MCP Performance Plastic Ltd., Kibbutz Hamaapil, Israel). These were wrapped with a 64μm of thickness PP film with a permeability to $O_2$ and $CO_2$ of 110 and 550$cm^3/m^2$/bar/d at 23 °C and 0% RH, respectively (Tecnopack SRL, Mortara, Italy) using a MAP machine (Ilpra Foodpack Basic V/G, Ilpra, Vigenovo, Italy). Weight to volume ratios of 1:10 and 2:10 (g:mL) were used for studies 3 and 4, respectively.

Four packaging conditions were established for study 3: (a) PP-HO: fresh-cut pineapple in PP trays filled with high oxygen concentration (38–40% $O_2$); (b) PP-LO: fresh-cut pineapple in PP trays filled with low oxygen concentration (10–12% $O_2$, 1% $CO_2$); (c) PP-AIR: fresh-cut pineapple in PP trays filled with air (20.9% $O_2$); (d) PP-ALG: fresh-cut pineapple coated with alginate and packaged in PP trays filled with air.

Similar initial conditions were used for study 4, but twice the fruit weight to volume ratio was used and trays were labeled as: (a) LO (low oxygen; 12% $O_2$ and 1% $CO_2$), (b) AIR (20.9% $O_2$), and (c) HO (high oxygen; 38 % $O_2$).

**Table 1.** Volatile compounds used as internal and external standards for fresh-cut pineapple aroma identification and quantification.

| ID [a] | RT [b] | Aroma compound | cas number [c] | OT [d] (µg/kg) |
|---|---|---|---|---|
| 1 | 2,804 | methyl 2-methyl propanoate | 547-63-7 | 6,3[iii] |
| 2 | 3,027 | ethyl propanoate | 105-37-3 | |
| 3 | 3,095 | methyl butanoate | 623-42-7 | 72[ii] |
| 4 | 3,506 | ethyl 2-methyl propanoate | 97-62-1 | |
| 5 | 3,686 | methyl 3-methyl butanoate | 556-24-1 | |
| 6 | 3,707 | methyl 2-methyl butanoate | 868-57-5 | 0.1[ii] |
| 7 | 3,917 | hexanal | 66-25-1 | |
| 8 | 4,047 | butyl acetate | 123-86-4 | |
| 9 | 4,450 | ethyl 2-methylbutanoate | 7452-79-1 | 0,006[ii] |
| 10 | 4,710 | 3-methylbutyl acetate | 123-92-2 | 2[i] |
| 11 | 4,853 | 2-heptanone | 110-43-0 | |
| 12 | 5,060 | methyl 5 hexenoate | 2396-80-7 | |
| 13 | 5,190 | methyl hexanoate | 106-70-7 | 77[i] |
| 14 | 5,870 | ethyl hexanoate | 123-66-0 | 1[i] |
| 15 | 5,990 | hexyl acetate | 142-92-7 | |
| 16 | 6,118 | methyl 3-(methylthio) propanoate | 13532-18-8 | 180[i] |
| 17 | 6,190 | limonene | 3338-55-4 | 10[i] |
| 18 | 6,220 | (Z)-beta-ocimene | 95327-98-3 | |
| 19 | 6,395 | 2,5-dimethyl-4-hydroxy-3(2H) furanone | 3658-77-3 | |
| 20 | 6,440 | 2,5-dimethyl 4 methoxy 3(2H) furanone | 4077-47-8 | 0.03[i] |
| 21 | 6,715 | ethyl heptanoate | 106-30-9 | 2,2[i] |
| 22 | 6,758 | ethyl 3-(methylthio) propanoate | 13327-56-5 | |
| 23 | 6,770 | linalool | 78–70–6 | 6[i] |
| 24 | 6,820 | nonanal | 124-19-6 | 1[i] |
| 25 | 6,940 | methyl octanoate | 111-11-5 | 200[i] |
| 26 | 7,278 | 4-ethyl phenol | 123-07-09 | |
| 27 | 7,310 | methyl (E) octenoate | 7367-81-9 | |
| 28 | 7,502 | ethyl octanoate | 106-32-1 | |
| IS [e] | 7,589 | methyl salicylate | 119-36-8 | |
| 29 | 7,954 | geraniol | 106-24-1 | |
| 30 | 8,185 | 4-ethyl-2-methoxy-phenol | 2785-89-9 | |
| 31 | 8,929 | ethyl decanoate | 110-38-3 | |
| 32 | 8,950 | alpha copaene | 3856-25-5 | |

(a): ID number used for this manuscript; (b): retention time (min); (c): Chemical Abstracts Service registry number; (d): Odor threshold concentration in water (µg/kg) from reference (i, ii or iii); (e): internal standard.

**Table 2.** Chemical structure and odor description of volatile compounds used as internal and external standards for fresh-cut pineapple aroma identification and quantification.

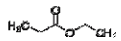
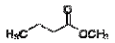
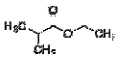
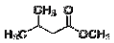
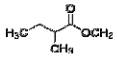
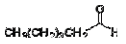
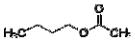
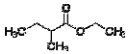
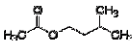
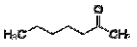
| ID [a] | aroma compounds | chemical structure | odor description | references [b] | linear structure | synonym |
|---|---|---|---|---|---|---|
| 1 | methyl 2-methyl propanoate |  | fruity, sweet | 5,8 | $(CH_3)_2CHCOOCH_3$ | methyl isobutyrate |
| 2 | ethyl propanoate |  | | 4,5,9 | $CH_3CH_2COOC_2H_5$ | ethyl propionate |
| 3 | methyl butanoate |  | apple, sweet, toast | 3,4,5,6,9,10 | $CH_3CH_2CH_2COOCH_3$ | methyl butyrate |
| 4 | ethyl 2-methyl propanoate |  | fruity, sweet | 5,8 | $(CH_3)_2CHCOOC_2H_5$ | ethyl isobutyrate |
| 5 | methyl 3-methyl butanoate |  | | 4,5,6,8 | $(CH_3)_2CHCH_2COOCH_3$ | methyl isovalerate |
| 6 | methyl 2-methyl butanoate |  | pineapple like, fruity, apple like | 3,4,5,6,8,9,10 | $CH_3CH_2CH(CH_3)COOCH_3$ | methyl 2-methyl butyrate |
| 7 | hexanal |  | green | 3,4,5,9,10,12 | $CH_3(CH_2)_4CHO$ | aldehyde $C_6$, caproaldehyde, hexyl aldehyde |
| 8 | butyl acetate |  | fruity | 3,4,6,14 | $CH_3COO(CH_2)_3CH_3$ | |
| 9 | ethyl 2-methylbutanoate |  | pineapple heart, fruity, fresh fruity odor note | 3,4,5,6,8,9,11 | $CH_3CH_2CH(CH_3)COOC_2H_5$ | |
| 10 | 3-methylbutyl acetate |  | | 5,9 | $CH_3COOCH_2CH_2CH(CH_3)_2$ | acetic acid 3-methylbutyl ester, isoamyl acetate, isopentyl acetate |
| 11 | 2-heptanone |  | banana | 4,14 | $CH_3(CH_2)_4COCH_3$ | methyl pentyl ketone |

**Table 2.** *(Continued).*

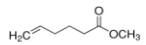
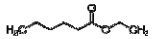
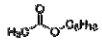
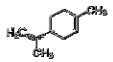
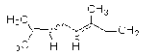
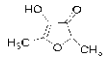
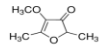
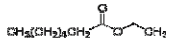
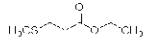
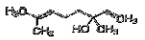
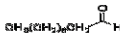
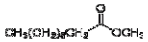
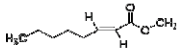
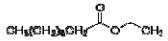
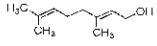
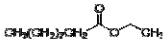
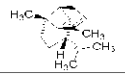
| ID [a] | aroma compounds | chemical structure | odor description | references [b] | linear structure | synonym |
|---|---|---|---|---|---|---|
| 12 | methyl 5 hexenoate |  | | 7,9 | $CH_2{:}CH(CH_2)_3COOCH_3$ | 5-hexenoic acid methyl ester |
| 13 | methyl hexanoate |  | pineapple, ether-like | 3,4,5,6,9,14 | $CH_3(CH_2)_4COOCH_3$ | caproic acid methyl ester, methyl caproate |
| 14 | ethyl hexanoate |  | fruity, apple, banana, fruity | 3,4,5,6,8,9,10 | $CH_3(CH_2)_4COOC_2H_5$ | caproic acid ethyl ester, ethyl caproate |
| 15 | hexyl acetate |  | grassy, fruity, appricot | 4,11,14 | $CH_3COO(CH_2)_5CH_3$ | capryl acetate |
| 16 | methyl 3-(methylthio) propanoate |  | penetrating, onion | 1,3,4,5,9 | $CH_3SCH_2CH_2COOCH_3$ | methyl 3-(methylmercapto)propionate |
| 17 | limonene |  | lemon, minty, orange | 3,4,6,9,12 | $C_{10}H_{16}$ | (+)-*p* -mentha-1,8-diene, (+)-carvene, (*R*)-4-isopropenyl-1-methyl-1-cyclohexene |
| 18 | (Z)-beta-ocimene |  | warm floral herb, sweet | 3,4 | | |
| 19 | 2,5-dimethyl-4-hydroxy-3(2H) furanone |  | fruity, sweet, sweet pineapple like, caramel like, caramelized pineapple | 1,3,4,5,6,8,9,10,13 | $C_6H_8O_3$ | 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, furaneol, strawberry furanone |
| 20 | 2,5-dimethyl-4-metoxy-3(2H) furanone |  | cherry, caramel like, fruity "sherry" like odor | 3,4,8,9 | $C_7H_{10}O_3$ | mesifuran |
| 21 | ethyl heptanoate |  | | 4,5, | $CH_3(CH_2)_5COOC_2H_5$ | ethyl enanthate |
| 22 | ethyl 3-(methylthio) propanoate |  | | 4 | $CH_3SCH_2CH_2CO_2C_2H_5$ | |

**Table 2.** *(Continued).*

| ID [a] | aroma compounds | chemical Structure | odor description | references [b] | linear structure | synonym |
|---|---|---|---|---|---|---|
| 23 | linalool |  | fruity, pineapple like | 4,12 | $(CH_3)_2C=CHCH_2CH_2C(CH_3)(OH)CH=CH_2$ | (±)-3,7-dimethyl-1,6-octadien-3-ol, (±)-3,7-dimethyl-3-hydroxy-1,6-octadiene |
| 24 | nonanal |  | piney | 3,4,9 | $CH_3(CH_2)_7CHO$ | aldehyde $C_9$, nonyl aldehyde, pelargonaldehyde |
| 25 | methyl octanoate |  | fruity, winy, orange | 3,4,5,6,9,10 | $CH_3(CH_2)_6COOCH_3$ | caprylic acid methyl ester, methyl caprylate |
| 26 | 4-ethyl phenol |  | | 9 | $C_2H_5C_6H_4OH$ | |
| 27 | methyl (E) octenoate |  | | 4,5 | $CH_3(CH_2)_4CH=CHCO_2CH_3$ | methyl *trans*-2-octenoate |
| 28 | ethyl octanoate |  | | 2,3,4,5,9 | $CH_3(CH_2)_6COOC_2H_5$ | ethyl caprylate |
| IS | methyl salicylate [c] |  | | | $2\text{-}(HO)C_6H_4CO_2CH_3$ | 2-hydroxybenzoic acid methyl ester, methyl 2-hydroxybenzoate, oil of wintergreen, Wintergreen oil |
| 29 | geraniol |  | floral, lemon, minty | 4,7 | $(CH_3)_2C=CHCH_2CH_2C(CH_3)=CHCH_2OH$ | *trans*-3,7-dimethyl-2,6-octadien-1-ol |
| 30 | 4-ethyl-2-methoxy-phenol |  | | | $C_2H_5C_6H_3\text{-}2\text{-}(OCH_3)OH$ | 4-ethylguaiacol |
| 31 | ethyl decanoate |  | | 4,5,9 | $CH_3(CH_2)_8COOC_2H_5$ | capric acid ethyl ester, ethyl caprate |
| 32 | alpha copaene |  | fruity, herbal | 3,5 | $C_{15}H_{24}$ | |

(a): identification number; (b): internal standard; (c): previously reported for one or several cultivars in fresh or processed pineapple (reference numbers as follow): 1. Badilla-Porras 2005; 2. Berger and others 1983; 3. Brat and others 2004; 4. Elss and others 2005; 5. Flath 1980; 6. Sinuco and others 2004; 7. Takeoka and others 1991; 8. Tokitomo and others 2005; 9. Umano and other 1992; 10. Wu and others 1991; 11. Ong and others 1998; 12. Qiao and others 2008; 13. Leffingwell 2009; 14. Nielsen and others 2008.

Trays were sealed using a vacuum sealer (ILPRA Foodpack Basic V/G, Ilpra, Vigenovo, Italy) and kept at 5 °C for up to 25 d. Trays (50 - 100g fresh-cut pineapple) from each packaging condition were randomly selected at each sampling date for headspace gas composition analysis, volatiles content and odor activity, SSC, TA, and pH, flesh color, juiciness, juice leakage, vitamin C, total phenolic content, antioxidant capacity, polyphenol oxidase and peroxidase enzymatic activity, mechanical properties and microbial analysis, as described for each particular study. At least two trays were used for each parameter evaluation.

## 5. PACKAGE HEADSPACE ANALYSIS

The headspace atmosphere composition of trays with pineapple pieces was analyzed using a gas chromatograph equipped with a thermal conductivity detector (Micro-GP CP 2002 gas analyzer, Chrompack International, Middelburg, The Netherlands) as described by Rojas-Graü et al. (2008). A 1.7mL aliquot was withdrawn through an adhesive septum stuck to the film cover, with a sampling needle directly connected to the injection module. The determination of the $O_2$ concentration was carried out by injecting a sample of 0.25µL to the a CP-Molsieve 5Å packed column (4m×0.32mm, d.f. = 10mm) at 60 °C and 100 kPa whereas a portion of 0.33µL was injected into a pora-PLOT Q column (10m×0.32mm, d.f. = 10mm) held at 75 °C and 200 kPa for $CO_2$, ethylene (C2H4), acetaldehyde ($C_2H_4O$) and ethanol ($C_2H_5OH$) determinations. Two trays from each packaging condition were randomly selected for gas analysis at each sampling date, during the 20 d storage time.

## 6. QUALITY EVALUATION

Fresh-cut pineapple characteristics were measured throughout storage. Titratable acidity, pH, soluble solids content (%), pulp color, juice leakage, juiciness, enzymatic activity and volatile aroma content and activity, texture, and microbiological stability were measured. Evaluation parameters varied among studies, according to their particular objectives.

### 6.1 Color measurement

Fresh-cut pineapple color was measured directly with a Minolta CR-400 chroma meter (Konica Minolta Sensing, Inc. Osaka, Japan), using the CIE scale *L*a*b**. The equipment was set up for illuminant $D_{65}$ and 10° observer angle and calibrated using a standard white reflector plate.

Three readings were obtained for each replicate by changing the position of the pineapple piece to get representative color measurements. Sixteen replicates were evaluated per each packaging condition. Color changes in *L** and *b** throughout storage at 5 °C were analyzed for studies 3 and 4.

Since no browning symptoms were observed in pineapple pieces during storage, but changes in tissue translucency were frequently observed, a side test was run with the aim to induce translucent appearance of fresh-cut pineapple pieces, and find out its relationship with changes in color parameters *L** and *b**. Translucent effect on color measurement was done by measuring the color of 50 fresh-cut pineapple pieces. Fruit pieces were submerged and held under water with the aid of an inverted funnel sealed with a septum in its thinner end. Air trapped within the funnel was removed with a syringe. A vacuum pressure for 2 min was applied to the whole system to remove internal gases from the fruit pieces using a laboratory vacuum pump. Then, vacuum was released, fresh-cut pineapple pieces were drained to remove excess water, and color was measured again, and compared with that before vacuum treatment.

## 6.2 Total soluble solids content, titratable acidity, pH, and SSC/TA

Fresh-cut fruit pieces (50 – 100 g) were homogenized using an Ultra Turrax T25 (IKA® WERKE, Germany) and filtered (Whatman paper no. 1). Soluble solids content was determined using an Atago RX- 1000 refractometer (Atago Company Ltd, Japan), pH was directly measured using a pH meter Crison 2001 (Crison Instruments S.A., Barcelona, Spain) and 10–15 g of filtered pulp were titrated with 0.1N NaOH to pH 8.1. Titratable acidity was expressed as grams of anhydrous citric acid in 100 g of fruit fresh weight. All measurements were carried out according to AOAC procedures (Horwitz, 2000). SSC/TA ratio was also calculated.

## 6.3 Water content

Water content of fresh-cut pineapple was measured following AOAC standard. Six 25 g samples of fresh cut pineapple were oven dried at 60 °C for 24 hours to constant weight for water content determinations.

## 6.4 Juice leakage

Juice leakage from pineapple pieces was measured according to the method of Marrero and Kader (2006) with some modifications. Juice leakage was assayed by tilting the packages at a 20° angle for 5 min and recovering accumulated liquid with a

5mL syringe. Results were reported as liquid volume recovered per 100 g of fresh-cut fruit in the package.

## 6.5 Juiciness

Juiciness was determined by a modification of the method used by González-Aguilar *et al.* [9]. Pineapple pieces (7 ± 1 g, 1.2 cm thick) were placed between two filter papers (Whatman No. 1, 10 cm diameter), compressed at 0.5 mm/s up to 25% strain and hold for 10 s before releasing. Initial and final weight were registered and reported as weight loss per 100 g of pineapple flesh.

## 6.6 Peroxidase and polyphenol oxidase activity

Peroxidase activity was determined according to the method used by Rojas-Graü, *et al.* [12]. Pulp tissue (50g) was homogenized in 50 mL of 0.2 M sodium phosphate buffer (pH 6.5) using an Ultra Turrax T25. The homogenate was centrifuged at 12500 rpm for 15 min (4 °C). Supernatant liquid was filtered (Whatman paper No 1), stored at 4 °C in darkness and used for the POD assay. Enzyme activity was determined adding 100 µL of this extract to a mix of 2.7 mL of sodium phosphate buffer (0.05 M, pH 6.5), 100 µL hydrogen peroxide (1.5%) and 200 µl of p-phenylendiamine (1%). The changes in absorbance were immediately measured at 446 nm at 10 sec intervals for 2 minutes at 25 °C using a Cecil CE 1010 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). The enzyme activity was expressed as the change in absorbance through time per volume of enzymatic extract (UA). The initial reaction rate was estimated from the linear portion of the plotted curve. Each determination was run in triplicate.

Polyphenol oxidase activity was determined according to the method used by Rojas-Graü, *et al.* (2008). A portion of 50 g of pineapple pieces was mixed with a McIlvaine buffer solution (1:1) at pH = 6.5 containing 1 M NaCl (Riedel-de-Haën AG, Seelze, Germany) and 5% polyvinylpolypyrrolidone (Sigma-Aldrich Chemie, Steinheim, Germany). The mixture was blended and homogenized using an Ultra Turrax T25 (IKAs WERKE, Germany). The homogenate was centrifuged at 12 500 rpm for 30 min at 4 °C (Centrifuge AVANTITM J-25, Beckman Instruments Inc., Fullerton, CA, USA). The supernatant was collected and filtered through Whatman number 1 paper, and the resulting solution constituted the enzymatic extract, which was used for enzyme activity determination. Enzyme activity was assayed spectrophotometrically by adding 3 mL of 0.05 M catechol (Sigma-Aldrich, Steinheim, Germany) and 75 µL of extract to a 4.5 mL quartz cuvette of 1 cm pathlength. The changes in absorbance at 400 nm were recorded every 5 s up to 3 min from the time the enzyme extract was

added using the spectrophotometer described above. All determinations were performed in triplicate.

## 6.7 Mechanical properties

Fresh-cut pineapple response to unidirectional compression, penetration, shear and extrusion forces was assessed at room temperature using a TA-TX2 Texture Analyzer (Stable Micro Systems LTD. Surrey, England) using a 5 kg load cell. Six resistance tests were used to find out which one better discriminate texture differences among pineapple pieces, three of them measured resistance to uniaxial single type forces (compression, penetration or shear) and to combined forces (compression, extrusion, and/or shear): a) *Uniaxial compression test*: 50 mm diameter aluminum cylindrical probe (P/50), test speed 0.5 mm/s, final target 25% strain, plus 10 s holding time at maximum strain; b) *Penetration test*: 2 mm diameter stainless cylindrical probe (P/2), test speed 5 mm/s, target 10 mm distance; c) *Shear test*: 7 cm length knife edge with slotted insert (HDP/BS), test speed 0.5 mm/s, target 15 mm distance. Cuts oriented along fiber direction; d) *Kramer shear press test*: mini-Kramer 5-bladed head shear cell (HDP/MKO5), test speed 3 mm/s, target 75% strain; e) *Ottawa press test*: mini-Ottawa cell (HDP/MKO5), test speed 3 mm/s, target 75% strain. f) *Texture Profile Analysis (TPA)*: 50 mm diameter cylindrical aluminum probe (P/50), test speed 5 mm/s, target 75% strain, 10 s holding time between two compression cycles.

Pineapple flesh wedges 1.2 cm thick, similar in shape and size, from the lower, middle and upper thirds of the fruit, were used for each of the texture assessment methods (16 repetitions per fruit section). Force-displacement-time data were registered and analyzed using Texture Exponent 32 software (Stable Micro Systems LTD. Surrey, England). Fracturability (N), hardness (N), probe displacement (mm), and areas under the curve (work, N mm) for each of these peaks were tabulated for compression, penetration and shear tests; maximum force and work of combined extrusion, shear and compression were reported for Ottawa and Kramer test, and for Texture Profile Analysis (TPA), fracturability (N), hardness (N), (first major failure and maximum peak load, respectively, N), probe displacement (mm) and areas under the curve (defined as work, N mm) for each of these peaks were tabulated for compression, penetration and shear tests; hardness (N) and work of combined extrusion, shear and compression were reported for Ottawa and Kramer test. For the Texture Profile Analysis (TPA), fracturability and hardness (measured during the first compression cycle, N), adhesiveness (negative area for the first compression cycle, N s), cohesiveness (ratio of positive force area during the second compression to that

during the first compression cycle, dimensionless), gumminess (hardness * cohesiveness, N), springiness (height recovered by the sample during the time elapsing between the end of the first compression cycle and the start of the second cycle), and resilience (ratio of the area for the first decompression stroke to the that of the first down stroke, dimensionless) were reported.

For all methods, force was applied along a single axis, main differences were the probes and type of force applied (single or combined): compression, penetration and shear forces were applied for the test with the same names, compression, shear and extrusion forces were combined for the Kramer test, and compression and extrusion for the Ottawa test. No similar results were expected between the tests, but differences were expected because contact area and type of the probe. The use of probes with larger flat surface area than the fruit pieces (compression, Kramer and Ottawa tests and TPA) were expected to give better results than those with the penetration and shear probes, since applied force was applied over the whole area of the sample, while penetration and shear test probes were expected to give larger variability, since force was applied with the aim to penetrate or cut, in single spot or line, and thus, it tissue morphology could be affected by fiber direction, type of tissues, etc.

Texture analysis of fresh-cut pineapple throughout storage was evaluated by running a Texture Profile Analysis along 20 d of storage at 5 °C in study 3. Fruit specimens were compressed twice to 50% of their original height (10 s interval) simulating mastication. A TA-TX2 Texture Analyzer (Stable Micro Systems LTD. Surrey, England) was used at room temperature and the following conditions were set according to the instrument manufacturer recommendations: $2 mms^{-1}$ pretest speed, $5.0 mms^{-1}$ test speed, $5.0 mms^{-1}$ post-test speed and 50% strain. A 50mm diameter cylindrical probe (P/50) was used to assure fresh-cut fruit surface area was completely covered by the probe. Force–distance–time data were registered for two cycle TPA test and texture parameters hardness (peak force during the first compression cycle, N/100 g), fragility or fracture force (peak of first fracture, $N/100\ g^{-1}$), adhesiveness (work required to overcome the attractive forces between the food and other surface, $Ns/100\ g^{-1}$), cohesiveness (ratio of positive force area during the second compression cycle to that during the first compression cycle, dimensionless), resilience (sample recover from deformation, dimensionless) and gumminess (hardness × cohesiveness, $N/100\ g^{-1}$) were calculated from force, distance and time data, using Texture Exponent 32 software (Stable Micro Systems LTD. Surrey, England). Force and energy results were calculated with respect to fresh-cut pineapple weight, to avoid the

effect of size and weight differences among fruit pieces on the results. Two trays were taken at each sampling time to perform the analysis, and no less than eight pineapple pieces were used for each packaging condition on each evaluation day.

## 6.8 Antioxidant characteristics

Pineapple vitamin C content, total phenolic compounds content, and antioxidant capacity were measured on duplicated samples. Vitamin C extraction procedure was based on the method validated by Odriozola-Serrano and others (2007). A portion of 25 g of fruit was added to a 25 mL of a 4.5% metaphosforic acid solution with 0.72% of DL-1,4-dithiothreitol (DTT) as reducing agent. The mixture was crushed, homogenized and centrifuged at 22,100g for 15 min at 4°C. The supernatant was vacuum-filtered through Whatman No.1 filter paper. The samples were then passed through a Millipore 0.45 µm membrane and injected into the HPLC system. Samples were introduced onto the column through a manual injector equipped with a sample loop (20 µL). Separation of ascorbic acid was performed using a reverse-phase C18 Spherisorb® ODS2 (5µm) stainless steel column (4.6 mm x 250 mm). The mobile phase was a 0.01% solution of sulfuric acid adjusted to 2.6 pH. The flow rate was fixed at 1.0 mL/min. Detection was performed with a 486 absorbance detector (Waters, Milford, MA) set at 245 nm. Identification of ascorbic acid was carried out by HPLC comparing the retention time with those of the standard solutions (up to 600 mg/kg). Results were expressed as mg of vitamin C in 100 g of pineapple flesh.

Total phenolic content were determined by the colorimetric method described by Singleton and others (1999) using the Folin-Ciocalteu reagent. Fresh-cut pineapple samples were homogenized using an Ultra Turrax T25. The homogenate was centrifuged at 6000 g for 15 min at 4 °C (Centrifuge Medigifer: Select, Barcelona, Spain) and filtered through a Whatman No 1 filter paper. Then, 0.5 mL of the extract was mixed with 0.5 mL of Folin-Ciocalteu reagent, 10 mL of saturated $Na_2CO_3$ solution and distilled water to complete 25 mL. Samples were allowed to stand for 1 h at room temperature before the absorbance at 725 nm was measured. Total phenolic content was determined by comparing the absorbance of duplicated samples with that of gallic acid standard solutions. Results were expressed as milligrams of gallic acid per 100 grams of pineapple flesh.

The antioxidant capacity of pineapple flesh was determined using the method described by Odriozola-Serrano and others (2007), by measuring the free radical-scavenging effect on DPPH (2,2-diphenyl-1-picrylhidrazyl) radical. Duplicated samples were homogenized using an Ultra Turrax T25. The homogenate was centrifuged at

6000g for 15 min at 4 °C (centrifuge Medigifer: Select, Barcelona, Spain); 0.01 mL aliquots of the supernatant were mixed with 3.9 mL of methanolic DPPH solution (0.025 g/L) and 0.090 mL of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. Absorption at 515 nm was measured on a spectrophotometer (CECIL CE 201; Cecil Instruments Ltd. Cambridge, UK) against a methanol blank. Results were expressed as percentage decrease with respect to the initial value.

## 6.9 Volatile compounds analysis

Volatile component of fresh-cut pineapple were extracted by headspace solid-phase micro-extraction (SPME) using a polydimethylsiloxane (PDMS) fiber with a 100 µm thickness coating from Supelco Co. (Park Bellefonte, PA, USA), followed by gas chromatography/mass spectrometry similar to that described by Lamikanra and Richard (2004). Duplicate samples of 50 g of pineapple flesh of the top, middle and bottom third of the fruit were used for volatiles determination in study 2, while two trays with 100 g fresh-cut pineapple were used in study 4 for each packaging condition and evaluation day.

Fruit pieces from each tray were homogenized using an Ultra Turrax T25; two 4 g samples of each homogenate were placed into 20 ml clear glass vials. Methyl salicylate (cas number 119-36-8) in water solution was added as internal standard (500 µg/kg). Vials were sealed and stirred for 15 min at 30 °C to achieve partition equilibrium of the analytes between the sample and the headspace; then the SPME fiber was inserted through a PTFE-faced butyl septum of cap into the headspace of the vial and hold for 15 min (sampling time) while stirring was continued.

Adsorbed substances were desorbed by inserting the PDMS fiber into the gas chromatograph-mass spectrophotometer (GC-MS) injection port at 250 °C. The desorbed compounds were separated using an Agilent 6890 N gas GC interfaced to a 5973 mass selective detector (Agilent Technologies España, S.L., Las Rozas, Spain) equipped with a Supelco Equity 5 capillary column of 30 m x 0.25 mm i.d. coated with 0.25 µm thick poly (5% diphenyl/95% dimethylsiloxane) phase (Supelco, Park Bellefonte, PA, USA). Extraction temperature (30 °C) was chosen with the aim to reproduce natural occurring aroma profile of fresh pineapple.

The GC was operated in a splitless mode using helium as the carrier gas at a constant rate of 1.5 mL/min. The oven temperature was programmed with an initial temperature of 40 °C, ramped to 250 °C at 20 °C/min rate and held for 10 min at the

final temperature. Mass spectra were obtained by electron ionization (EI) at 70 eV, and spectra range from 40 to 450 m/z.

The SPME fiber was preconditioned at 200 °C for 15 min before each use, and blank runs were done to check the absence of residual compounds on the fiber, which might bias the results.

Identification of volatile compounds in pineapple was performed by comparison of mass spectral and chromatographic retention data of target compounds with that of authentic reference substances. Thirty two authentic reference compounds were used to identify and quantify volatile components in fresh-cut pineapple (Table 1 and 2). Aqueous solutions with known concentration of reference volatiles and the internal standard (methyl salicylate) were analyzed a using headspace solid-phase microextraction with a 100 µm PDMS coating fiber, followed by GC-MS analysis using identical conditions to those used for pineapple samples.

Quantification was done by the calculation of average relative response factors (RRF) for each volatile compound, using the chromatographic data of prepared water solutions of reference substances with respect to the internal standard (methyl salicylate), as follows:

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Aroma profile was defined by the volatiles detected in fresh-cut pineapple under extraction and analysis conditions. Most abundant volatile components were selected as those with the largest concentration. Volatile contribution was reported as Odor activity values (OAV), calculated as the ratio of actual volatile concentration to its odor threshold concentration in water, given by Leffingwell 2009, Takeoka and others 2008, Tokitomo and others 2005. Volatiles concentrations throughout storage were determined along the fruit in study 2 and for all packaging conditions in study 4

## 6.10 Microbiological analysis

Changes in the microbial population of fresh-cut pineapple was studied by mesophilic and psychrophilic aerobic counts, and yeast and mould counts were carried out during the 20 d of storage, as described by Rojas-Graü et al. (2008).

Mesophilic and psychrophilic bacteria counts were made according to the ISO 4833:1991 guideline using Plate Count Agar (PCA) (Biokar Diagnostics, Beauvais, France) and the pour plate method. The plates of psychrophilic bacteria were incubated at 5 °C for 10–14 days, whereas mesophilic bacteria were incubated at 35°C for 48 h. Yeast and mould counts were made according to the ISO 7954:1987 guideline using Chloramphenicol Glucose Agar (CGA) (Biokar Diagnostics, Beauvais, France) and the spread plate method. The plates were incubated at 25 °C for 2–5 d. Analyses were carried out in randomly sampled pairs of trays, with two replicate counts per tray.

STUDIES

# MECHANICAL AND CHEMICAL PROPERTIES OF GOLD CULTIVAR PINEAPPLE FLESH (*Ananas comosus*)

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## ABSTRACT

Pineapple flesh cut from three cross-sections along the central axis were used to determine mechanical response to compression, penetration, shear and extrusion forces, color and related enzymes activity, antioxidant properties and other quality attributes and how they vary along the central axis of the fruit in order to determine the key factors to define Gold cultivar pineapple quality requirements for fresh-cut processing. Hardness, fracturability and associated work did not significantly vary among fruit pieces from different sections of the fruit, except for shear hardness (from 6.5 ± 1.2 to 10.0 ± 3.5 N) and related work (from 19 ± 6 to 41 ± 24 N mm). Color parameters, L*and b*, increased from the lower to the upper third, while a* and POD activity (6.70 ± 0.15 to 6.02 ± 0.11 /min/ mL) significantly decreased while PPO activity was not detected. Vitamin C and total phenol content to acidity ratio were lower in the upper third of the fruit (305 ± 40 mg/100 $g_{fw}$ and 40.3 ± 1.0 mg $_{gallic\ acid}$/100 $g_{fw}$, respectively), contrary to titratable acidity (0.45 ± 0.05 to 0.70 ± 0.05 g/100 $g_{fw}$) and water content (81.2 ± 0.8 to 85.7 ± 1.4 %). POD activity, water content, total phenolic compounds and the ratio soluble solids to acidity were the four parameters which allowed better discrimination between Gold cultivar pineapple flesh-cut from the three cross-sections along the central axis of the fruit and showed the highest correlation coefficients between each pair of parameters.

# 1. INTRODUCTION

Pineapple quality attributes are very well appreciated all around the world. They combine good flavor, aroma, juiciness, sweetness and texture together with high nutritional content, as it is a good source of vitamin C, fiber and minerals [1-2]. It is a large fruit composed of multiple fruitlets fused together and arranged in 8 spirals around a central axis. Its flesh is complex and anisotropic, composed of different types of tissues and cavities, with an upwards ripening pattern moving from the basal part of the fruit to the top [3-4]. On the other hand, fresh-cut pineapples have a good potential as a value-added product, for which homogeneity is a key attribute, and thus, it is important to determine raw fruits characteristics and their variability throughout the fruit for proper selection, processing and quality assessment. Such information is not available for pineapple flesh cross-sections along the central axis though some authors have reported average values for some whole fruit attributes.

Texture, color, antioxidant and other biochemical properties are important for fruit acceptability [5], being the first two very much associated with product appearance. Texture depends on geometrical, surface and mechanical attributes of the sample, tissue composition and turgidity, and the structure response to physical stresses. It is perceived by a combination of tactile, visual and hearing senses and its determination is complex and influenced by assessment methods, instruments and operation conditions. [6]. Objective methods, developed for engineering materials, have been widely used to describe and compare mechanical attributes of fresh-cut fruits and vegetables [7], even though these products are composed by cells arranged in different patterns, with different composition, internal structure and degree of homogeneity, susceptible to changes during handling These determinations involve single-point measurements with different types of probes, and the application of unidirectional compression, penetration, shear forces and extrusion forces, or a combination of them, and their utility will depend on how each methodology could discriminate among different fruit pieces. Average response to compression, penetration and shear forces have been measured for pineapple slices by Eduardo *et al.* [8] González-Aguilar *et al.* [9] and Hernández et al. [10] who had reported their results as hardness or maximum force, while texture profile analysis fracturability, hardness, adhesiveness, gumminess and cohesiveness have been determined for pineapple pieces through 5 °C storage by Montero-Calderón *et al.* [11]. However, there are no studies on how flesh texture attributes vary along the fruit.

Color, antioxidant characteristics and other quality properties have been also reported as average values for the whole fruit or not mention has been made of the specific sections from which samples have been cut. The same is true for vitamin C, although Paull and Chen [1] suggested that vitamin C can be larger near the surface of the fruit than close to its core.

The objective of this study was to determine mechanical properties, color and related enzymes activity, antioxidant properties and other quality attributes of pineapple flesh and how they vary among three cross-sections along the central axis of the fruit and how they vary along the central axis of the fruit in order to determine the key factors to define Gold cultivar pineapple quality requirements for fresh-cut processing.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Twenty Gold cultivar pineapples (*Ananas comosus* L. Merrill) imported from Costa Rica were bought at a local supermarket in Lleida, Spain (approximately 15 to 20 days after harvesting, 7-10 °C during transport). Free from defects pineapples, with uniform stage of maturity determined by external color were used. Shell had several to most of their eyes partially filled with yellow color, all of them surrounded by green. Fruits were stored at 11 $\pm$ 1 °C overnight prior to processing.

### 2.2. Sample preparation

Working area, cutting boards, knives, containers and other utensils and surfaces in contact with the fruit during processing were washed and sanitized with 200 μL/L sodium hypochlorite solution at pH 7 to have a maximum sanitizing effect prior to processing. Pineapple crown leaves were removed and the fruit was washed twice in two 200 μL/L sodium hypochlorite solutions for 5 minutes, excess water was drained for 3 to 5 min after each dip. Pineapple fruits were peeled, cross-cut into three sections along the central core of the fruit and separated into different groups labeled as lower, middle and upper third (Figure 1). Then they were sliced (1.2 cm thick) using sharp knives, cored and cut into wedges (6 to 7 g each). Fresh-cut pineapple pieces were washed in 20 μL/L sodium hypochlorite solutions for 2 minutes, drained and packed in 500 mL trays and stored at 5 °C for less than two hours before their analysis. For every cross-section of the fruit (lower, middle and upper thirds), two trays, each with eight fruit pieces were used for each of the

mechanical resistance measurements, antioxidant properties, enzyme activity, and other physicochemical attributes, as described ahead.

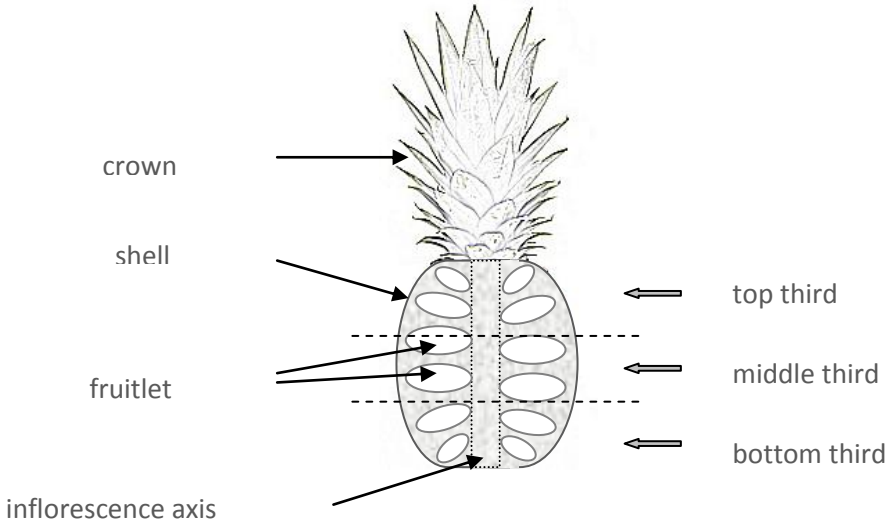


**Figure 1.** Schematic representation of pineapple morphology and three cross-sections of the fruit. Dotted lines represent cuts dividing upper, middle and lower sections.

## 2.3. Basic fruit composition characteristics

Juiciness was determined by a modification of the method used by González-Aguilar *et al.* [9]. Pineapple pieces (7 ± 1 g, 1.2 cm thick) were placed between two filter papers (Whatman No. 1, 10 cm diameter), compressed at 0.5 mm/s up to 25% strain and hold for 10 s before releasing. Initial and final weight were registered and reported as weight loss per 100 g of pineapple flesh. Titratable acidity, pH, soluble solids content (%) were determined from duplicate 50 g samples of fresh-cut fruit, homogenized using an Ultra Turrax T25 (IKA® WERKE, Germany) and filtered (Whatman paper No 1). Soluble solids content was determined using an Atago RX-1000 refractometer (Atago Company Ltd, Japan), pH was directly measured using a pHmeter Crison 2001 (Crison Instruments S.A., Barcelona, Spain) and flesh acidity was assessed by titration with 0.1 N NaOH to pH 8.1, and it was expressed as grams of anhydrous citric acid per 100 g of fruit fresh weight. All measurements were carried out according to AOAC procedures. SSC/TA ratio was calculated for all measurements as another quality parameter.

Water content of fresh-cut pineapple was measured following AOAC standard. Six 25 g samples of fresh cut pineapple were oven dried at 60 °C for 24 hours to constant weight for water content determinations.

## 2.4. Mechanical properties

Fresh-cut pineapple response to unidirectional compression, penetration, shear and extrusion forces was assessed at room temperature using a TA-TX2 Texture Analyzer (Stable Micro Systems LTD. Surrey, England) using a 5 kg load cell. Six resistance tests were used to find out which one better discriminate texture differences among pineapple pieces, three of them measured resistance to uniaxial single type forces (compression, penetration or shear) and to combined forces (compression, extrusion, and/or shear): a) *Uniaxial compression test*: 50 mm diameter aluminum cylindrical probe (P/50), test speed 0.5 mm/s, final target 25% strain, plus 10 s holding time at maximum strain; b) *Penetration test*: 2 mm diameter stainless cylindrical probe (P/2), test speed 5 mm/s, target 10 mm distance; c) *Shear test*: 7 cm length knife edge with slotted insert (HDP/BS), test speed 0.5 mm/s, target 15 mm distance. Cuts oriented along fiber direction; d) *Kramer shear press test*: mini-Kramer 5-bladed head shear cell (HDP/MKO5), test speed 3 mm/s, target 75% strain; e) *Ottawa press test*: mini-Ottawa cell (HDP/MKO5), test speed 3 mm/s, target 75% strain. f) *Texture Profile Analysis (TPA)*: 50 mm diameter cylindrical aluminum probe (P/50), test speed 5 mm/s, target 75% strain, 10 s holding time between two compression cycles.

Pineapple flesh wedges 1.2 cm thick, similar in shape and size, from the lower, middle and upper thirds of the fruit, were used for each of the texture assessment methods (16 repetitions per fruit section). Force-displacement-time data were registered and analyzed using Texture Exponent 32 software (Stable Micro Systems LTD. Surrey, England). Fracturability (N), hardness (N), probe displacement (mm), and areas under the curve (work, N mm) for each of these peaks were tabulated for compression, penetration and shear tests; maximum force and work of combined extrusion, shear and compression were reported for Ottawa and Kramer test, and for Texture Profile Analysis (TPA), fracturability (N), hardness (N), (first major failure and maximum peak load, respectively, N), probe displacement (mm) and areas under the curve (defined as work, N mm) for each of these peaks were tabulated for compression, penetration and shear tests; hardness (N) and work of combined extrusion, shear and compression were reported for Ottawa and Kramer test. For the Texture Profile Analysis (TPA), fracturability and hardness (measured during the first compression cycle, N), adhesiveness (negative area for the first compression cycle, N

s), cohesiveness (ratio of positive force area during the second compression to that during the first compression cycle, dimensionless), gumminess (hardness * cohesiveness, N), springiness (height recovered by the sample during the time elapsing between the end of the first compression cycle and the start of the second cycle, , and resilience (ratio of the area for the first decompression stroke to the that of the first down stroke, dimensionless) were reported.

For all methods, force was applied along a single axis, main differences were the probes and type of force applied (single or combined): compression, penetration and shear forces were applied for the test with the same names, compression, shear and extrusion forces were combined for the Kramer test, and compression and extrusion for the Ottawa test. No similar results were expected between the tests, but differences were expected because contact area and type of the probe. The use of probes with larger flat surface area than the fruit pieces (compression, Kramer and Ottawa tests and TPA) were expected to give better results than those with the penetration and shear probes, since applied force was applied over the whole area of the sample, while penetration and shear test probes were expected to give larger variability, since force was applied with the aim to penetrate or cut, in single spot or line, and thus, it tissue morphology could be affected by fiber direction, type of tissues, etc.

## 2.5. Color and related enzymes

Color was measured directly with a Minolta CR-400 chroma meter (Konica Minolta Sensing, INC. Osaka, Japan), using the CIE color space L*a*b*. The equipment was set up for illuminant $D_{65}$ and 10° observer angle and calibrated using a standard white reflector plate. Sixteen color readings were registered for each section of the fruit. Results were reported as L*, a*, b*.

Peroxidase activity was determined according to the method used by Rojas-Graü, *et al.* [12]. Pulp tissue (50g) was homogenized in 50 mL of 0.2 M sodium phosphate buffer (pH 6.5) using an Ultra Turrax T25. The homogenate was centrifuged at 12500 rpm for 15 min (4 °C). Supernatant liquid was filtered (Whatman paper No 1), stored at 4 °C in darkness and used for the POD assay. Enzyme activity was determined adding 100 µL of this extract to a mix of 2.7 mL of sodium phosphate buffer (0.05 M, pH 6.5), 100 µL hydrogen peroxide (1.5%) and 200 µl of p-phenylendiamine (1%). The changes in absorbance were immediately measured at 446 nm at 10 sec intervals for 2 minutes at 25 °C using a Cecil CE 1010 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). The enzyme activity was expressed as the change in absorbance

through time per volume of enzymatic extract (UA). The initial reaction rate was estimated from the linear portion of the plotted curve. Each determination was run in triplicate.

Polyphenol oxidase activity was determined according to the method used by Rojas-Graü, *et al.* (2008). A portion of 50 g of pineapple pieces was mixed with a McIlvaine buffer solution (1:1) at pH = 6.5 containing 1 M NaCl (Riedel-de-Haën AG, Seelze, Germany) and 5% polyvinylpolypyrrolidone (Sigma-Aldrich Chemie, Steinheim, Germany). The mixture was blended and homogenized using an Ultra Turrax T25 (IKAs WERKE, Germany). The homogenate was centrifuged at 12 500 rpm for 30 min at 4 °C (Centrifuge AVANTITM J-25, Beckman Instruments Inc., Fullerton, CA, USA). The supernatant was collected and filtered through Whatman number 1 paper, and the resulting solution constituted the enzymatic extract, which was used for enzyme activity determination. Enzyme activity was assayed spectrophotometrically by adding 3 mL of 0.05 M catechol (Sigma-Aldrich, Steinheim, Germany) and 75 μL of extract to a 4.5 mL quartz cuvette of 1 cm pathlength. The changes in absorbance at 400 nm were recorded every 5 s up to 3 min from the time the enzyme extract was added using the spectrophotometer described above. All determinations were performed in triplicate.

## 2.6. Antioxidant characteristics

Pineapple vitamin C, total phenolic compounds content and antioxidant capacity were measured on duplicated samples. Vitamin C extraction procedure was based on the method proposed by Odriozola-Serrano *et al.* [13]. A portion of 25 g of fruit was added to a 25 mL of a 4.5% metaphosforic acid solution with 0.72% of DL-1,4-dithiothreitol (DTT) as reducing agent. The mixture was crushed, homogenized and centrifuged at 22100g for 15 min at 4 °C. The supernatant was vacuum-filtered through Whatman No.1 filter paper. The samples were then passed through a Millipore 0.45 μm membrane and injected into the HPLC system. Samples were introduced onto the column through a manual injector equipped with a sample loop (20 μL). Separation of ascorbic acid was performed using a reverse-phase C18 Spherisorb® ODS2 (5μm) stainless steel column (4.6 mm x 250 mm). The mobile phase was a 0.01% solution of sulfuric acid adjusted to 2.6 pH. The flow rate was fixed at 1.0 mL/min. Detection was performed with a 486 absorbance detector (Waters, Milford, MA) set at 245 nm. Identification of ascorbic acid was carried out by HPLC comparing the retention time with those of the ascorbic acid standard. Results were expressed as mg of vitamin C in 100 g of pineapple flesh.

Total phenol content were determined by the colorimetric method of Singleton *et al.* [14] using the Folin-Ciocalteu reagent. Fresh-cut pineapple samples were homogenized using an Ultra Turrax T25. The homogenate was centrifuged at 6000 g for 15 min at 4 °C (Centrifuge Medigifer: Select, Barcelona, Spain) and filtered through a Whatman No 1 filter paper. Then, 0.5 mL of the extract was mixed with 0.5 mL of Folin-Ciocalteu reagent, 10 mL of saturated $Na_2CO_3$ solution and distilled water to complete 25 mL. Samples were allowed to stand for 1 h at room temperature before the absorbance at 725 nm was measured. Total phenol content was determined by comparing the absorbance of duplicated samples with that of the standards. Results were expressed as milligrams of gallic acid per 100 grams of pineapple flesh.

The antioxidant capacity of pineapple flesh was determined using the method described by Odriozola-Serrano *et al.* [13], by measuring the free radical-scavenging effect on DPPH (2,2-diphenyl-1-picrylhidrazyl) radical. Duplicated samples were homogenized using an Ultra Turrax T25. The homogenate was centrifuged at 6000g for 15 min at 4 °C (centrifuge Medigifer: Select, Barcelona, Spain); 0.01 mL aliquots of the supernatant were mixed with 3.9 mL of methanolic DPPH solution (0.025 g/L) and 0.090 mL of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. Absorption at 515 nm was measured on a spectrophotometer (CECIL CE 201; Cecil Instruments Ltd. Cambridge, UK) against a methanol blank (with DPPH). Results were expressed as percentage decrease with respect to the initial value.

### 2.7. Statistical analysis

A completely random design was used for three cross sections of pineapple along the fruit: lower, middle and upper thirds. Statistical analyses were performed using Statgraphics Plus version 5.1. (Statistical Graphics Co., Rockville, MD, USA). Analysis of variance (ANOVA) was performed to compare quality attributes in different parts of the fruit and Duncan test to compare means at a 5% significance level. Correlations between pineapple flesh quality parameters were also evaluated.

## 3. RESULTS AND DISCUSSION

### 3.1. Basic fruit composition characteristics

All physicochemical parameters, except pH, varied throughout the pineapple sections (Table 6) as a result of fruit anisotropy and morphology. SSC and SSC/TA

were significantly larger near the lower of the fruit, while titratable acidity and flesh moisture content were sensible bigger in the upper third, because of the ripening pattern from the lower to the top of the fruit; such differences could affect flavor perception and acceptability an should be considered for product processing and quality assessment of pineapple processed foods. Gold cultivar flesh SSC was similar to those reported for Perola, Red Spanish, and Josepine cultivars, larger than those for Smooth cayenne pineapples and smaller than those for Flhoran41 cultivar [2, 11, 15, 16, 17, 18, 19]. Water content also showed significant differences ($p<0.05$) along the fruit, from 81.2 ± 0.8 in the lower third to 85.7 ± 1.4% in the upper third. On the other hand, juiciness, measured as released fluids during compression tests, was significantly larger in the middle third of pineapple (12.1 ± 1.2 g/100g). Differences were attributed to fruit morphology since fruitlets size, shape and orientation vary along the fruit [3] because of the shell restrain to growth; fruitlets in the middle third of the fruit are generally larger and their internal structure could vary and favor juice leakage, as explained by Harker *et al.* [20], who correlated released fluids from fruit tissues with cell size, structure, arrangement and failure mechanism. Our results suggested that fluids inside pineapple tissue are easily released as the sample is compressed, even though pineapple flesh structure can withstand larger forces without size or shape changes for up to 20 days at 5 °C, with considerable fluids loss [11].

## 3.2 Mechanical properties

***Uniaxial compression, penetration and shear resistance.***

Table 1 show mechanical properties obtained from uniaxial compression, penetration and shear tests for pineapple flesh samples. In general, force-displacement curves showed a steady increase in force up to a point when the tissue suddenly fractured, followed by multiple peaks due to subsequent pineapple tissues failures, as the probe advanced. Hardness was always larger than fracturability, and both of them were larger for compression test as compared with penetration and shear test results.

For uniaxial compression test, flesh samples from different sections of the fruit were deformed up to 25% strain without reaching neither fracture point (fracturability) nor maximum resistance force (hardness); however, juice leakage was observed throughout each test. Such results suggested small and continuous failures probably occurred causing losses in membrane integrity together with an increase in membrane permeability as flesh tissues were pressed down, these could had leaded to formation of microscopic channels, increased cell interspaces and larger water

diffusion rates, as previously reported by Paull and Chen [1] for water-soaked tissues observed on translucent pineapple flesh.

**Table 1.** Mechanical properties of pineapple flesh from different sections of the fruit for compression, penetration and shear tests.

| Force type and cross-section | Fracturability (N) | Fracture work (N mm) | Hardness (N) | Hardness work (N mm) |
|---|---|---|---|---|
| **Compression** | | | | |
| Bottom | nd | nd | 25.2 ± 7.8 | 35 ± 16 |
| Middle | nd | nd | 26.7 ± 5.7 | 41 ± 15 |
| Top | nd | nd | 30.5 ± 9.6 | 42 ± 14 |
| **Penetration** | | | | |
| Bottom | 4.0 ± 1.1 | 5.5 ± 2.7 | 5.2 ± 1.3 | 20 ± 10 |
| Middle | 3.0 ± 0.7 | 4.1 ± 1.9 | 4.5 ± 0.8 | 20 ± 7 |
| Top | 3.8 ± 1.0 | 6.4 ± 3.1 | 4.9 ± 1.2 | 24 ± 8 |
| **Shear** | | | | |
| Bottom | 8.7 ± 4.0 | 16.9 ± 11.6 | 10.0 ± 3.5 $^{b}$ | 41 ± 24 $^{b}$ |
| Middle | 6.0 ± 1.5 | 10.4 ± 4.1 | 8.5 ± 1.2 $^{ab}$ | 19 ± 6 $^{a}$ |
| Top | 6.2 ± 2.1 | 11.4 ± 4.4 | 6.9 ± 2.2 $^{a}$ | 24 ± 18 $^{ab}$ |

nd: not detected. Data shown are mean values ± standard deviation. Different letters for sets of three values within a column indicate statistically significant differences (Duncan, $p<0.05$). Letters not included when non significant differences exist.

Damages became larger as the probes advanced, disrupting cell walls and other structural support of flesh tissue. Maximum force obtained during compression test underestimate actual value, since instrument was set for deformations up to 25% strain, but it was considered as flesh hardness for comparison purposes. No significant differences ($p<0.05$) were found for neither pineapple flesh hardness (for 25% strain) throughout the fruit (from 25.2 ± 7.8 to 30.5 ± 9.6 N) nor for compression work (from 35 ± 16 to 42 ± 14 N mm); however, a small increasing trend was found from the lower to the upper thirds of the fruit. High variability among fruit pieces from the same third of the fruit was large for all mechanical properties of the fruit and overlapped differences along pineapple fruit. The 50 mm diameter probe was used for uniform force distribution on flesh sample surface as well as average

response of the whole sample, but variability of results was as high as those obtained with other probes.

For penetration test, fracturability, hardness and work for each of them with the 2 mm diameter probe, did not show significant differences among different thirds of the fruit. Fracturability ranged from 3.0 ± 0.7 to 4.0 ± 1.1 N and hardness from 4.5 ± 0.8 to 5.2 ± 1.3 N. They were up to 6 times smaller than those obtained by uniaxial compression test, because of contact area differences between the probes (2 and 50 mm diameter) and the flesh samples. Hardness high variability and little changes through time were reported by Hajare *et al.* [21] for fresh and gamma irradiated pineapple slices stored at 8 °C and Gil *et al.* [22] and Eduardo *et al.* [8] for Tropical Gold and Smooth cayenne cultivars using probes from 3 to 13.5 mm diameter, while Chonhenchob *et al.* [23] found some changes during 10 °C storage for Phuket cultivar fresh-cut pineapple.

A slight increase in shear fracturability (8.7 ± 4.0 N) and hardness (10.0 ± 3.5 N) of pineapple flesh from the lower third of the fruit was apparent ($p<0.05$), though no significant differences were found between the flesh from the middle and upper sections of the pineapple. This was attributed to ripening stage difference on pineapple fruitlets along the fruit; since as the fruitlets ripen, tissue elasticity could increase due to compositional changes, and thus fracture and maximum force resistance of flesh samples also increase.

Results show a steady gain in force and work ($p<0.05$) as flesh samples were compressed, penetrated or cut until the maximum force was reached (Table 2), significant differences were found as the probe got into the fruit pieces, but not among the three cross sections of pineapple, except for 3 mm or less penetration and compression depths, but were not useful to discriminate flesh samples from each section of the fruit.

### *Combined forces resistance*

Combined resistance force and work required for compression, shear, and extrusion forces determined by the Kramer test (Table 3) resulted in hardness values similar to those obtained for the compression test, while total required work was much larger, explained by the extra work needed to force pineapple pieces to pass through the mini Kramer cell. Similar results were obtained for combined compression and extrusion forces in Ottawa test, where total work was even higher due to larger contact area with the product and the use of a flat plate probe as compared with that with knife edges of mini-Kramer cell.

**Table 2.** Force and work of compression, penetration and shear for pineapple flesh from three cross-sections of the fruit, as a function of probe movement.

| Test | Distance (mm) | Force (N) | | | Work (N mm) | | |
|---|---|---|---|---|---|---|---|
| | | Bottom | Middle | Top | Bottom | Middle | Top |
| Uniaxial compression | 2 | 4.4 ± 2.4 $^{aA}$ | 6.6 ± 2.8 $^{aAB}$ | 8.8 ± 3.4 $^{aB}$ | 1.8 ± 1.2 $^{aA}$ | 3.2 ± 1.6 $^{aAB}$ | 4.7 ± 2.1 $^{aB}$ |
| | 3 | 14.4 ± 6.3 $^{b}$ | 17.0 ± 6.3 $^{b}$ | 20.8 ± 6.6 $^{b}$ | 10.9 ± 5.5 $^{bA}$ | 14.9 ± 6.2 $^{bAB}$ | 19.3 ± 7.0 $^{bB}$ |
| | 4 | 22.3 ± 8.4 $^{c}$ | 24.1 ± 5.8 $^{c}$ | 21.9 ± 6.2 $^{b}$ | 27.7 ± 14.6 $^{c}$ | 33.1 ± 12.8 $^{c}$ | 21.7 ± 8.0 $^{b}$ |
| Penetration | 2 | 2.9 ± 1.1 $^{a}$ | 1.9 ± 0.6 $^{a}$ | 2.5 ± 0.8 $^{a}$ | 2.6 ± 1.1 $^{aB}$ | 1.7 ± 0.6 $^{aA}$ | 2.0 ± 0.7 $^{aAB}$ |
| | 3 | 3.7 ± 1.2 $^{ab}$ | 2.6 ± 0.5 $^{b}$ | 3.4 ± 0.9 $^{bc}$ | 5.8 ± 2.0 $^{bB}$ | 4.0 ± 1.1 $^{bA}$ | 5.0 ± 1.5 $^{bAB}$ |
| | 4 | 3.8 ± 1.1 $^{ab}$ | 3.2 ± 0.5 $^{bc}$ | 3.7 ± 1.0 $^{ab}$ | 9.6 ± 3.1 $^{c}$ | 7.0 ± 1.3 $^{c}$ | 8.7 ± 2.3 $^{c}$ |
| | 6 | 4.3 ± 1.3 $^{b}$ | 3.6 ± 0.8 $^{c}$ | 4.2 ± 0.8 $^{bc}$ | 17.3 ± 4.9 $^{d}$ | 13.8 ± 1.6 $^{d}$ | 16.3 ± 3.5 $^{d}$ |
| | 8 | 4.3 ± 1.5 $^{b}$ | 3.8 ± 0.8 $^{c}$ | 4.7 ± 1.3 $^{c}$ | 26.3 ± 7.0 $^{e}$ | 21.3 ± 2.2 $^{e}$ | 24.9 ± 5.0 $^{e}$ |
| | 10 | 3.2 ± 0.9 $^{a}$ | 3.5 ± 0.8 $^{c}$ | 3.5 ± 0.8 $^{ab}$ | 34.0 ± 8.0 $^{f}$ | 28.9 ± 3.1 $^{f}$ | 33.4 ± 6.1 $^{f}$ |
| Shear | 2 | 2.9 ± 1.0 $^{a}$ | 2.7 ± 1.5 $^{a}$ | 2.5 ± 1.5 $^{a}$ | 2.3 ± 0.9 $^{a}$ | 2.1 ± 1.6 $^{a}$ | 2,0 ± 1,5 $^{a}$ |
| | 3 | 4.9 ± 1.6 $^{ab}$ | 4.4 ± 1.7 $^{b}$ | 4.5 ± 2.0 $^{bc}$ | 6.3 ± 2.1 $^{a}$ | 5.,7 ± 3.2 $^{ab}$ | 5,6 ± 3,3 $^{ab}$ |
| | 4 | 6.8 ± 2.6 $^{bc}$ | 5.5 ± 1.2 $^{b}$ | 5.8 ± 2.3 $^{d}$ | 12.1 ± 3.6 $^{a}$ | 10.6 ± 4.6 $^{b}$ | 10.8 ± 5.4 $^{b}$ |
| | 6 | 7.7 ± 3.7 $^{c}$ | 5.3 ± 1.0 $^{b}$ | 6.0 ± 2.2 $^{d}$ | 28.0 ± 8.9 $^{b}$ | 22.1 ± 5.8 $^{c}$ | 22.5 ± 9.2 $^{c}$ |
| | 8 | 6.5 ± 3.3 $^{bc}$ | 4.2 ± 1.8 $^{b}$ | 5.3 ± 2.3 $^{cd}$ | 42.2 ± 13.9 $^{c}$ | 32.6 ± 7.8 $^{d}$ | 33.8 ± 13.2 $^{d}$ |
| | 10 | 6.1 ± 3.5 $^{bc}$ | 4.5 ± 2.0 $^{b}$ | 4.7 ± 2.6 $^{bc}$ | 54.7 ± 18.6 $^{d}$ | 41.6 ± 10.1$^{e}$ | 44.0 ± 17.9 $^{e}$ |
| | 12 | 5.6 ± 3.4 $^{bc}$ | 4.3 ± 2.2 $^{b}$ | 4.0 ± 2.5 $^{b}$ | 66.6 ± 22.5 $^{f}$ | 50.4 ± 15.2$^{f}$ | 52.9 ± 22.7 $^{f}$ |

Data shown are mean ± standard deviation. Different letters indicate statistically significant differences (Duncan, $p<0.05$); upper case letters compare among lower, middle and upper thirds of the fruit; lower case letter compare among deformation, penetration and shear depths. Letters not included when non significant differences exist.

Total work determined with Ottawa test was significantly bigger for flesh samples from the middle third of the fruit. Explanation of such differences is difficult because of the simultaneous use of different types of forces as well as pineapple structure heterogeneity. Hardness and work variability among flesh samples overlapped differences along the fruit when Ottawa and Kramer tests were applied, as it was the case for penetration, compression and shear force tests; these results suggested little influence of maturity stage differences along the pineapple fruit on the flesh response to mechanical forces. In addition, even though López-Malo and Palou [24] did not differentiate fruit flesh samples location inside pineapple, they found large

variability (up to 30% standard deviations) among fresh and blanched pineapple slices hardness.

**Table 3.** Mechanical properties of pineapple flesh from different sections of the fruit using Kramer and Ottawa tests using a TA-TX2 texture analyzer.

| Test name | Type of forces | Cross-section along the central axis | Hardness (N) | Total work [1] (N mm) |
|---|---|---|---|---|
| **Kramer** | shear | Bottom | 27.1 ± 5.7 | 133 ± 21 |
| | compression | Middle | 29.0 ± 6.2 | 153 ± 52 |
| | extrusion | Top | 24.5 ± 3.1 | 117 ± 29 |
| **Ottawa** | compression | Bottom | 37.2 ± 6.8 | 220 ± 43 [a] |
| | extrusion | Middle | 41.5 ± 8.8 | 273 ± 48 [b] |
| | | Top | 33.6 ± 5.7 | 213 ± 33 [a] |

1: total work for 75% strain of fruit flesh sample. Data shown are mean values ± standard deviation. Different letters for sets of three values within a column indicate statistically significant differences (Duncan, $p<0.05$). Letters not included when non significant differences exist.

### *Texture profile analysis (TPA)*

TPA results are shown in Table 4. Pineapple flesh fracturability and hardness slightly increased from the lower to the upper third of the fruit, but differences were not significant ($p>0.05$). Flesh hardness range from 76 to 79 N and doubled those of fracturability, which ranged from 30 to 34 N. Adhesiveness, springiness, cohesiveness, gumminess and resilience did not significantly vary among the lower, middle and upper thirds of the fruit. Low adhesiveness was found, ranging from -0.4 to -0.5 N, while pineapple flesh samples also showed poor elastic behavior, as resilience (0.045-0.046) and springiness (0.28-0.35) results were low. Low cohesiveness value results (0.11-0.12) indicated much less work had to be done during the second bite as compared with the first. TPA parameters also showed large variability because of product heterogeneity. Such results agree with those found in our previous work with Gold cultivar fresh-cut pineapple [11], in which packaging conditions and storage time at 5 °C did not significantly affect TPA texture parameters in pineapple flesh, and with those reported by Kingsly *et al.* [25] who use the same procedure to evaluate high-pressure effect on pineapple slices texture attributes during processing, and did not find significant differences for hardness,

cohesiveness and springiness. Pineapple apparent texture (visual and tactile) was maintained for over 20 d at 5 °C without noticeable changes by Montero-Calderón *et al.* [11]. Our results showed that TPA test is not useful to discriminate among pieces cut from the lower, middle and upper thirds of the fruit.

**Table 4.** Texture profile analysis parameters for pineapple flesh from different sections of the fruit.

| | Position inside the fruit | | |
|---|---|---|---|
| **Test** | **Bottom third** | **Middle third** | **Top third** |
| **Fracturability** (N) | 30 ± 8 | 33 ± 11 | 34 ± 10 |
| **Hardness** (N) | 76 ± 21 | 79 ± 22 | 79 ± 19 |
| **Adhesiveness** (N s) | -0.5 ± 0.1 | -0.4 ± 0.2 | -0.4 ± 0.1 |
| **Springiness** (dimensionless) | 0.35 ± 0.03$^{b}$ | 0.28 ± 0.05$^{a}$ | 0.33 ± 0.09$^{b}$ |
| **Cohesiveness** (dimensionless) | 0.12 ± 0.02 | 0.11 ± 0.02 | 0.12 ± 0.01 |
| **Gumminess** (N) | 9.5 ± 4.0 | 8.7 ± 3.5 | 8.8 ± 2.9 |
| **Resilience** (dimensionless) | 0.045 ± 0.008 | 0.046 ± 0.007 | 0.045 ± 0.008 |

Data shown are mean values ± standard deviation. Different letters within the same line indicate statistically significant differences (Duncan, $p<0.05$). Letters not included when non significant differences exist.

Even though texture has been recognized as a very important quality parameter for many fruits, no significant differences ($p>0.05$) were found among fruit pieces along the pineapple with any of the six measuring procedures, explained by intrinsic large variability overlapping possible differences.

Table 5 shows correlation coefficients between mechanical properties of pineapple flesh. Shear hardness and fracturability showed a high correlation (0.96) indicating maximum resistance force was directly related to first irreversible damage force, but that correlation was low for penetration test results and not significant for other measurements approaches. No significant correlations were found between mechanical properties obtained by different methods, since different probe geometries, operations conditions, and force types were used. On the other hand, TPA parameters were significantly correlated, though some correlations were low, suggesting non linear correlations. The largest correlations were found between fracturability and hardness (0.80) or gumminess (0.87).

**Table 5.** Correlation coefficients among pineapple mechanical properties of Gold cultivar pineapple flesh

| | Shear hardness | Shear fracturability | Compression hardness | Penetration hardness | Penetration fracturability | Ottawa hardness | Kramer hardness | TPA hardness | TPA fracturability | TPA Adhesiveness | TPA Springiness | TPA Cohesiveness | TPA Gumminess | TPA Resilience |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Shear** hardness | 1 | | | | | | | | | | | | | |
| fracturability | 0,96 | 1 | | | | | | | | | | | | |
| **Compression** hardness | - | - | 1 | | | | | | | | | | | |
| **Penetration** hardness | 0,51 | 0,61 | - | 1 | | | | | | | | | | |
| fracturability | - | - | - | 0,59 | 1 | | | | | | | | | |
| **Ottawa** hardness | - | - | - | - | - | 1 | | | | | | | | |
| **Kramer** hardness | - | - | - | - | - | - | 1 | | | | | | | |
| **TPA** hardness | - | - | - | - | - | - | - | 1 | | | | | | |
| fracturability | - | - | - | - | - | - | - | 0,80 | 1 | | | | | |
| adhesiveness | - | - | - | - | - | - | - | 0,70 | 0,43 | 1 | | | | |
| springiness | - | - | - | - | - | - | - | 0,44 | 0,52 | 0,48 | 1 | | | |
| cohesiveness | - | - | - | - | - | - | - | 0,64 | - | 0,67 | 0,47 | 1 | | |
| gumminess | - | - | - | - | - | - | - | 0,69 | 0,87 | - | - | - | 1 | |
| resilience | - | - | - | - | - | - | - | 0,70 | - | 0,44 | - | 0,77 | - | 1 |

$p < 0.05$

## 3.3. Color and related enzymes

### *Color of fresh-cut pineapple*

Color values L*, a* and b* of fresh-cut pineapple pieces showed a slight but significant increase ($p \leq 0.05$) from the lower to the upper section. Value a* significantly varied along the fruit, from -4.8 ± 0.9 to -5.7 ± 0.4 in the lower and upper thirds, respectively. L* increased from 66.8 ± 4.4 to 70.5 ± 2.1, and b* from 46.0 ± 4.9 to 49.0 ± 2.1, from the lower to the upper third of the fruit (Table 6). Large variability among the color values was observed with no browning symptoms, as previously reported by Montero-Calderón *et al.* [11] and explained by fruit tissue heterogeneity,

translucency phenomenum, fruitlet maturation stage along the fruit and among individual pineapples. Range of color parameters found in this study agree with those reported by other authors [10, 11, 22, 26] though no references were done about section of the fruit from which pineapple flesh pieces were cut.

**Table 6.** Physicochemical characterization of Gold cultivar fresh-cut pineapple pieces cut from different sections of the fruit.

| | Position inside the fruit | | |
|---|---|---|---|
| **Physicochemical attribute** | **Bottom third** | **Middle third** | **Top third** |
| **Color parameters** | | | |
| **L*** | 66.8 ± 4.4 $^{a}$ | 68.7 ± 3.0 $^{b}$ | 70.5 ± 2.1 $^{b}$ |
| **a*** | -4.8 ± 0.9 $^{c}$ | -5.3 ± 0.6 $^{b}$ | -5.7 ± 0.4 $^{a}$ |
| **b*** | 46.0 ± 4.9 $^{a}$ | 46.7 ± 3.2 $^{a}$ | 49.0 ± 2.3 $^{b}$ |
| **POD activity, UA** (UA/min/ml) | 6.70 ± 0.15 $^{c}$ | 6.50 ± 0.25 $^{b}$ | 6.02 ± 0.11 $^{a}$ |
| **pH** | 3.49 ± 0.04 | 3.45 ± 0.03 | 3.45 ± 0.02 |
| **SSC** (%) | 12.7 ± 0.7 | 13.0 ± 2.2 | 12.6 ± 0.5 |
| **TA** ($g_{citric\ acid}$/100 $g_{fw}$) | 0.45 ± 0.05 $^{a}$ | 0.56 ± 0.12 $^{ab}$ | 0.70 ± 0.05 $^{b}$ |
| **SSC/TA** | 28.9 ± 4.0 $^{c}$ | 23.3 ± 1.6 $^{b}$ | 17.9 ± 0.7 $^{a}$ |
| **Water content** (%) | 81.2 ± 0.8 $^{a}$ | 82.4 ± 0.5 $^{b}$ | 85.7 ± 1.4 $^{c}$ |
| **Juiciness** (g/100 $g_{fw}$) | 10.4 ± 0.8 $^{a}$ | 12.1 ± 1.2 $^{b}$ | 10.9 ± 0.8 $^{a}$ |

SSC: soluble solids content; TA: titratable acidity; SSC/TA: soluble solid content to acidity ratio. Data shown are mean ± standard deviation. Different letters within the same line indicate statistically significant differences (Duncan, $p<0.05$). Letters not included for non significant differences. Letters not included when non significant differences exist.

### *Peroxidase and Polyphenol oxidase Activity*

Peroxidase activity (POD) results are shown in Table 7. It significantly decreased ($p<0.05$) from the lower of the fruit (6.70 ± 0.15 UA/min/mL), through the middle (6.50 ± 0.25 UA/min/mL) up to the top of the fruit (6.02 ± 0.11UA /min/mL). Differences were attributed to differences in maturity degree of the fruitlets along the pineapple. POD activity has been associated with flavor changes in raw fruits and vegetables (off-flavors and off-odors), discoloration, ripening and cell wall degradation, however, for Smooth cayenne pineapple, several authors [27, 28, 29] results showed no evidence of POD relation to such changes. Furthermore, Dahler *et al.* [30] and Zhou *et al.* [27] found constant POD activity on the same cultivar stored at 13 °C for 3-5 weeks and discarded its relation with browning reactions, while Avallone *et al*. [28] discarded its participation on enzymatic browning associated with

decay tissue caused by *Penicillium funicosum* Thom., as POD activity did not change through time in both healthy and decay tissue. Chitarra and da Silva [31] observed increased POD activity throughout storage on Smooth cayenne pineapples at higher temperature (20 °C). In previous work with Gold cultivar fresh-cut pineapple, we found POD activity throughout the 20 days storage at 5 °C, with no browning symptoms of fruit flesh [11]. Polyphenol oxidase (PPO)activity was not detected in fresh-cut pineapple prepared from Gold cultivar in any of the three sections of the fruit evaluated, which is a positive attribute for fresh-cut processing, and agrees with light color changes on fruit pieces, which did not include tissue browning nor darkening, generally associated to this enzyme. PPO activity has been reported for other cultivars [9, 30, and 31] which associated it with tissue darkening, but not for Gold cultivar.

**Table 7.** Antioxidant characterization of Gold cultivar fresh-cut pineapple pieces cut from different sections of the fruit.

| Antioxidant characteristic | Bottom third | Middle third | Top third |
|---|---|---|---|
| **Vitamin C** ($mg/kg_{fw}$) | $333 \pm 32^{ab}$ | $351 \pm 15^{b}$ | $305 \pm 40^{a}$ |
| **Total phenol content** ($mg_{gallic\ acid}/100g_{fw}$) | $50.8 \pm 5.1^{c}$ | $44.6 \pm 0.3^{b}$ | $40.3 \pm 1{,}0^{a}$ |
| **Antioxidant capacity** (%DPPH inhibition) | $43.1 \pm 2.7$ | $42.9 \pm 5.1$ | $45.6 \pm 5.6$ |

Data shown are mean ± standard deviation. Different letters indicate statistically significant differences (Duncan, $p<0.05$). Letters not included when non significant differences exist.

## 3.4. Antioxidant properties

### *Vitamin C*

Vitamin C ranged from 305 ± 40 to 351 ± 15 $mg/kg_{fw}$ with no statistical differences among the different parts of the fruit. Hajare *et al.* [21] and Miller and Schaal [32] reported differences up to 150% in ascorbic acid content between individual pineapple fruits, without making differences of the section of the fruit used for the experimental determinations. Average vitamin C content for Gold cultivar pineapple ranges from 310 to 790 mg/100 $g_{fw}$, compared with 260 to 350 $mg/kg_{fw}$ for Smooth cayenne cultivar [22, 26, 33]. In addition to cultivar effect, large variability on vitamin C content can be affected by multiple factors, like the clone, solar radiation, air temperature and acidity, and it could be negatively related to internal browning

symptoms [1]. Since pineapple is a good source of vitamin C, further studies should be made to maximize and preserve its content in pineapple flesh from the field to the consumer table, through the optimization of pre- and postharvest handling practices, processing, packaging and storage conditions.

***Total phenol content***

Total phenol content (TPC) significantly varied ($p \leq 0.05$) along the fruit, it decreased from 50.8 ± 5.1 $mg_{gallic\ acid}$ / 100 $g_{fw}$ in the lower third of the fruit where the fruitlets are more mature, to 44.6 ± 0.3 to 40.3 ± 1.0 $mg_{gallic\ acid}$ / 100 $g_{fw}$ in the middle and upper thirds of the pineapple, respectively (Table 7). These results are explained by Dahler *et al.* [30] who observed TPC increased as Smooth cayenne pineapple ripened (25 to 39 mg/100$g_{fw}$) and during storage at 10 °C (37 to 51 mg/100 $g_{fw}$). TPC has also been associated to physiological response to infections or injuries [34] and pre-harvest soil application of potassium [35] since TPC decreased as potassium application in the field increased and also as pineapple fruit ripens, thus, TPC content could vary among different fruit batches, growing area and agricultural practices.

***Antioxidant Capacity***

Antioxidant capacity of pineapple flesh through the DPPH radical scavenging method is shown in Table 7 for three cross sections along the fruit. Not significant differences ($p<0.05$) were found among the different parts of the fruit, but it was slightly higher on the upper third of the fruit, near the crown. Values ranged from 43.1 ± 2.7 and 42.0 ± 5.1 % of DPPH inhibition for the lower and middle thirds, respectively, up to 45.6 ± 5.6 % at the top. Leong and Shui [36], reported pineapple antioxidant capacity as 85.6 ± 21.3 mg/100g, for fruit bought at a local market in Singapore ( cultivar not reported) and classified it as a medium antioxidant capacity, with similar values to that of apple and lemon, and 2.5 times that of tomato.

Table 8 presents results of the correlation analysis among pineapple flesh quality parameters except mechanical attributes. Highly significant correlation coefficients ($p<0.05$) were found between pineapple flesh POD activity, total phenol content, water content and SSC/TA ratio, which were the only quality parameters which could clearly differentiate pineapple flesh from the three cross-sections of the fruit. Color parameter a* significantly varied along the fruit but only showed low but significant correlation coefficients with POD activity, antioxidant capacity measured as DPPH inhibition and SSC/TA ratio. High correlations with fruit flesh water content suggests internal quality attributes could be strongly influenced by climate conditions and water management pre-harvest practices. POD high correlation coefficients with total phenol, acidity and SSC/TA ratio (0.87 and above), agree with Chitarra and da

Silva [31] results, who reported an increase of phenol content with peroxidase activity throughout storage at 20 °C. Vitamin C correlation coefficients with total phenol content, titratable acidity and water content were also high (0.94, -0.88 and -0.88, respectively), and possibly affected by pre-harvest practices. L* and b* did not significantly correlate with any of the antioxidant, mechanical or physicochemical parameters, suggesting color appearance independence with flavor attributes, though no sensorial evaluation has been done. Juiciness correlation coefficients with other quality parameters were not significant.

**Table 8.** Correlation coefficients among pineapple flesh quality parameters.

| | L* | a* | b* | POD activity | Vitamin C | Total phenolic compounds | AOX capacity | TA | SSC/TA | Water content | Juiciness |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **L*** | 1 | | | | | | | | | | |
| **a*** | - | 1 | | | | | | | | | |
| **b*** | - | - | 1 | | | | | | | | |
| **POD activity** | - | 0,50 | - | 1 | | | | | | | |
| **Vitamin C** | - | - | - | 0,73 | 1 | | | | | | |
| **Total phenolic compounds** | - | - | - | 0,87 | 0,94 | 1 | | | | | |
| **AOX capacity** | - | -0,47 | - | -0,59 | -0,73 | -0,70 | 1 | | | | |
| **TA** | - | - | - | -0,91 | -0,88 | -0,99 | 0,64 | 1 | | | |
| **SSC/TA** | - | 0,40 | - | 0,91 | 0,70 | 0,89 | -0,50 | -0,95 | 1 | | |
| **Water content** | - | - | - | -0,88 | -0,88 | -0,98 | 0,62 | 0,99 | -0,94 | 1 | |
| **Juiciness** | - | - | - | - | - | - | - | - | - | - | 1 |

p< 0.05; TA: titratable acidity, SSC/TA: soluble solids content to acidity ratio; AOX capacity measured as % DPPH inhibition.

## 4. CONCLUSIONS

Mechanical properties variations among Gold cultivar pineapple flesh from the lower, middle and upper thirds of the fruit were overlapped by high variability among flesh samples for compression, penetration, Kramer, Ottawa and TPA procedures. Shear hardness and its related work were useful to partially differentiate pineapple flesh from the lower third from that from other parts of the fruit. Hardness assessed by compression, Kramer and Ottawa tests was larger than that obtained with the shear and penetration tests because of probe geometry, types of forces involved and operation parameter differences, but none of them permit to discriminate among fruit cross-sections. Vitamin C, juiciness, titratable acidity and color parameters L* and b* can only partially discriminate among pineapple sections flesh, while antioxidant capacity cannot.

SSC/TA ratio, total phenol content, POD activity, color parameter a* and water content were the only quality parameters that clearly allow discrimination between pineapple flesh pieces cut from the three cross-sections of the fruit and also showed a high correlation between each pair of parameters. While SSC/TA ratio, TPC, POD activity and parameter a* increased from the lower to the upper third of the fruit, the water content increased. Vitamin C larger content and bigger juiciness were found in the middle section of the fruit. SSC/TA ratio, water content and juiciness, vitamin C and TPC are the recommended as the key quality parameters to determine for product development, tolerance limits definition and quality control purposes of this Gold cultivar pineapple for fruits to be directly consumed or fresh-cut processed.

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# AROMA PROFILE AND VOLATILES ODOR ACTIVITY ALONG GOLD CULTIVAR PINEAPPLE FLESH

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## ABSTRACT

Physicochemical attributes, aroma profile and odor contribution of pineapple flesh were studied for the top, middle and bottom cross-sections cut along the central axis of Gold cultivar pineapple. Relationships between volatile and nonvolatile compounds were also studied. Aroma profile constituents were determined by headspace solid-phase microextraction at 30 °C, followed by gas chromatography-mass spectrometry analysis.

Twenty volatile compounds were identified and quantified. Among them, esters were the major components which accounted for 90% of total extracted aroma. Methyl butanoate, methyl 2-methyl butanoate and methyl hexanoate were the three most abundant components representing 74% of total volatiles in pineapple samples. Most odor active contributors were methyl and ethyl 2-methyl butanoate, and 2,5-dimethyl 4-methoxy 3(2H)-furanone (mesifuran).

Aroma profile components did not varied along the fruit, but volatile compounds content significantly varied ($p<0.05$) along the fruit, from 7560 to 10910 µg/kg, from the top to the bottom cross-sections of the fruit, respectively. In addition, most odor active volatiles concentration increased from the top to the bottom third of the fruit, concurrently with SSC and TA differences attributed to fruitlets distinct degree of ripening.

Large changes in SSC/TA ratio and volatiles content throughout the fruit found through this study are likely to provoke important differences among individual fresh-cut pineapple trays, compromising consumer perception and acceptance of the product. Such finding highlighted the need to include volatiles content and SSC/TA ratio and their variability along the fruit as selection criteria for pineapples to be processed and quality assessment of the fresh-cut fruit.

# 1. INTRODUCTION

Pineapple (*Ananas comosus* (L,) Merr.) is an exotic fruit very well appreciated by its aroma, juiciness and flavor (taste and aroma). There are many cultivars, with varied colors, shapes, sizes, odor and flavors. Among them, Gold cultivar (MD2) has stood out in the international markets because of its sensory characteristics, highlighting flavor, sweetness to acidity balance and juiciness.

Pineapple is a rather large fruit, composed of multiple fruitlets, with a progressive maturation pattern, starting from those located in the bottom section of the fruit, up to those in the top, near the crown. It is also a non-climacteric fruit, and consequently, its eating quality is determined at the time of harvest, with little variation thereafter, despite the fact that the fruit flesh quality attributes vary along the fruit (Montero-Calderón and others 2008, Zhou and others 2003). Moreover, Baldwin (2004) signaled that flavor quality of non-climacteric products may decline after harvest, but little has been reported on volatiles produced during fruit development.

Flavor refers to taste and odor perception. Sweetness, sourness and aroma are the main components of fruit flavor, given by the balance among sugars, acids and volatiles. In fact, the perception of sweetness can be modified by the acid content and aroma compounds (Baldwin 2004). Nowadays, flavor quality is being addressed as key elements for consumer acceptance, of both intact and fresh-cut fruits, as emphasized by Kader (2008) who pointed out the need to optimize the eating quality at the time of consumption, by maintaining optimal flavor and nutritional quality.

Pineapple aroma is the result of a complex mixture of volatiles. Over 400 compounds have been identified for several fresh and processed pineapple products (Elss and others 2005, Tokitomo and others 2005, Takeoka and others 1991, Brat and others 2004, Lamikanra and Richard 2004), but only some of them are odor active, and contribute to the overall aroma of the fruit. There is limited information about Gold cultivar pineapple aroma profile; how it varies along the fruit as well as what is the contribution of each volatile compound to the odor of the fruit.

Volatiles can be extracted by solvent liquid extraction, vacuum distillation, solid-phase microextraction (SPME) and other methods. Among these, SPME followed by GC-MS analysis have been widely used for fruits (Lamikanra and Richard 2004, Ong and other 1998, Azondalou and other 2003, Augusto and other 2000), because they allow volatiles extraction without solvents or heating, identification and analysis with

very little modification of the sample. Hence, aroma profile determined by this methodology, closely resemble the natural occurring aroma of the fruit.

The main objective of this study was to determine aroma profile and odor activity values of Gold cultivar pineapple flesh and how they are affected by position inside de fruit, with the aim to provide key elements to define tolerance and processing strategies for a better quality of fresh-cut pineapple.

# 2. MATERIALS AND METHODS

## 2.1. Materials

Gold cultivar pineapples (*Ananas comosus* L. Merrill) imported from Costa Rica, were bought at a local supermarket in Lleida, Spain and stored at 11 $\pm$ 1 °C overnight prior to processing. Fruits were free from mechanical injuries, insects, pathogens or other defects. Shell had several to most of their eyes partially filled with yellow color, all of them surrounded by green.

***Reference compounds***

Volatile compounds used as internal and external standards for fresh pineapple aroma analysis are included in Table 1. They were chosen from previous studies with pineapple products and preliminary assays. Regents were purchased from Sigma-Aldrich Química SA, Madrid, Spain.

## 2.2. Sample preparation

Working area, cutting boards, knives, containers and other utensils and surfaces in contact with the fruit during processing were washed and sanitized. Fruits were washed in 200 µL/L sodium hypochlorite solution (pH 7), shelled and cut into three cross-sections along the fruit marked as bottom, middle and top cross-sections being the last, the closest to the fruit crown. Ten samples (50g each) were taken from each section of the fruit for volatile compounds analysis. Samples were homogenized using an Ultra Turrax T25 and rapidly frozen at -18 °C.

**Table 1.** Volatile compounds used as internal and external standards for identification and quantification of pineapple aroma composition.

| ID [a] | Aroma compound | CAS[b] | RT (min) [c] |
|---|---|---|---|
| 1 | methyl 2-methyl propanoate | 547-63-7 | 2,804 |
| 2 | ethyl propanoate | 105-37-3 | 3,027 |
| 3 | methyl butanoate | 623-42-7 | 3,136 |
| 4 | ethyl 2-methyl propanoate | 97-62-1 | 3,506 |
| 5 | methyl 3-methyl butanoate | 556-24-1 | 3,686 |
| 6 | methyl 2-methyl butanoate | 868-57-5 | 3,707 |
| 7 | hexanal | 66-25-1 | 3,917 |
| 8 | butyl acetate | 123-86-4 | 4,047 |
| 9 | ethyl 2-methylbutanoate | 7452-79-1 | 4,450 |
| 10 | 3-methylbutyl acetate | 123-92-2 | 4,710 |
| 11 | 2-heptanone | 110-43-0 | 4,853 |
| 12 | methyl 5 hexenoate | 2396-80-7 | 5,060 |
| 13 | methyl hexanoate | 106-70-7 | 5,190 |
| 14 | ethyl hexanoate | 123-66-0 | 5,870 |
| 15 | hexyl acetate | 142-92-7 | 5,990 |
| 16 | methyl 3-(methylthio) propanoate | 13532-18-8 | 6,118 |
| 17 | limonene | 3338-55-4 | 6,190 |
| 18 | (Z)-beta-ocimene | 95327-98-3 | 6,220 |
| 19 | 2,5-dimethyl-4-hydroxy-3(2H) furanone | 3658-77-3 | 6,395 |
| 20 | 2,5-dimethyl 4 methoxy 3(2H) furanone | 4077-47-8 | 6,440 |
| 21 | ethyl heptanoate | 106-30-9 | 6,715 |
| 22 | ethyl 3-(methylthio) propanoate | 13327-56-5 | 6,770 |
| 23 | linalool | 78–70–6 | 6,758 |
| 24 | nonanal | 124-19-6 | 6,820 |
| 25 | methyl octanoate | 111-11-5 | 6,940 |
| 26 | 4-ethyl phenol | 123-07-09 | 7,278 |
| 27 | methyl (E) octenoate | 7367-81-9 | 7,310 |
| 28 | ethyl octanoate | 106-32-1 | 7,502 |
| IS[d] | methyl salicylate | 119-36-8 | 7,589 |
| 29 | geraniol | 106-24-1 | 7,954 |
| 30 | 4-ethyl-2-methoxy-phenol | 2785-89-9 | 8,185 |
| 31 | ethyl decanoate | 110-38-3 | 8,929 |
| 32 | alpha copaene | 3856-25-5 | 8,950 |

(a): volatile compounds identification number used in this study; (b): Chemical Abstracts Service registry number; (c): retention time; (d): internal standard

## 2.3. Fresh fruit characteristics

Physicochemical quality attributes were used to characterize the pineapple fruits used for volatile analysis.

### *2.3.1. Non volatile components of pineapple flavor*

TA, pH, SSC were determined from duplicate 50 g samples of fresh-cut fruit, homogenized using an Ultra Turrax T25 (IKA® WERKE, Germany) and filtered (Whatman paper No 1). SSC was determined using an Atago RX-1000 refractometer (Atago Company Ltd, Japan); pH was directly measured using a pH-meter Crison 2001 (Crison Instruments S.A., Barcelona, Spain) and TA was assessed by titration with 0.1 N NaOH to a pH end-point of 8.1, and its results were expressed as grams of anhydrous citric acid per 100 g of fruit fresh weight. All measurements were carried out according to AOAC procedures. SSC/TA ratio was calculated for all measurements.

### *2.3.2. GC-MS Aroma analysis*

Volatile component of fresh-cut pineapple were extracted by headspace solid-phase micro-extraction (SPME) using a polydimethylsiloxane (PDMS) fiber with a 100 µm thickness coating from Supelco Co. (Park Bellefonte, PA, USA), combined with gas chromatography/mass spectrometry (similar to Lamikanra and Richard 2004). Four grams of pineapple flesh homogenate were placed into 20 ml clear glass vials. Methyl salicylate (cas number 119-36-8) in water solution was added as internal standard (500 µg/kg). Vials were sealed and stirred for 15 min at 30 °C to achieve partition equilibrium of the analytes between the sample and the headspace; then the SPME fiber was inserted through a PTFE-faced butyl septum of cap into the headspace of the vial and exposed for 15 min (sampling time) while stirring was continued. Adsorbed substances were desorbed by inserting the PDMS fiber into the gas chromatograph-mass spectrophotometer (GC-MS) injection port at 250 °C. The desorbed compounds were separated using an Agilent 6890 N gas GC interfaced to a 5973 mass selective detector (Agilent Technologies España, S.L., Las Rozas, Spain) equipped with a Supelco Equity 5 capillary column of 30 m x 0.25 mm i.d. coated with 0.25 µm thick poly (5% diphenyl/95% dimethylsiloxane) phase (Supelco, Park Bellefonte, PA, USA). The GC was operated in a splitless mode using helium as the carrier gas at a constant rate of 1.5 mL/min. The oven temperature was programmed with an initial temperature of 40 °C, ramped to 250 °C at 20 °C/min rate and held for 10 min at the final temperature. Mass spectra were obtained by electron ionization (EI) at 70 eV, and spectra range from 40 to 450 m/z. Compounds were identified by

comparing collected mass spectra and chromatographic retention data of the fruit samples with that of external standard compounds described in section 2.1.

The SPME fiber was preconditioning at 200 °C for 15 min before each use, and blank runs were done to check the absence of residual compounds on the fiber, which might bias the results.

Identification of volatile compounds in pineapple was performed by comparison of mass spectral and chromatographic retention data of target compounds with that of authentic reference substances. Quantification was carried out using the relative response factors (RRF) calculated from GC-MS data of a water solution with known concentrations of the internal (methyl salicylate) and external standards run at the same chromatographic conditions given above.

Preliminar runs were done to verify fiber and chromatographic method suitability to achieve good resolution peaks for all volatile standards.

Most abundant volatile components were selected as those with the largest concentration. Volatile contribution was reported as Odor activity values (OAV). They were calculated as the ratio of actual volatile concentration to its odor threshold concentration in water (Leffingwell 2009, Takeoka and others 2008, Tokitomo and others 2005).

### 2.4. Statistical analysis

Significance of the results and statistical differences were analyzed using Statgraphics Plus version 5.1. (Statistical Graphics Co., Rockville, MD, USA). Analysis of variance (ANOVA) was performed to compare volatiles and physicochemical attributes of Gold cultivar flesh samples cut from different sections of the fruit. Duncan test was used to determine differences among means, with a level of significance of 0.05 and principal component analysis (PCA) was carried out to study correlations among variables.

## 3. RESULTS AND DISCUSSION

### 3.2. Nonvolatile components of pineapple flavor

SSC, TA, pH, and the ratio of SSC/TA for Gold cultivar pineapple flesh are shown in Table 2. Significant differences were found throughout the fruit for all nonvolatile

components ($p<0.05$). SSC, SSC/TA and pH increased from the top to the bottom cross-sections of the fruit, while the opposite was true for TA. These results are explained by fruit morphology and maturity differences along the fruit, since aggregated fruitlets of pineapple progressively ripen, starting from the fruitlets near the bottom end of the fruit and moving up to those near the crown until the fruit is harvested. From then on, the pineapple neither continues to ripen nor exhibits many changes, but still maintains a gap on the maturity stage and quality attributes along the fruit. Such differences can directly affect sweetness perception of the fruit, as it depends on the sugars content and acidity balance.

**Table 2.** Physicochemical parameters of Gold cultivar pineapple flesh cut from three cross-sections along the central axis of the fruit.

| Section | SSC | pH | TA | SSC/TA |
|---|---|---|---|---|
| Top | 11,4 ± 0,4 [a] | 3,41 ± 0,04 [a] | 0,79 ± 0,01 [c] | 14,3 ± 0,6 [a] |
| Middle | 13,0 ± 0,5 [b] | 3,49 ± 0,04 [b] | 0,66 ± 0,05 [b] | 19,6 ± 2,0 [b] |
| Bottom | 14,0 ± 0,3 [c] | 3,58 ± 0,04 [c] | 0,59 ± 0,04 [a] | 23,9 ± 1,7 [c] |

SSC: soluble solids content (%); pH: pulp pH; TA: titratable acidity (mg $_{\text{citric acid}}$/100 $_{\text{g fresh weight}}$).
Values with the same lower case letter are not significantly different (Duncan $p<0.05$)

From the stand point of the fresh-cut processing industry, such differences directly affect final product homogeneity and consistency, emphasizing the need to consider pineapple sugar to acid ratio (SSC/TA) variations along the fruit and among the production lot as a quality parameter to select the fruits to be used for processing.

## 3.3. Volatile components of pineapple

Naturally occurring volatiles in Gold cultivar pineapple were identified and quantified. Most abundant volatile components were defined as those with the greatest concentrations, whereas those with the highest OAV were addressed as the largest contributors to pineapple aroma.

### *Aroma profile and odor contribution*

The aroma profile for Gold cultivar pineapple flesh is shown in Table 3. A total of twenty volatile compounds were identified and quantified from pineapple flesh samples by headspace SPME. Fifteen of them were esters, accounting for roughly 90 % of total aroma content, but terpenes, alcohols and adehydes were also found.

Most abundant volatile compounds identified in pineapple flesh were methyl butanoate, methyl 2-methyl butanoate, and methyl hexanoate with concentrations above 1000 µg/kg, accounting for 74% of total volatiles in pineapple samples in the three cross sections of the fruit. They were followed by 2,5-dimethyl-4-methoxy-3(2H) furanone (DMMF), methyl 2-methyl propanoate, and methyl 3-(methylthio) propanoate, with concentrations above 500 µg/kg.

Pineapple aroma profile found at 30 °C was consistent along the fruit cross-sections, and with previous reports on volatile compounds found in pineapple (Akioka and Umano 2008, Elss and others 2005, Spanier and others 1998, Lamikanra and Richard 2004), although relative concentration varied, explained by differences in cultivars, growing conditions and volatiles extraction methods.

Such differences could also justify why ethyl hexanoate , ethyl 3-(methylthio) propanoate and DMHF (2,5 dimethyl-4-hydroxy-3(2H) furanone) were not detected on pineapple samples throughout this study, despite it has been reported as a key odorants in pineapple (Tokitomo and others 2005, Brat and others 2004, Umano and others 1992 and Elss and others 2005). In fact, Cadwallader (2005) pointed out that DMHF naturally appears in fruits as mesifuran, whereas Belitz and others (2004) explained that extraction yields of furanone compounds in a liquid matrix are poor because of their high solubility and easy decomposition. Furthermore, Lee and Nagy (1987) reported very small content of DMHF in pineapples grown in Costa Rica, as compared with those from Hawaii (cultivar not reported).

It should be highlighted that aroma profile found in this study corresponds to the natural occurring balance of volatiles in pineapple flesh, because of the direct and low temperature extraction method used. Although, some heavier and less volatile compounds could pass undetected.

The effect of flesh position inside the fruit on aroma profile of pineapple is shown in Table 3. Volatile constituents of aroma profile were the same for the three cross-sections of the fruit, but total volatile concentration varied from 7560 to 10910 µg/kg, from the top to the bottom third of the fruit, which roughly corresponds to an increase of 45%. Such results showed that more immature fruitlets in the top third of the fruit released less volatiles compounds than those with more advanced degree of maturity.

**Table 3.** Headspace concentration of volatile compounds in Gold cultivar pineapple cut from the top, middle and bottom cross-sections of the fruit.

| ID | Aroma compounds | Odor threshold[1] | Average concentration (µg/kg) | | |
|---|---|---|---|---|---|
| | | | Top section | Middle section | Bottom section |
| 1 | methyl 2-methyl propanoate | 6,3[iii] | 520 [a] | 571 [a] | 860 [b] |
| 2 | ethyl propanoate | 10[i] | t | t | t |
| 3 | methyl butanoate | 72[ii] | 2531 [a] | 2902 [ab] | 3597 [b] |
| 4 | ethyl 2-methyl propanoate | 0.02[i] | t | t | t |
| 5 | methyl 3-methyl butanoate | | t | t | t |
| 6 | methyl 2-methyl butanoate | 0.1[ii] | 1966 [a] | 2427 [a] | 3263 [b] |
| 7 | hexanal | 4.5[i] | t | t | t |
| 8 | butyl acetate | 66[i] | t | t | t |
| 9 | ethyl 2-methylbutanoate | 0.006[ii] | 23,5 [a] | 36,7 [b] | 49,4 [b] |
| 10 | 3-methylbutyl acetate | 2[i] | 6,6 [a] | 3,1 [a] | 5,2 [a] |
| 11 | 2-heptanone | | t | t | t |
| 12 | methyl 5 hexenoate | | 0,6 [a] | 1,2 [a] | 2,4 [a] |
| 13 | methyl hexanoate | 77[i] | 1083 [a] | 1204 [ab] | 1248 [b] |
| 14 | ethyl hexanoate | 1[i] | 52 [a] | 129 [b] | 357 [c] |
| 15 | hexyl acetate | | t | t | t |
| 16 | methyl 3-(methylthio) propanoate | 180[i] | 682 [b] | 623 [b] | 507 [a] |
| 17 | limonene | 10[i] | 3,1 [a] | 3,2 [a] | 3,9 [a] |
| 18 | (Z)-beta-ocimene | | 1,2 [a] | 2,6 [a] | 4,2 [a] |
| 19 | 2,5-dimethyl-4-hydroxy-3(2H) furanone | | t | t | t |
| 20 | 2,5-dimethyl-4-methoxy-3(2H) furanone | 0.03[i] | 619 [a] | 797 [ab] | 934 [b] |
| 21 | ethyl heptanoate | 2,2[i] | 0,9 [a] | 1,6 [a] | 1,7 [a] |
| 22 | ethyl 3-(methylthio) propanoate | 7[i] | 0,0 [a] | 9,7 [a] | 5,0 [a] |
| 23 | linalool | 6[i] | t | t | t |
| 24 | nonanal | 1[i] | 1,6 [ab] | 2,2 [b] | 0,5 [a] |
| 25 | methyl octanoate | 200[i] | 46,8 [a] | 49,6 [a] | 43,0 [a] |
| 26 | 4-ethyl phenol | | t | t | t |
| 27 | methyl (E) octenoate | | 0,9 [a] | 1,2 [ab] | 1,5 [b] |
| 28 | ethyl octanoate | | 0,7 [a] | 2,3 [a] | 1,9 [a] |
| IS [2] | methyl salicylate | | | | |
| 29 | geraniol | | t | t | t |
| 30 | 4-ethyl-2-methoxy-phenol | | t | t | t |
| 31 | ethyl decanoate | | 1,0 [a] | 1,5 [a] | 1,5 [a] |
| 32 | alpha copaene | | 19,8 [a] | 28,8 [a] | 25,5 [a] |
| | *Total volatiles content* (µg/kg) | | 7560 | 8796 | 10912 |

t: not detected in pineapple samples under selected analysis conditions; 1: Odor threshold concentration in water (µg/kg) from: i. Leffingwell 2009, ii. Takeoka and others 2008, iii. Tokitomo and others 2005; 2: IS internal standard. Means with the same lower case letter did not show significant differences ($p<0.05$).

Increase in volatile compounds concentration can be explained by changes occurring during ripening, which starts several weeks before harvest. It begins with an increase in sugars accumulation in conjunction with a depletion of the acid content, followed by volatiles production, as the activity of various enzymes and pathways switch. Volatile compounds become synthesized from free amino acids, carbohydrates and through β-oxidation of fatty acids (Cadwallader 2005, Beaulieu and Baldwin, 2002). Moreover, some of the important esters found for pineapple as most abundant components, have been reported as the product of the transformation of amino acids or fatty acids. Ethyl and methyl-2-methyl butanoate compounds are produced from isoleucine, while 2-methyl propanoate from valine, whereas butanoates and hexanoates are synthetized from free fatty acids. The other major compound found in this study for Gold cultivar flesh was mesifuran, produced from *D*-glucose or *D*-fructose.

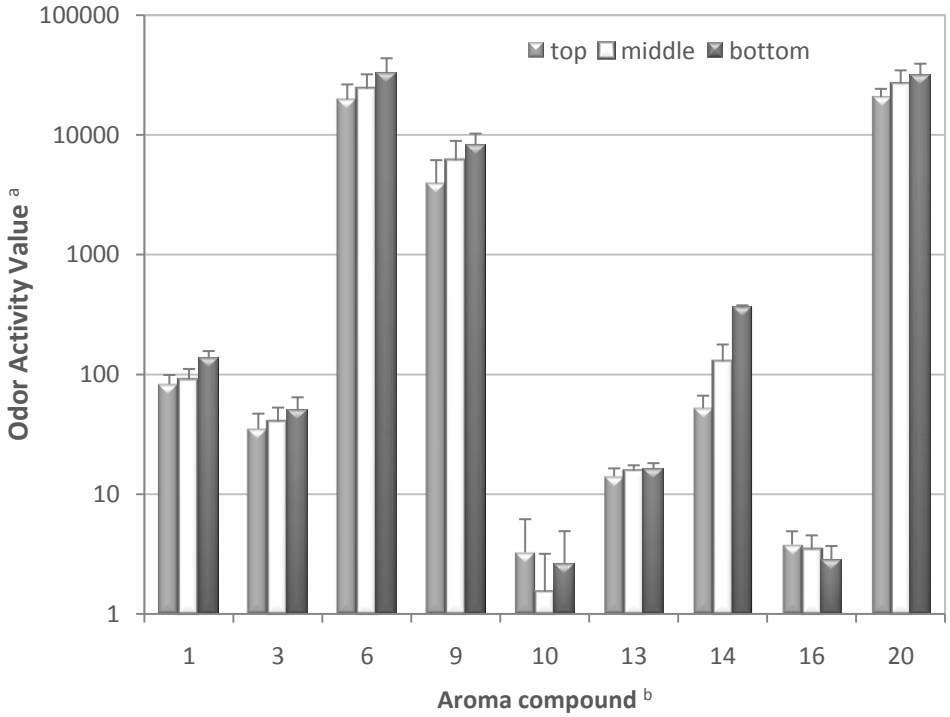
Volatiles changes associated to fruit ripening have been reported for Flhoran41, Smooth cayenne and other non declared pineapple cultivar (Brat and others 2004, Umano and others 1992). They found increased content of most volatiles as the fruit ripens, yet comparisons were made among average concentrations for the whole fruit, without discriminating between different sections of pineapple.

Our results also showed that the magnitude of the changes in volatiles content varied among compounds. Volatiles methyl 2-methyl propanoate, methyl butanoate, methyl 2-methyl butanoate, methyl hexanoate and mesifuran increased from 15 to 66% from the top to the bottom of the fruit, but the largest changes were observed for ethyl 2-methylbutanoate and ethyl hexanoate, which increased 110 and 585%, respectively. In contrast, methyl 3-(methylthio) propanoate concentration depleted 25% from the top to the bottom cross-sections of the fruit. Despite the gap among relative content of volatiles, the top, middle and bottom cross-sections of pineapple fruits contained the same volatiles constituents in its aroma profile; consequently, differences in pineapple aroma along individual fruits are essentially quantitative, rather than qualitative.

***Most odor active volatiles***

Besides concentration, volatile contribution to pineapple flavor and aroma is an important quality parameter, which is useful to identify individual impact of volatile compounds on the fruit flesh aroma perception. Odor activity values (OAV) of volatile compounds along Gold cultivar pineapple flesh are shown in Figure 1.

It was found that nine volatiles compounds had OAV higher than one (concentration levels above their detection limit), with enormous differences among them. By far, the two largest contributors to pineapple aroma in the fresh Gold cultivar were an ester, methyl 2-methyl butanoate and a furanone, mesifuran, which showed OAV´s between 19 to 32 thousand times their threshold concentrations along the fruit. They were followed by ethyl 2-methyl butanoate, ethyl hexanoate, methyl 2-methyl propanoate, methyl butanoate, methyl hexanoate, methyl 3-(methylthio) propanoate and 3-methylbutyl acetate. Among these volatiles, ethyl 2-methyl butanoate and mesifuran were also reported as impact volatiles for Flhoran41 cultivar (Brat and others 2004) and Super Sweet F2000 cultivar (Tokitomo and others 2005).




A: Only aroma compounds with OAV values larger than one were included in the graph.
b: aroma compounds numbers correspond to: 1) methyl 2-methyl propionate; 3) methylbutanoate; 6) methyl 2-methylbutanoate; 9) ethyl 2-methylbutanoate; 10) 3-methylbutyl acetate; 13) methyl hexanoate, 14) ethyl hexanoate; 16) methyl 3-(methylthio)propanoate; 20) 2,5-dimethyl-4-methoxy-3(2H) furanone.

**Figure 1.** Odor activity of most odor active volatiles in Gold cultivar pineapple flesh cut from three cross-sections along the fruit.

Due to differences in detection limits (threshold concentrations) of volatile compounds, some major contributors to pineapple aroma, like ethyl 2-methylbutanoate, had high OAV (3915 – 8232) but relatively low concentration (23.5 to 49.4 μg/kg); conversely, some of the most concentrated volatiles, such as methyl butanoate and methyl hexanoate, have lower OAV (Table 3, Figure 1).

Our results show that odor activity of volatiles compounds significantly varied ($p<0.05$) along the pineapple cross-sections. For most volatiles, OAV increased from the top third to the bottom third of the fruit, except methyl 3-(methylthio) propanoate, for which it decreased.

It was observed, that OAV differences along the fruit did not alter the order of importance of the three main contributors of aroma (methyl and ethyl 2-methyl butanoate, and mesifuran), with values of 3900 and beyond. In contrast, odor activity of methyl hexanoate surpassed that of ethyl 2-methyl propanoate, despite the fact that OAV´s of both compounds increased form the top to the bottom third of the fruit. Consequently, these results showed differentiated balance of volatiles activity for each section of the fruit, which might affect aroma perception of pineapple flesh.

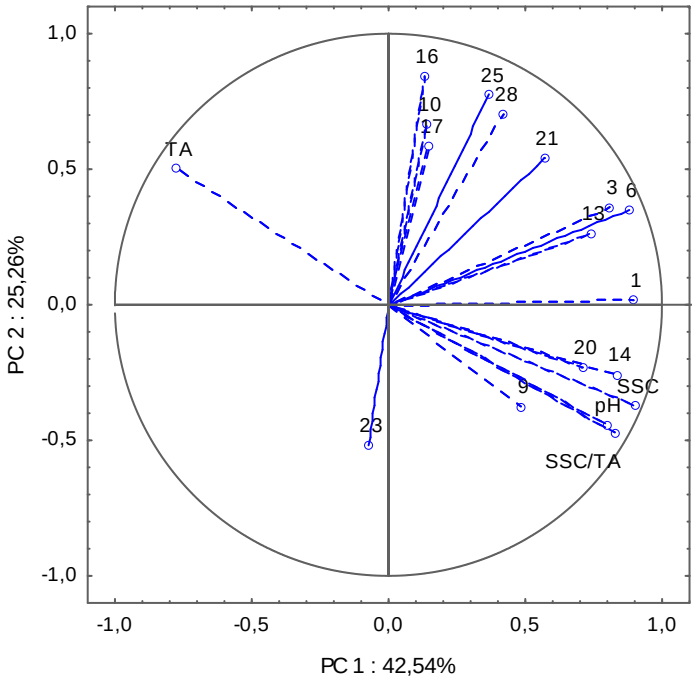
Two particularities should be addressed here, on one hand, OAV values were calculated with odor thresholds concentrations of individual compounds in water. They do not consider synergistic effect or masking behavior that volatiles could have in a complex matrix like pineapple flesh, neglecting any possible interaction among volatile compounds, which could enhance or minimize their individual contribution to pineapple aroma. On the other hand, there are not studies reported on how OAV values are related to odor contribution, once the threshold limit is surpassed, or whether a saturation limit for volatiles perception can be reached for specific compounds.

Furthermore, volatile variations along pineapple cross-sections point up the need to consider them for fresh-cut products and other processing industries, since those differences will affect finished product perception and uniformity.

Consequently, even though volatiles with the highest OAV are likely to have a marked effect on pineapple aroma, those with concentration above their limit of detection should also be considered for quality assessment of pineapple.

### *Principal component analysis*

A principal component analysis (PCA) was performed on all samples and variables (OAV, SSC, TA, pH, SSC/TA) to determine relationships among volatile compounds activity throughout pineapple flesh. Two principal components (PC1 and PC2) were obtained, accounting for 67.8% of the variability in the original data (Figure 2). Statistical results showed little relation among most of the odor active volatiles (methyl 2-methyl propanoate, methyl butanoate, methyl 2-methyl butanoate, 3-methylbutyl acetate, methyl hexanoate and methyl 3-(methylthio) propanoate) and non-volatile attributes of pineapple flesh. However, SSC, pH, mesifuran and ethyl hexanoate were directly related, whereas TA was negatively related to ethyl 2-methyl butanoate content and other non-volatile attributes.




SSC: soluble solids content, TA: titratable acidity, pH: pineapple flesh pH, SSC/TA: ratio of SSC to TA, Numbers correspond to volatile compounds ID, as described in Table 1.

**Figure 2.** Principal components plot with the projection of volatiles content and physicochemical attributes of Gold cultivar pineapple

The score plot of PC1 versus PC2 obtained from the full-data PCA model (Figure 3) clearly discriminate among pineapple flesh from different cross-section of the fruit. It can be observed that most of the samples from the bottom third of the fruit, are located in the right-hand side of the score plot, whereas those samples from the top third appear on the left-hand side. Therefore, the content of most concentrated and the most odor active volatile compounds concurred with largest soluble solids content, pH and low TA at the bottom section of Gold cultivar pineapple fruit flesh.

These results demonstrated that variation of nonvolatile and volatile components content along Gold cultivar pineapple is strongly related to the position inside the fruits from where the flesh pieces are cut. Such differences are the result of the progressive ripening pattern of pineapple, as well as the growing conditions and pre-harvest practices.

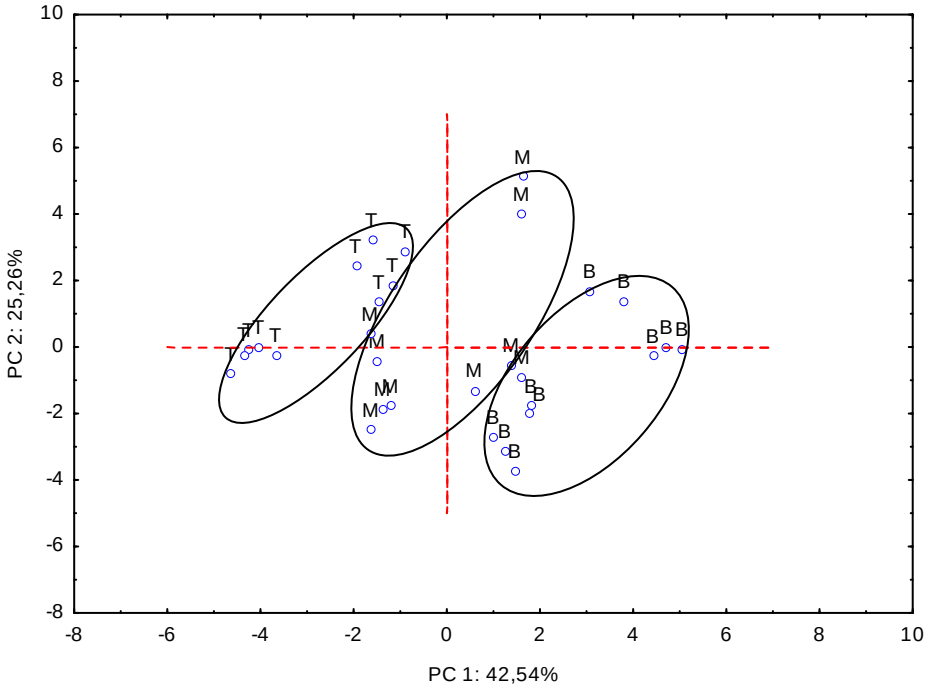



**Figure 3:** Score plot of PC1 versus PC2 for pineapple from the top (T), middle (M) and bottom (B) cross-sections of the fruit.

On the other hand, meanwhile all components associated with flavor (sugar, acids and volatiles content) vary along the fruit at different rates, consumer acceptance of fresh-cut fruits most often relies upon the inherent flavor of the product.

Consequently, most odor active volatiles content (methyl and ethyl 2-methyl butanoate, and mesifuran), SSC and TA (or SSC/TA ratio) should be controlled for consistent and uniform quality products, fixing required levels and tolerance limits. Pineapple fruits with low variability along the fruit and within individual batches is the best choice.

Results are also useful to define processing strategies addressed to obtain consistent and uniform quality products and minimize the differences between individual trays of fresh-cut pineapple, such as rejection of over or under mature fruits, and mixing procedures.

## 4. CONCLUSIONS

Natural occurring aroma profile of Gold cultivar pineapple flesh at 30 °C consisted in 20 volatile components, from which esters represented 90% of total extracted compounds. Aroma profile constituents are maintained along the fruit, though volatiles content vary. Methyl butanoate, methyl 2-methyl butanoate and methyl hexanoate were the most concentrated volatile components of Gold cultivar pineapple. Methyl and ethyl 2-methyl butanoate, and mesifuran were the most odor active contributors to pineapple aroma. Consequently, our results pointed out the importance to include odor active volatiles determinations, SSC and TA on quality assessment of fresh like product, as a tool for consistent and uniform aroma characteristics within individual packages, and for shelf-life studies throughout storage.

Further studies on pre-harvest practices and harvesting indices addressed to reduce the gap between volatile compounds content and physicochemical attributes of pineapple flesh along the fruit are recommended. Additionally sensorial evaluation of pineapple aroma could be used to validate the relative importance of odor active compounds and their contribution to pineapple aroma.

## ACKNOWLEDGEMENTS

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# EFFECT OF PACKAGING CONDITIONS ON QUALITY AND SHELF-LIFE OF FRESH-CUT PINEAPPLE (*Ananas comosus*)

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## ABSTRACT

Influence of packaging conditions on fresh-cut 'Gold' pineapple shelf-life were studied during 20 d of storage at 5 °C. Fresh-cut fruit pieces were packed in polypropylene trays (PP) and wrapped with 64μm polypropylene film under active (high 40% or low oxygen, 11.4%) or passive modified atmospheres (air or cut fruit coated with 1%, w/v alginate). Changes in headspace composition, titratable acidity, pH, soluble solids content, juice leakage, color, texture, and microbial growth were evaluated over time. For all packaging conditions, oxygen concentration continuously decreased below its initial concentration over 20 d storage, but never reached levels below 2% $O_2$. Meanwhile, $CO_2$ concentration inside all packages continuously increased over time up to 10.6–11.7% from the initial conditions. Ethylene concentrations were always less than 0.4 μL $L^{-1}$ while ethanol was detected only after 13 d of storage. Color parameters $L^*$ and $b^*$ significantly decreased over time in all packaging conditions and were directly attributed to the translucency phenomenon in the fruit flesh. When alginate coating was used, juice leakage was significantly reduced in contrast with the substantial juice accumulation observed in the rest of the packaging conditions. Texture profile analysis (TPA) parameters, did not significantly change over time, suggesting that structural characteristics of fresh-cut pineapple pieces were preserved throughout storage. From the microbial point of view, the shelf-life of 'Gold' fresh-cut pineapple was limited to 14 d by mesophilic bacterial growth. Further studies are needed to evaluate the sensory aspects, as well as to characterize the flesh translucency phenomenon and reduce juice leakage of fresh-cut pineapple.

# 1. INTRODUCTION

Pineapple (*Ananas comosus*) is the world's most popular noncitrus tropical and subtropical fruit. Currently, 'Gold' is the most accepted cultivar around the world. This cultivar has cylindrical shape, square shoulders, an intense orange-yellow shell color and a medium to large size (1.3–2.5 kg), and stands out for its excellent quality and sensory characteristics. The flesh is clear yellow, very sweet, compact and fibrous and has a high ascorbic acid content but low total acidity, when compared with other varieties such as 'Smooth Cayenne' (Chan et al., 2003).

Consumer demand for tropical fresh-cut products is increasing rapidly in the world market, and fresh-cut pineapple is already found in many supermarkets and food service chains (González- Aguilar et al., 2004; Marrero and Kader, 2006). Fresh-cut pineapple fruit is appreciated for its taste, flavor and juiciness. However, its shelf-life is limited by changes in color, texture, appearance, off-flavors and microbial growth which are affected by packaging conditions and storage temperature as well as cultivar and maturity stage (Soliva-Fortuny and Martín-Belloso, 2003; Marrero and Kader, 2006). Several treatments have been studied to maintain quality and extend shelf-life of fresh-cut fruit (García and Barret, 2002; Soliva-Fortuny and Martín-Belloso, 2003; Rojas-Graü et al., 2007a) but little has been reported on fresh-cut pineapple. Modified atmosphere packaging and refrigeration are the main tools used to slow undesirable quality changes and increase the shelf-life of fresh-cut pineapples. Marrero and Kader (2001, 2006) reported on post-cutting life of fresh-cut 'Smooth Cayenne' pineapple pieces from 4 d at 10 °C to over two weeks at 0 °C (10% $CO_2$ combined with a maximum of 8% oxygen) with no chilling injury symptoms, while González-Aguilar et al. (2004) reported 14 d at 10 °C for the same cultivar (2–5% $CO_2$, and 12–15% $O_2$). Chonhenchob et al. (2007) found fungi as the limiting factor for fresh-cut pineapple (no cultivar reported) in different plastic containers after 6–13 d at 10 °C. Differences are also reported for color, juice leakage and browning of fresh-cut pineapple, which can be explained by differences among cultivars and packaging conditions.

Usually, low $O_2$ levels combined with moderate to high $CO_2$ levels are applied to extend the shelf-life of fresh-cut commodities and the optimal storage conditions depend on the metabolic characteristics of the specific product (Kader et al., 1989). Permeability characteristics of the packaging containers and lids, the initial gas concentration, storage temperature and mass of fresh-cut pineapple lead to changes

in the internal headspace gas concentration over time and thus, could influence the quality attributes of fresh-cut pineapple.

Texture is an important attribute for fresh-cut fruit that determines the acceptance or rejection by the consumers and it generally changes over time as a result of tissue stresses during processing (Soliva-Fortuny et al., 2002). Little is known about pineapple structure and how it changes throughout time in fresh-cut products. Its non-uniform flesh makes measurement of texture attributes very difficult to determine. Several authors have reported pineapple firmness and rupture force during storage (González-Aguilar et al., 2004; Gil et al., 2006; Chonhenchob et al., 2007; Ramsaroop and Saulo, 2007), but no prior research has been reported about the texture profile analysis (TPA) on fresh-cut pineapples. Studies on TPA response of fresh-cut pineapple pieces to processing and storage conditions are needed for a better understanding of quality changes during storage, since this analysis is based on the determination of texture multi-parameter attributes rather than a single one such firmness and cutting tests.

On the other hand, edible coatings have also been used to protect fresh-cut fruit from dehydration and water loss. The coating acts as a gas barrier around each fruit piece and creates sort of a modified atmosphere in each coated piece. It is expected to reduce water losses and extend shelf-life of the fresh-cut product at optimum temperature and relative humidity (Rojas-Graü et al., 2008). Some polysaccharides have been used as edible coatings to improve the quality of different fresh-cut fruit (Tapia et al., 2007; Rojas-Graü et al., 2007b; Rojas-Graü et al., 2008). However, there are no published data on edible coatings used in fresh-cut pineapple.

The objective of this study was to evaluate the effect of different packaging conditions on the quality attributes and shelf-life of 'Gold' fresh-cut pineapple.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Fresh 'Gold' pineapples (*Ananas comosus* L. Merrill) imported from Costa Rica were bought at a local supermarket in Lleida, Spain (approximately 15–20 d after harvesting, 7–10 °C during transport). Shell color stage was where several to most of the shell eyes were partially filled with yellow color, all of them surrounded by green (De la Cruz-Medina and García, 2007). Fruit were stored at 11 ± 1 °C overnight prior to processing.

An ascorbic acid (1%) and citric acid (1%) solution was used to keep a low pH level on the fresh-cut pineapple surface and as an anti-browning agent. Food grade sodium alginate (Keltone® LV, ISP, San Diego, CA, USA) was used as the carbohydrate biopolymer for coating formulation. Glycerol (Merck, Whitehouse Station, NJ, USA) and sunflower oil (La Española, Spain) were added as plasticizer and emulsifier, respectively. Calcium chloride (Sigma–Aldrich Chemic, Steinhein, Germany) was used to induce cross-linking reactions.

## 2.2. Fresh-cut processing

Working area, cutting boards, knives, containers and other utensils and surfaces in contact with the fruit during processing were washed and sanitized with 200µL $L^{-1}$ sodium hypochlorite solution at pH 7 to have a maximum sanitizing effect prior to processing. Pineapple crown leaves were removed and the fruit was washed twice in two 200µL $L^{-1}$ sodium hypochlorite solutions for 5 min each, letting excess water drain for 3–5 min after each dip. Fruit were peeled and cut into 1 cm-thick slices using an electric slicing machine (Food Slicer-6128: Toastmaster Corp, Elgin, USA). Slices were then cored and cut into wedges (6–8 g, each) with sharp knives. Fresh-cut pineapple pieces were immersed in 1% citric acid and 1% ascorbic acid solution for 2 min as antibrowning agents and to keep the surface pH low enough to reduce microbial growth; excess water was drained for 2 min. When alginate edible coating was used as a protective barrier, ascorbic and citric acid were incorporated directly into the calcium chloride solution to reduce excessive handling of fresh-cut produce. The treated pieces were packaged as detailed in Section 2.4 and stored at 5 °C.

## 2.3. Fresh-cut fruit coating

Alginate coating was prepared as described by Rojas-Graü et al. (2008). Alginate powder (1%, w/v) was dissolved in distilled water under controlled heating (80 °C) and stirred until the mixtures became clear. Glycerol was added as plasticizer (1.5%, w/v). The solution was emulsified with 0.025% (w/v) sunflower oil, using an Ultra Turrax T25 (IKA® WERKE, Germany) with a S25N-G25G device for 5 min at 24,500 rpm and degassed under vacuum. Fresh-cut pineapple pieces were submerged for 2 min in the coating solution, drained for 2 min and submerged for another 2 min in a 2% (w/v) calcium chloride bath for carbohydrate polymer cross linking. Ascorbic acid (1%) and 1% citric acid were also added to the latter solution.

## 2.4. Packaging conditions and storage

Portions of 50 g of treated fresh-cut pineapples were placed into PP trays (500 cm3, MCP Performance Plastic Ltd., Kibbutz Hamaapil, Israel). These were wrapped with a 64µm of thickness PP film with a permeability to $O_2$ and $CO_2$ of 110 and 550$cm^3\ m^{-2}\ bar^{-1}\ d^{-1}$ at 23 °C and 0% RH, respectively (Tecnopack SRL, Mortara, Italy) using a MAP machine (Ilpra Foodpack Basic V/G, Ilpra, Vigenovo, Italy).

Four packaging conditions were established: (a) PP-HO: fresh-cut pineapple in PP trays filled with high oxygen concentration (38–40% $O_2$); (b) PP-LO: fresh-cut pineapple in PP trays filled with low oxygen concentration (10–12% $O_2$, 1% $CO_2$); (c) PP-AIR: fresh-cut pineapple in PP trays filled with air (20.9% $O_2$); (d) PP-ALG: fresh-cut pineapple coated with alginate and packaged in PP trays filled with air.

Trays were randomly taken at 0, 4, 6, 8, 11, 13, 15, 18 and 20 d for internal atmosphere analyses and color (2 trays), physicochemical determinations (2 trays), texture measurements (2 trays) and microbiological analysis (2 trays).

## 2.5. Headspace gas analysis

The internal atmosphere of each single tray was analyzed using a gas chromatograph equipped with a thermal conductivity detector (Micro-GP CP 2002 gas analyzer, Chrompack International, Middelburg, The Netherlands) as described by Rojas-Graü et al. (2008). A 1.7mL aliquot was withdrawn through an adhesive septum stuck to the film cover, with a sampling needle directly connected to the injection module. The determination of the $O_2$ concentration was carried out by injecting a sample of 0.25µL to the a CP-Molsieve 5Å packed column (4m×0.32mm, d.f. = 10mm) at 60 °C and 100 kPa whereas a portion of 0.33µL was injected into a pora-PLOT Q column (10m×0.32mm, d.f. = 10mm) held at 75 °C and 200 kPa for $CO_2$, ethylene (C2H4), acetaldehyde ($C_2H_4O$) and ethanol ($C_2H_5OH$) determinations. Two trays from each packaging condition were randomly selected for gas analysis at each sampling date, during the 20 d storage time.

## 2.6. Quality evaluation

Fresh-cut pineapple characteristics were measured throughout storage. Titratable acidity, pH, soluble solids content (%), pulp color, juice leakage, texture, and microbiological stability were measured.

### *2.6.1. Total soluble solids content, pH and titratable acidity*

Fresh-cut fruit pieces (50 g) were homogenized using an Ultra Turrax T25 (IKA® WERKE, Germany) and filtered (Whatman paper no. 1). Soluble solids content was determined using an Atago RX- 1000 refractometer (Atago Company Ltd, Japan), pH was directly measured using a pH meter Crison 2001 (Crison Instruments S.A., Barcelona, Spain) and 10–15 g of filtered pulp were titrated with 0.1N NaOH to pH 8.1. Titratable acidity was expressed as grams of anhydrous citric acid in 100 g of fruit fresh weight. All measurements were carried out according to AOAC procedures (Horwitz, 2000).

### *2.6.2. Color measurement*

Fresh-cut pineapple color was measured directly with a Minolta CR-400 chroma meter (Konica Minolta Sensing, Inc. Osaka, Japan), using the CIE scale $L^*a^*b^*$. The equipment was set up for illuminant D65 and 10° observer angle and calibrated using a standard white reflector plate.

Three readings were obtained for each replicate by changing the position of the pineapple piece to get representative color measurements. Sixteen replicates were evaluated per each packaging condition. Color changes in $L^*$ and $b^*$ throughout 20 d storage at 5 °C were analyzed.

Since no browning symptoms were observed in pineapple pieces during storage, but changes in tissue translucency were frequently observed, a side test was run with the aim to induce translucent appearance of fresh-cut pineapple pieces, and find out its relationship with changes in color parameters $L^*$ and $b^*$. In the first place, the color of 50 fruit pieces was measured; then they were collected in water using an inverted funnel sealed with a septum. Vacuum pressure was applied for 2 min to remove internal gases using a laboratory vacuum pump. Then, vacuum was released, fresh-cut pineapple pieces were drained to remove excess water, and color was measured again.

### *2.6.3. Juice leakage*

Juice leakage from pineapple pieces was measured according to the method of Marrero and Kader (2006) with some modifications. Juice leakage was assayed by tilting the packages at a 20° angle for 5 min and recovering accumulated liquid with a 5mL syringe. Results were reported as liquid volume recovered per 100 g of fresh-cut fruit in the package.

### *2.6.4. Texture evaluation*

Texture analysis of fresh-cut pineapple was evaluated by running a Texture Profile Analysis along 20 d of storage at 5 °C. Fruit specimens were compressed twice to 50% of their original height (10 s interval) simulating mastication. A TA-TX2 Texture Analyzer (Stable Micro Systems LTD. Surrey, England) was used at room temperature and the following conditions were set according to the instrument manufacturer recommendations: 2 $mms^{-1}$ pretest speed, 5.0 $mms^{-1}$ test speed, 5.0$mms^{-1}$ post-test speed and 50% strain. A 50mm diameter cylindrical probe (P/50) was used to assure fresh-cut fruit surface area was completely covered by the probe. Force–distance–time data were registered for two cycle TPA test and texture parameters hardness (peak force during the first compression cycle, N/100 g), fragility or fracture force (peak of first fracture, N/100 $g^{-1}$), adhesiveness (work required to overcome the attractive forces between the food and other surface, Ns/100 $g^{-1}$), cohesiveness (ratio of positive force area during the second compression cycle to that during the first compression cycle, dimensionless), resilience (sample recover from deformation, dimensionless) and gumminess (hardness × cohesiveness, N/100 $g^{-1}$) were calculated from force, distance and time data, using Texture Exponent 32 software (Stable Micro Systems LTD. Surrey, England). Force and energy results were calculated with respect to fresh-cut pineapple weight, to avoid the effect of size and weight differences among fruit pieces on the results. Two trays were taken at each sampling time to perform the analysis, and no less than eight pineapple pieces were used for each packaging condition on each evaluation day.

### *2.6.5. Microbiological analysis*

Changes in the microbial population of fresh-cut pineapple was studied by mesophilic and psychrophilic aerobic counts, and yeast and mould counts were carried out during the 20 d of storage, as described by Rojas-Graü et al. (2008). Mesophilic and psychrophilic bacteria counts were made according to the ISO 4833:1991 guideline using Plate Count Agar (PCA) (Biokar Diagnostics, Beauvais, France) and the pour plate method. The plates of psychrophilic bacteria were incubated at 5 °C for 10–14 days, whereas mesophilic bacteria were incubated at 35°C for 48 h. Yeast and mould counts were made according to the ISO 7954:1987 guideline using Chloramphenicol Glucose Agar (CGA) (Biokar Diagnostics, Beauvais, France) and the spread plate method. The plates were incubated at 25 °C for 2–5 d. Analyses were carried out in randomly sampled pairs of trays, with two replicate counts per tray.

### 2.7. Statistical analysis

A completely random design was used with four packaging conditions (PP-HO, PP-LO, PP-AIR, PP-ALG), 9 evaluations throughout storage, two repetitions with 50 g fresh-cut pineapple package as the experimental unit. Experimental data were analyzed using Statgraphics Plus version 5.1. (Statistical Graphics Co., Rockville, MD, USA). Analysis of variance (ANOVA) was performed to compare packaging conditions results. Duncan's test was used to compare means at the 5% significance level.

## 3. RESULTS AND DISCUSSION

### 3.1. Headspace gases

Changes in headspace gas composition inside fresh-cut pineapple containers are shown in Figs. 1 and 2. Headspace $O_2$ content significantly decreased over time ($p \leq 0.05$) in all packaging conditions, showing a steady decreasing pattern up to day 20 (about 0.6% per d) without reaching an equilibrium concentration throughout storage (Fig. 1). Slow changes in headspace $O_2$ composition could be explained by the low respiration rate of pineapple at 5 °C (2–4μL $kg^{-1}$ $h^{-1}$ at 7 °C, Kader, 2006), film permeability characteristics, and the low ratio of fruit weight to container volume used (1g:10mL). In fact, during the entire period of storage, $O_2$ concentration was never below 2%, avoiding anaerobic conditions and possible formation of off-flavors and off-odors. Soliva-Fortuny et al. (2004) indicated that 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-flavors and off-odors.

In the other hand, the $CO_2$ level significantly increased during storage ($p \leq 0.05$) at a similar rate (0.5% per d) for all packaging conditions (Fig. 1). These results showed that $CO_2$ headspace concentrations were not dependent on initial oxygen content (10–40%) for fresh-cut pineapple packed in PP trays and stored at 5 °C. Modified atmospheres of 8% $O_2$ and 10% $CO_2$ for fresh-cut 'Smooth Cayenne' pineapple have been recommended by Marrero and Kader (2006) to achieve 12 d of storage life at 5°C and more than 15 d at 2.2 and 0 °C. In our study, such oxygen levels were only achieved inside PP-LO containers during storage, whereas high $CO_2$ levels were achieved inside all PP containers after 8–11 d of storage at 5 °C, with no undesirable changes in quality attributes for over 15 d. However, 'Gold' pineapple shelf-life was marked by microbial spoilage and juice leakage after 2 weeks of storage, as will be

discussed below. These results suggest that headspace gas composition might not be a key parameter to extend shelf-life of fresh-cut pineapple, in the range from 2% to 40% $O_2$ and up to 15% $CO_2$, and agree with those obtained by Marrero and Kader (2006) for the 'Premium select' cultivar and by Chonhenchob et al. (2007) for the 'Phuket' cultivar.

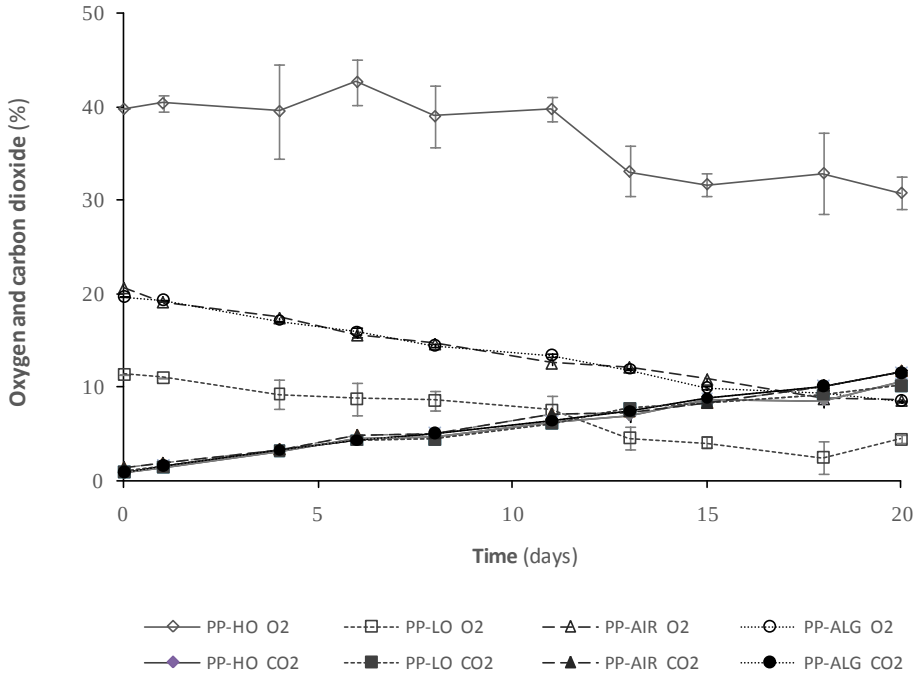


**Fig. 1.** Evolution of the headspace oxygen (full symbols) and carbon dioxide (empty symbols) composition in fresh-cut pineapple stored at 5 °C under different packaging conditions: PP-HO: PP trays filled with high $O_2$ concentration (38–40%); PP-LO: PP trays filled with low $O_2$ concentration (10–12%); PP-AIR: PP trays filled with air; PP-ALG: PP trays filled with air and containing fresh-cut pineapple coated with alginate. Data shown are mean ± standard deviation.

Ethylene concentrations inside the packages were very low, showing maximum values at the fourth day of storage (0.2–0.4 µL $L^{-1}$), decreasing for the next few days down to zero and later increasing to levels between 0 and 0.4µL $L^{-1}$ after 12 d of storage (Fig. 2a). This behavior could be attributed to a temporary increase in ethylene production due to processing damages which resulted in an accumulation of this gas inside the package. Later on, ethylene production of healthy tissue of fresh-cut pineapple decreased and stayed close to zero during storage at 5 °C. After 12 d of storage fresh-cut fruit became older, and tissue deterioration started. As a consequence, ethylene production increased again and with increasing accumulation inside the package throughout time. However, ethylene concentration did not

exceed 1µL $L^{-1}$ under any packaging conditions, suggesting that cut pineapple has low physiological activity. Similar behavior has been reported by Marrero and Kader (2006) for fresh-cut pineapple ('Smooth Cayenne' cultivar PRI 36-20) who observed an increased production after cutting, followed by a decrease and later increase up to 0.5µL $L^{-1}$ after 10 d of storage at 7.5 °C.

Fresh-cut pineapple packaged in PP-LO did not register any ethylene presence after 8 d storage, probably due to the inhibition of ethylene production under low oxygen conditions. In fact, the inhibition of ethylene in the absence of or at low $O_2$ concentrations has been reported by some authors in fresh-cut fruit (Qi et al., 1999; Soliva-Fortuny et al., 2004; Oms-Oliu et al., 2008). It is well known that oxygen participates in the conversion of 1-amino-cyclopropane-1-carboxylic acid (ACC) to ethylene (Yang, 1981). Contrary to what was expected, fresh-cut pineapple packaged in PP-HO had higher rates of ethylene production than in the other packaging conditions (Fig. 2a). Kader and Ben-Yehoshua (2000) indicated that 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. Currently, knowledge about the effect of high $O_2$ atmospheres on postharvest physiology and quality of fresh-cut fruit is limited and its basic biological mechanisms are not completely understood (Oms-Oliu et al., 2007).

Ethanol gas was detected in all package headspace only after the 15th day of storage (Fig. 2b). Significant differences ($p < 0.05$) were found among packaging conditions; fresh-cut pineapple stored under PP-LO or PP-ALG had the higher ethanol concentrations, reaching values of 46µL $L^{-1}$ after 20 d storage. Wszelaki and Mitcham (2000) indicated that low $O_2$ atmospheres seem to promote the production of anaerobic metabolites due to anaerobic metabolism. On the contrary, pineapple pieces stored under PPHO show the least ethanol concentration (13µL $L^{-1}$) up the 20th storage day at 5 °C. The application of high $O_2$ levels in packages of fresh-cut fruit could be particularly effective in preventing anaerobic fermentative reactions promoted by low $O_2$ atmospheres (Allende et al., 2004). This is in agreement with the hypothesis of Day (1996) which declares that under high $O_2$ atmospheres there would be less fermentative metabolites than under high $CO_2$. Since ethanol can be associated with undesirable fermentation reactions, these studies showed that such reactions did not occur in any of the fresh-cut fruit packaging conditions during the first two weeks of storage.

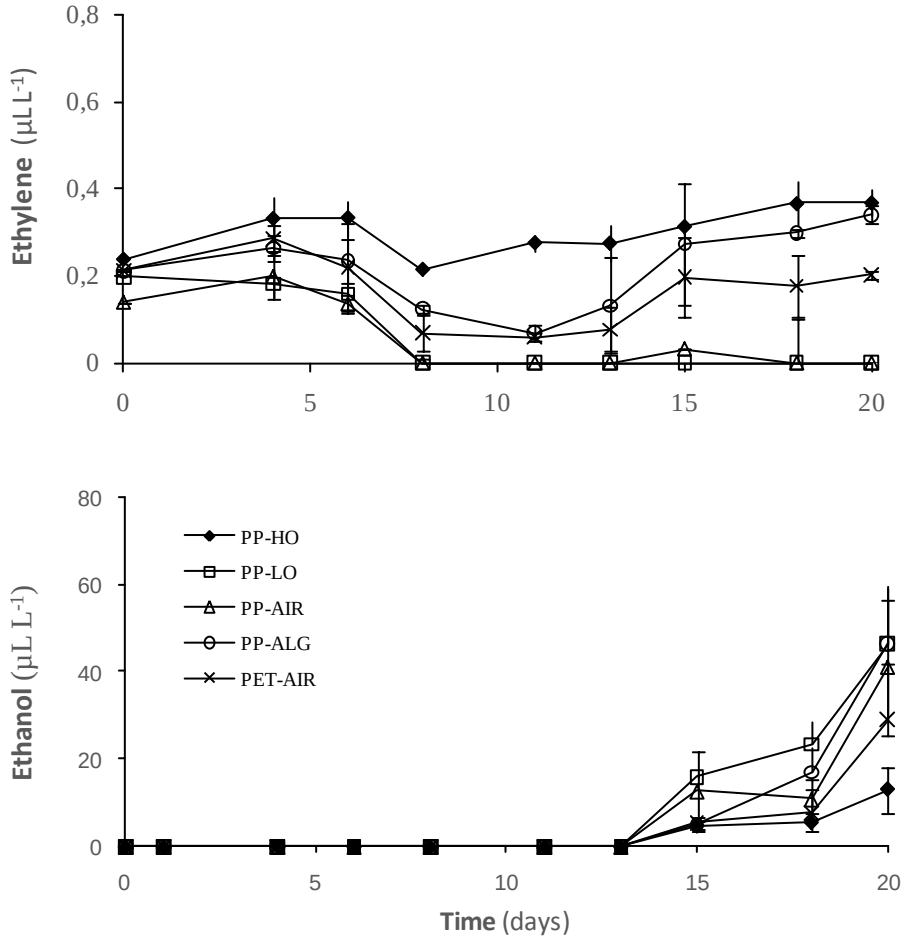


**Fig. 2.** Evolution of the headspace ethylene (a) and ethanol (b) composition in fresh-cut pineapple stored at 5 °C under different packaging conditions. PP-HO: PP trays filled with high $O_2$ concentration (38–40%); PP-LO: PP trays filled with low $O_2$ concentration (10–12%); PP-AIR: PP trays filled with air; and PP-ALG: PP trays filled with air and containing fresh-cut pineapple coated with alginate. Data shown are mean ± standard deviation.

## 3.2. Changes in quality parameters

Titratable acidity (TA), soluble solids content (SS) and pH showed little changes during storage and no significant differences were found either over time or among packaging conditions. Average values for these parameters were 0.68±0.02 g/100 g,

13.9±0.2% SS and 3.58±0.04, for TA, SS and pH, respectively. Gil et al. (2006) found similar results for fresh-cut pineapple ('Gold' cultivar) stored under modified atmosphere conditions (2% $O_2$ and 10% $CO_2$), while Ramsaroop and Saulo (2007) reported slightly lower TA and SS values and higher pH for whole fruit of the same cultivar. Santos et al. (2005) also observed little changes in fresh-cut pineapple ('Perola' cultivar) during storage for these parameters, for fruit stored under several modified atmosphere conditions at 8 °C. Bartolomé et al. (1995) found higher TA values and lower SS and pH for 'Red Spanish' and 'Smooth Cayenne' cultivars. The variability of TA, SS and pH values found in these studies could be explained by several factors such as the type of cultivar, maturity stages, and even the position inside the fruit.

### *3.2.1. Color changes in fresh-cut pineapple*

Changes in color parameters *L** (luminosity) and *b** (−blue to ±yellow) of fresh-cut pineapple were studied throughout 20 d storage at 5 °C. Significant differences for both, *L** and *b** values were found among all packaging conditions over time. Fruit pieces stored under PP-LO or PP-AIR had higher *L** and *b** values than those packaged under PP-HO or PP-ALG conditions. Since changes in *L** and *b** values occurred at approximately the same rate for all packaging conditions, differences among fresh-cut fruit were attributed to the normal color variability of individual pineapple pieces. Pineapple fruit is composed of multiple fruitlets (up to 200, depending on the cultivar) and each of them with various types of tissues (Paull and Chen, 2003). In addition, maturation pattern of the fruit starts from the fruitlets at the base of the fruit and moves up to the crown, which results in different stages of maturity of the fruitlets throughout the whole fruit. Because of such a complex fruit anatomy and maturity pattern, fruit flesh is non-uniform in color and texture, and this explains why flesh *L** and *b** values are very variable among fresh-cut pineapple pieces.

Even though color differences among packaging conditions were attributed to inherent pineapple fruit characteristics, it was found that there were significant differences in *L** and *b** through storage time. Average *L** values changed from 63.9 to 71.5 at the beginning of the experiment to 50.1 to 62.1 after 20 d 5 °C storage, while *b** values changed from 32.4 to 41.9 down to 23.0 to 36.3. These color differences in *L** and *b** were mostly attributed to observed changes in translucent appearance of the fruit flesh, which changed from a yellow-white opaque color to a translucent yellow color. Neither browning nor dry surface appearance were observed in fresh-cut pineapple pieces during storage.

When translucency was induced in fresh-cut pineapple pieces, *L** and *b** decreased an average of 15 and 12 units, respectively, with no changes in *a** (−3.3±0.7). When such findings were compared with changes of *L** and *b** values obtained in this study, it became clear that the registered changes on color parameters at all the packaging conditions were due to translucency development, rather than tissue browning. Chen and Paull (2001) found an increase in electrolyte leakage in 'Smooth Cayenne' pineapple translucent tissue and considered that its development was related to the diffusion rate of water and solutes across fruit flesh cell membranes, changes in membrane permeability, sugar content and differences in internal osmotic pressure which could promote the removal of water from the phloem into the apoplast.

The magnitude of color changes in this study for *L** and *b** agree with those obtained by others during storage (González-Aguilar et al., 2004; Marrero and Kader, 2006; Gil et al., 2006), even though they did not relate such changes to translucent appearance of the tissues. For instance, Marrero and Kader (2006) reported small changes in *L** values for 'Smooth Cayenne' pineapples stored at 5 °C, and 'Premium Select' pineapple pieces stored at 0 and 5 °C for 14 d; Gil et al. (2006) reported changes in *b** parameter of about 9 units, for fresh-cut 'Gold' pineapple after 9 d of storage at 10 °C, while González-Aguilar et al. (2004) also found rather small changes in *L** and *b** values for fresh-cut 'Smooth Cayenne' pineapple stored at 10 °C during 14 d, but they considered that such changes were due to browning reactions and related them to polyphenol oxidase (PPO) activity in that cultivar. In contrast, no PPO activity was found in fresh-cut 'Gold' pineapple used in this study and no browning symptoms were observed along storage (data not shown).

### *3.2.2. Juice leakage*

Results of accumulated juice leakage inside the container, throughout 20 d of storage of fresh-cut pineapple pieces at 5 °C are shown in Fig. 3. The liquid inside the package significantly increased over time for all packaging conditions ($p < 0.05$). Fresh-cut pineapple stored in PP-ALG packaging conditions had less liquid leakage per fruit weight than those packed under PP-HO, PPLO and PP-AIR through time. No significant differences ($p < 0.05$) were found between the later three packaging conditions, thus indicating that headspace gas composition did not affect juice leakage during storage. Santos et al. (2005) reported similar results for fresh-cut 'Perola' cultivar pineapple, stored under passive and active modified atmospheres. Reduced juice leakage in PP-ALG packages was attributed to the effect of alginate coating application, which increase the surface water vapor resistance of the fresh-

cut pineapple pieces. When juice leakage of fruit under PP-ALG and PP-AIR packaging conditions were compared, the contribution of alginate coating was evident; after 15 d of storage at 5 °C, the juice leakage was almost four times less for coated fresh-cut pineapple pieces (1.0 and 3.6 mL/100 g for coated and uncoated pieces, respectively). The good barrier water properties exhibited by alginate coating has been previously reported by Rojas-Graü et al. (2008) who found that 2% alginate edible coating applied to fresh-cut 'Fuji' apples was effective in preventing water losses. Neither off-flavors nor off-odors were noticed on alginate coated pineapple pieces, so no evidence was found that retaining liquid inside the cut pieces could have accelerated product deterioration.

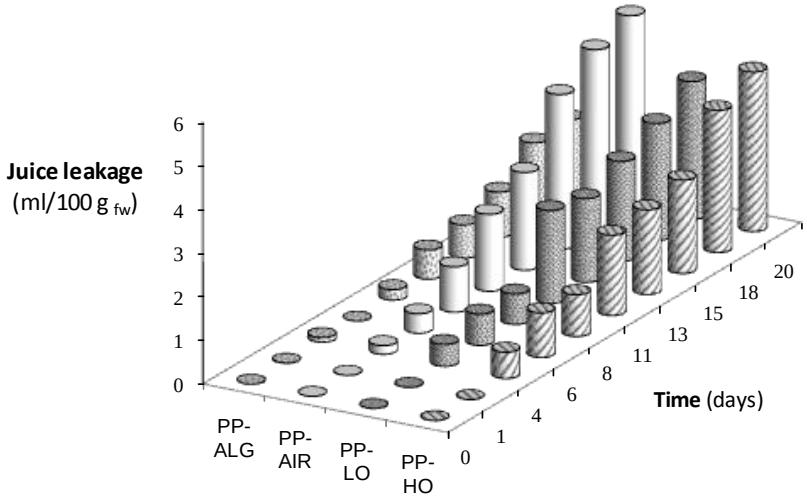


**Fig. 3.** Effect of packaging conditions on juice leakage volume of fresh-cut pineapple pieces stored at 5 °C. PP-HO: PP trays filled with high $O_2$ concentration (38–40%); PP-LO: PP trays filled with low $O_2$ concentration (10–12%); PP-AIR: PP trays filled with air; and PP-ALG: PP trays filled with air and containing fresh-cut pineapple coated with alginate. Data shown are mean ± standard deviation.

### *3.2.3. Texture profile analysis*

Results of TPA are reported as force and work per 100 g of fresh weight, to avoid any distortion due to the effect of size differences among pineapple pieces (Table 1). TPA curves registered multiple fracture peaks as the probe advanced into the fresh-cut

pineapple piece during the first compression cycle; most of the time the first fracture peak (fragility) occurred before the maximum peak force was achieved, but no uniform pattern was found for maximum peak location or for the number of fracture peaks along the first compression cycle. This behavior could be explained by the non-uniform structural characteristics of pineapple flesh.

No significant differences were found either among fresh-cut pineapple packaging conditions or throughout the 20 d of storage at 5 °C for any of the TPA parameters studied (Table 1). Average hardness and fragility forces for pineapple pieces were 337±55 and 320±59 N/100 g of fresh-cut fruit, respectively. Average adhesiveness was −3.4±2.7N s/100 g of fresh-cut fruit, gumminess values were 37.4±7.8 N/100 g of fresh-cut fruit, and dimensionless parameters cohesiveness and resilience were 1.8±1.5 and 0.115±0.015, respectively. Non-uniform flesh characteristics of pineapple flesh and maturation pattern of the fruit, contribute to large variability among individual fruit pieces mechanical attributes.

Absence of significant changes in TPA parameters of fresh-cut pineapple pieces over time at 5 °C indicates that fruit structure of pineapple pieces properly resist storage time and conditions. This observation coincides with the appearance of the pineapple pieces, which kept their shape and size throughout the 20 d of storage. Similar behavior have been reported by Gil et al. (2006) who did not find significant differences in whole and fresh-cut pineapples firmness (3mm tip penetration test) for the 'Tropical Gold' cultivar after 9 d of storage at 5 °C. By contrast, González-Aguilar et al. (2004) and Chonhenchob et al. (2007) found some tissue firmness reduction during storage for fresh-cut pineapple ('Smooth Cayenne' and 'Phuket' cultivars, respectively) but in both studies fresh-cut pineapple was stored at 10 °C, and at such temperatures, larger changes could be expected in fresh-cut fruits as compared with 5 °C storage.

TPA results suggested that juice leakage of fresh-cut pineapple pieces does not affect their response to the unidirectional compression force (hardness and fragility). Our results for TPA, color and translucency of fresh-cut pineapple pieces through time agreed with the results obtained by Lana et al. (2006) in fresh-cut tomatoes. These authors reported very small changes in fresh-cut tomato firmness during storage at low temperature (5 °C) even though translucent tissue appeared after 1 or 2 d of storage. They did not find evidence of cell membrane or cell wall degradation and suggested that translucency could be explained by physical phenomena (diffusion of water and solutes) and the response to stress-related compounds produced.

**Table 1.** Texture profile analysis (TPA) of fresh-cut pineapple fruits stored under different packaging conditions. PP-HO: PP trays filled with high $O_2$ (38 – 40%); PP-LO: PP trays filled with low $O_2$ (10 – 12%); PP-AIR: PP trays filled with air; PP-ALG: PP trays filled with air and containing fresh-cut pineapple coated with alginate, and PET-AIR: PET trays filled with air.

| **Packaging conditions** | **Days at 5 °C** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **HARDNESS (N/100 g fw)** | 0 | 4 | 6 | 8 | 11 | 13 | 15 | 18 | 20 |
| PP-HO | 325 ± 61$^{a}$ | 376 ± 103$^{a}$ | 323 ± 62$^{a}$ | 324 ± 32$^{b}$ | 346 ± 55$^{a}$ | 325 ± 57$^{a}$ | 353 ± 68$^{a}$ | 369 ± 60$^{b}$ | 369 ± 60$^{b}$ |
| PP-LO | 319 ± 84$^{a}$ | 390 ± 43$^{a}$ | 338 ± 63$^{a}$ | 317 ± 54$^{a,b}$ | 335 ± 46$^{a}$ | 336 ± 77$^{a}$ | 327 ± 46$^{a}$ | 326 ± 57$^{b}$ | 308 ± 58$^{a}$ |
| PP-AIR | 300 ± 68$^{a}$ | 378 ± 46$^{a}$ | 354 ± 56$^{a}$ | 313 ± 47$^{a,b}$ | 353 ± 50$^{a}$ | 345 ± 48$^{a}$ | 351 ± 52$^{a}$ | 357 ± 28$^{b}$ | 343 ± 55$^{a,b}$ |
| PP-ALG | 306 ± 24$^{a}$ | 350 ± 41$^{a}$ | 318 ± 44$^{a}$ | 280 ± 26$^{a}$ | 328 ± 50$^{a}$ | 306 ± 72$^{a}$ | 311 ± 40$^{a}$ | 276 ± 29$^{a}$ | 324 ± 36$^{a,b}$ |
| **FRAGILITY (N/100 g fw)** | | | | | | | | | |
| PP-HO | 303 ± 43$^{a,b}$ | 362 ± 119$^{a}$ | 305 ± 64$^{a}$ | 311 ± 54$^{a}$ | 309 ± 43$^{a}$ | 322 ± 56$^{a}$ | 340 ± 60$^{a}$ | 356 ± 66$^{b}$ | 357 ± 69$^{b}$ |
| PP-LO | 297 ± 83$^{a,b}$ | 349 ± 79$^{a}$ | 328 ± 65$^{a}$ | 302 ± 51$^{a}$ | 301 ± 69$^{a}$ | 323 ± 85$^{a}$ | 322 ± 48$^{a}$ | 273 ± 94$^{a}$ | 285 ± 66$^{a}$ |
| PP-AIR | 264 ± 52$^{a}$ | 361 ± 57$^{a}$ | 331 ± 71$^{a}$ | 293 ± 44$^{a}$ | 325 ± 63$^{a}$ | 344 ± 51$^{a}$ | 337 ± 62$^{a}$ | 344 ± 34$^{b}$ | 334 ± 63$^{a,b}$ |
| PP-ALG | 287 ± 40$^{a}$ | 334 ± 56$^{a}$ | 309 ± 47$^{a}$ | 274 ± 35$^{a}$ | 313 ± 41$^{a}$ | 304 ± 76$^{a}$ | 290 ± 31$^{a}$ | 257 ± 36$^{a}$ | 323 ± 36$^{a,b}$ |
| **ADHESIVENESS (N s/100 g fw)** | | | | | | | | | |
| PP-HO | -2.9 ± 2.7$^{a}$ | -4.0 ± 3.2$^{a}$ | -3.8 ± 3.1$^{a}$ | -3.0 ± 2.7$^{a}$ | -1.8 ± 0.8$^{a}$ | -4.2 ± 3.2$^{a}$ | -3.8 ± 2.5$^{a}$ | -3.8 ± 3.0$^{a}$ | -3.2 ± 2.8$^{a}$ |
| PP-LO | -2.9 ± 2.4$^{a}$ | -4.1 ± 3.5$^{a}$ | -3.4 ± 2.7$^{a}$ | -2.7 ± 2.8$^{a}$ | -2.2 ± 2.1$^{a}$ | -2.7 ± 2.7$^{a}$ | -4.0 ± 3.2$^{a}$ | -3.9 ± 3.0$^{a}$ | -3.7 ± 3.3$^{a}$ |
| PP-AIR | -4.1 ± 3.3$^{a}$ | -3.0 ± 2.6$^{a}$ | -4.9 ± 3.2$^{a}$ | -2.8 ± 2.3$^{a}$ | -1.6 ± 0.5$^{a}$ | -5.2 ± 3.0$^{a}$ | -5.1 ± 2.6$^{a}$ | -2.3 ± 2.0$^{a}$ | -2.6 ± 2.4$^{a}$ |
| PP-ALG | -3.8 ± 3.3$^{a}$ | -3.6 ± 3.2$^{a}$ | -2.1 ± 2.3$^{a}$ | -4.0 ± 3.1$^{a}$ | -1.8 ± 1.9$^{a}$ | -4.5 ± 3.6$^{a}$ | -3.8 ± 2.9$^{a}$ | -3.5 ± 2.6$^{a}$ | -2.5 ± 2.4$^{a}$ |
| **GUMMINESS (N/100 g fw)** | | | | | | | | | |
| PP-HO | 31.7 ± 7.8$^{a}$ | 37.8 ± 8.3$^{a,b}$ | 35.1 ± 7.8$^{a}$ | 32.9 ± 3.0$^{a}$ | 34.6 ± 5.6$^{a}$ | 54.7 ± 14.2$^{a}$ | 36.4 ± 5.9$^{a}$ | 36.5 ± 5.5$^{b}$ | 37.0 ± 6.2$^{a}$ |
| PP-LO | 34.6 ± 13.0$^{a}$ | 43.5 ± 8.5$^{b}$ | 39.3 ± 14.5$^{a}$ | 31.6 ± 6.5$^{a}$ | 37.8 ± 7.2$^{a}$ | 57.7 ± 13.8$^{a}$ | 33.2 ± 8.0$^{a}$ | 36.4 ± 7.1$^{b}$ | 31.6 ± 6.1$^{a}$ |
| PP-AIR | 31.8 ± 43.1$^{a}$ | 43.1 ± 12.0$^{b}$ | 36.6 ± 7.2$^{a}$ | 32.9 ± 6.4$^{a}$ | 38.5 ± 8.8$^{a}$ | 56.8 ± 10.1$^{a}$ | 35.8 ± 4.3$^{a}$ | 37.2 ± 6.9$^{b}$ | 35.4 ± 6.1$^{a}$ |
| PP-ALG | 31.3 ± 34.6$^{a}$ | 34.6 ± 3.8$^{a}$ | 33.3 ± 5.4$^{a}$ | 29.5 ± 6.9$^{a}$ | 32.2 ± 8.4$^{a}$ | 50.8 ± 13.7$^{a}$ | 31.3 ± 7.2$^{a}$ | 30.0 ± 3.0$^{a}$ | 31.0 ± 5.5$^{a}$ |
| **RESILIENCE (adimensional)** | | | | | | | | | |
| PP-HO | 1.5 ± 1.9$^{a}$ | 2.3 ± 1.3$^{a}$ | 1.7 ± 2.0$^{a}$ | 3.0 ± 1.9$^{a}$ | 0.9 ± 1.6$^{a}$ | 1.2 ± 0.8$^{a}$ | 1.5 ± 1.4$^{a}$ | 2.0 ± 1.4$^{a}$ | 1.8 ± 1.6$^{a}$ |
| PP-LO | 0.9 ±1.3$^{a}$ | 2.1 ± 1.8$^{a}$ | 2.1 ± 1.6$^{a}$ | 2.1 ± 1.8$^{a}$ | 1.3 ± 1.3$^{a}$ | 1.1 ± 0.7$^{a}$ | 1.8 ± 1.5$^{a}$ | 1.4 ± 1.6$^{a}$ | 1.5 ± 1.2$^{a}$ |
| PP-AIR | 1.6 ± 1.8$^{a}$ | 1.8 ± 1.8$^{a}$ | 1.6 ± 1.6$^{a}$ | 1.8 ± 2.0$^{a}$ | 0.9 ± 1.2$^{a}$ | 1.4 ± 0.4$^{a}$ | 1.51± 1.5$^{a}$ | 2.0 ± 1.4$^{a}$ | 2.2 ± 1.1$^{a}$ |
| PP-ALG | 2.2 ± 2.0$^{a}$ | 2.8 ± 1.9$^{a}$ | 1.7 ± 1.8$^{a}$ | 2.9 ± 1.7$^{a}$ | 2.4 ± 2.2$^{a}$ | 1.4 ± 0.9 | 1.9 ± 1.5$^{a}$ | 1.1 ± 1.4$^{a}$ | 2.7 ± 1.2$^{a}$ |
| **COHESIVENESS (adimensional)** | | | | | | | | | |
| PP-HO | 0.097 ± 0.011$^{a}$ | 0.103 ± 0.020$^{a}$ | 0.108 ± 0.006$^{a}$ | 0.102 ± 0.008$^{a}$ | 0.101 ± 0.015$^{a}$ | 0.168 ± 0.029$^{a}$ | 0.104 ± 0.007$^{a}$ | 0.101 ± 0.023$^{a}$ | 0.101 ± 0.013$^{a}$ |
| PP-LO | 0.107 ± 0.015$^{a}$ | 0.111 ± 0.017$^{a}$ | 0.116 ± 0.035$^{a}$ | 0.100 ± 0.012$^{a}$ | 0.113 ± 0.014$^{a}$ | 0.173 ± 0.026$^{a}$ | 0.102 ± 0.018$^{a}$ | 0.113 ± 0.023$^{a}$ | 0.104 ± 0.021$^{a}$ |
| PP-AIR | 0.106 ± 0.011$^{a}$ | 0.113 ± 0.021$^{a}$ | 0.104 ± 0.012$^{a}$ | 0.105 ± 0.012$^{a}$ | 0.108 ± 0.014$^{a}$ | 0.164 ± 0.014$^{a}$ | 0.103 ± 0.014$^{a}$ | 0.104 ± 0.017$^{a}$ | 0.104 ± 0.013$^{a}$ |
| PP-ALG | 0.103 ± 0.009$^{a}$ | 0.099 ± 0.009$^{a}$ | 0.104 ± 0.008$^{a}$ | 0.105 ± 0.023$^{a}$ | 0.097 ± 0.015$^{a}$ | 0.166 ± 0.014$^{a}$ | 0.100 ± 0.015$^{a}$ | 0.109 ± 0.009$^{a}$ | 0.096 ± 0.014$^{a}$ |

*Different letters indicate statistically significant differences ($p<0.05$)*

The few changes in fruit TPA parameters at 5 °C highlight the need to identify other quality parameters that could properly measure changes occurring in the fresh-cut pineapple pieces during storage. In the other hand, the packaging containers and covers used in this study properly protected the integrity of fresh-cut pineapple pieces.

### *3.2.4. Microbiological analysis*

Fig. 4 shows the development of moulds and yeast, mesophilic and psychrophilic bacteria on fresh-cut pineapple during cold storage of at 5 °C. No significant differences ($p < 0.05$) were found among packaging conditions for the microbial growth; however, significant differences were observed through storage time. Initial populations ranged from 3 to 4 log CFU $g^{-1}$ for moulds and yeast at day 0 and reached 7–7.5 log CFU $g^{-1}$ after 18 d of storage. Similar increase was observed for mesophilic and psychrophilic bacteria reaching values of 7–8.5 log CFU $g^{-1}$, respectively after 18 d at 5 °C.

Spanish hygienic regulations for processing, distribution and commerce of prepared meals (Real Decreto 3484/2000) include a maximum limit for mesophilic bacteria of 7 log CFU $g^{-1}$ for meals prepared from raw vegetables (BOE, 2001). Mesophilic bacteria in fresh-cut pineapple containers reached that level on the 14th day of storage, whereas psychrophilic bacteria and yeast and moulds reached it at 18th day for all packaging conditions. Mesophilic bacteria counts were used to define the shelf-life of fresh-cut pineapple, since these microorganisms were the first to exceed regulation limits. Packaging in modified atmosphere prolonged the shelf-life of 'Gold' fresh-cut pineapple by 14 d of storage. After 13 d, headspace gas concentration of ethanol starts to be noticed, which is also a sign of undesirable changes and degradation processes which lead to off-flavors and off-odors.

Good manufacturing practices were used during fresh-cut pineapple preparation in this study, and even so, relatively high microbial counts were found at day 0. This is explained by the fact that pineapple fruit contained multiple fruitlets, which can trap some microorganisms during fruit development (Rohrbach and Johnson, 2003). The characteristics of shape and rough surface in pineapples make difficult an effective sanitizing of the fruit, and usually lead to fresh-cut fruit with larger microbial counts than other temperate fruit, such us apples and pears. However, most of these microorganisms are bacteria and fungi which cause postharvest diseases of the fruit, principally *Penicillium funiculosum*, which causes fruitlet core rot or green eye, leathery pocket and interfruitlet corking, but they are normally safe for consumer (Rohrbach and Pfeiffer, 1976).

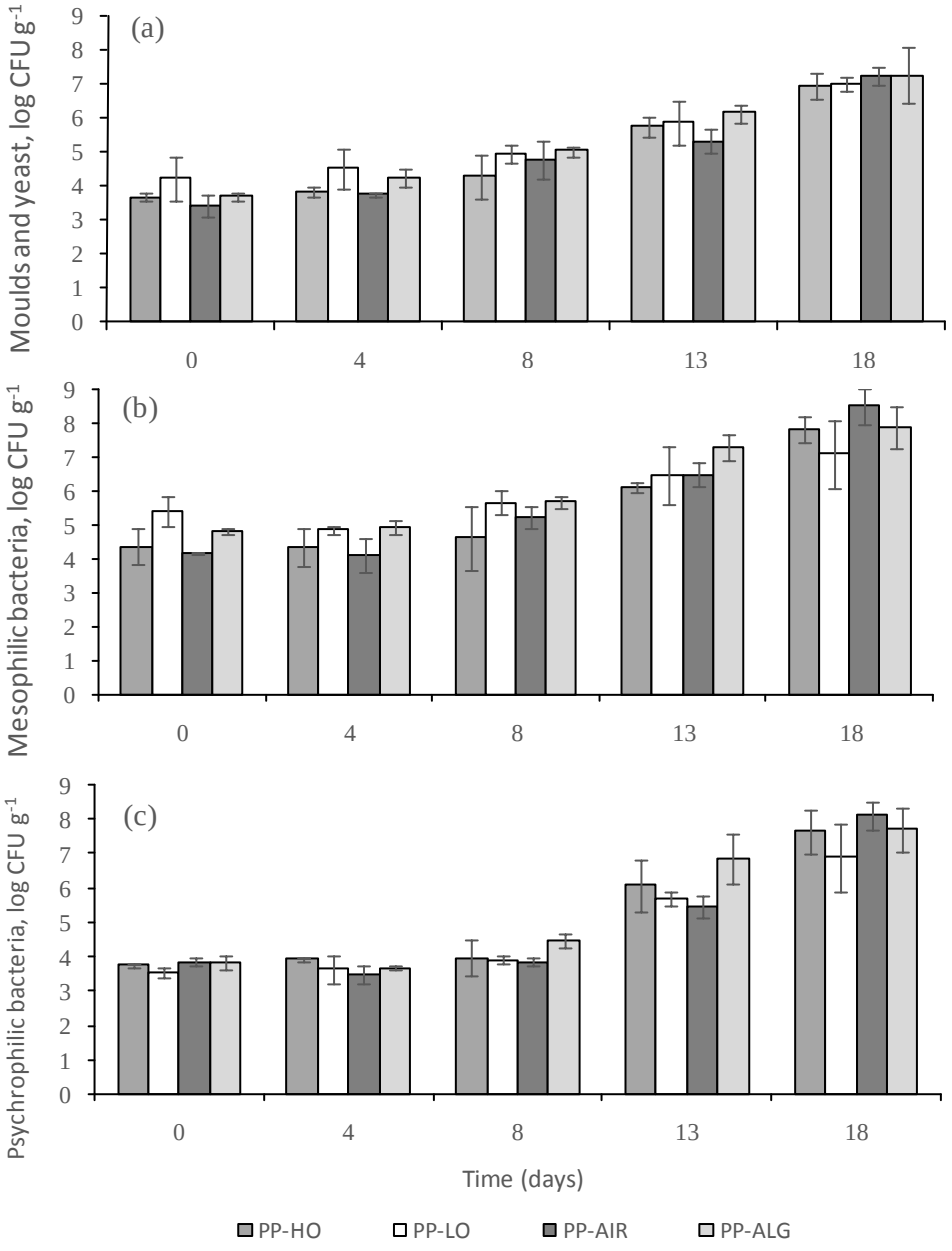


**Fig. 4.** Effect of packaging conditions on growth of moulds and yeast(a), mesophilic bacteria(b) and psychrophilic bacteria(c) in fresh-cut pineapple pieces stored at 5◦C. PP-HO:PP trays filled with high $O_2$ concentration(38–40%); PP-LO:PP trays filled with low $O_2$ concentration(10–12%);PP-AIR:PP trays filled with air; and PP-ALG:PP trays filled with air and containing fresh-cut pineapple coated with alginate. Data shown are mean ± standard deviation.

Neither PP-HO nor PP-LO packaging conditions were effective in reducing microbial counts, although, the shelf-life of pineapple pieces was extended above 11 d refrigerated storage. Santos et al. (2005) did not find significant differences ($p < 0.05$) among fresh-cut 'Perola' pineapple stored under passive modified atmosphere, and under low oxygen (5 and 2%) and high carbon dioxide concentration (5 and 10%) and stored at 5 °C for 10 d. Alginate coating, did not improve fresh-cut pineapple resistance to microbial growth, as it was previously reported by Rojas-Graü et al. (2007b) for 'Fuji' apples using alginate and gellan coatings. These different behaviors could be attributed to the morphological characteristic of the fruit. Soliva-Fortuny and Martín-Belloso (2003) reported that physicochemical attributes of the fruit have an important effect on microbiological shelf-life of fresh-cut fruit.

## 4. CONCLUSIONS

Modified atmosphere packaging allowed conservation of fresh-cut pineapples without undesirable changes in quality parameters during refrigerate storage. The end of shelf-life was signaled by mesophilic bacterial growth at 14 d storage. In addition, all packaging conditions studied avoided both fermentation and deterioration symptoms (ethanol concentration, off-odors and off-flavors) during the first two weeks of storage. Fruit pH, titratable acidity, and soluble solids content did not significantly change, neither among packaging conditions nor the throughout storage time. Texture parameters did not significantly change over time, suggesting that structural characteristics of fresh-cut pineapple pieces were preserved throughout 20 d at 5 °C, without being affected by the packaging conditions. The main color changes observed in fresh-cut pineapple pieces were only attributed to the translucency phenomenon in the fruit flesh. The use of alginate coating significantly improved shelf-life of the cut-pineapple, as reflected in higher juice retention in contrast with the substantial juice leakage observed in the rest of packaging conditions.

Further studies are recommended to evaluate the effect of other edible coatings, maturity grade of fruit, storage time between harvest and processing on juice leakage, flesh color and translucency, sensory attributes and consumer perception of fresh-cut pineapple.

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Content

# INFLUENCE OF MODIFIED ATMOSPHERE PACKAGING ON VOLATILE COMPOUNDS, PHYSICOCHEMICAL AND ANTIOXIDANT ATTRIBUTES OF FRESH-CUT PINEAPPLE *(Ananas comosus)*

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## ABSTRACT

The effects of modified atmosphere packaging on volatile compound content and physicochemical and antioxidant attributes of Gold cultivar fresh-cut pineapples were assessed throughout storage at 5 °C. Fresh-cut pineapple pieces were packed under LO (low oxygen, 12% $O_2$, 1% $CO_2$), AIR (20.9% $O_2$) and HO (high oxygen, 38% $O_2$) headspace atmospheres. Methyl butanoate, methyl 2-methylbutanoate, and methyl hexanoate were the most abundant volatiles regardless of the packaging atmosphere and days of storage; whereas most odor active volatiles were methyl and ethyl 2-methylbutanoate, 2,5-dimethyl-4-methoxy-3(2H)-furanone and ethyl hexanoate. Physicochemical attributes of pineapple did not significantly vary, whereas vitamin C content and total antioxidant capacity were lower for fresh-cut pineapple in HO (488 ± 38 mg/100 mg $_{fw}$ and 54.4 ± 5.7%, respectively) than for LO and AIR packages. Storage life of fresh-cut pineapple was limited to 14 days by volatile compounds losses, and fermentation processes.

## INTRODUCTION

Pineapple is one of the most popular tropical fruits. Its flesh is nutritious, juicy, aromatic and very tasty. However, it is a large fruit which requires labor and space for processing and storage. This inconvenience can be avoided by fresh-cut pineapple products, ready-to-eat, with the freshness of the intact fruit. Nonetheless, the quality of fresh-cut fruits rapidly deteriorates after processing.

Modified atmospheres have been used as alternative treatments to increase the shelf life of fresh-cut products. Reduced oxygen and increased carbon dioxide levels in package headspace can help to slow down respiration reactions as well as changes in color, texture and other quality attributes, but they have been shown to cause changes in flavor volatile content in whole citrus, apples and mangoes and their fresh-cut derivatives *(1)*. Although some data are available on the effect of the use of modified atmospheres on pineapple *(2-4)*, no information has been published on the effects of storage atmosphere on the volatile compounds emitted by pineapple.

Most efforts to preserve the quality of fresh-cut products have been done on appearance and safety attributes, but flavor has become a key factor in consumer preferences and buying decisions. Moreover, Kader *(5)* suggested that flavor attributes are usually lost before other deterioration symptoms appear. Flavor has two components: aroma and taste. Aroma is the result of the combined effect of the presence of various volatiles in the fruit, and taste, the result of content of nonvolatile compounds, thus it is necessary to understand how they change throughout storage.

Pineapple aroma has been studied for many years; most works have been focused on compound identification, which has led to over 400 compounds identified in fresh and processed products *(6-9)*. There is some information on changes with maturity stage and stress treatments for some cultivars *(10 - 14)*; however, cultivars are not always reported, extraction procedures and analyses vary and quantification of volatiles is reported using different relative units, making it difficult to compare. Odor activity values were reported by Tokitomo et al. *(9)*, who found 4-hydroxy-2,5 dimethyl-3(2H)-furanone, ethyl 2-methyl propanoate, ethyl 2-methylbutanoate as the main contributors to pineapple aroma for the super sweet cultivar (F-2000), with odor activity values above 1000. In preliminary tests, we found methyl butanoate, methyl 2-methylbutanoate, and methyl hexanoate were the most abundant components of Gold cultivar pineapple flesh; whereas the largest contributors to pineapple aroma were methyl 2-methylbutanoate, mesifurane (2,5 dimethyl-4-

methyoxy-3(2H)-furanone) and ethyl 2-methylbutanoate. Nonetheless, there is limited information on volatile composition variations of fresh-cut pineapple throughout storage.

The objective of this study was to evaluate the effect of modified atmosphere packaging on the volatile compounds content and odor activity, physicochemical and antioxidant attributes of Gold cultivar fresh-cut pineapple throughout storage at 5°C.

## MATERIALS AND METHODS

**Materials.** Gold cultivar pineapples (*Ananas comosus* L. Merrill) imported from Costa Rica were bought at a local supermarket in Lleida, Spain, and stored at 11 $\pm$ 1 °C overnight prior to processing. Fruits were free from mechanical injuries, insects, pathogens or other defects. Shells had several to most of their eyes partially filled with yellow color, all of them surrounded by green.

Polypropylene trays (500 $cm^3$, MCP Performance Plastic Ltd., Kibbutz Hamaapil, Israel) were sealed with a 64 µm thickness polypropylene film (Tecnopack SRL, Mortara, Italy) with permeability to $O_2$ and $CO_2$ of 110 and 550$cm^3/m^2$/bar/day at 23 °C and 0% relative humidity, respectively.

**Chemicals**. Authentic volatile compounds were used as internal and external standards for fresh-cut pineapple aroma analysis. They were chosen from previous studies with pineapple products. The list of chemicals is given ahead, followed by the odor threshold concentration in water (µg/kg) of each volatile compound, when available. Methyl salicylate (internal standard), and the following external standards: **(1)** methyl 2-methylpropanoate, 6.3 µg/kg *(9)*; **(2)** ethyl propanoate; **(3)** methyl butanoate, 72 µg/kg *(6)*; **(4)** ethyl 2-methylpropanoate; **(5)** methyl 3-methylbutanoate; **(6)** methyl 2-methylbutanoate, 0.1 µg/kg *(6)*; **(7)** hexanal; **(8)** butyl acetate; **(9)** ethyl 2-methylbutanoate, 0.006 µg/kg *(6)*; **(10)** 3-methylbutyl acetate, 2 µg/kg *(15)*; **(11)** 2-heptanone; **(12)** methyl 5-hexenoate; **(13)** methyl hexanoate, 77 µg/kg *(15)*; **(14)** ethyl hexanoate, 1 µg/kg *(15)*; **(15)** hexyl acetate; **(16)** methyl 3-(methylthio)propanoate, 180 µg/kg *(15)*; **(17)** limonene, 10 µg/kg *(15)*; **(18)** (Z)-beta-ocimene; **(19)** 2,5-dimethyl-4-hydroxy-3(2H)-furanone; **(20)** 2,5-dimethyl-4-methoxy-3(2H)-furanone, 0.03 µg/kg *(15)*; **(21)** ethyl heptanoate, 2.2 µg/kg *(15)*; **(22)** ethyl 3-(methylthio)propanoate; **(23)** linalool; **(24)** nonanal, 1 µg/kg *(15)*; **(25)** methyl octanoate, 200 µg/kg *(15)*; **(26)** 4-ethyl phenol; **(27)** methyl (E)-2-octenoate; **(28)** ethyl octanoate; **(29)** geraniol; **(30)** 4-ethyl-2-methoxyphenol; **(31)** ethyl decanoate;

**(32)** alpha copaene. Reagents were purchased from Sigma-Aldrich Química SA, Madrid, Spain.

**Fresh-cut processing.** Working area, cutting boards, knives, containers and other utensils and surfaces in contact with the fruit during processing were washed and sanitized with 200 µL/L sodiumhypochlorite solution at pH 7 to have a maximum sanitizing effect before processing. Pineapple crown leaves were removed, and the fruit was washed twice in two 200 µL/L sodiumhypochlorite solutions for 5 min each, letting excess water drain for 3-5 min after each dip. Fruits were peeled and cut into 1.2 cm thick slices using an electric slicing machine (Food Slicer-6128: Toastmaster Corp, Elgin, IL). Slices were cored and cut into wedges (6-7 g, each) with sharp knives.

Fruit pieces from the bottom, middle and top sections of the fruit were carefully mixed before packaging to minimize the effect of flesh quality differences along the fruit. Fresh-cut pineapple pieces were immersed in 1% citric acid and 1% ascorbic acid solution for 2 min as antibrowning agents and to keep the surface pH low enough to reduce microbial growth. Excess water was drained for 2 min, and 100g pineapple pieces were packaged under the following initial conditions: (a) LO (low oxygen; 12% $O_2$ and 1% $CO_2$), (b) AIR (20.9% $O_2$), and (c) HO (high oxygen; 38 % $O_2$).

Trays were sealed using a vacuum sealer (ILPRA Foodpack Basic V/G, Ilpra, Vigenovo, Italy) and kept at 5 °C for up to 25 days. For each tray, a fruit weight to volume ratio of 2:10 g/mL was used. Two trays (100g of fresh-cut pineapple) from each packaging condition were randomly selected at each sampling date for headspace gas composition analysis, volatiles content, SSC (soluble solids content), TA (titratable acidity), and pH, flesh color, juice leakage, vitamin C, total phenolic content and antioxidant capacity.

**Quality evaluation.** Packages' internal atmosphere, headspace volatile compounds, nonvolatile content and microbiological stability were evaluated on fresh-cut pineapple along storage.

***Package headspace analysis.*** The head space oxygen, carbon dioxide, ethylene, ethanol and acetaldehyde composition of each single tray was analyzed using a gas chromatograph equipped with a thermal conductivity detector (Micro-GP CP 2002 gas analyzer, Chrompack International, Middelburg, The Netherlands) as described by Rojas-Graü et al. *(13).* A 1.7 mL aliquot was withdrawn through an adhesive septum stuck to the film cover, with a sampling needle directly connected to the injection module. The determination of the $O_2$ concentration was carried out by injecting a sample of 0.25 µL to the a CP-Molsieve 5Å packed column (4m×0.32mm,

d.f. = 10mm) at 60 °C and 100 kPa, whereas a portion of 0.33µL was injected into a pora-PLOT Q column (10m×0.32mm, d.f. = 10mm) held at 75 °C and 200 kPa for $CO_2$, ethylene, acetaldehyde and ethanol determinations.

***Volatile components of fresh-cut analysis.*** Volatile component of fresh-cut pineapple were extracted by headspace solid-phase microextraction (SPME) using a polydimethylsiloxane (PDMS) fiber with a 100 µm thickness coating from Supelco Co. (Bellefonte, PA), followed by gas chromatography/mass spectrometry similar to that described by Lamikanra and Richard *(14).* Two trays with 100 g of fresh-cut pineapple packaged in LO, AIR and HO atmospheres were evaluated after 0, 7, 14 and 21 days of storage at 5 °C.

Fruit pieces from each tray were homogenized using an Ultra Turrax T25; two 4 g samples of each homogenate were placed into 20 mL clear glass vials. Methyl salicylate (CAS number 119-36-8) in water solution was added as internal standard (500 µg/kg). Vials were sealed and stirred for 15 min at 30 °C to achieve partition equilibrium of the analytes between the sample and the headspace; then the SPME fiber was inserted through a PTFE-faced butyl septum of cap into the headspace of the vial and held for 15 min (sampling time) while stirring was continued.

Adsorbed substances were desorbed by inserting the PDMS fiber into the gas chromatograph-mass spectrometer (GC-MS) injection port at 250 °C. The desorbed compounds were separated using an Agilent 6890N gas GC coupled to a 5973 mass selective detector (Agilent Technologies España, S.L., Las Rozas, Spain) equipped with a Supelco Equity 5 capillary column of 30 m x 0.25 mm i.d. coated with 0.25 µm thick poly (5% diphenyl/95% dimethylsiloxane) phase (Supelco, Bellefonte, PA). Extraction temperature (30 °C) was chosen with the aim to reproduce naturally occurring aroma profile of fresh pineapple.

The GC was operated in a splitless mode using helium as the carrier gas at a constant rate of 1.5 mL/min. The oven temperature was programmed with an initial temperature of 40 °C, followed by a ramp up to 250 at 20 °C/min and held for 10 min at the final temperature. Mass spectra were obtained by electron ionization (EI) at 70 eV, and spectra range from 40 to 450 m/z.

The SPME fiber was preconditioned at 200 °C for 15 min before each use, and blank runs were done to check the absence of residual compounds on the fiber.

Identification of volatile compounds in pineapple was performed by comparison of mass spectra and retention times of target compounds with that of authentic

reference substances. Thirty-two authentic reference compounds were used to identify and quantify volatile components in fresh-cut pineapple. Aqueous solutions with known concentration of reference volatiles were analyzed using headspace solid-phase microextraction with a 100 µm PDMS coating fiber, followed by GC-MS analysis using identical conditions to those used for pineapple samples.

Quantification was done by the calculation of average relative response factors (RRF) for each volatile compound, using the chromatographic data of prepared water solutions with respect to methyl salicylate, used as internal standard (RRF = peak $area_{analyte}$ x concentration $_{int.std.}$/peak $area_{int.std.}$ x concentration $_{analyte}$).

Aroma profile was defined by the volatiles detected in fresh-cut pineapple under extraction and analysis conditions. Volatiles concentrations throughout storage were determined for all packaging conditions. Volatiles odor contribution to pineapple aroma was assessed by odor activity values (OAVs), calculated as the ratio of actual volatile content to odor threshold concentration in water, given by the literature *(9, 15, 16)*.

***Nonvolatile components of pineapple***. Titratable acidity, pH, and soluble solids content (%) were determined from duplicate 100 g samples of fresh-cut fruit, homogenized using an Ultra Turrax T25 (IKA WERKE, Germany) and filtered (Whatman paper No 1). Soluble solids content was determined using an Atago RX-1000 refractometer (Atago Company Ltd., Japan), pH was directly measured using a pH-meter Crison 2001 (Crison Instruments S.A., Barcelona, Spain) and flesh acidity was assessed by titration with 0.1 N NaOH to a pH end-point of 8.1, and its results were expressed as grams of anhydrous citric acid per 100 g of fruit fresh weight. All measurements were carried out according to AOAC procedures. SSC/TA ratio was calculated for all packaging conditions and evaluation date.

Color was measured directly with a Minolta CR-400 chroma meter (Konica Minolta Sensing, INC. Osaka, Japan), using the CIE color space L*a*b*. The equipment was set up for illuminant $D_{65}$ and 10° observer angle and calibrated using a standard white reflector plate. Sixteen color readings were registered for each section of the fruit. Results were reported as L*, a*, b*.

Juice leakage was determined as described by Montero-Calderón et al. (*2)*. Trays were tilted at a 20° angle for 5 min and accumulated drained juice collected with a 5 mL syringe. Results were reported as liquid volume recovered per 100 g of fresh-cut fruit in the package.

Pineapple vitamin C content, total phenolic compounds content, and antioxidant capacity were measured on duplicated samples. Vitamin C extraction procedure was based on the method proposed by Odriozola-Serrano et al. *(17)*. A portion of 25 g of fruit was added to 25 mL of a 4.5% metaphosforic acid solution with 0.72% of DL-1,4-dithiothreitol (DTT) as reducing agent. The mixture was crushed, homogenized and centrifuged at 22100g for 15 min at 4 °C. The supernatant was vacuum-filtered through Whatman No. 1 filter paper. The samples were then passed through a Millipore 0.45 µm membrane and injected into the HPLC system. Samples were introduced onto the column through a manual injector equipped with a sample loop (20 µL). Separation of ascorbic acid was performed using a reverse-phase C18 Spherisorb ODS2 (5µm) stainless steel column (4.6 mm x 250 mm). The mobile phase was a 0.01% solution of sulfuric acid adjusted to pH 2.6. The flow rate was fixed at 1.0 mL/min. Detection was performed with a 486 absorbance detector (Waters, Milford, MA) set at 245 nm. Identification of ascorbic acid (Scharlau Chemie, SA, Barcelona, Spain) was carried out by HPLC comparing the retention time with those of the standards. Results were expressed as mg of vitamin C in 100 g of pineapple flesh.

Total phenolic content was determined by the colorimetric method described by Singleton et al. *(18)* using the Folin-Ciocalteu reagent. Fresh-cut pineapple samples were homogenized using an Ultra Turrax T25. The homogenate was centrifuged at 6000 g for 15 min at 4 °C (Centrifuge Medigifer: Select, Barcelona, Spain) and filtered through a Whatman No. 1 filter paper. Then, 0.5 mL of the extract was mixed with 0.5 mL of Folin-Ciocalteu reagent, 10 mL of saturated $Na_2CO_3$ solution and distilled water to complete 25 mL. Samples were allowed to stand for 1 h at room temperature before the absorbance at 725 nm was measured. Total phenolic content was determined by comparing the absorbance of duplicated samples with that of gallic acid standard solutions. Results were expressed as milligrams of gallic acid per 100 g of pineapple flesh.

The antioxidant capacity of pineapple flesh was determined using the method described by Odriozola-Serrano et al. *(17)*, by measuring the free radical-scavenging effect on DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. Duplicated samples were homogenized using an Ultra Turrax T25. The homogenate was centrifuged at 6000g for 15 min at 4 °C (centrifuge Medigifer: Select, Barcelona, Spain); 0.01 mL aliquots of the supernatant were mixed with 3.9 mL of methanolic DPPH solution (0.025 g/L) and 0.090 mL of distilled water. The homogenate was shaken vigorously and kept in the darkness for 30 min. Absorption at 515 nm was measured on a

spectrophotometer (CECIL CE 201; Cecil Instruments Ltd. Cambridge, U.K.) against a methanol blank. Results were expressed as percentage decrease with respect to the initial value.

**Data analysis.** Significance of results and statistical differences were analyzed using Statgraphics Plus version 5.1 (Statistical Graphics Co., Rockville, MD). Analysis of variance (ANOVA) was performed to compare quality attributes of fresh-cut pineapple throughout 5 °C storage, using the Duncan test to compare means at a 5% significance level.

## RESULTS AND DISCUSSION

**Package headspace gases.** Oxygen and carbon dioxide headspace concentration throughout storage at 5 °C are shown in Figure 1. Initial atmosphere concentration significantly affected the headspace atmosphere. During the first two weeks of storage, oxygen concentration decreased whereas carbon dioxide content increased, as a result of the metabolic activity of the fresh-cut fruit, together with some gases exchange through the package sealed film.

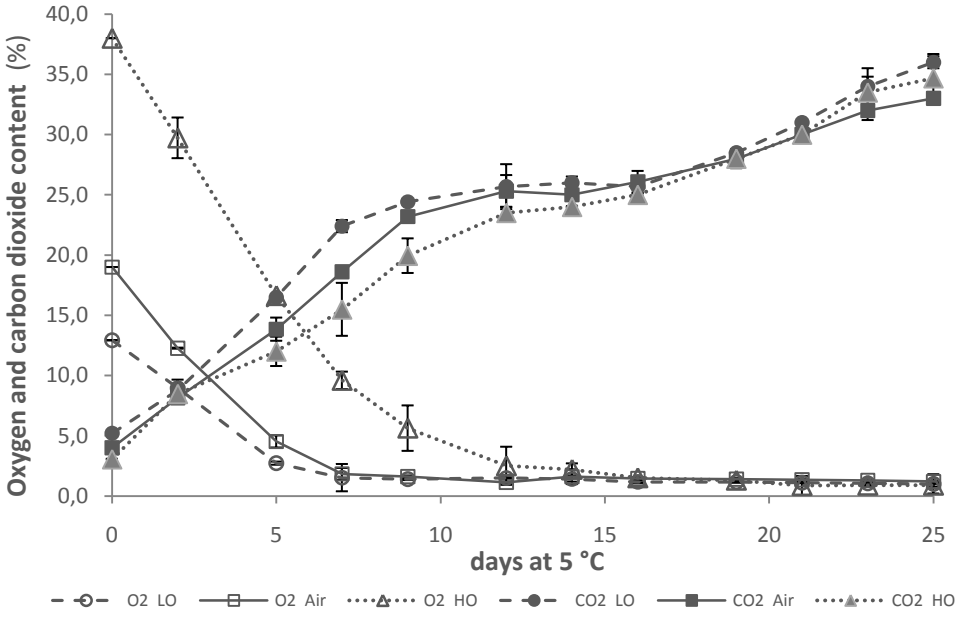



**Figure 1.** Headspace oxygen and carbon dioxide concentrations of Gold cultivar fresh-cut pineapple packaged under three initial atmospheres and stored at 5 °C. LO: 12% $O_2$, 1% $CO_2$; AIR: 20.9% $O_2$; and HO: 38 % $O_2$. Each point in the graph is the mean value of four measurements.

Fresh-cut fruits packed under LO atmospheres, were the fastest to reach oxygen contents close to 2% (5 days) and they were followed by fruit pieces under AIR (7 days) and HO (15 days) atmospheres. In contrast, carbon dioxide content showed a steady increase in the headspace atmosphere until a plateau was reached during the second week of storage, which was later followed by an abrupt increase by the 19$^{th}$ day of storage, attributed to pineapple tissues switch to anaerobic respiration. Changes occurred faster for LO and AIR packages, as compared with those with HO atmospheres.

Our findings showed that fresh-cut pineapple was able to tolerate low oxygen (2% or less) and high carbon dioxide concentrations (up to 25%) for several days, before switching to anaerobic respiration. Fruits packed under LO and AIR tolerated such conditions from the seventh to the 19$^{th}$ day of storage, without ethanol or acetaldehyde production increase. Thus, it is likely that the use of alternative packages with higher permeability characteristics could be used to reduce $O_2$ depletion and $CO_2$ accumulation inside the package headspace, and consequently retard fermentation reactions due to anaerobic behavior.

Ethanol and acetaldehyde headspace content are shown in **Figures 2 and 3**. Small accumulations of both gases were observed during the first weeks of storage. This was attributed to natural occurrence of both compounds in almost every fruit even under aerobic conditions *(19)*, though larger accumulation of both gases also revealed some fermentation process.

Ethanol production in the trays was triggered and showed a sudden increase after 19 days of storage (**Figure 2**), regardless of the packaging atmosphere. Likewise, acetaldehyde content rose markedly (**Figure 3**), with no significant differences among packaging atmospheres.

Concurrent increase of carbon dioxide, ethanol and acetaldehyde accumulation inside all packages after 15 to 20 days of storage (**Figures 1 to 3**) confirmed anaerobic respiration reactions of fresh-cut pineapple. Several authors have reported that low concentrations of $O_2$ and/or high $CO_2$ promote anaerobic respiration which results in the accumulation of acetaldehyde, ethanol and further increase of carbon dioxide content, which is also an intermediate product of fermentation (3, 4, 20-22). The major function of fermentative metabolism is to allow an alternative production of ATP through substrate phosphorylation, which permits the plant tissue to temporarily survive (20), but such changes can affect

flavor and other sensorial attributes and might allow the growth of undesirable anaerobic microorganisms which can be harmful for consumer health. Hence, anaerobiosis is the product response to stress caused by low oxygen or high carbon dioxide atmospheres and/or internal damage of the product. In fact, Pesis (19) suggested that tissue deterioration of overmature fruits may cause an increase in anaerobic respiration because of reduced mitochondrial activity associated with membrane damage and the losses in cells ability to produce enough energy.

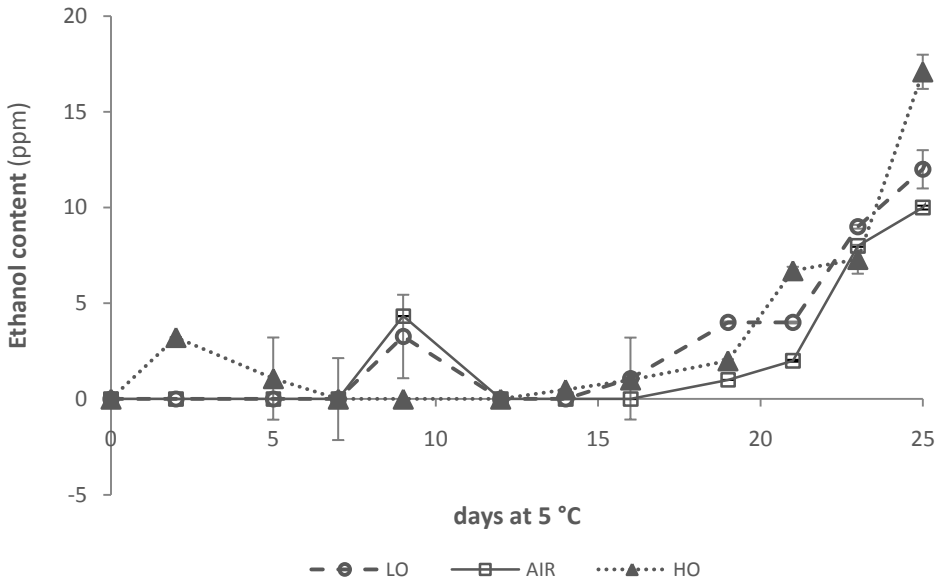



**Figure 2.** Ethanol content in packages' headspace of Gold cultivar fresh-cut pineapple throughout storage at 5 °C. LO: 12% $O_2$, 1% $CO_2$; AIR: 20.9% $O_2$; and HO: 38% $O_2$. Each point in the graph is the mean value of four measurements.

**Volatile compounds of pineapple. Table 1** shows volatile constituents identified and quantified by headspace solid-phase microextraction for Gold cultivar fresh-cut pineapple during 21 days of storage at 5 °C, packed under three initial internal atmospheres (LO, AIR, HO).

*Aroma profile and major components*. Twenty volatile constituents of Gold cultivar pineapple were detected in Gold cultivar pineapple aroma, for fresh-cut fruits packaged under the three atmospheres studied. Esters accounted for 95% of total volatile compounds emitted at 30 °C, methyl butanoate, methyl 2-methylbutanoate, and methyl hexanoate being the most abundant volatile components (roughly 75% of total volatiles). They were followed by another two esters, methyl 3-(methylthio)

propanoate and methyl 2-methylpropanoate, and a furanone, 2,5-dimethyl-4-methoxy-3(2H)-furanone (mesifurane) (**Table 1**).

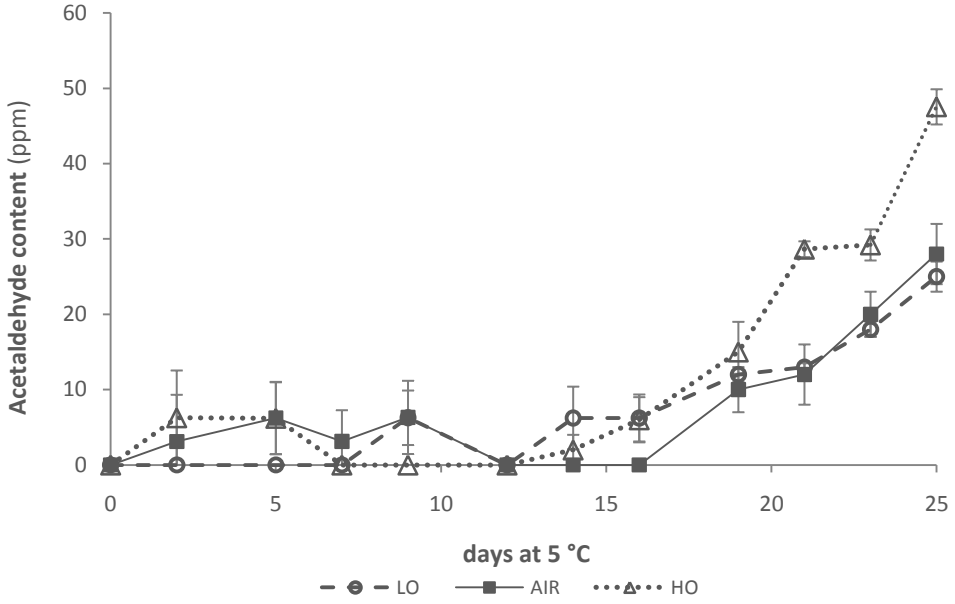



**Figure 3.** Acetaldehyde content in packages headspace of Gold cultivar fresh-cut pineapple throughout storage at 5 °C. LO: 12% $O_2$, 1% $CO_2$; AIR: 20.9% $O_2$; and HO: 38 % $O_2$.

Total volatile compounds content of fresh-cut pineapple was larger for fruit pieces packed in AIR atmospheres during the first two weeks of storage, than for LO and HO. In general, it was observed that volatile compounds content reached maximum concentrations during the second week of storage, regardless of the packaging atmosphere, and decreased thereafter. By day 21, volatiles content decreased in all samples but those packed in air showed the lowest levels of the major components (methyl butanoate, methyl 2-methylbutanoate, and methyl hexanoate) and the total volatiles content, suggesting faster product deterioration beyond 14 days of storage.

In contrast, methyl hexanoate, methyl 3-(methylthio)propanoate, and mesifurane emission decreased throughout the 21 days of storage in fruit pieces stored in AIR. Thus, initial package headspace atmosphere affected total content and individual content of volatile compounds in pineapple pieces, as well as their relative composition, since volatiles content varied throughout storage, although the aroma profile constituents were the same along storage.

**Table 1.** Changes in volatiles content (µg/kg) of Gold cultivar fresh-cut pineapple packed in LO, AIR and HO atmospheres throughout storage at 5 °C.

| | Volatile compound | Storage time (days) | | | |
|---|---|---|---|---|---|
| | | 0 | 7 | 14 | 21 |
| LO (12% $O_2$, 1% $CO_2$) | methyl 2-methyl propanoate | 383 a A | 728 b B | 495 a A | 485 a A |
| | methyl butanoate | 2435 a A | 3481 b A | 2687 a B | 2460 b B |
| | methyl 2-methyl butanoate | 2105 a A | 2271 b A | 2064 a A | 2161 ab B |
| | ethyl 2-methylbutanoate | 23,0 a A | 191,7 c B | 46,6 ab B | 67,7 b B |
| | 3-methylbutyl acetate | 3,4 a A | 55,0 b A | 7,8 A | 27,8 ab A |
| | methyl 5 hexenoate | 1,8 a A | 0,0 a A | 2,4 a B | 0,7 a A |
| | methyl hexanoate | 1163 b A | 899 a A | 913 a B | 773 a A |
| | ethyl hexanoate | 101 a B | 1091 c C | 286 a B | 605 b B |
| | methyl 3-(methylthio) propanoate | 500 a B | 433 a A | 451 a A | 439 a B |
| | limonene | 11,9 a A | 12,9 a A | 11,5 a AB | 11,7 a A |
| | (Z)-beta-ocimene | 3,9 a A | 4,0 a A | 3,8 a A | 3,2 a A |
| | 2,5-dimethyl-4-metoxy-3(2H)-furanone | 357 a A | 319 a B | 350 a C | 345 a B |
| | ethyl heptanoate | 2,1 a A | 17,7 b B | 6,7 a B | 14,8 b B |
| | ethyl 3-(methylthio) propanoate | 7,6 a A | 58,1 c B | 13,6 a A | 32,7 b B |
| | nonanal | 2,2 a B | 1,7 a A | 2,8 a C | 2,8 a A |
| | methyl octanoate | 36,1 a A | 58,4 b A | 44,7 ab B | 61,7 b C |
| | methyl (E) octenoate | 0,6 a A | 1,3 b A | 1,0 ab A | 1,4 b B |
| | ethyl octanoate | 1,7 a B | 41,6 b B | 16,4 a AB | 43,5 b B |
| | ethyl decanoate | 0,7 a A | 7,8 b C | 2,1 a A | 5,8 b A |
| | alpha copaene | 9,5 a A | 14,5 a A | 11,2 a A | 21,7 b A |
| | *Total extracted volatile compounds in LO* | 7148 | 9687 | 7415 | 7564 |
| AIR (20.0% $O_2$) | methyl 2-methyl propanoate | 561 a B | 580 a A | 699 b B | 604 a B |
| | methyl butanoate | 3113 b B | 3135 b A | 3559 c C | 1250 a A |
| | methyl 2-methyl butanoate | 2464 c B | 2276 b A | 2437 bc B | 1034 a A |
| | ethyl 2-methylbutanoate | 39,4 a B | 76,4 b A | 222,7 c C | 22,9 a A |
| | 3-methylbutyl acetate | 8,1 a A | 13,8 a B | 273,4 b B | 2,9 a A |
| | methyl 5 hexenoate | 2,2 a A | 2,3 a B | 0,0 a A | 0,8 a A |
| | methyl hexanoate | 1452 c B | 1147 b B | 494 a A | 536 a A |
| | ethyl hexanoate | 213 a C | 648 b B | 1272 c C | 119 a A |
| | methyl 3-(methylthio) propanoate | 644 c C | 582 bc B | 455 b A | 241 a A |
| | limonene | 8,9 a A | 24,9 a A | 7,6 a A | 13,8 a A |
| | (Z)-beta-ocimene | 7,2 b A | 2,7 a B | 1,3 a A | 2,6 a A |
| | 2,5-dimethyl-4-metoxy-3(2H)-furanone | 487 c B | 367 b C | 198 a A | 217 a A |
| | ethyl heptanoate | 3,5 a A | 12,8 b B | 12,6 b C | 4,1 a A |
| | ethyl 3-(methylthio) propanoate | 13,4 a B | 31,3 b A | 97,6 c B | 10,0 a A |
| | nonanal | 1,0 a AB | 3,3 b B | 1,9 a B | 1,9 a A |
| | methyl octanoate | 99,9 c B | 80,6 b AB | 13,9 a A | 24,6 a A |
| | methyl (E) octenoate | 0,8 a A | 1,7 b B | 0,7 a A | 0,8 a A |
| | ethyl octanoate | 8,8 a C | 36,4 b B | 24,2 b B | 10,1 a A |
| | ethyl decanoate | 1,1 a A | 4,0 b B | 6,3 c B | 1,7 a A |
| | alpha copaene | 13,3 a AB | 24,3 b A | 11,8 a A | 13,0 a A |
| | *Total extracted volatile compounds in AIR* | 9142 | 9048 | 9788 | 4110 |
| HO (38% $O_2$) | methyl 2-methyl propanoate | 606 b B | 777 c B | 643 b B | 463 a A |
| | methyl butanoate | 2270 b A | 3363 c A | 2238 b A | 1403 a A |
| | methyl 2-methyl butanoate | 2056 a A | 2646 b B | 1990 a A | 2186 a B |
| | ethyl 2-methylbutanoate | 16,3 a A | 99,8 b A | 12,5 a A | 83,1 b B |
| | 3-methylbutyl acetate | 8,1 a A | 10,3 a A | 10,3 a A | 20,3 a A |
| | methyl 5 hexenoate | 1,33 a A | 1,51 a AB | 1,33 a AB | 0,82 a A |
| | methyl hexanoate | 1197 b A | 1427 c C | 1040 b B | 638 a A |
| | ethyl hexanoate | 37,3 a A | 335,0 b A | 30,7 a A | 515,1 c B |
| | methyl 3-(methylthio) propanoate | 312 ab A | 353 b A | 307 ab A | 268 a AB |
| | limonene | 16,9 a A | 11,5 a A | 13,1 a B | 10,5 a A |
| | (Z)-beta-ocimene | 4,9 a A | 1,9 a A | 3,8 a A | 2,4 a A |
| | 2,5-dimethyl-4-metoxy-3(2H)-furanone | 318 c A | 196 a A | 313 c B | 267 b A |
| | ethyl heptanoate | 1,5 a A | 3,8 a A | 1,3 a A | 8,8 b AB |
| | ethyl 3-(methylthio) propanoate | 7,3 a A | 14,1 a A | 7,7 a A | 29,3 b C |
| | nonanal | 0,3 a A | 1,0 ab A | 0,6 a A | 2,1 b A |
| | methyl octanoate | 34,9 a A | 100,4 b B | 30,4 a B | 43,8 a B |
| | methyl (E) octenoate | 0,7 ab A | 1,9 c B | 0,5 a A | 1,1 b B |
| | ethyl octanoate | 0,8 a A | 11,2 ab A | 0,7 a A | 20,6 ab A |
| | ethyl decanoate | 1,1 a A | 1,4 a A | 0,9 a A | 4,6 b A |
| | alpha copaene | 25,9 a B | 19,5 a A | 19,7 a A | 12,9 a A |
| | *Total extracted volatile compounds in HO* | 6916 | 9376 | 6664 | 5979 |

Values are means of four replicate pineapple samples, for each compound and atmosphere; concentration means along storage with the same lowercase letters are not significantly different

(Duncan $p < 0.05$); likewise, means with the same uppercase letters reveal not significant differences between packaging atmosphere concentration for each specific compound (Duncan $p < 0.05$).

The largest reduction of volatiles emission, observed in AIR packages during the third week of storage, was concurrent with the carbon dioxide, ethanol and acetaldehyde production increase inside the packages. These observations suggested that anaerobic metabolism speeded up volatile losses and other deteriorative reactions, and confirm the differentiated effect of the initial headspace atmosphere. In fact, Beaulieu and Baldwin *(1)*, indicated that ester formation in apples originates from oxygen-dependent reactions, thus, depletion of that gas could negatively affect production of esters, which could be the case for some pineapple volatiles, since oxygen concentration rapidly decreased.

Comparison among packaging atmosphere treatments showed that volatiles content of the three major volatiles (methyl butanoate, methyl 2-methylbutanoate and methyl hexanoate) was smaller for LO and HO atmospheres on day zero. Differences were attributed to the effect of the packaging procedures, since the sequence of the system used in this study included three steps: vacuum extraction of air from the package headspace, gas mixture flush over the fresh-cut fruit and heat seal of the tray lids. Thus, vacuum pressure used to replace air also produced the physical extraction of some volatiles.

During the following days, the content of the volatiles built up to maximum levels for all packaging conditions, and later depleted sooner for LO and HO than for AIR. Similar results were found by Beaulieu and Baldwin *(1)*, who also reported a temporary increase in ester accumulation in apples during the first days after processing, explained by the product response to wound stress and reduced resistance for volatiles escaping from fruit tissues once the fruit skin is removed. For pineapple, Lamikanra *(14)* observed significant changes in volatiles content in Gold cultivar pineapple after one day of storage, for thin slices (1-2mm) cut from damaged fruit flesh close to exposed cut surfaces, demonstrating stress effect due to fresh-cut processing.

It was interesting to notice that, despite the fact that carbon dioxide and oxygen composition in the package headspace of all trays become very similar along the second week of storage, volatile composition varied in different proportions and rates. Volatiles content in fresh-cut pineapple packed in LO and HO atmospheres decreased earlier (during the second week of storage) than those packed in AIR (during the third week), but an abrupt decrease was observed in AIR packages by the 21$^{st}$ day of storage for methyl butanoate, methyl and ethyl 2-methylbutanoate, methyl hexanoate, and mesifurane. Thus, in general, volatiles in fresh-cut pineapple

pieces packed in AIR were better withheld through the first two weeks of storage, whereas volatiles content in fruit pieces under LO and HO atmospheres showed important losses from the seventh to the 14$^{th}$ day of storage, with little variation thereafter.

On the other hand, even though only one packaging film and one volume to product ratio were used, it is likely that they contributed to protect losses of volatiles, since volatile emission of fruit pieces was maintained for at least 2 weeks.

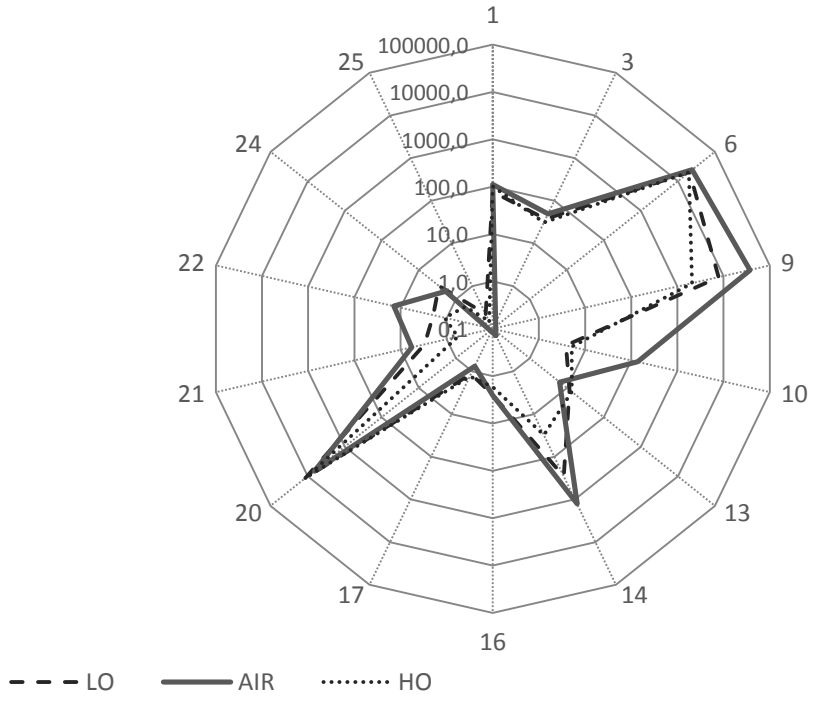
Package headspace composition and volatiles content showed little signs of deterioration during the first two weeks of storage, whereas symptoms of fermentation processes and losses of volatiles were evident during the following days, suggesting 14 days as the maximum storage period for fresh-cut pineapple at 5 °C in AIR atmospheres.

*Most odor activity volatiles.* Contribution of volatile compounds to pineapple aroma was determined as odor activity values (OAV) at the end of the second week of storage (**Figure 4**). The most active volatile compounds in Gold cultivar fresh-cut pineapple were methyl 2-methylbutanoate, ethyl 2-methylbutanoate, ethyl hexanoate, and mesifurane, regardless of the packaging atmosphere. In addition, it was observed that despite the finding that methyl butanoate and methyl hexanoate concentrations in pineapple flesh were high, their contribution to the fruit aroma was much smaller than that of other volatiles.

Odor activity values of volatile compounds in LO, AIR and HO atmospheres showed the same quality profile from a qualitative point of view (**Figure 4**). However, OAVs of volatiles in pineapple samples packaged in AIR, were similar to or exceeded those of the fruit packed in LO or HO, for most of the odor active volatiles. In example, OAVs of volatile methyl 2-methylpropanoate, methyl 2-methylbutanoate and mesifurane showed that they have a similar impact on pineapple aroma for all three atmosphere conditions, whereas OAVs of ethyl 2-methylbutanoate, 3-methylbutyl acetate, ethyl hexanoate and ethyl 3-(methylthio)propanoate were larger for fruit pieces packed under AIR than for those under LO and HO atmospheres, indicating larger contribution of such volatiles to fresh-cut pineapple aroma packed in AIR headspace atmosphere on the 14$^{th}$ day of storage.

On the other hand, it should be highlighted that, despite the fact that OAVs are useful to determine relative contribution of volatile compounds to aroma perception, they are based on individual behavior of volatile compounds in water solutions, hence, they do not consider any synergetic effect among odor active volatiles and how aroma perception could be altered by changes in volatile concentrations. In that sense, Ferreira *(23)* pointed out that perception of

volatiles in complex mixtures such as wines could be affected by alcohols and other volatile compounds, because they affect the solubility of other volatiles and their real contribution to aroma. Some odors can be enhanced while some others can be hidden in a complex mixture, and the mix of volatile compounds can act as an aromatic buffer with little changes in perceptions when one or several constituents' content varies. The above suggests the need to complement our results with sensory evaluations to determine the actual effect of observed changes in volatiles on pineapple aroma perception throughout storage.




Numbers around the graph correspond to pineapple volatile compounds (Table 1); LO: 12% $O_2$, 1% $CO_2$; AIR: 20.9% $O_2$; and HO: 38 % $O_2$.

**Figure 4.** Odor activity values (OAVs) of volatiles compounds in fresh-cut pineapple (Gold cultivar) stored under LO, AIR, and HO modified atmosphere conditions after 14 days of storage at 5 °C. LO: 12% O2, 1%CO2. AIR: 20.9% O2. HO: 38% O2. Numbers around the graph correspond to pineapple volatile compounds: **(1)** methyl 2-methylpropanoate; **(3)** methyl butanoate; **(6)** methyl 2-methylbutanoate; **(9)** ethyl 2-methylbutanoate; **(10)** 3-methylbutyl acetate; **(13)** methyl hexanoate; **(14)** ethyl hexanoate; **(16)** methyl 3-(methylthio)propanoate; **(17)** limonene; **(20)** 2,5-dimethyl-4-methoxy-3(2H)-furanone; **(21)** ethyl heptanoate; **(22)** ethyl 3-(methylthio)propanoate; **(24)** nonanal; **(25)** methyl octanoate.

**Non volatile components of pineapple. Table 2** shows average physicochemical and antioxidant characteristics of fresh-cut pineapple packed under LO, AIR and HO initial headspace concentrations.

*Physicochemical parameters*. Soluble solids content (SSC), titratable acidity (TA), pH, the ratio of soluble solids to acidity (SSC/TA) and color parameter L*a*b* did not show significant changes ($p < 0.05$) among either packaging atmosphere nor storage period at 5 °C. These results were explained by the fact that pineapple is a nonclimacteric fruit and, as such, shows little changes in its properties, once it is harvested, and because storage at 5 °C slowed down deterioration processes and microbiological growth *(12)*. Soluble solids content was maintained at 13.3 ± 0.3 %, titratable acidity at 0.78 ± 0.03 $mg_{citric\ acid}$/100 mg $_{fresh\ weight}$, and fruit pH at 3.43 ± 0.08.

**Table 3.** Average physicochemical and antioxidant properties of nonvolatile components of fresh-cut pineapple quality stored under LO, AIR and HO atmospheres throughout 21 days at 5 °C.

| Quality attribute | LO | AIR | HO |
|---|---|---|---|
| ***Physicochemical properties*** | | | |
| SSC (%) | 13.3 ± 0.3 a | 13.2 ± 0.3 a | 13.4 ± 0.3 a |
| TA (mg $_{citric\ acid}$/100 mg $_{fw}$) | 0.79 ± 0.04 a | 0.79 ± 0.04 a | 0.77 ± 0.02 a |
| SSC/TA | 17.0 ± 0.8 a | 16.7 ± 1.0 a | 17.5 ± 0.6 a |
| pH | 3.45 ± 0.07 a | 3.42 ± 0.07 a | 3.45 ± 0.08 a |
| Color | | | |
| L* | 68.3 ± 3.9 a | 67.3 ± 4.6 a | 67.4 ± 5.7 a |
| a* | -3.9 ± 0.8 a | -3.6 ± 0.8 a | -3.3 ± 0.9 a |
| b* | 33.5 ± 3.5 a | 33.3 ± 3.7 a | 32.1 ± 4.2 a |
| ***Antioxidant properties*** | | | |
| Vitamin C (mg/100 mg $_{fw}$) | 548 ± 34 b | 561 ± 39 b | 488 ± 38 a |
| Antioxidant capacity (%DPPH inhibition) | 59.0 ± 4.1 a | 58.9 ± 4.3 a | 54.4 ± 5.7 a |

Means with the same lower case letters are not significantly different (Duncan $p<0.05$). LO: 12% $O_2$, 1% $CO_2$; AIR: 20.9% $O_2$; and HO: 38 % $O_2$.

Color parameters L*, a* and b* for Gold cultivar fresh-cut pineapple showed some variability among samples due to fruit heterogeneity, but not significant differences among packaging conditions or storage time. Additionally, fresh-cut fruit did not show any browning symptom throughout the first 21 days of storage, corroborating color stability of this cultivar flesh attributed to absence of

PPO (polyphenoloxidase) activity found in previous studies *(2)*. Average L*, a* and b* for pineapple flesh were kept at 67.7 ± 4.7, -3.9 ± 0.8, and 33.0 ± 3.8, respectively. Furthermore, it should be highlighted that color stability throughout time and among packaging conditions is a positive attribute for fresh-cut processing, since it can largely contribute to preserve freshness appearance of the finished product.

In spite of little variation of other physical parameters, juice leakage from pineapple pieces significantly increased ($p < 0.05$) during storage and changed with the different initial atmospheres (**Figure 5**). Fresh-cut fruits in the trays initially flushed with HO atmosphere did not lose juices during the first 9 days of storage, and showed less juice accumulation throughout storage. In contrast, fresh-cut pineapple in LO packages exhibited the largest juice built up.

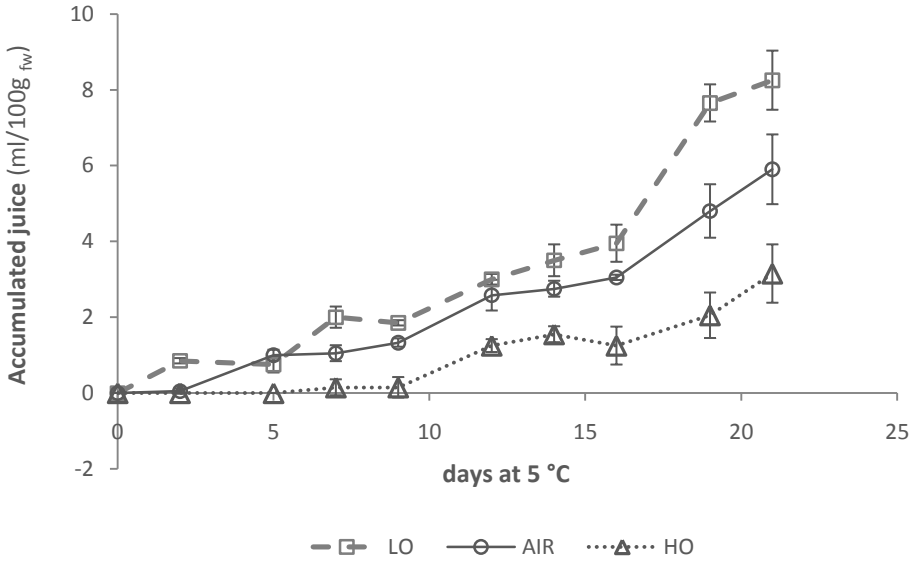



**Figure 5.** Accumulated juice in fresh-cut pineapple packages throughout storage at 5°C. LO: 12% $O_2$, 1% $CO_2$; AIR: 20.9% $O_2$; and HO: 38 % $O_2$. Each point in the graph is the mean value for two fresh-cut pineapple trays.

Juice drainage from pineapple pieces can be explained by the physical damage caused during processing. As the fruit shell is removed and further cuts are performed, tissues are injured, cell structure is disrupted and membranes are weakened. These damages reduce internal fluid withholding capacity of fruit tissues, increase the product surface area in contact with the surrounding atmosphere and

favor tissues' deterioration during storage, which further reduce tissues' ability to retain juices.

In addition, our results suggested that headspace $CO_2$ concentrations contribute to increase juice leakage. Elevated concentrations of this gas could have caused a toxic effect on tissues' physiology or at least accelerated them. This effect was also observed by Budu and Joyce *(3)* in fresh-cut slices of Smooth cayenne cultivar. Our results also agree with those found in previous studies *(2)*, for which juice leakage rapidly increased after 6-8 days of storage, when internal concentration went beyond 20% $CO_2$. The use of packages more permeable to $CO_2$ is suggested to avoid internal atmosphere build up of high concentrations of this gas.

*Antioxidant characteristics.* Vitamin C content of fresh-cut pineapple was very stable throughout the 20 days storage for all packaging conditions, but differences were found among fruits stored in AIR or LO atmospheres and those under HO (**Table 2**). For fresh-cut pineapple pieces stored under AIR and LO headspace atmospheres, the average concentration was nearly 555 ± 36 mg of vitamin C/100 $mg_{fw}$, whereas that under HO atmosphere was significantly lower (488 ± 38 mg/100 $mg_{fw}$) ($p < 0.05$).

Lower concentration of vitamin C in fresh-cut pineapple stored under HO atmosphere was explained by larger oxygen headspace concentration and lower carbon dioxide content, which favored vitamin C oxidation, as observed by Soliva-Fortuny et al. *(24)* and Odriozola-Serrano et al. *(25, 26)* who found increased vitamin C degradation of fresh-cut pears and tomato slices, for higher oxygen content in the package headspace. The same authors observed very small changes in vitamin C content for tomato slices during 11 days of storage at 5 and 10 °C and over 21 days at 4 °C for slices stored under modified atmosphere packaging (5kPa $O_2$ and 5 kPa $CO_2$), and attributed increased stability to low oxygen concentration. Pineapple is recognized as a good source of vitamin C, thus high content and stability of this vitamin in fresh-cut pineapple are important for consumer acceptability.

**Figure 6** shows the effect of packaging conditions on total phenolic compounds (TPC) of fresh-cut pineapple during storage at 5 °C. An increase in TPC was observed during the first days of storage under LO and AIR atmospheres, followed by a steady decrease throughout storage. Initial increase of TPC could be explained by the increase of phenolic compounds produced as a response to injuries occurring during processing, with the aim to repair wound damage and resist microbial invasion *(*26).

The same authors observed enhanced oxidative stress induced by too low $O_2$ and high $CO_2$ concentrations inside tomato slice packages and attributed it to increase of phenylalanine lyase (PAL) activity, which is the key enzyme that uses phenylalanine to synthesize phenolic compounds. In contrast, TPC of fresh-cut pineapple stored under HO atmosphere did not increase during the first storage days, but continually decreased throughout storage. TPC were significantly different in HO atmospheres as compared with LO and AIR atmospheres, explained by larger oxygen concentration in the package headspace during the first two weeks of storage, which could have favor oxidative processes in the fruit.

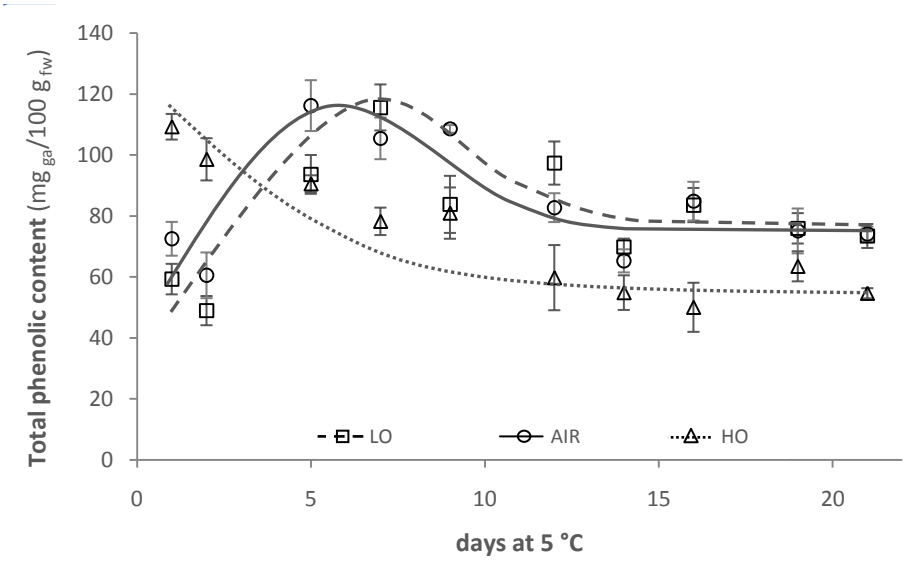



**Figure 6.** Total phenolic compounds changes during storage of fresh-cut pineapple at 5 °C. LO: 12% $O_2$, 1% $CO_2$; AIR: 20.9% $O_2$; and HO: 38 % $O_2$. Each point in the graph is the mean value for two fresh-cut pineapple trays.

On the other hand, total antioxidant capacity, given as %DPPH inhibition, is shown in **Table 2**. It was found that antioxidant capacity was very stable along storage, and results showed similar behavior as vitamin C, since fruit pieces packed in AIR and LO atmosphere showed a larger antioxidant capacity (58.9 ± 4.1%), compared with those in HO atmospheres (54.4 ± 5.7%), which was also explained by larger oxygen availability inside the packages.

Then, passive modified atmosphere packaging (AIR) allowed the preservation of volatile compounds and nonvolatile components in fresh-cut pineapple of the cultivar Gold during storage at 5 °C for at least 14 days of storage and permitted

longer withholding of volatile emission and antioxidant attributes than LO and HO atmospheres. The use of an oxygen enriched atmosphere (HO) reduced juice leakage from pineapple pieces, but favored losses in volatile compounds content and antioxidant characteristics and accelerated acetaldehyde production after the second week of storage. Methyl 2-methylbutanoate, ethyl 2-methylbutanoate, 2,5-dimethyl-4-methoxy-3(2H)-furanone and ethyl hexanoate were the most active volatiles in pineapple aroma throughout storage and could be used as quality indicators of fresh-cut pineapple throughout storage. High concentrations of $CO_2$ promoted volatile losses, juice leakage, and anaerobic respiration.

Vitamin C content and antioxidant capacity did not vary throughout time, but they were better preserved under LO and AIR atmospheres, whereas mechanical properties, color parameters L*, a* and b*, SSC, TA and pH did not significantly change over time, under any of the packaging conditions.

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# DISCUSSION

# GENERAL DISCUSSION

This work was focus on the evaluation of Gold cultivar pineapple flesh quality profile along the fruit and the influence of the use of passive (with and without an edible coating) and active modified atmospheres on the quality of fresh-cut pineapple throughout storage, with the aim to identify key elements to improve product consistency, homogeneity and durability.

The purpose of this section is to integrate and globally discuss the results obtained throughout the studies and highlight the most relevant findings.

It was divided in two parts:

1. Internal quality profile of physicochemical, mechanical, and antioxidant properties, in addition to the aroma volatile compounds characterization of Gold cultivar pineapple flesh.

2. Effect of packaging conditions on the quality and shelf-life of fresh-cut pineapple.

## 1. INTERNAL QUALITY PROFILE OF GOLD CULTIVAR PINEAPPLE FLESH.

Pineapple internal quality profile was assessed considering the fruit morphology, since it is a composited fruit, containing multiple fruitlets fused together and arranged around a central axis (core). Gold cultivar pineapple flesh quality attributes along the fruit were studied. Physicochemical, mechanical, and antioxidant attributes, were determined for fruit flesh samples from the bottom, middle and top cross-sections of the fruit of the fruit, which were later completed with the determination of the pineapple aroma profile and the odor activity of volatile compounds. Objective methods were used for quality assessment to avoid the effect of subjective appreciation of the analyst.

Moreover, it should be highlighted that pineapple has a progressive ripening pattern, which starts near the base of the fruit and moves upward to the crown; consequently, within each fruit, it is likely to find fruitlets at distinct stages of ripeness with differentiated quality attributes.

The following discussion is focused on the comparison of pineapple quality attributes of three cross-sections cut along the central axis of the fruit, as well as the identification those which better discriminate among fruit pieces from different sections of the fruit.

## 1.1 Physicochemical characteristics

Color, soluble solids content (SSC), titratable acidity (TA), pH, water content, juiciness and enzymatic activity of polyphenol oxidase (PPO) and peroxidase (POD) were determined for pineapple flesh prepared from three cross-sections cut along the central axis of the fruit (bottom, middle and top thirds). Little changes were found in physicochemical attributes of pineapple, though some showed significant differences.

***Color:***

CIE Lab color parameters, L*, a*, and b*, slightly varied along pineapple fruit, but differences were overlapped by a high intrinsic variability (standard deviation of the order of 5 to 10%). Differences were attributed to tissue heterogeneity of the flesh, evident to the naked eye. Multiple fruitlets are composed of carpellary and non-carpellary tissues, with three seed cavities or locules, ovules, placenta, seed, sepals and a blossom cup (Rohrbach and Johnson, 2003). Since color measurements are based on reflectance characteristics of the surface area, it is likely that observed differences among tissues influenced instruments readings, and, in spite of the large number of color measurement repetitions (8 to 16 readings per sample), kept a high variability.

Gold cultivar pineapple flesh luminosity, measured as L* values ranged from 64 to 71; whereas a* values varied from -3.3 to -5.7, revealing the presence of chlorophyll compounds, and b* values were in the range between 32 and 49, because of yellowish internal color of this cultivar. Range of color parameters found in this study agree with those reported by other authors (Gil and others 2006, Hernández and other 2006, Marrero and Kader 2006, Montero-Calderón and others 2007), however no references were done about section of the fruit from which pineapple flesh pieces were cut.

In general, L* and b* values were higher and a* lower for fruit pieces cut from the top third of the fruit ($p \leq 0.05$). It should be noticed, that results corresponded to average characteristics of specific fruit batches used for processing, which could be larger or smaller along individual fruits or batches, and could also be affected by the

place of origin where the fruit is grown, pre- and postharvest factors and even the size of the fruits. During preliminary tests, it was observed that color variability differences from one extreme of the fruit to the other largely varied among individual fruits and batches of the same cultivar, which highlight the importance for the fresh-cut industry to understand such differences and to establish tolerance limits for the fruit to be processed, in order to reduce color differences in the final fresh-cut pineapple products offered to the consumer.

Some color related alterations are flesh darkening and translucency, described as water soaked appearance of the tissues. No browning symptoms were observed on pineapple flesh regardless of the cross-section of the fruit from which it was cut, but some translucency was observed throughout storage, as discussed ahead.

***SSC, TA, SSC/TA, pH:***

SSC and TA significantly varied along the fruit ($p<0.05$), whereas the SSC and SSC/TA ratio increased from the top to the bottom of the fruit, the opposite was true for titratable acidity. Differences were explained by fruit morphology and ripening stage differences of the fruitlets along the pineapple. pH parameter showed small or no variations along the fruit.

SSC in Gold cultivar flesh used in this study varied from 12.6 to 14.0%, titratable acidity varied from 0.45 to 0.80 mg $_{\text{citric acid}}$/100 $g_{fw}$, pH from 3.41 to 3.58, whereas SSC/TA ratio from 14 to 29. In general, SSC, pH and SSC/TA increased from the top to the bottom third of the fruit, in contrast with acidity, which decreased. It was also observed that SSC/TA ratio was useful to magnify differences between fruit flesh SSC and acidity, making it easier to discriminate among individual pineapple fruit pieces.

On the other hand, SSC and acidity are directly related to fruit taste perception. They are the nonvolatile components of flavor, and they have shown to have a synergistic effect, in the sense that sweetness of two fruits with identical SSC but different acidity (TA) can be easily differentiated and could contribute to product acceptance or rejection.

Gold cultivar flesh SSC were similar to those reported for Perola, Red Spanish, and Josepine cultivars, larger than those for Smooth cayenne pineapples, and smaller than those for Flhoran41 cultivar (Brat and others 2004, Montero-Calderón and others 2008, Santeso and others 2005, Santos and others 2005, Sarzi and Durigan 2002, Shamsudin and others 2007, Torres-Prado and others 2003).

***Water content and juiciness:***

Water content significantly decreased from the top to the bottom third of the fruit from 86 to 81%,, explained by differences in stages of maturity. These results contrasted with flesh juiciness, measured as released fluids during compression tests, which resulted significantly larger in the middle third of pineapple (12.1 $g_{juice}/100g_{fw}$). Differences were attributed to fruit morphology, since fruitlets size, shape and orientation vary along the fruit (Py and others 1987) because of the shell restrain to growth. Fruitlets in the middle third of the fruit are generally larger and their internal structure could vary and favor juice leakage. In fact, Harker and others (1997) correlated released fluids from fruit tissues with cell size, structure, arrangement and failure mechanism.

***Enzymatic activity:***

Peroxidase (POD) activity increased nearly 10% ($p<0.05$) from 6.02 ± 0.11 to 6.70 ± 0.15 UA/min/mL, from the top to the bottom third of the fruit). Differences were attributed to the variation in the maturity stage of the fruitlets along the pineapple. However, the influence of this enzyme activity on other fruit quality characteristics and changes is not clear.

POD activity has been associated with flavor and color changes in raw fruits and vegetables (off-flavors and off-odors), ripening and cell wall degradation. In this study, good correlations coefficients were found between POD activity and water content, total phenolic compounds content, and SSC/TA ratio, but not with the color parameters or the mechanical characteristics of the flesh, regardless of the position inside the fruit. In addition, no off-flavors or off-odors were found in the fruit flesh, for any of the positions inside the fruit. Variation of POD activity found in Gold cultivar pineapple is congruent with that reported by Chitarra and da Silva (1999) for Smooth cayenne cultivar near the central axis and close to the shell of the fruit, but the participation of this enzyme on browning and other deterioration reactions have been discarded by several authors (Avallone and others 2003, Dahler and others 2002, Lamikanra 2002, Zhou and others 2006).

Polyphenol oxidase (PPO) activity was not detected in fresh-cut pineapple prepared from Gold cultivar in any of the three sections of the fruit evaluated. This is a remarkable characteristic for fresh-cut processing purposes, since PPO activity is generally associated with tissue darkening for other pineapple cultivars (Dahler and others 2002, Chitarra and da Silva 1999, Eduardo and others 2008), but not for the Gold cultivar.

## 1.2 Mechanical characteristics

Pineapple texture attributes were assessed by six different approaches, measuring the response of pineapple flesh samples to different types of forces (compression, penetration, shear and combined forces), with the purpose to determine the test that could better discriminated among fruit pieces from different parts of the pineapple.

Little changes were observed in pineapple flesh response to mechanical forces, but a large variability was observed for all mechanical properties of the fruit, which overlapped differences along pineapple fruit. Such results were explained by pineapple flesh mixed tissues, resulting in very heterogeneous fruit pieces.

***Uniaxial compression, penetration and shear resistance***

Pineapple flesh responded to uniaxial compression, penetration and shear tests in similar way. Resistance force increased as the probe (cylinder, flat end needle or knife, respectively) moved into the flesh sample, up to the point when the tissue suddenly fractured; then, it continued increasing, passing through multiple peaks due to subsequent pineapple tissues failures, as the probe advanced forward into the fruit sample. Hardness, fracturability and associated work (force-deformation curve) did not significantly vary among fruit pieces from different sections of the fruit, except for the shear test, which showed larger values for pineapple flesh cut from the bottom third of the fruit. Shear force ranged from 6.5 ± 1.2 to 10.0 ± 3.5 N, whereas shear work from from 19 ± 6 to 41 ± 24 N mm). Maximum shear force and work increase were attributed to ripening stage differences on pineapple fruitlets along the fruit; since as the fruitlets ripen, tissue elasticity could increase due to compositional changes, and consequently, their resistance to shear force.

For uniaxial compression test, flesh samples from different sections of the fruit were deformed up to 25% strain. Multiple peaks were observed on the force-deformations curves as the probe pushed down the sample, but maximum resistance force was not reached. Such results suggested small and continuous failures of the flesh tissues, provoking losses in the membrane integrity. Damages became larger as the probes advanced, disrupting cell walls and other structural support of flesh tissue.

The high variability and little changes found for hardness was reported by Hajare and others (2006) for fresh and gamma irradiated pineapple slices stored at 8 °C and Gil and others (2006) and Eduardo and others (2008) for Tropical Gold and Smooth cayenne cultivars using probes from 3 to 13.5 mm diameter, while Chonhenchob and

others (2007) found some changes during 10 °C storage for Phuket cultivar fresh-cut pineapple.

***Combined forces resistance***

Resistance to combined forces in mini-Ottawa (compression and extrusion) and Kramer (compression, shear and extrusion) tests were similar to those from compression tests, with large variability for hardness and total work overlapping differences between the response of pineapple flesh cut from different parts of the fruit. Large variability on pineapple flesh hardness was also reported by López-Malo and Palou (2009), for both fresh and blanched pineapple slices.

***Texture profile analysis***

Texture profile analysis was run for pineapple flesh, in order to determine if texture properties could be explained by one or several texture parameters of this test. Results show little differences among fruit samples which were overlapped by variability among fruit pieces, as for the other mechanical properties tests, and also explained by fruit flesh heterogeneity. Similar results were reported by Kingsly and others (2009) who use the same procedure to evaluate high-pressure effect on pineapple slices texture attributes during processing, and did not find significant differences for hardness, cohesiveness and springiness. Results indicated that TPA test is not the best tool to discriminate among pieces cut from different cross-sections of pineapple fruit.

Even though texture has been recognized as a very important quality parameter for many fruits, no significant differences ($p>0.05$) were found among fruit pieces along the pineapple with any of the six measuring procedures, explained by intrinsic large variability overlapping possible differences.

## 1.3 Antioxidant characteristics

Vitamin C, total phenol content and total antioxidant capacity where assessed as important nutritional quality parameters of pineapple, and to follow up how they change throughout storage.

***Vitamin C***

Vitamin C ranged from 305 ± 40 to 351 ± 15 mg/$kg_{fw}$ with no statistical differences among the different parts of the fruit. Though differences were found when compared among pineapple fruit batches used for different experiments, which varied from 488 ± 38 to 561 ± 39 mg/$kg_{fw}$. Differences among fruits from the same cultivar can be explained by pre- and postharvest factors. Hajare and others (2006)

and Miller and Schaal (1951) reported differences up to 150% in ascorbic acid content between individual pineapple fruits, without making differences of the section of the fruit used for the experimental determinations. Average published values for vitamin C content for Gold cultivar pineapple range from 310 to 790 mg/100 $g_{fw}$, compared with 260 to 350 mg/$kg_{fw}$ for Smooth cayenne cultivar (Gil and others 2006, Marrero and Kader 2006, Ramsaroop and Saulo 2007). In addition to cultivar effect, large variability on vitamin C content can be affected by multiple factors, like the clone, solar radiation, air temperature and acidity, and it could be negatively related to internal browning symptoms (Paull and Chen 2003).

***Total phenol content (TPC)***

TPC significantly varied along the fruit, it decreased roughly 20% from the more mature fruitlets in the bottom third (50.8 ± 5.1 $mg_{gallic\ acid}$/100 $g_{fw}$) to those in the top third (40.3 ± 1.0 $mg_{gallic\ acid}$/100 $g_{fw}$). These results agree with changes reported Dahler and others (2002) for Smooth cayenne pineapple as the fruit ripened, who also reported changes throughout storage at 10 °C (37 to 51 mg/100 $g_{fw}$).

***Antioxidant Capacity***

Antioxidant capacity of pineapple flesh, determined on the basis of the DPPH radical scavenging, did not significantly varied ($p<0.05$) among pineapple flesh samples from the different parts of the fruit. It ranged from 42.0 ± 5.1 % to 45.6 ± 5.6 % of DPPH inhibition. Variations were found among different pineapple batches, from 54.4 ± 5.7 to 59.0 ± 4.1%, as they do between cultivars. Leong and Shui (2002), reported pineapple antioxidant capacity of 85.6 ± 21.3 mg/100g, for fruit bought at a local market in Singapore (cultivar not reported).

## 1.4 Aroma profile and odor contribution

Fruit aroma is the result of the balance of a few or many volatile compounds; it is among one of the most valuable attributes for fresh-cut fruits, because it plays an important role on consumer perception and product acceptability. The aroma profile of Gold cultivar pineapple flesh was identified and quantified at 30 °C, to resemble the natural occurring volatiles balance at consumption temperature.

***Most abundant volatile compounds:***

Large differences were observed in the relative response factors (RRF) of volatile compound standards, as it was expected, because of the disparity size, shape, mass, volatility of individual compounds, and affinity to PDMS fiber.

Most abundant volatile compounds were defined as those with the highest concentrations, whereas those with the biggest OAV´s were addressed as the greatest contributors to pineapple aroma.

Twenty volatile compounds were identified and quantified as constituents of fresh-cut pineapple aroma. Fifteen of them were esters, which accounted for 90 to 95 % of total aroma content. Methyl butanoate, methyl 2-methyl butanoate, and methyl hexanoate were the major components of pineapple aroma profile, with concentrations above 1000 $\mu g/kg_{fw}$, followed by 2,5-dimethyl-4-methoxy-3(2H) furanone (mesifuran), methyl 2-methyl propanoate, and methyl 3-(methylthio) propanoate, which together took for over 97% of total volatiles.

All of the Gold cultivar aroma profile constituents were previously reported (Elss and others 2005, Brat and others 2004, Tokitomo and others 2005) as important components in various pineapple products (fresh and processed) and cultivars, though their composition and relative importance varied. Also, a few compounds, previously reported as significant components, were not detected for the fresh flesh of the Gold cultivar, either because of their low content, cultivar differences and /or extraction procedures and conditions.

From a qualitative point of view, aroma profile constituents were consistent along the pineapple cross-sections. However, their concentration significantly varied ($p<0.05$) among pineapple flesh from the three cross-section of the fruit. However, total content of pineapple volatiles increased from the top to the bottom third of the fruit, changing from 7560 to 10910 $\mu g/kg_{fw}$.

Increase in volatile compounds concentration was attributed to changes occurring during ripening in pineapple fruits, which starts several weeks before harvest. Sugar accumulation increases while the acid content is depleted, enzymes activity varies, and volatiles production increases. Synthesis of volatile compounds from free amino acids, carbohydrates and through β-oxidation of fatty acids occur (Cadwallader 2005, Beaulieu and Baldwin, 2002). In fact, some of the important esters found for pineapple as major components, have been reported as the product of the transformation of amino acids or fatty acids. Ethyl and methyl-2-methyl butanoate compounds are produced from isoleucine, while 2-methyl propanoate from valine, whereas butanoates and hexanoates are synthetized from free fatty acids. The other major compound found in this study for Gold cultivar flesh was mesifuran (20), produced from *D*-glucose or *D*-fructose.

Methyl 2-methyl propanoate, methyl butanoate, methyl 2-methyl butanoate, ethyl 2-methyl butanoate and mesifuran increased from 15 to 66% from the top to the bottom third of the fruit, but the largest changes were observed for ethyl 2-methylbutanoate and ethyl hexanoate, which increased 110 and 585%, respectively, fin the same direction. In contrast, methyl 3-(methylthio) propanoate concentration decreased 25% from the top to the bottom cross-section of the fruit. Such results suggested volatile production and concentration increase with maturity stage from the fruitlets from the top to the bottom sections.

***Most odor active volatiles:***

Volatile compounds have differentiated threshold concentrations; some can be detected in very small concentrations, while others require much larger content. Volatile compounds contribution to pineapple aroma was calculated as the ratio of actual volatile concentration to its odor threshold concentrations in water, known as OAV or Odor Active Value (Tokitomo and others 2005).

Most odor active volatiles in Gold cultivar pineapple flesh are methyl 2-methyl butanoate, ethyl 2-methyl butanoate and mesifuran, with OAV above 10000 times their limit of detection; they were followed by ethyl hexanoate, methyl 2-methyl propanoate, 3-methylbutyl acetate, methyl hexanoate, and methyl 3- (methylthio) propanoate.

It was also found that odor activity values (OAV) varied throughout three cross-sections along the central axis of the fruit, in most of the cases they increase from the top to the bottom third of the fruit, although a reduction was observed for methyl 3 (methylthio) propanoate. Such changes results in a modification on the balance among volatiles compounds, which could alter the pineapple aroma perception.

Results showed that nonvolatile components of pineapple, measured as SSC and SSC/TA varied along the fruit, and were directly relates to volatiles ethyl 2-methyl butanoate, mesifuran and ethyl hexanoate, but not to other volatile compounds.

Thus, quality profile of pineapple flesh varied along the top, middle and bottom cross-sections of the fruit for most quality attributes, that is, color, SSC, TA, water content, juiciness, enzymatic activity, vitamin C, total phenolic content, volatiles compounds concentration and their odor activity, and hardness assessed by the shear test. Small or no differences were observed for other mechanical responses to compression, shear, penetration, and combined forces.

Intrinsic variation of the quality profile of pineapple along its length showed the gaps in the attributes of the edible portion of the fruit existing from one extreme of the fruit to the other. This natural variability of the fruit cannot be ignored, but on the contrary, it should be considered to establish fruit selection and processing criteria, leading to more reproducible and homogeneous quality of fresh-cut pineapple packages.

Once the quality profile was assessed, the stability of pineapple flesh quality attributes was studied throughout storage.

## 2. INFLUENCE OF PACKAGING CONDITIONS ON FRESH-CUT PINEAPPLE FLESH QUALITY

This part of the work focus on the effect of the packaging conditions on the fresh-cut pineapple processed using the whole edible portion of pineapple fruits. Alternative packaging conditions were used, including active (LO: 12 % $O_2$, 1% $CO_2$) and HO: 38% $O_2$) and passive (AIR: 20.9% $O_2$) modified atmospheres. The effect of the use of an alginate edible coating on pineapple flesh was also studied, as a complement to passive modified atmosphere.

Package headspace, physicochemical and antioxidant characteristics, as well as the changes in volatile compounds of fresh-cut pineapple at 30 °C were evaluated through storage.

### 2.1 Package headspace concentration

Fresh-cut pineapple respiration activity changed headspace concentration inside individual packages, as oxygen was consumed and carbon dioxide produced.

Oxygen content significantly decreased over time ($p \leq 0.05$) in every package showing a steady decreasing pattern up to the 20$^{th}$ day of storage (about 0.6% per day) without reaching an equilibrium concentration throughout storage, when 50g/100ml product-to-package ratio was used. During the entire period of storage, oxygen headspace concentration was never below 2%, avoiding anaerobic conditions and the formation of off-flavors and off-odors. Slow changes in headspace $O_2$ composition could be explained by the low respiration rate of pineapple at 5 °C (2 – 4 µl kg $^{-1}$ h$^{-1}$ at 7 °C, Kader, 2006), product-to-package ratio and the permeability characteristics of the sealed film covers.

On the other hand, the $CO_2$ level significantly increased during storage ($p \leq 0.05$) at a similar rate in all packaging conditions (0.5% per day), with no significant differences, explained by oxygen availability inside the packages, which preserved aerobic metabolism of the fruits during the first two weeks of storage. Similar results were found for the alginate coated fruit pieces in fresh-cut pineapple, which did not altered package headspace concentration of oxygen, carbon dioxide, ethylene or ethanol either. Moreover, neither off-flavors nor off-odors were detected in fresh-cut pineapples during the first 15 days of storage at any of the packaging conditions.

In addition, an increase in the headspace ethanol concentration was detected in all packages headspace from the 15$^{th}$ day of storage on. It was concurrent with the appearance of off-odors, suggesting fermentation reactions associated with anaerobic metabolism, favoured by increased carbon dioxide concentration inside the fresh-cut fruit.

Changes in oxygen concentration were faster when a larger product-to-package ratio was used (2:10), resulting in marked differences among packaging conditions ($p<0.05$) during the first two weeks of storage.

Oxygen depletion rate decreased as concentration reached levels near 2%, by the seventh day of storage inside LO and AIR packages, but after 15 days for HO packages. In contrast, carbon dioxide content increased in all packages until a plateau was reached at levels near 25% $CO_2$, during the second week of storage at 5°C, which was followed by an abrupt increase by the 19$^{th}$ day, attributed to the switch to anaerobic metabolism. The gap between oxygen consumption and carbon dioxide production curves were explained by differences in initial oxygen headspace concentrations.

Small concentrations of ethanol and acetaldehyde gases in the package headspace were observed during the first two weeks of storage. These two compounds naturally occur in almost every fruit, even under aerobic conditions and are precursors of natural aroma compounds (Pesis, 2005). However, ethanol and acetaldehyde headspace concentrations showed an abrupt increase after the second week of storage, regardless of the packaging atmosphere. Concurrent increase of carbon dioxide, ethanol and acetaldehyde accumulation inside all packages confirmed anaerobic respiration reactions of fresh-cut pineapple, promoted by low concentrations of $O_2$ and/or high $CO_2$. Anaerobic metabolism stimulates the accumulation of acetaldehyde, ethanol and further increase of carbon dioxide content, which is also an intermediate product of fermentation (Ke and others 1994,

Lange and others 1997, Budu and others 2005, 2007, Oms-Oliu and others 2007). However, such changes can affect flavor and other sensorial attributes and might allow the growth of undesirable anaerobic microorganisms which can be harmful for consumer health. In fact, Pesis (2005) suggested that tissues deterioration of over-mature fruits may cause an increase in anaerobic respiration because of reduced mitochondrial activity associated with membrane damage and the losses in cells ability to produce enough energy.

## 2.2 Physicochemical characteristics

Little changes were found in physicochemical attributes of pineapple, but some of them were significant throughout storage at 5 °C:

***Color***

CIE Lab color parameters, L*, a*, and b*, show no significant differences among packaging condition, though some changes were observed throughout storage. Differences were overlapped by color variability due to tissue heterogeneity. However, it should be pointed out, that color differences and variability were dependent on the batch of fruits, which could be due to ripeness stage of the fruit, pre- and postharvest factors and others. Alginate coating did not affect color parameters values. Color stability, given by little color differences along storage, is a very convenient quality attribute of Gold cultivar attribute, which can largely favor fresh-cut pineapple products appearance during marketing and distribution.

Changes in pineapple flesh color can be affected by other changes, such as the appearance of translucent tissues, which affect the reflection characteristics of the sample. Translucent or water soaked tissues were observed for fresh-cut pineapple used for the first study of changes throughout storage. Development of translucent tissues is not completely understood, but they appear to be associated with the pre-harvest factors and as a response to stress caused by processing. In fact, some color differences reported in literature for several cultivars, for L* and b* could be associated with translucent phenomena, since they are not concurrent to typical changes in a* values associated to tissue browning.

***SSC, TA, SSC/TA, pH :***

Titratable acidity (TA), soluble solids content (SSC) and pH showed little changes during storage and not significant differences were found neither over time nor among packaging conditions, regardless of the product-to-package ratio. These results were attributed to non-climacteric behavior of pineapple fruits.

***Juice leakage:***

The quality of fresh-cut pineapple might be affected by an excessive leak of fruit juice accumulated inside the packages. Juice leakage significantly increased over time regardless of the use of active or passive modified atmospheres packaging. Small quantities of juice were accumulated during the first 8 days of storage, but an abrupt increase was observed from the 11$^{th}$ day on for passive and active atmosphere packages. In contrast, alginate coated fruit pieces showed much less juice leakage throughout storage (up to fourfold reduction), attributed to an increase in the water vapor resistance provided by the edible coating, which protected the fruit piece from leaking and reduced accumulated juice. The good barrier water properties exhibited by alginate coating has been previously reported by Rojas-Graü and others (2008) who found that 2% alginate edible coating applied to fresh-cut apples was effective in preventing water losses.

On the other hand, neither off-flavors nor off-odors were noticed on alginate coated pineapple pieces up to the 15$^{th}$ day of storage, so no evidence was found that retaining liquid inside the cut pieces could have accelerated product deterioration.

Juice leakage was small during the first 9 days of storage regardless of the packaging condition for the second essay, where 100 g fruit were packed in 500 mL trays. However, from then on, juice leakage rapidly increased. Fresh-cut fruits in the trays initially flushed with HO atmosphere did not lose juices during the first 9 days of storage, and showed less juice accumulation throughout storage. In contrast, fresh-cut pineapple in LO packages exhibited the largest juice built up.

Juice losses from pineapple pieces can be explained by the physical damage caused during processing and removal of the fruit shell. Tissues are injured, cell structure disrupted and membranes are weakened, reducing the internal fluids withhold capacity of fruit tissues. The above is aggravated by larger exposed surface to the surrounding atmosphere and tissues deterioration during storage, which further decreases tissue ability to retain juices.

It was interesting to note an apparent relation between carbon dioxide headspace concentration and fresh-cut pineapple juice leakage, which could have caused a toxic effect on tissues physiology or at least accelerated them. This effect was also observed by Budu and Joyce (2005) in fresh-cut slices of Smooth cayenne cultivar. Such observation agree with results of previous study with fewer fresh-cut pineapple were top package (1:10 ratio), though the relation was not noticed before, since juice

leakage rapidly increased after 6-8 days of storage, when internal concentration went beyond 20% $CO_2$.

The use of packages with higher permeability to $CO_2$ is suggested to avoid internal atmosphere build up of high concentrations of this gas.

## 2.3 Antioxidant characteristics

***Vitamin C.***

Content of vitamin C was very stable throughout the 20 days fresh-cut pineapple. Fresh-cut pineapple pieces stored under AIR and LO headspace atmospheres had an average concentration of 555 ± 36 mg vitamin C/100 $mg_{fw}$, respectively, while that under HO atmosphere had significant ($p<0.05$) lower (488 ± 38 mg/100 $mg_{fw}$). Lower concentration of vitamin C in fresh-cut pineapple stored under HO atmosphere were explained by larger oxygen headspace concentration and lower carbon dioxide content, which favored vitamin C oxidation, as reported by Soliva-Fortuny and others (2002) and Odriozola-Serrano and others (2008 a,b).

***Total phenolic compounds (TPC).***

An increase in TPC was observed during the first days of storage under LO and AIR atmospheres, followed by a steady decrease throughout storage. Initial increase of TPC could be explained by the increase of phenolic compounds produced as a response to injuries occurred during processing, with the aim to repair wounds damage and resist microbial invasion (Odriozola-Serrano and others 2008a). In contrast, TPC of fresh-cut pineapple stored under HO atmosphere did not increase during the first storage days, but continually decreased throughout storage. TPC were significantly different in HO atmospheres as compared with LO and AIR atmospheres, explained by larger oxygen concentration in the package headspace during the first two weeks of storage, which could have favor oxidative processes in the fruit.

***Antioxidant capacity.***

The antioxidant capacity, given as %DPPH inhibition, was very stable along storage, and results showed similar behavior to vitamin C. Fruit pieces packed in AIR and LO atmosphere showed a larger antioxidant capacity (58.9 ± 4.3 and 59.0 ± 4.1%, respectively), compared with those in HO atmospheres (54.4 ± 5.7), which was also explained by larger oxygen availability inside the packages.

## 2.4 Mechanical characteristics.

A texture profile analysis (TPA) was performed to evaluate mechanical characteristics changes of fresh-cut pineapple throughout storage. This method was chosen because of its multiple parameter response, as a strategy for better assessment of possible changes.

Results of TPA were given as force and work per 100 grams of fresh weight, to avoid any distortion due to the effect of size differences among pineapple pieces. TPA curves registered multiple fracture peaks as the probe advanced into the fresh-cut pineapple piece during the first compression cycle; most of the time the first fracture peak (fracturability) occurred before the maximum peak force was achieved, but no pattern was found for maximum peak location or for the number of fracture peaks along the first compression cycle. This behavior was explained by the non uniform structural characteristics of pineapple flesh tissues, as discussed before.

No significant differences were found either among fresh-cut pineapple packaging conditions or throughout the 20 days of storage at 5 °C for any of the TPA parameters studied, because fruit heterogeneity made results variability to overlap differences among treatments. Average hardness and fragility forces for pineapple pieces were 337 ± 55 and 320 ± 59 N/100 g, respectively. Average adhesiveness was 3.4 ± 2.7 N/100 g, gumminess values were 37.4 ± 7.8 N/100 g, and dimensionless parameters cohesiveness and resilience were 1.8 ± 1.5 and 0.115 ± 0.015, respectively.

Nonetheless, absence of significant changes in TPA parameters of fresh-cut pineapple pieces over time at 5 °C also indicates that fruit structure of pineapple pieces was maintained throughout storage and was not affected by packaging conditions, and that packaging material properly protected the integrity of fresh-cut pineapple pieces. This observation coincides with the appearance of the pineapple pieces, which kept their shape and size throughout the 20 days of storage. Similar behavior have been reported by Gil and others (2006) who did not find significant differences in whole and fresh-cut pineapples firmness (3mm tip penetration test) for Tropical Gold cultivar after 9 days of storage at 5 °C.

## 2.5 Aroma profile

Volatile constituent of pineapple aroma profile were evaluated along storage for fresh-cut pineapple pieces. The effect of passive (air) and active modified

atmospheres (LO and HO) was studied, once the natural barrier of the fruit (shell) was removed, and the product surface area increased during processing.

Packaging conditions did not affect aroma volatiles profile found for the fresh fruit, the same components were present throughout storage, with no additional peaks revealing the presence of other compounds.

***Most abundant volatile components***

Esters constitute 95% of the total volatile compounds emitted at 30 °C for fresh-cut pineapple. Methyl butanoate, methyl 2-methyl butanoate and methyl hexanoate were the major volatile components of fresh-cut pineapple for all packaging atmospheres and all throughout storage, accounting for roughly 75% of the total volatiles content. They were followed by methyl 3-(methylthio) propanoate, methyl 2-methyl propanoate, and 2-5-dimethyl-4-methoxy-3 (2H) furanone.

Total volatile compounds extracted from fresh-cut pineapple were larger for fruit pieces packed in AIR atmospheres during the first two weeks of storage, than for LO and HO. In general, volatile compounds content reached a maximum concentration during the second week of storage, regardless of the packaging atmosphere, and decreased by the 21$^{st}$ day of storage. Fruit pieces packed in air retained their volatiles longer than in other atmospheres, but showed an abrupt decrease during the third week, concurrent with an increase in carbon dioxide, ethanol and acetaldehyde content of the packages headspace. Such changes suggested that anaerobic metabolism speeded up volatile losses and other deteriorative reactions.

Packaging system for active modified atmospheres can also affect volatiles content due to vacuum pressure exerted while individual trays are sealed. Apparent volatile losses were observed for LO and HO atmospheres.

On the other hand, little differences among aroma volatiles content packed under different conditions were explained by packaging permeability to volatile compounds and the ratio of free volume to product mass inside each tray, which caused a rapid oxygen consumption and carbon dioxide production inside the packages, leveling up the internal gas concentrations of all trays to low oxygen and high carbon dioxide content after 5 to 12 days. In fact, it is likely that volatile compounds content changes along storage were associated to low oxygen and high carbon dioxide content, as well as to low temperature storage, as suggested by Beaulieu and Baldwin (2002), who indicated that ester formation in apples originates from oxygen-dependent reactions.

This results confirmed the importance of packaging material permeability characteristics to oxygen and carbon dioxide, but also to volatile compounds; in the first case, to provide proper atmosphere conditions able to prevent anaerobic respiration, and in the latter case, to act as a barrier to reduce volatiles diffusion and losses, which trigger fruit taste and aroma losses.

***Most odor active volatile compounds***

The same volatiles were identified as major contributors to pineapple aroma than those found for fruit quality profile assessment. Methyl 2-methyl butanoate, ethyl 2-methyl butanoate, ethyl hexanoate and mesifuran were the most active volatile contributors to fresh Gold cultivar pineapple aroma, regardless of the packaging atmosphere.

In general, OAV's of most odor active volatiles were larger for fresh-cut pineapple packed in AIR than for that packed under LO or HO. Methyl 2-methyl propanoate, methyl 2-methyl butanoate and mesifuran were alike for the three atmospheres, at the $14^{th}$ day of storage. Whereas, ethyl 2-methyl butanoate, 3-methylbutyl acetate, hexyl acetate, ethyl heptanoate and ethyl 3(methylthio) propanoate were larger for fresh-cut pineapple packed under AIR than LO or HO atmospheres. These results indicated that AIR atmospheres better withhold volatile compounds activity up to the $14^{th}$ day of storage at 5 °C.

In consequence, volatiles with the highest OAV and largest variability among packaging conditions are recommended as quality indicators throughout storage, together with the total volatile content, which give a quick indication of the changes occurring with volatile compounds in pineapple samples.

## 2.6 Microbial stability

Development of moulds-yeast, mesophilic and psychrophilic bacteria on fresh-cut pineapple during cold storage of at 5 °C was studied. No significant differences ($p<0.05$) were found among packaging conditions for microbial growth; however, significant differences were observed through storage time. Initial population ranged from 3 to 4 log CFU $g^{-1}$ for moulds and yeasts at day 0 and reached 7 to 7.5 log CFU $g^{-1}$ after 18 days of storage. Similar increase was observed for mesophilic and psychrophilic bacteria reaching 7 to 8.5 log CFU $g^{-1}$, respectively after 18 days at 5 °C.

Mesophilic bacteria in fresh-cut pineapple containers reached the maximum permitted limit (7 log CFU/g, BOE, 2001) after the $13^{th}$ day of storage, whereas

psychrophilic bacteria and yeast and moulds reached it at the 15$^{th}$ and 18$^{th}$ day, respectively for all packaging conditions.

Mesophilic bacteria counts were used to define the shelf-life of fresh-cut pineapple, since these microorganisms were the first to overpass regulation limits. Packaging in active modified atmosphere prolonged the shelf-life of Gold fresh-cut pineapple by 15 days of storage compared with the rest of packaging conditions which limited their shelf-life to 11 days by mesophilic bacterial growth.

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# CONCLUSIONS

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Gold cultivar pineapple flesh quality attributes vary along the top, middle and bottom cross-sections of the fruit for the majority of the quality attributes. The content of the most abundant volatile compounds in pineapple aroma and the most odor active volatiles increased from the top to the bottom third of the fruit, as well as the total volatile compounds content. The color parameters L*, a* and b*, SSC, TA, vitamin C, phenolic compounds, water content, POD activity are also affected by the position from which the flesh is cut.

Mechanical properties variations among Gold cultivar pineapple flesh from the bottom, middle and top thirds of the fruit were overlapped by intrinsic high variability among flesh samples for compression, penetration, Kramer, Ottawa and Texture profile analysis procedures. Shear test hardness and work was the only texture parameters able to discriminate among fruit pieces from different parts of the fruit.

Natural occurring aroma profile of Gold cultivar pineapple flesh at 30 °C consisted of 20 volatile components, and did not change along the fruit from a qualitative point of view. Methyl butanoate, methyl 2-methyl butanoate, methyl hexanoate and mesifuran were the most concentrated volatile components of Gold cultivar pineapple; their concentration increased 15 to 66% from the top to the bottom of the fruit. The most odor active volatiles of pineapple aroma of this cultivar were methyl and ethyl 2-methyl butanoate, mesifuran and ethyl hexanoate, whose concentration increased from 15 to 585% along the fruit, from top to bottom.

The most abundant and the most odor active volatiles of fresh-cut pineapple stored under modified atmosphere packages changed over time, reaching a maximum value during the second week of storage, and rapidly decreasing thereafter. Passive atmospheres (AIR) preserve volatile compounds longer than LO and HO packaging.

Juice leaked from the pineapple pieces increased along the three weeks at 5 °C. It was better withhold by using an alginate coating and under HO atmospheres. High $CO_2$ and/or low $O_2$ atmospheres are likely to promote juice leakage and fermentation processes.

Vitamin C content and antioxidant capacity did not vary throughout time, but they were better preserved under LO and AIR atmospheres. Whereas mechanical

properties, color parameters L*, a* and b*, SSC, TA and pH did not significantly change over time, under any of the packaging conditions.

The end of fresh-cut pineapple shelf-life was signaled by mesophilic bacterial growth at 14th day of storage at 5 °C, rapid volatile compounds losses and juice accumulation inside the packages.

Thus, volatile and nonvolatile components should be assessed for fruits for processing selection and homogeneous and reproducible fresh-cut pineapple products, whereas odor active volatiles content, juice leakage and microbial stability should be used for quality evaluation throughout storage.

## FINAL REMARKS

The results of this research work highlighted the need of adequate selection of the fruits for processing and fresh-cut packaging, and the use of alginate coating to reduce juice leakage. Volatile losses, juice leakage and microbial growth were identified as the limiting factors of fresh-cut pineapple quality preservation.

Future research should focus on sensory evaluation essays to validate the relative impact of odor active volatile compounds (OAV larger than 1) on fresh-cut pineapple perception of aroma and taste and how it changes throughout storage.

Further studies on the effect of fruit size, pre- and postharvest handling addressed to reduce the gap of the quality attributes of pineapple flesh along the fruit, the juice leakage and volatile compound losses. Studies could include plant nutrition, ripening induction, seasonal changes, harvesting indices, packages with increased permeability to $O_2$ and $CO_2$ and alternative cultivars.

# GLOSSARY

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| | |
|---|---|
| AIR | headspace atmosphere with initial 20.9% $O_2$ iconcentration |
| ALG | alginate edible coating |
| CFU | colony forming units |
| CGA | chloramphenicol glucose agar |
| DMHF | 2,5-dimethyl-4-hydroxy-3(2H) furanone |
| DPPH | 2,2-diphenyl-1-picrylhidrazyl (cas: 1898-66-4) |
| DTT | DL-1,4-dithiothreitol |
| F | force, N |
| GC | gas chromatography |
| GC-MS | gas chromatography-mass spectrometry |
| HO | high oxygen atmosphere, 38% $O_2$ |
| HOX | high oxygen atmosphere, 38-42% $O_2$ |
| HPLC | high resolution liquid chromatography |
| ID | identification number |
| IS | internal standard |
| LO | low oxygen atmosphere, 1% $CO_2$, 12 % $O_2$ |
| LOX | low oxygen atmosphere, 1% $CO_2$, 12 % $O_2$ |
| OAV | odor activity value |
| PDMS | polidimethylsiloxane |
| PCA | principal component analysis |
| PCA | plate count agar |
| POD | Peroxidase |
| PP | polypropylene |
| PPO | polyphenol oxidase |
| RH | relative humidity, % |
| RRF | relative response factor |
| RT | retention time, min |
| SPME | solid-phase microextraction |

| | |
|---|---|
| SSC | soluble solids content, % |
| SSC/TA | soluble solids content to acidity ratio |
| T | temperature, °C |
| TA | Titratable acidity, % |
| TC | threshold concentrations, µg/kg |
| TPA | texture profile analysis |
| TPC | total phenolic content |
| cas | Chemical Abstracts Service |
| fw | fresh weight |
| nd | not detected |
| p | probability |
| t | time |

### *Volatile compounds*

| | |
|---|---|
| 1 | methyl 2-methyl propanoate |
| 2 | ethyl propanoate |
| 3 | methyl butanoate |
| 4 | ethyl 2-methyl propanoate |
| 5 | methyl 3-methyl butanoate |
| 6 | methyl 2-methyl butanoate |
| 7 | hexanal |
| 8 | butyl acetate |
| 9 | ethyl 2-methylbutanoate |
| 10 | 3-methylbutyl acetate |
| 11 | 2-heptanone |
| 12 | methyl 5-hexenoate |
| 13 | methyl hexanoate |
| 14 | ethyl hexanoate |
| 15 | hexyl acetate |

16 methyl 3-(methylthio) propanoate
17 limonene
18 (Z)-beta-ocimene
19 2,5-dimethyl-4-hydroxy-3(2H) furanone
20 2,5-dimethyl 4 methoxy 3(2H) furanone
21 ethyl heptanoate
22 ethyl 3-(methylthio) propanoate
23 linalool
24 nonanal
25 methyl octanoate
26 4-ethyl phenol
27 methyl (E) octenoate
28 ethyl octanoate
29 geraniol
30 4-ethyl-2-methoxy-phenol
31 ethyl decanoate
32 alpha copaene

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