Calidad del tomate fresco cortado tratado por pulsos de luz (PL)

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Universitat de Lleida

TESI DOCTORAL

Calidad del Tomate Fresco Cortado Tratado por Pulsos de Luz (PL)

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Que presenta:

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Para obtener el título de Dr. en Ciencia y Tecnología Agraria y Alimentaria

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SUMARIO

El tomate es una de las hortalizas más cultivadas y de suma importancia económica a nivel mundial. Su elevado valor nutritivo y características organolépticas han generado una creciente demanda de alimentos procesados que mantengan las propiedades del producto fresco. Sin embargo, el procesado mínimo del tomate puede ocasionar la disminución y pérdida de muchas de sus propiedades. Esto ha motivado el estudio de tratamientos que sean capaces de asegurar la inocuidad y la retención de la calidad del tomate fresco cortado. Los tratamientos por pulsos de luz (PL) han demostrado buenos resultados en la inactivación microbiana con un bajo impacto en las características fisicoquímicas y nutricionales de algunos alimentos frescos cortados. De este modo, el objetivo primordial de este trabajo fue evaluar los efectos de los PL en la calidad global del tomate fresco cortado.

El proyecto se realizó en 4 etapas, abarcando aspectos microbiológicos, fisicoquímicos, nutricionales y sensoriales del tomate cortado. Los resultados indicaron una clara influencia de los PL en la reducción tanto de la microbiota nativa como de la inoculada. Los modelos cinéticos relacionaron estos patrones tanto con los incrementos de la fluencia (Φ) aplicada como con el tiempo de almacenamiento (t). Estos descensos en los recuentos microbianos ayudaron a prolongar la vida útil del tomate cortado hasta por 4 días más que en los no tratados.

Las características fisicoquímicas se vieron afectadas tanto por la aplicación de los tratamientos de PL como como consecuencia de los fenómenos deletéreos acontecidos durante el almacenamiento. Se observó un incremento de los azucares (SST), reducción de pH y acidez (AT), cambios de color e incremento del color rojo ($>a^*$, $<L^*$, b^*, $>^o h$), pérdidas de peso (> 7 %) y agua (> 10 %), descenso de firmeza y resistencia al corte. Estas alteraciones fisicoquímicas fueron asociadas a la senescencia del tomate y en gran medida al estrés fisiológico causado por los PL. Además, dicho estrés es capaz de desencadenar cambios metabólicos, causando variaciones en el contenido de compuestos bioactivos y afectando la calidad nutricional del tomate cortado.

El aumento de la Φ de tratamiento resultó en mayores descensos del contenido de vitamina C y originando un declive de la capacidad antioxidante. Este resultado podría atribuirse en gran medida a la fotosensibilidad y fotoxidación de la vitamina C. El incremento en el contenido de licopeno y compuestos fenólicos también fue atribuido al desencadenamiento de reacciones metabólicas inducidas por los PL. Los modelos matemáticos utilizados fueron capaces de describir y explicar el

impacto de la fluencia y su repercusión en los contenidos de compuestos bioactivos durante el almacenamiento del tomate cortado.

Durante la evaluación sensorial se identificaron algunos desordenes negativos como astringencia y harinosidad, que fueron relacionados tanto con el incremento de compuestos fenólicos como con el desequilibro de las enzimas pectinmetilesterasa (PME) y poligalacturonasa (PG) inducido por los tratamientos aplicados. Independientemente de los cambios fisicoquímicos negativos reportados durante la evaluación sensorial, la aceptabilidad del tomate fresco cortado fue favorecida por los PL. De esta manera, un incremento en la fluencia resultó en una mayor aceptabilidad del tomate fresco cortado. El sabor (aroma y gusto) y el color resultaron ser los atributos sensoriales con mayor peso en la preferencia de los consumidores. Estos resultados fueron bien correlacionados con los parámetros analíticos. Además, los cambios resultantes de las determinaciones analíticas fueron positivamente vinculados con los resultados sensoriales. Los parámetros fisicoquímicos que influyeron en la aceptación de un modo más determinante fueron el color rojo y la pérdida de agua.

Por tanto, se puede decir que los tratamientos de PL ayudaron a incrementar la calidad del tomate cortado, prolongando su vida útil y mejorando sus atributos sensoriales durante el almacenamiento.

SUMARI

El tomàquet és una de les hortalisses més cultivades i amb molta importància econòmica al voltant del món. Tant l'alt valor nutritiu com la seva frescor han influït en la creixent demanda de tomàquet fresc tallat. No obstant això, durant el processament mínim del tomàquet es poden desenvolupar la reducció i pèrdua de moltes de les seves característiques. Aquesta situació ha motivat el desenvolupament i estudi de tractaments capaços de assegurar la innocuïtat i retenció de la qualitat del tomàquet laminat. Els tractaments per llum polsada (PL) han mostrat resultats positius en la inactivació microbiana i un baix impacte en les característiques fisicoquímiques i nutricionals d'alguns aliments frescos tallats. D'aquesta manera, l'objectiu principal en aquest treball va ser l'avaluació dels efectes dels PL sobre la qualitat total del tomàquet fresc laminat.

Aquest projecte es va fer en quatre etapes comprenent aspectes microbiològics, físics, nutricionals i sensorials del tomàquet tallat. Els resultats van indicar una clara influència dels PL en la reducció tant de la microflora nativa com de la inoculada. Els models cinètics van relacionar aquests patrons amb els increments de la fluència (Φ) aplicada així com també amb el temps d'emmagatzematge (t). Els descens de la microbiota van ajudar a allargar la vida útil de les rodanxes de tomàquet fins a quatre dies més que en els no tractats.

Les característiques fisicoquímiques es van veure afectades tant per els PL com per la senescència pròpia del tomàquet durant el emmagatzematge. Així, es van observar increments en els sucres (SST), baixades del pH i l'acidesa (AT), augment del color roig intens (+a*,-L*,b*,>ºhue), pèrdues de pes (>7 %) i aigua (>10 %), disminució de la fermesa i de la resistència al tall.

Aquestes alteracions fisicoquímiques es van associar a la senescència del tomàquet, però també l'estrès fisiològic causat pels PL. A més a més, aquest estrès va desencadenar canvis metabòlics, que van provocar variacions en el contingut dels compostos bioactius, afectant la qualitat nutricional de la tomata tallada.

Durant el transcurs de l'avaluació sensorial es van identificar alguns desordres negatius tals com astringència i farinositat, els quals van ser relacionats tant amb el increment de compostos fenòlics com amb un desequilibri dels enzims pectinmetilesterasa (PME) i poligalacturonasa (PG) originat pels PL. En realitat, la acceptació del tomàquet tallat va ser afavorida pels tractaments de llum independentment dels efectes negatius reportats en el transcurs de l'avaluació sensorial. D'aquesta manera, l'increment de la fluència aplicada va resultar en una millor acceptació del tomàquet tallat. El sabor (olor i gust) i el color es van relacionar positivament

amb els resultats obtinguts instrumentalment. A més, l'acceptabilitat es va correlacionar bé amb la pèrdua d'aigua i una coloració roja més intensa.

D'aquesta manera, es pot concloure que els tractaments de llum van ajudar a incrementar la qualitat del tomàquet tallat, allargant la seva vida útil i millorant els seus atributs sensorials durant l'emmagatzematge.

SUMMARY

Tomato is one of the most cultivated vegetables worldwide with a strong economic impact. The growing demand for fresh-cut tomatoes has been influenced by their high nutritional value and fresh-like characteristics. However, those attributes can be negatively affected during minimal processing. This fact has motivated the study of effective treatments that guarantee the safety and quality retention of tomato slices. Pulsed light treatments (PL) have shown a great efficacy to reduce microbial loads with a minimal impact on the quality of fresh-cut commodities. Hence, the main objective of the present work was to evaluate the effects of PL treatments on the overall quality of fresh-cut tomatoes.

This project was divided into 4 blocks, thus covering microbiological, physical, nutritional and sensorial aspects. Results seem to indicate the influence of PL on inactivation of the native and inoculated microorganisms. Gompertzian models described accurately those patterns as a function of the increasing fluence applied (Φ) and the storage period (t). Decreases in the microbial populations allowed an additional shelf-life extension of up to 4 days compared to untreated samples.

PL treatment conditions as well as the incidence if deleterious processes occurring in the product over storage influenced thephysicochemical characteristics of fresh-cut tomato slices. Increased sugars content , reduction of pH and acidity values, changes on red-color (+a*,-L*,b*,>ºhue), weight and juice losses as well as declines of firmness and shearing strength were all attributed to senescence and physiological stress caused by PL. This stress accounts for the triggering of metabolic changes, thus boosting changes on the physicochemical integrity of fresh-cut tomatoes. Moreover, those modifications provoked alteration of the bioactive compounds, hence affecting the nutritional quality of fresh-cut tomatoes.

Declines of the antioxidant capacity were related to losses of vitamin C, which seems to be influenced by the increasing Φ applied. This effect can be attributed to sensibility and photo-oxidation of vitamin C. Increases in the lycopene and phenolic compounds contents were also attibuted to those metabolic reactions induced by PL. The effect of the fluence and its impact on the bioactive compounds along the storage of tomato was well fitted by Gompertzian functions.

Some negative disorders as astringency and mealiness were reported during the sensory evaluation. Those alterations were related to increments in the content of phenolic compounds as well as to unbalances on the pectin-methylesterase (PME) and polygalacturonase (PG) enzymes caused

by PL. Indeed, the sensory acceptability of tomato slices was increased when PL were applied, irrespectively of the previously mentioned deleterious effects. Acceptability seemed to be directly influenced by flavor (taste and aroma) and color attributes. Those sensorial results correlated well with values obtained through instrumental assays. Relationship among acceptability and the analytical determinations were positively correlated, being the increases of red color and water losses the most determinant parameters. In conclusion, quality of fresh-cut tomatoes was favored by PL treatments, by extending the shelf-life and improving the sensory quality of the product.

INTRODUCCIÓN

1.1 El tomate

El tomate (*Lycopersicon esculentum* Mill.) es una fruta con orígenes en América central, donde era ampliamente cultivado en México por los Aztecas. Su nombre proviene del náhuatl “tomatl” que significa “fruta rechoncha” y posteriormente en el siglo XVI el nombre fue castellanizado a “tomate”. Este fruto llegó a Europa al tiempo que los españoles llegaron al nuevo mundo (Jenkins, 1948). Inicialmente el tomate se utilizó como planta ornamental y no fue hasta el siglo XVIII que fue consolidado como una hortaliza en Europa (Coronel y Castillo, 2009).

Según el Servicio de Investigación Agrícola (ARS) del Departamento de Agricultura de los Estados Unidos (USDA), existen más de 10,000 tipos diferentes de variedades de tomate y cada una de ellas presenta características físicas, nutritivas y sensoriales distintas a las otras. A pesar de la amplia gama de tomates existentes, menos del 20% son cultivadas, distribuidas y procesadas para su consumo (USDA, 2017).

Las variedades comerciales de tomate se pueden clasificar según su forma (redondos, asurcados, oblongos y cereza o cherry), color (de color verde a rojo intenso conforme su madurez avanza) y calibre (puede ser en base al diámetro o al peso, dependiendo del país productor) (FAO, 2007). Estas variedades se caracterizan por tener un sabor ligeramente dulce y una acidez alta, un bajo contenido de calorías y un elevado porcentaje de fitonutrientes (FEN, 2017).

1.1.1 Producción y consumo

La producción de tomate representa el 15 % de producción de frutos frescos en el mundo. Su cultivo y producción se extiende mundialmente, siendo países con climas cálidos y soleados los que tienen la mayor producción de este fruto. El tomate es la hortaliza más cultivada en el mundo, alcanzando una superficie total de 4,083 hectáreas y una producción anual cercana a las 165 millones de toneladas (Figura 1).

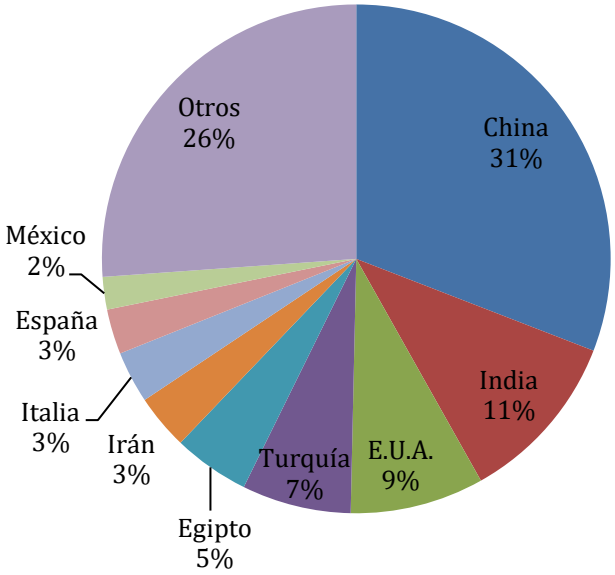

Figura 1. Distribución de la producción mundial de tomate. (FAOSTAT, 2017)

Diez países producen casi el 80 % de la producción anual de tomate en el mundo. El mayor productor de tomate es china con 52 millones de toneladas, seguida de India con 18 millones de toneladas y Estados Unidos de América con 14 millones de toneladas. Sin embargo, hay muchos países con una alta producción de tomate como Turquía, Egipto, Irán, Italia, España, Brasil y México (FAOSTAT, 2017).

España es el octavo productor de tomate fresco en el mundo (>2.5 % de la producción anual mundial) y el primer productor en la Unión Europea (UE) con casi 55,000 hectáreas cultivadas y una producción de casi 5 millones de toneladas anuales (FAOSTAT, 2017). El tomate es la segunda hortaliza más cultivada en España con un 17% del volumen total de la producción anual de frutos frescos. Además, el tomate es la principal hortaliza exportada por España con casi 1 millón de toneladas en 2016 (FEPEX).

Dos terceras partes de la producción mundial de tomate son consumidas en fresco, siendo las variedades de mesa las más demandadas

por los consumidores. En la última década, se ha incrementado el consumo de tomate fresco en el mundo, siendo China, India y Estados Unidos de América los países en los que el consumo es más elevado. Dentro del territorio español también se ha reportado un ligero incremento en el consumo de tomate fresco en los últimos años, alcanzando un total de 624 mil toneladas anuales y de 13.98 kg por habitante al año (MAPAMA, 2015).

Las características sensoriales y nutritivas del tomate lo convierten en uno de los frutos de mayor demanda para su consumo, tanto en fresco como en productos procesados. Su calidad está dada por la suma total de todos aquellos atributos físicos, químicos, nutricionales y sensoriales que impactan de forma directa e indirecta en la aceptación del producto.

Tabla 1. Factores relacionados con la calidad del tomate fresco.

Factores	Componentes
Apariencia (visual)	Tamaño (dimensión, peso, volumen)
	Forma
	Color
	Defectos (físicos, fisiológicos y patológicos)
Textura (tacto)	Firmeza
Sabor (gusto, olor)	Dulzura
	Acidez
	Aroma (compuestos volátiles)
	Sabores
Valor nutritivo	Carbohidratos
	Vitaminas
	Proteínas
	Minerales

Fuente: Salunkhe et al. 1991

Los atributos más valorados por los consumidores son aquellos relacionados con sus características fisicoquímicas, aunque la inocuidad y el contenido nutritivo también juegan un papel importante en la calidad del tomate fresco (Tabla 1). Por este motivo, los procesos postcosecha deben evitar la aparición de alteraciones de la calidad en el tomate.

1.1.2 Características fisicoquímicas y sensoriales

Las características visuales, texturales y químicas del tomate son de gran interés en la industria alimentaria debido a su impacto sobre la aceptación de los consumidores. Estas características están influenciadas por la variedad, el estado de madurez y por la forma de procesamiento.

El *color* es comúnmente utilizado como índice de la calidad general del tomate, siendo preferidos aquellos frutos con un color rojo intenso y brillante (D'Souza et al., 1992; Tijskens y Evalo, 1994). Según Shewfelt et al. (1987) el tomate de alta calidad se caracteriza por un valor de tono (ºh) cercano a 0º, lo que indica un color cercano al rojo puro.

La *textura* es otro factor que influye en la calidad del tomate fresco. Este atributo está dado por la firmeza, la consistencia interna y la dureza de la piel. Los consumidores prefieren tomates con una firmeza alta, sin ablandamientos exteriores y un interior solido y jugoso. Algunos autores reportan la relación entre los cambios de firmeza, ablandamiento y cambios de color en el tomate (Pangaribuam, 2005). La textura pude verse comprometida durante el procesamiento postcosecha, lo que ocasiona pérdidas de calidad y una baja aceptación por parte del consumidor.

La composición química es la responsable del *sabor* y *aroma* característicos del tomate. En la madurez máxima del tomate se alcanza el equilibrio en el contenido de azúcares y ácidos, así como una alta concentración de compuestos volátiles. Los consumidores prefieren tomates dulces y ligeramente ácidos. La dulzura está relacionada con la cantidad de sólidos solubles (SS), predominantemente azúcares, que constituyen cerca del 60% de la materia seca del tomate. Los SS pueden ser determinados por refractometría y expresados como % de sólidos solubles o ºBrix. La

mayoría de las variedades de tomate presentan entre un 3.5 y 7.0 % de sólidos solubles en la madurez de consumo (Dorais et al., 2001).

La acidez también se relaciona con la percepción del sabor y está asociada al contenido de ácidos en el tomate, particularmente de acido cítrico. El descenso de la acidez es debido a la actividad metabólica de los frutos durante la maduración. Los cambios metabólicos convierten los ácidos orgánicos de reserva en azúcares, los cuales serán consumidos durante la respiración celular (Badui, 1996). La acidez se determina mediante titulación (AT) y se reporta en porcentaje (%) de ácido cítrico. El tomate de alta calidad generalmente está caracterizado por una acidez titulable superior a 0.32 %, un contenido de sólidos solubles de 3 % y un radio de SS/AT superior a 10 (Kader et al., 1978).

Por otra parte, el perfil aromático del tomate está dado por una alta concentración de compuestos químicos como terpenos y lactonas, ésteres, carbonilos y alcoholes, ácidos libres y compuestos heterocíclicos (Marcovic et al., 2007).

1.1.3 Valor nutritivo

El consumo de tomates está considerado como un buen indicador de buenos hábitos alimenticios y de un estilo de vida saludable (Odriozola-Serrano et al., 2008). El tomate está compuesto principalmente por agua, tiene un bajo contenido calórico y es rico en vitaminas y minerales (Adalid, 2011) (Tabla 1). Además, el consumo de tomate ejerce un efecto benéfico en la defensa contra el estrés oxidativo, lo que se ha relacionado con una menor predisposición a padecer cáncer y algunas enfermedades cardiovasculares (Ames, et al., 1993). De esta manera, la capacidad antioxidante del tomate representa uno de los mayores beneficios de este fruto a la salud humana.

La capacidad antioxidante del tomate está determinada por una amplia cantidad de compuestos que presentan estructuras químicas y mecanismos de acción muy variados. La concentración de estos compuestos depende de la madurez, la variedad, las condiciones de

crecimiento del fruto, la zona geográfica y la época de cosecha del tomate (Borguini et al., 2013). En general, su capacidad antioxidante está relacionada con el contenido de compuestos bioactivos, tales como vitaminas y algunos compuestos fenólicos como los flavonoides y carotenoides, especialmente licopeno y beta-caroteno (Vallverdú-Queralt et al., 2012).

Tabla 1. Composición nutricional del tomate (*Lycopersicon esculentum* Mill.).

Por 100 g de porción comestible		
Agua	94.5	g
Carbohidratos	3.9	g
Azúcares	2.6	g
Fibra	1.2	g
Grasas	0.2	g
Proteinas	0.9	g
β-caroteno	449	µg
Retinol (Vit. A)	42	µg
Tiamina (Vit. B1)	0.037	µg
Niacina (Vit. B3)	0.594	µg
Vitamina B6	0.08	µg
Vitamina C	14	mg
Vitamina E	0.54	mg
Vitamina K	7.9	µg
Magnesio	11	mg
Fósforo	24	mg
Potasio	237	mg

Fuente: USDA (2017).

La vitamina C es la que se encuentra en mayor proporción en el tomate. Esta vitamina protege las membranas celulares contra los efectos de la peroxidación (Traver y Stevens, 2011). El contenido de ácido ascórbico varía entre 12.5 y 31.8 mg kg^{-1}, aunque estas concentraciones decrecen con la maduración y procesamiento del tomate (Klein 1987; Watada et al. 1976).

Los compuestos fenólicos influyen en el poder antioxidante, pero además juegan un papel importante en el sabor y olor del tomate. Existen alrededor de 8000 compuestos fenólicos y se caracterizan por tener un anillo fenólico en su estructura molecular (Harbone y Williams, 2000). Estos compuestos son productos secundarios del metabolismo de defensa de las plantas contra el daño causado durante y después de su cosecha (Lewinsohn et al., 2001). Entre los compuestos fenólicos presentes en el tomate destacan los flavonoles (kaempferol, quercitina y miricetina) y ácidos fenólicos como el acido clorogénico (0.14-8 mg /100g) (Odriozola-Serrano, 2009).

El licopeno es el carotenoide presente en mayor concentración en el tomate y el causante de su color rojo (Odriozola-Serrano et al., 2009a). Este carotenoide representa entre un 50 y 83 % del contenido total de pigmentos en el tomate y su concentración se incrementa paulatinamente con la maduración del fruto (Thompson et la., 2000). La cantidad de licopeno es mayor en el pericarpio que en la pulpa, siendo aproximadamente de 540 mg kg^{-1} en el pericarpio y de 110 mg kg^{-1} en la pulpa (MacGlasson y Lee, 1993).

1.2 Frutos frescos cortados (FFC)

El consumo de frutas y hortalizas representa entre un 10 % y un 50 % del consumo diario de alimentos en el mundo (FAO, 2015). El estilo de vida actual ha llevado a la población a la búsqueda de alimentos fáciles de preparar y de consumir. Estos productos deben ser seguros para la salud, mantener una apariencia fresca, libre de productos químicos y unas características organolépticas y nutritivas similares a las de los productos frescos. La industria ha buscado satisfacer esta necesidad mediante la producción de alimentos frescos cortados, los cuales son definidos como cualquier tipo de fruta o vegetal o alguna combinación de ellos, que han sido alterados físicamente de su forma original pero que aún mantienen su estado fresco (IFPA, 1999). A los productos frescos cortados también se los conoce como productos mínimamente procesados (PMP) o de IV gama.

1.2.1 Producción y consumo de frutas frescas y mínimamente procesadas

La producción anual de frutas y hortalizas ha ido en aumento en los últimos años, superando así los 2,000 millones de toneladas en 2015. La UE es uno de los mayores productores de hortalizas en el mundo con una producción que supera los 40 millones de toneladas anuales. El primer productor mundial de frutas y hortalizas es China con un 27 % de la producción mundial, seguido por India (7 %) y Estados Unidos (3 %). Según la Federación Española de Asociaciones de Productores Exportadores de Fruta, Hortalizas, Flores y Plantas (FEPEX) en España, la superficie dedicada al cultivo de frutas y hortalizas es de 800,000 hectáreas aproximadamente, de las cuales 156,000 corresponden al cultivo de hortalizas. La producción española de frutas y hortalizas fue de 13 millones de toneladas, lo que la convierte en el primer productor de la UE y el noveno país productor a nivel mundial (MAPAMA, 2015). Además, España es el primer exportador de frutas y hortalizas frescas en el mundo con 11.6 millones de toneladas seguido por los Países Bajos, México y Estados Unidos de América con 8.3, 7.4 y 6.5 millones de toneladas, respectivamente (FEPEX, 2016).

La Organización Mundial de la Salud (OMS) recomienda el consumo de un mínimo de 400 g diarios de frutas y verduras para prevenir algunas enfermedades cardiovasculares y la carencia de micronutrientes en los seres humanos. De acuerdo a los datos procedentes de la FAOSTAT, China es el principal consumidor de frutas y hortalizas frescas, representando casi el 60% de la dieta promedio, mientras que en Reino Unido, Estados Unidos de América y México representa el 33, 27 y 25 % de la dieta diaria promedio (FAOSTAT, 2017). En España el consumo per cápita es de 180.8 kg lo que representa casi el 24% de la dieta diaria (FEPEX, 2015).

Por otro lado, el consumo de frutas y hortalizas frescas cortadas se ha incrementado en los últimos años. En Estados Unidos de América el sector de los productos cortados está bien consolidado, alcanzando hasta

casi el 20 % de la producción total de frutas y hortalizas frescas y con un consumo de casi 30 kilos por persona anualmente. En la mayoría de países del centro y norte de la UE se ha visto un incremento importante en la producción y consumo (>3 kilos anuales por habitante) de productos frescos cortados. En España, la implantación de los productos frescos cortados está en plena expansión. La producción de frutas y verduras frescas cortadas en el mercado español es menor, pero la demanda crece cada año. Según la asociación de Frutas y Hortalizas Lavadas Listas para su Empleo (AFHORFRESH), que reúne el 90% de los productores del territorio español, la producción de frutos frescos cortados fue apenas superior a las 70 toneladas en el año 2015 (FEPEX).

1.2.2 Aspectos legislativos

La seguridad alimentaria es el aspecto más importante a cuidar en la elaboración de productos frescos cortados y la mayoría de regulaciones de producción se basan en criterios de seguridad y calidad microbiológica. En España, estos productos están regulados por el Real Decreto 3484/2000 en el que se establecen las normas de higiene en la elaboración, distribución y comercio de comidas preparadas. No obstante, los criterios microbiológicos han sido sustituidos por un reglamento de la UE (CE 2072/2005) que establece límites para distintos microorganismos relevantes en materia de seguridad alimentaria. Esta regulación exige la ausencia de microorganismos del género Salmonella, además de establecer restricciones en relación a la presencia de *Escherichia coli* y *Listeria monocytogenes*.

Debido a que el procesado de frutos frescos puede conllevar riesgos microbiológicos, es necesaria la implantación de sistemas de análisis de puntos de control crítico (APCC) durante los procesos de elaboración y en el producto final. Éstos son obligatorios para garantizar la seguridad microbiológica (CE 2073/2005). La comisión del Codex Alimentarius elaboró un documento titulado “Código de prácticas de higiene para frutas y hortalizas frescas” que incluye un anexo sobre frutas y verduras frescas

cortadas (FAO, 2003a). En este documento se recomienda la implantación de buenas prácticas de fabricación para el control de riesgos microbiológicos y establece los límites máximos de niveles de residuos químicos y tóxicos que pueden poner en riesgo la salud de los consumidores (FAO, 2003b).

De esta manera, el mercado de productos frescos cortados en Europa se ciñe a normativas derivadas del Codex Alimentarius (135/2010) conformada por diferentes reglamentos orientados a garantizar la seguridad alimentaria a lo largo de toda la cadena de producción alimentaria y estableciendo los límites máximos de agentes tóxicos y microbiológicos permitidos en un alimento preparado. Así mismo, en Estados Unidos de América la U.S. Food and Drug Administration (FDA) ha publicado "Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial Hazards on Fresh and Fresh-Cut Produce" en el que se proponen métodos de control de riesgos microbiológicos en alimentos frescos cortados (FDA, 2000).

1.2.3 Preparación de frutos frescos cortados

El incremento en la demanda de FFC ha influido en el desarrollo de tecnologías de procesado que no tengan un impacto negativo en la calidad final del producto. La calidad de los FFC depende directamente de la calidad del producto entero y de otros factores relacionados al procesado, almacenamiento y distribución (Martín-Belloso et al., 2012). En general, los FFC deben mantener una apariencia fresca y sin defectos físicos, mantener sus características organolépticas y no representar un riesgo para la salud del consumidor (Watada y Qi, 1999). Sin embargo, estos productos son altamente perecederos, por lo que es necesaria la implantación de técnicas que eviten su deterioro durante el procesado, transporte, distribución y almacenamiento (Figura 2).

El primer paso en la elaboración de los FFC, es la selección de la materia prima. Cuando el fruto es separado de la planta ocurren una serie de cambios fisiológicos como transpiración, respiración y maduración, que

afectan negativamente la calidad sensorial del producto cortado (Soliva-Fortuny et al., 2003). Una buena elección de la variedad, tamaño, firmeza y estado de madurez del producto minimiza el impacto de los procesos de posteriormente aplicados (Oms-Oliu, 2009). Es importante seleccionar frutos sin defectos y en estado de madurez fisiológica, lo que no necesariamente tiene que ser la madurez comercial o de consumo, para que el sabor y el aroma propios del producto se hayan desarrollado (Gorny et al., 2000).

La maquinaria, instrumentos y área de procesado deben estar provistos de sistemas que permitan su limpieza evitando la acumulación de restos con el consiguiente riesgo de contaminación microbiológica del alimento (Odriozola-Serrano, 2009).

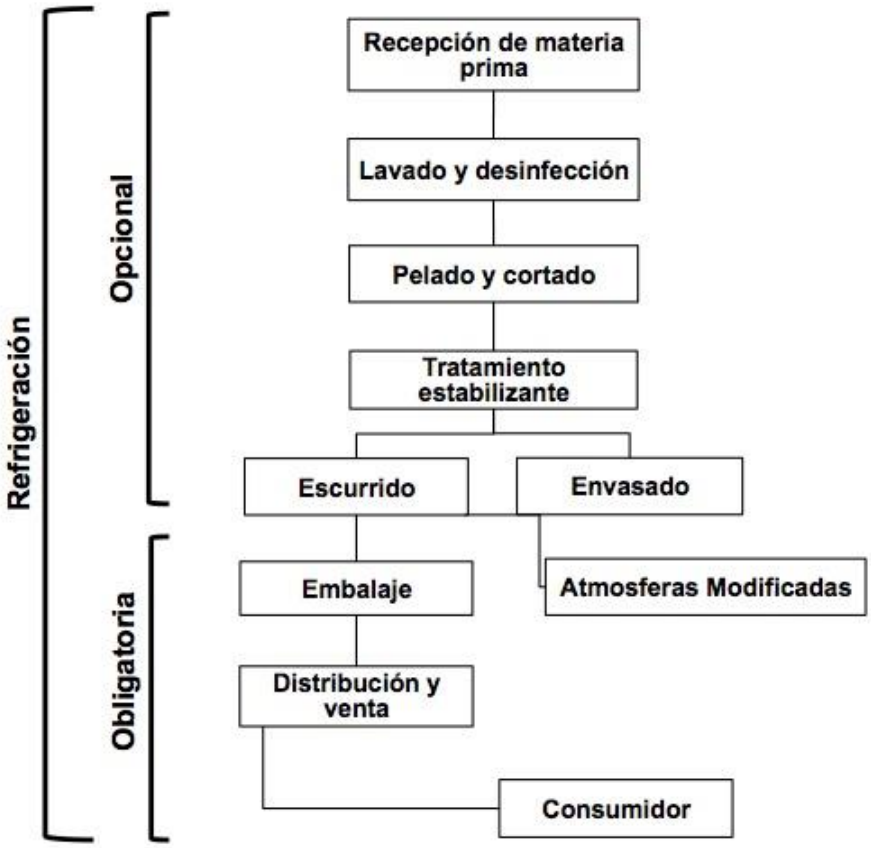

Figura 2. Esquema de preparación de fruta fresca cortada.

El tipo de procesado también influye de manera importante en la modificación de la calidad de los frutos frescos cortados durante el almacenamiento. Las principales alteraciones relacionadas con el procesado de los FFC son incremento de la respiración tisular, deterioro bioquímico, pérdida de la integridad física y proliferación microbiana. Durante el procesado de los frutos se liberan sustancias ricas en nutrientes, lo que propicia condiciones idóneas para el crecimiento microbiano (Martín-Belloso y Rojas-Graü, 2005). El pelado y el corte del fruto genera una pérdida de la integridad física, promoviendo la producción de etileno y el incremento de la respiración, acelerando el deterioro y reduciendo así la vida útil del alimento (Perera y Baldwin, 2001). De hecho algunos autores han reportado un fuerte impacto de diferentes condiciones de procesado, tales como el tipo de corte, el envasado, la temperatura y el tiempo de almacenamiento, en la calidad del tomate fresco (Hakim et al. 2002; Aguayo et al. 2004).

La pérdida de la calidad nutricional y sensorial se asocia a los cambios metabólicos desencadenados durante el procesado y almacenamiento de estos productos (Toivonen et al., 2003). El contenido de compuestos bioactivos en los FFC está afectado por la exposición a la luz, por la temperatura y por su interacción con otros compuestos presentes en el medio (Gil et al., 2006; Lee y Kader, 2000). Por este motivo, los tratamientos estabilizantes aplicados durante el procesado tienen como objetivo principal alargar la vida útil, reduciendo la proliferación de microorganismos, y evitar que el producto sufra alteraciones fisicoquímicas, nutricionales y sensoriales que afecten su calidad final (Martín-Belloso et al., 2006). Además, cada producto fresco cortado presenta cambios fisiológicos, bioquímicos y nutricionales distintos, por lo que el método de control es distinto para cada uno de ellos (Wiley, 1994).

La selección de las condiciones de conservación marca el último punto del procesado de los FFC. La elaboración, almacenamiento, distribución y comercio de estos productos requiere del mantenimiento ininterrumpido de la cadena refrigeración. El uso de temperaturas de

refrigeración permite un mejor control sobre el desarrollo de microorganismos, evita cambios drásticos en las características fisicoquímicas y nutricionales e influye también sobre la actividad de algunas enzimas que pueden causar pérdidas de calidad durante el almacenamiento de los frutos frescos cortados (Martin-Belloso et al., 2012).

1.2.4 Factores que afectan la calidad de los productos frescos cortados

Algunas alteraciones como pardeamiento y decoloración, ablandamiento, deshidratación, pérdida de agua, translucidez, desarrollo de aromas y sabores, así como el crecimiento de microorganismos son algunas de las principales causas de pérdida de calidad de los FFC (Rojas-Graü et al., 2009). Los FFC son propensos a sufrir degradación y oxidación de pigmentos y fitonutrientes y algunos cambios físicos, como consecuencia de la senescencia del producto y de las operaciones de procesado (Oms-Oliu, 2009).

1.2.4.1 Estabilidad microbiológica

La estabilidad microbiológica es el principal limitante de la calidad y determina la vida útil de los productos frescos cortados. Las fuentes de contaminación más importantes son el uso de agua contaminada, así como la poca profilaxis durante el procesado del producto. Sin embargo, el procesamiento mínimo también favorece la liberación de nutrientes al exterior del producto provocando condiciones óptimas para el desarrollo de microorganismos.

El crecimiento de algunos microorganismos como mohos y levaduras puede deberse al bajo pH que presentan las frutas. Así, los hongos *Penicillium spp*., *Alternaria spp*. y *Trichoderma spp*. y levaduras como *Metschnikowia spp*., *Debaryomyces spp*., *Candida spp*., y *Hanseniaspora* son los microorganismos comúnmente desarrollados en los productos frescos cortados (Nguyen-yhe y Carlin, 1994; Jay, 2002)

Además, se ha reportado la presencia de algunas bacterias durante el almacenamiento de los productos frescos cortados (Lanciotti et al., 1999). Algunos microorganismos patógenos como *Salmonella spp*., *Shigella spp*., *Escherichia coli* O157:H7 y *Listeria monocytogenes* se han reportado en productos con un alto nivel de acidez (Oms-Oliu et al., 2009).

El crecimiento de microorganismos también puede dar lugar a podredumbres en los FFC, causando alteraciones fisicoquímicas y afectando su calidad (Duran, 2006). Entre éstas cabe destacar: blanda bacteriana (*Erwinia carotova*), fúngica gris (*Botrytis cinérea*), blanda por Rhyzopus (*Rhyzopus stolonifer, nigricans*) y podredumbres por *Phypophora* (*Phypophora cactorum*), *Alternaria* y *Fosarium*.

1.2.4.2 Cambios fisicoquímicos y nutritivos.

El procesado también puede producir cambios fisicoquímicos, alterando la calidad visual, nutritiva y sensorial de los FFC. Durante el procesado del fruto se daña la compartamentalización celular del tejido vegetal, liberando compuestos fenólicos que son oxidados por acción de enzimas oxidativas tales como polifenol oxidasa (PPO) y peroxidasa (POD). En el primer caso, se producen quinonas incoloras, las cuales se polimerizan formando melaninas (Gil et al., 2006). Estas melaninas son las responsables del **pardeamiento enzimático** que pueden sufrir algunos productos frescos. En respuesta a este tipo de alteraciones, los tejidos vegetales pueden acelerar el mecanismo secundario de defensa, produciendo una mayor cantidad de compuestos que actúan contra la oxidación. La presencia de PPO y POD se considera un índice de deterioro de los frutos (Vamos-Vigyazo, 1981). Estas enzimas participan en los procesos de defensa de los tejidos vegetales llevando acabo la lignificación (Morkunas y Gmerek, 2007). La síntesis de metabolitos secundarios tales como ácido ascórbico y algunos compuestos fenólicos es la principal barrera de defensa contra la oxidación de los tejidos vegetales (Iyengar y McEvil, 1992; Ke y Salveit, 1989).

La **translucidez** tiene un fuerte impacto en la apariencia de los productos frescos cortados. Este tipo de alteración se caracteriza por la presencia de zonas oscuras y vítreas y juega un papel importante en la calidad del tomate fresco cortado (Artés et al., 1999; Gil et al., 2002; Lana et al., 2006). La **decoloración** se ha relacionado con reacciones enzimáticas, causadas por la deshidratación y por formación de ligninas que oxidan las melaninas en los tejidos vegetales (Cisneros-Zevallos et al., 1995; Artés et al., 1999).

Por otro lado, la **textura** de los productos frescos cortados puede ser fuertemente afectada por las operaciones de procesado. La ruptura de la integridad celular induce la hidrólisis enzimática de las sustancias pécticas que forman la pared celular (Varoquaux, 1991). Las enzimas pectinmetilesterasa (PME) y poligalacturonasa (PG) juegan un papel esencial en el *ablandamiento* de los frutos. La pectina es hidrolizada por acción de la PME, formando metanol y ácido péctico, los cuales son despolimerizados por acción de la PG, provocando la desestabilización de la estructura celular de los frutos (Alandes et al., 2006). Esta pérdida de la adhesión entre las células produce *cambios osmóticos*, *evaporación*, *deshidratación* y *liberación de agua* intracelular al exterior, causando la formación de exudados superficiales y pérdidas de *firmeza* (Tatsumi et al., 1991; Burton, 1982; Cisneros-Zevallos et al., 1995). Así mismo, se produce un *incremento de la velocidad de respiración*, lo que incrementa la concentración de dióxido de carbono (CO_2) y disminución del oxígeno (O_2) (Gorny et et., 2000). El incremento en la tasa respiratoria promueve la transpiración, que da lugar a *deshidratación* y *pérdida de agua* y cambios en la actividades enzimáticas pectinolíticas (Soliva-Fortuny et al., 2004; Saladié et al., 2007).

Los cambios en el **contenido nutritivo** y de los **compuestos bioactivos** también son de gran importancia en la calidad de los productos frescos cortados (Prior y Cao, 2000). La deshidratación y pérdida de agua influyen en el incremento del contenido de azúcares. Sin embargo, los ácidos orgánicos descienden debido a la oxidación, lo que ocasiona un

descenso del pH. El contenido de aminoácidos varía dependiendo de la temperatura de conservación. De hecho, los FFC mantenidos a menores temperaturas presentan menores pérdidas en el contenido de algunos aminoácidos (Kim et al., 1993).

Los *compuestos bioactivos* son importantes debido a su función antioxidante y el efecto protector contra algunas enfermedades relacionadas con la oxidación celular (Shi et al., 2001). De hecho, la evaluación de la capacidad antioxidante es importante para conocer el valor comercial y nutricional de los productos vegetales (Sánchez-Moreno, 2012). Ésta viene determinada por el contenido en distintos grupos de compuestos con capacidad de prevenir o retardar los fenómenos de oxidación en los tejidos del fruto. El procesado mínimo puede afectar el contenido, composición, actividad y biodisponibilidad de los compuestos antioxidantes en los frutos frescos cortados (Robles-Sánchez et al., 2007). Además, estas moléculas son altamente sensibles al oxígeno, la luz y las altas temperaturas (Gil et al., 2002). Sin embargo, la capacidad antioxidante no presenta la misma tendencia en todos los frutos frescos cortados. Lana y Tijskens (2006) reportan una reducción de la capacidad antioxidante en el tomate fresco cortado, pero en mango, naranjas y zanahorias frescas cortadas no se han reportado cambios en su actividad (Robles-Sánchez et al., 2007; Alasalvar et al., 2005). Algunos autores reportan que la capacidad antioxidante en naranjas y manzanas frescas cortadas está relacionada con el contenido de vitamina C (Del Caro et al., 2004; Cocci et al., 2006). Por otra parte, estudios realizados en nectarinas, melocotones, ciruelas (Gil et al., 2002), cítricos (Gorinstein et al., 2004), melón y pera (Oms-Oliu et al., 2008) reportan una correlación positiva entre la capacidad antioxidante y el contenido de compuestos fenólicos. Aunque algunos de estos compuestos son oxidados por las enzimas polifenoloxidasa (PPO) y peroxidasa (POD), otros compuestos fenólicos como los tocoferoles y los derivados del ácido cinámico y flavonoides como la quercetina y el kaempferol, pueden incluso inhibir la actividad oxidativa de las enzimas PPO y POD (Macheix et al., 1990; Ashie et al., 1996).

1.2.4.3 Cambios sensoriales

El desarrollo de microorganismos y el cambio en las propiedades fisicoquímicas son los principales factores que afectan los atributos sensoriales de los FFC. El cambio en la concentración de los sólidos solubles totales (SST), acidez titulable (AT), firmeza y compuestos volátiles afectan directamente la percepción sensorial de los alimentos (Shewfelt et al., 1987; Watada, 1996). Los FFC normalmente son consumidos sin otros ingredientes, por lo que conservar su sabor característico es esencial para mantener su calidad sensorial. El sabor se ve afectado directamente por el contenido de azúcares (dulzor), ácidos orgánicos (acidez) y compuestos fenólicos (astringencia) y volátiles (aroma) (Kader, 2002). Según Salvador et al. (2007), la pérdida de calidad sensorial se puede producir incluso antes que el deterioro por microorganismos, lo que afecta su comercialización. De hecho, algunos estudios sensoriales muestran que la mayoría de los FFC pierden la calidad de comercialización después de una semana de almacenados en refrigeración (Oms-Oliu et al., 2009). La calidad sensorial de los FFC está formada por la percepción de la apariencia, la textura y el sabor.

El contenido de ácidos orgánicos también se ve afectado durante el procesado, la oxidación y reducción de estos compuestos genera una disminución de la acidez que impacta en la aceptación del alimento. Este fenómeno está acompañado por el incremento de azúcares y liberación de compuestos fenólicos que también afectan el sabor de los FFC.

Por otro lado, la presencia de agua y un pH bajo originan el desarrollo de hongos (Chen et al., 2002) y bacterias (Heard et al., 2002) en los frutos, lo que puede generar una inminente reducción de la aceptabilidad del producto. Además, los microorganismos generan enzimas extracelulares como PME y PG, que degradan los polisacáridos de la pared celular, liberando agua y nutrientes del fruto (Miedes y Lorences, 2004).

El color también tiene un papel importante en la aceptabilidad de los FFC (Shewfelt, 1992). Durante el procesado y almacenamiento de los FFC

se producen cambios físicos que pueden promover algunas alteraciones como decoloración o la pérdida de compuestos coloreados como carotenoides, clorofilas o flavonoides (Barret et al., 2010). La liberación de agua intracelular también produce alteraciones físicas como deshidratación y zonas húmedas en la superficie de los FFC y afecta negativamente a su aceptabilidad. El daño en la integridad física causado durante el procesado de los FFC también puede desencadenar reacciones metabólicas que generan desequilibrios de las concentraciones de las enzimas PME y PG, lo que altera la estructura física del alimento (Waldrom et al., 2003).

1.2.5 Conservación de frutos frescos cortados

El desarrollo de microorganismos y la rápida pérdida de las propiedades fisicoquímicas son los principales limitantes de la vida útil de los productos frescos cortados. Se han estudiado diferentes tipos de tratamientos para el control de estas alteraciones. Los métodos térmicos son capaces de reducir considerablemente la carga microbiana. Sin embargo, la aplicación de estos procedimientos es responsable de la pérdida de la mayoría de propiedades organolépticas, nutritivas y físicas características de los productos frescos (Caminiti et al., 2011). Ello ha propiciado el estudio de diferentes tecnologías alternativas capaces de garantizar la inocuidad de los FFC sin afectar a su calidad (Allende et al, 2006).

Las tecnologías no-térmicas han surgido como una alternativa para mantener la calidad en los FFC lo más cercana a la de un producto fresco, asegurando la estabilidad microbiológica y manteniendo altos niveles de compuestos nutritivos (Guerrero-Beltrán y Barbosa-Cánovas, 2004). Dentro de los métodos de procesado no-térmico destacan los tratamientos de lavado e inmersión, bio-películas, envasado en atmósferas modificadas y tecnologías emergentes de conservación.

Los lavados con agentes antimicrobianos y antioxidantes son usados comúnmente para mejorar la calidad de los FFC (Oms-Oliu et al., 2010). Algunos estudios proponen el empleo de lavados por inmersión con agentes

como ácido cítrico, dióxido de cloro, ozono y ácido peroxi-acético para inducir una reducción de la carga microbiana (Odriozola-Serrano, 2009a). Sin embargo, los resultados revelan algunos efectos adversos sobre la calidad nutricional y sensorial del los productos cortados (Laurila y Ahvenainen, 2002). Algunos tratamientos de lavado con agentes antimicrobianos, sales de calcio, minerales, proteínas, vitaminas, y antioxidantes, solos o combinados, han demostrado reducir los recuentos microbianos y mantener las características fisicoquímicas durante el almacenamiento del tomate fresco cortado (Ahmed et al., 2012; Antunes et al., 2013). El uso de 1-metil-ciclopropano (1-MCP) es capaz de retener las características fisicoquímicas del tomate fresco cortado durante el almacenamiento a 5 ºC (Jeong et al., 2004). Los tratamientos de inmersión en peróxido de hidrógeno han mostrado un efecto positivo en la reducción de los conteos microbianos, sin embargo, tiene un efecto negativo en la capacidad antioxidante y el contenido de compuestos bioactivos en el tomate fresco cortado (Kim et al, 2007). El uso de compuestos volátiles como jasmonato y etanol reduce la carga microbiana y mantiene el contenido de compuestos bioactivos durante el almacenamiento en refrigeración (5 ºC) del tomate fresco cortado (Ayala-Zavala et al., 2008).

La aplicación de películas comestibles también es efectiva en la conservación de los FFC (Tamer, 2010). La capacidad de estos tratamientos para mantener la calidad de los FFC se atribuye a la formación de una película semipermeable en el producto, alterando la permeabilidad del agua, oxígeno y dióxido de carbono, reduciendo la respiración y retrasando la senescencia del fruto (Thumula, 2006).

Así mismo, el envasado bajo atmósfera protectora ha cobrado importancia debido a su efecto beneficioso en la inhibición del pardeamiento enzimático y en el control de microorganismos durante el almacenamiento de frutas y hortalizas frescas cortadas (Day et al., 2000). La exposición del tomate cortado a algunos gases como óxido nítrico, ozono y envasado en atmósferas modificadas también ha mostrado buenos resultados en la reducción de los conteos microbianos y en el mejoramiento de las

características físicas del tomate (Aguayo et al., 2006; Kim et al., 2007; Aguayo et al., 2004). Estos mismos autores observaron un mejor resultado de estos tratamientos al utilizar temperaturas de conservación menores a 5 ºC. Además, en los últimos años se ha estudiado la aplicación combinada de tratamientos de inmersión en disoluciones de agentes químicos y envasado en atmósfera modificada. Estos procedimientos pueden tener un efecto aditivo sinérgico o antagonista que puede contribuir a la reducción de la carga microbiana así como en la conservación de las características propias de los alimentos frescos (Rajkowski y Baldwin, 2003).

Por otra parte, las tecnologías emergentes de conservación de alimentos incluyen a los pulsos eléctricos, ultrasonidos, altas presiones hidrostáticas, irradiación, plasma frío y pulsos de luz (PL). En los últimos años se ha estudiado la aplicación combinada de tecnologías físicas y químicas, mostrando buenos resultados tanto en la inactivación de microorganismos como en el mantenimiento de las características fisicoquímicas y sensoriales de los productos frescos cortados (Mahajan et al., 2014).

1.3 Pulsos de luz (PL)

Los pulsos de luz son utilizados de forma general en la reducción de los recuentos microbiológicos en la superficie de alimentos, equipos y materiales de envasado de alimentos (Oms-Oliu et al., 2010b). Es una tecnología respetuosa con el medioambiente y que no genera residuos durante su utilización. A los PL también se les conoce como pulsos de luz blanca, pulsos lumínicos, pulsos de luz de alta intensidad, pulsos de luz intensa y luz ultravioleta (Ramos-Villarroel et al., 2013).

1.3.1 Principios básicos de la tecnología de PL

Los equipos de PL están constituidos principalmente por un condensador que almacena la energía durante un periodo corto y un elemento de control que libera esta energía de forma súbita en una cámara

de tratamiento mediante una lámpara capaz de generar un impulso de luz de alta intensidad comprendido entre 100-1100 nm (Figura 3).

En los primeros estudios de esta tecnología se utilizaban lámparas de mercurio y láseres capaces de proveer altas intensidades de luz, pero el incremento de temperatura, la producción cruzada de ozono y un excesivo gasto energético motivaron la búsqueda de otras fuentes radiantes (Seubert y Nichols, 2004).

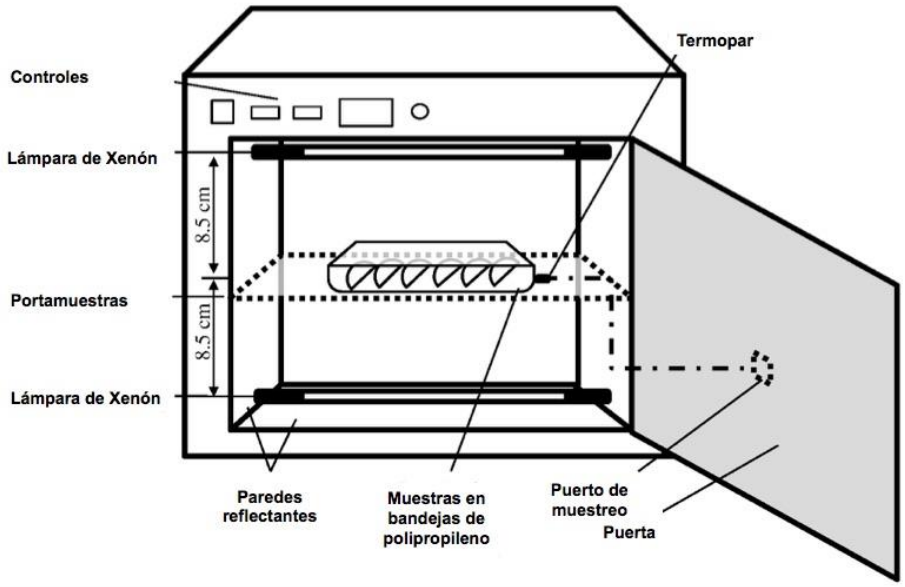

Figura 3. Equipo de pulsos de luz XeMaticA-2 (SteriBeam Systems GmbH. Kehl, Alemania).

En la actualidad, las lámparas de gas xenón son ampliamente utilizadas en los equipos de PL. Estas lámparas son capaces de producir pulsos intensos de irradiación a temperatura ambiente y cubrir amplias superficies. Los equipos de PL permiten la aplicación de pulsos a frecuencias de entre 1 y 20 destellos por segundo, con una duración entre ellos de entre 1μs y 1 s y con una densidad de energía (*DE*; *Φ*) de entre 0.01 y 50 J cm^{-2} (Barbosa-Canovas et al., 1998).

La alta intensidad del pulso permite la rápida generación de especies reactivas, mientras que el periodo de oscuridad entre pulsos posibilita la disipación del calor y la luz (Kim et al., 2009). La distribución de las longitudes de onda abarca la luz ultravioleta (UV) (100-400 nm), luz visible (400-700 nm) y el infrarrojo cercano (700-1100 nm) (Figura 4) (Om-Oliu et al., 2010b).

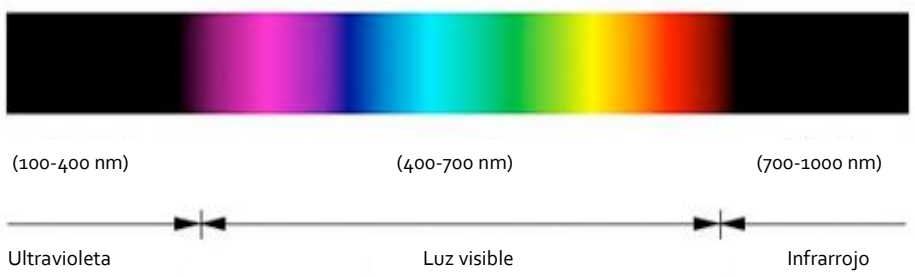

Fig. 4 Espectro luminoso. Longitudes de onda comprendidas por los tratamientos por PL.

En el área de alimentos, la intensidad de los tratamientos de PL es más frecuentemente expresada como densidad de energía, la cual es definida como la cantidad de energía emitida por la lámpara y que incide en la muestra por unidad de área. La *DE* es comúnmente reportada como Φ= J cm^{-2}, aunque muchos estudios utilizan el tiempo de tratamiento, el número de pulsos de luz, la frecuencia, el voltaje utilizado o simplemente la energía total emitida por la fuente para referirse a la intensidad del tratamiento. Por este motivo es complicado realizar una comparación directa de los resultados obtenidos en diferentes estudios (Gómez-López et al., 2007).

La efectividad de los tratamientos de PL depende de varios factores críticos los cuales están relacionados tanto con el tratamiento como con el producto mismo. Los factores relacionados con el tratamiento de PL son la *DE* emitida por la lámpara, la distancia que hay entre la muestra y la fuente emisora de energía y el medio de propagación de dicha energía. La distribución espectral también se ha reportado como un factor que

determina el efecto de los pulsos de luz sobre los alimentos (Woodling y Moraru, 2007).

El efecto de inactivación microbiana ha sido atribuido mayoritariamente a la región UV-C (Ramos-Villaroel et al., 2012). Por otro lado, la composición química (Gómez-López et al. 2005; Moraru y Uesegui, 2009:) y topografía (Lagunas-Solar et al., 2006) del alimento son los principales factores que pueden afectar la eficacia de estos tratamientos.

1.3.2 Efectos de los PL en la inactivación microbiana

La capacidad microbicida de los PL depende principalmente de la interacción entre los microorganismos y la luz, pero también de la interacción de la luz con el substrato (Ramos Villarroel et al., 2013). La magnitud del efecto de inactivación microbiana se ha atribuido mayoritariamente a las longitudes de onda correspondientes a la región del UV-C (200-290 nm) (Wang et al., 2005). Debido a las características de tratamiento y de los equipos de PL, se han identificado efectos fotoquímicos, fototérmicos y fotofísicos en el producto tratado (Ramos-Villarroel et al. 2013):

1.3.2.1 Efecto fotoquímico

La capacidad de reducción bacteriana de los PL ha sido mayoritariamente atribuida a la transformación fotoquímica de las bases pirimidínicas del ADN (citosina y timina) y a la formación de dímeros de timina (Giese y Darby, 2000). Estos dímeros inhiben la generación de nuevas cadenas de ADN durante el proceso de replicación, lo que resulta en la inactivación (muerte clonogénica) de los microorganismos (Bolton y Linden, 2003). En el caso de esporas, se ha reportado la formación de 5-timin-5-,6-dihidro-timina, también llamado un ”fotoproducto de espora”, y rupturas sencillas y dobles de los dímeros de pirimidina, lo que ocasiona un daño en la replicación (Slieman y Nicholson, 2000). En levaduras se ha observado una reacción similar en los cambios de las hebras individuales y formación de dímeros de pirimidina (Takeshita et al., 2003).

Wang et al. (2005) defienden la hipótesis de que el efecto fotoquímico producido como consecuencia de la absorción de las longitudes de onda UV por el ADN es la principal causa de la inactivación microbiana por PL. Sin embargo, algunos estudios afirman que la inactivación microbiana por PL no es totalmente dependiente del daño directo al ADN. Gómez-López et al. (2007) reportó daños en la pared celular de levaduras. Estos daños en las membranas podrían ocasionar la destrucción de los ácidos nucleicos.

1.3.2.2 Efecto fototérmico

La aplicación de altas *DE* parece generar un trastorno celular debido a la absorción de la luz. Este efecto fue descrito en 1984 por Hiramoto, quien propuso que la absorción de los rayos incidentes en los hongos provocaba una esterilización de tipo térmico. Algunos autores como Dunn et al. (1989) propusieron que el efecto germicida de los PL se debía tanto al efecto fototérmico como al fotoquímico, indicando que el calentamiento que migraba del exterior al interior del producto era relativamente bajo por lo que ambos efectos eran necesarios para tener un impacto significativo. Wekhof et al. (2000) plantearon que una *DE* de 0.5 J cm^{-2} era necesaria para registrar un sobrecalentamiento de la pared de una espora, provocando un ruptura de la membrana, liberación del contenido intracelular al exterior y la consecuente pérdida de la capacidad de duplicación celular. Este sobrecalentamiento puede ser atribuido a la diferencia en la absorción de luz UV tanto por la bacteria como por el sustrato donde se encuentra. Takeshita et al. (2003) observaron que la ruptura de las membranas celulares provocado tanto por el efecto fotoquímico como fototérmico causaba daños en el ADN de las levaduras. Este efecto también puede relacionarse con la vaporización del agua en el interior de la célula, lo que provoca trastornos en las membranas (Fine y Gervais, 2004). Además, es conocido que las proteínas son sensibles al incremento de temperatura, por lo que el incremento de la *DE* puede provocar la pérdida de la función proteica y así causar la muerte celular (Panico, 2005).

1.3.2.3 Efecto fotofísico

Aunque los efectos fotoquímico y fototérmico parecen ser los responsables directos de la inactivación microbiana, algunos autores afirman que este mecanismo también se ve influenciado por el efecto fotofísico ejercido por los PL.

Elmanasser et al. (2007) mencionan que los PL pueden inducir cambios estructurales que provocan una distorsión de la membrana celular, lo cual produce cambios en la forma de las células. Esta deformación celular ha sido reportada tanto en esporas (Wekhof, 2001) como en bacterias (Krishnamurthy et al., 2008; Takeshita et al., 2003). Este tipo de destrucción física podría ser el resultado de la pérdida de integridad causada tanto por efecto fotoquímico como fototérmico. Ramos-Villarroel et al. (2012) reportaron una alteración pronunciada de la membrana citoplasmática de células de *E. coli* y *L. Innocua* inoculadas en champiñón y expuestas a 12 J cm^{-2}. Estos autores señalaron que los daños estructurales, observados mediante microscopía electrónica de transmisión (TEM), eran debidos al efecto fotofísico infringido por los PL. Así, estas deformaciones celulares, podrían relacionarse con la disfuncionalidad de las proteínas y destrucción de ácidos nucleicos, lo que terminaría ocasionando la muerte celular.

1.3.3 Efectos de los PL en las características fisicoquímicas

En la literatura se reportan algunos efectos de los PL en las características fisicoquímicas de los FFC y que impactan de forma indirecta en su percepción sensorial y calidad. El incremento en la intensidad de tratamiento puede provocar mayores cambios en las características fisicoquímicas del producto. Sin embargo, el efecto de los PL puede variar dependiendo del tipo de fruto, la madurez, la constitución fisicoquímica, la microbiota presente y las condiciones de conservación del alimento. Algunos autores reportan que los PL son capaces de inducir un incremento en la tasa respiratoria o una acelerada producción de dióxido de carbono y consumo de oxígeno en envases con patatas, lechugas, champiñones, aguacate, espinacas y tomates frescos cortados (Dunn et al. 1989; Gómez-López et al. 2005; Oms-Oliu et al. 2010; Ramos-Villarroel et al. 2011;

Agüero et al. 2016; Valdivia-Nájar et al. 2017). Estos autores señalan que este cambio metabólico puede ser asociado a un estrés y/o daño fisiológico del producto vegetal como consecuencia de la exposición a los PL.

El incremento en la respiración celular de los productos frescos cortados se asocia con la aparición de fenómenos de tipo osmótico y enzimático que pueden llegar a alterar la textura. Oms-Oliu et al. (2010) estudiaron el efecto de los PL en champiñón laminado y observaron pardeamiento y un ligero cambio en la textura, atribuyendo estos cambios al efecto fototérmico causado por Φ de entre 12 y 28 J cm^{-2}. Ramos-Villarroel et al. (2011) reportaron que la exposición de aguacate fresco cortado a Φ de 12 J cm^{-2}, afectó gravemente el color y la textura, provocando pardeamiento y ablandamiento. Similarmente, Izquier y Gómez-Lopez et al. (2011) reportaron pardeamiento y deterioro de la integridad celular de manzanas frescas cortadas expuestas a PL. Algunos estudios realizados en manzanas frescas cortadas sujetas a PL también coinciden en que la aplicación de *DE* superiores a 16 J cm^{-2} induce el pardeamiento debido al incremento de la interacción enzima-sustrato provocado principalmente por el daño celular (Ignat et al., 2014; Avalos et al., 2016). Sin embargo, estos autores no pudieron determinar un efecto directo de los PL sobre la textura de la manzana fresca cortada. Hoornstra et al. (2002) no detectaron cambios en la textura de lechuga iceberg pero reportaron alteraciones en el color (decoloración) después de los tratamientos por PL. En este sentido, los PL pueden provocar daños estructurales en los cloroplastos, los cuales son los encargados de realizar la fotosíntesis y de la producción de clorofila, provocando la decoloración del producto.

Por otro lado, hay autores que señalan que los PL no producen cambios importantes en el color o en la textura de frambuesas, fresas, tomates, manzana frutos frescos cortados (Bialka y Demirci, 2008; Aguiló-Aguayo et al., 2013; Gómez et al., 2012). Similarmente Agüero et al. (2016) reportaron que después de una evaluación sensorial no se detectaron cambios significativos en el color de espinacas frescas cortadas y sujetas a bajas dosis de PL (8-20 J cm^{-2}).

1.3.4 Efectos de los PL en las características nutrimentales

Hasta el día de hoy existen muy pocos trabajos enfocados en los cambios nutrimentales de los productos frescos cortados sujetos a tratamientos de PL. Algunos estudios reportan un bajo impacto en el contenido de proteínas y vitaminas en productos cárnicos, pollo, pescados y quesos sujetos a dosis reducidas de PL (<30 J cm^{-2}) (Dunn et al., 1989; Dunn et al., 1995; Shuwaish et al., 2000; Ozer y Demirci, 2006).

La gran mayoría de reportes relacionados a los compuestos bioactivos en los frutos frescos cortados y expuestos a PL, concuerdan en un incremento acelerado del contenido de algunos compuestos provocado por el daño a la estructura celular, la liberación de sustancias intracelulares y a una rápida síntesis de compuestos con poder antioxidante como respuesta al estrés fotoquímico y fototérmico infligido por los PL (Aguiló-Aguayo et al., 2013; Rodov, et al., 2012; Solovchenko y Merzliak, 2008). Sin embargo, estos incrementos iniciales pueden ser favorecidos o mermados durante el almacenamiento de los productos frescos cortados. Agüero et al., (2016) obtuvieron incrementos iniciales de la capacidad antioxidante y de los compuestos fenólicos, aunque estos valores descendieron drásticamente durante el almacenamiento de espinacas frescas cortadas y sujetas a PL. Artés-Hernández et al. (2009) observaron importantes descensos de la capacidad antioxidante y los compuestos fenólicos durante el almacenamiento de espinacas sujetas a PL (UV-C; 10 kJ cm^{-2}). Sin embargo, estos últimos autores no observaron cambios significativos en la capacidad antioxidante ni en el contenido fenólico justo después del tratamiento por PL. Así mismo, Rodov et al. (2012) y Avalos-Llanos et al. (2016) reportaron aumentos tanto de la actividad antioxidante como del contenido fenólico de higos y manzanas cortadas y sujetas a PL. Estos autores señalan que el estrés causado por los PL es capaz de inducir la activación del metabolismo fenilpropanoide, incrementado así el contenido de compuestos fenólicos y por tanto aumentado la capacidad antioxidante del alimento. Murugesan et al. (2012) sugieren que el incremento de la

temperatura en la superficie de las bayas de saúco puede inducir la síntesis de compuestos fenólicos a causa de la exposición a PL. De hecho, la producción de metabolitos secundarios ha sido atribuida a un mecanismo de defensa de los tejidos vegetales contra daños causados por la luz UV (Dixon y Pavia, 1995).

La vitamina C es altamente sensible a la luz, por lo que es lógico deducir que los tratamientos de luz causan un impacto negativo en el contenido de este compuesto. Oms-Oliu et al. (2010a) observaron un descenso en la concentración de vitamina C de champiñón laminado expuesto a PL y sugirieron que el incremento de la *DE* de tratamiento incitaba a mayores reducciones del contenido de vitamina C. Estos mismos autores señalan que un alto contenido de CO_2 en el envase guarda relación con la concentración de vitamina C en champiñón laminado. Este fenómeno se puede relacionar bien con el incremento en la respiración de los productos frescos cortados y expuestos a PL. En este sentido, algunos autores reportaron que el incremento de la Φ de tratamiento produjo incrementos del consumo de O_2 y de producción de CO_2 en los espacios de cabeza de tomate (Valdivia-Nájar et al., 2017) y espinaca (Agüero et al., 2016) cortada y expuesta a PL (0-30 J cm^{-2}). Estos autores señalan que estos cambios se relacionan con un aumento de la actividad metabólica inducida por los PL.

1.3.5 Conservación de alimentos mediante PL

La utilización de tecnologías físicas como método de conservación de FFC ha cobrado gran importancia debido en gran medida a su bajo impacto en la calidad de estos alimentos. Entre estos tratamientos se encuentran los PL, los cuales se han utilizado ampliamente en la industria alimentaria para la desinfección de envases y equipos de procesado. En 1984 se patentó el primer equipo de desinfección de alimentos utilizando luz pulsada (Hiramoto, 1984), pero no es hasta la década del 2000 que los PL (100-1100 nm) cobraron importancia en la conservación de alimentos (Artés y Allende, 2005; Wekfof, 2000).

Los mejores resultados en la inactivación de microorganismos se han obtenido utilizando altas *DE* en un periodo de tiempo más corto (Garvey et al., 2014). El código 21-CFR179.41 publicado por la FDA limita el uso de los PL para la desinfección de alimentos a la utilización de lámparas de gas xenón como fuente de luz, una emisión espectral entre 200-110nm y una energía máxima incidente en el alimento que no exceda los 12 J cm^{-2} (FDA, 2016). En base a estudios previos, la FDA considera que estas condiciones son suficientes para asegurar la eficacia en la reducción microbiana con un mínimo impacto sobre las características químicas y nutritivas del alimento (Koutcha, 2014).

Los estudios realizados en los últimos años, señalan a los PL como una alternativa a los métodos térmicos para la desinfección de alimentos, puesto que además no generan un impacto importante en sus características sensoriales y nutritivas. Hoy en día, la aplicación de los tratamientos de PL ha sido reportada en diferentes tipos de alimentos (Tabla 4). La mayoría de los estudios se centran en el efecto germicida de los PL y muy pocos están orientados a determinar el impacto de los tratamientos aplicados sobre las características fisicoquímicas, nutritivas y sensoriales de los alimentos tratados.

Tabla 4. Investigaciones recientes acerca de la aplicación de luz pulsada en alimentos de origen vegetal y animal.

Sustrato	Observaciones	DE (Φ)	Resultados	Referencia
Origen Vegetal				
Liquidos				
Zumo de naranaja Zumo de manzana Zuma de manzana		1.8-5.5 J cm^{-2}	Inactivación de *E. Coli*: 4.0 log, y de *L. innocua*: 2.98 log	Pataro et al. (2011)
Zumo de fresa Zumo de naranja		71.6 J cm^{-2}	Inactivación de flora nativa: 0.3-2.6 log y de *L. innocua*: 5-8 log	Ferrario et al. (2015)
Solidos				
Campiñón	Laminado	4,8,12 y 28 J cm^{-2}	Inactivacion de flora nativa de 0.6-2.2 log, Efectos negativos en color y textura	Oms-Oliu et al. (2010)
Aguacate	Troceado	6,12 J cm^{-2}	Inactivación de *E. Coli*:>2.90 log y de *L. Innocua*:>2.61 log. Efectos negativos en color y firmeza	Ramos-Villarroel et al. (2011)
Lechuga Calabacín Zanahoria	Troceados	0-12 J cm^{-2}	Inactivación de flora nativa de 0.7-3.4 log	Izquier y Gómez-López (2011)

Tabla 4. Investigaciones recientes acerca de la aplicación de luz pulsada en alimentos de origen vegetal y animal (continuación).

Sustrato	Observaciones	DE (Φ)	Resultados	Referencia
Champiñón	Laminado	3.3-10.3 J cm^{-2}	Incremento del contenido de vitamina D2	Koyyalamundi et al. (2011)
Melón Champiñón	Laminados	12 J cm^{-2}	Reducción de *E. Coli*: 3.0 log, y de *L. innocua*: 2.0 log. Efectos negativos en color y firmeza	Ramos-Villarroel et al. (2012 a y b)
Mango	Troceado	8 J cm^{-2}	Retención de caracteristicas fisicoquimicas y nutrimentales	Charles et al. (2013)
Tomate	Entero y cortado	Entero: 2.68-5.36 J cm^{-2} Secc.: 1-30 J cm^{-2}	Reducción de *Saccharomyces cerevisiae*: 2.3 log Pérdida de textura. Incremento de bioactivos	Aguiló-Aguayo et al. (2013)
Frambuesas Arándanos	Enteros	0.10-0.29 J cm^{-2}	Reducción de *Salmonella* de 4.0 a >5.6 log	Huang et al. (2015)
Setas Lentinula	Enteras y secas	26.2, 52.4 y 78.5 kJ m^{-2}	Incremento de vitamina D2, compuestos fenolicos y capacidad antioxidante	Chien et al. (2016)
Mango	Troceado	0.6 J cm^{-2}	Incremento de compuestos biactivos, capacidad antioxidante y color	Lopes et al. (2016)

Tabla 4. Investigaciones recientes acerca de la aplicación de luz pulsada en alimentos de origen vegetal y animal (continuación).

Sustrato	Observaciones	Intensidad	Resultados	Referencia
Melón	Trocead	2.7-15.6 J cm^{-2}	Inactivación de mohos y levaduras: >1.4 log Retardo del aumento de respiración	Koh et al. (2016)
Fresas	Enteras	2.4-47.8 J cm^{-2}	Inactivación de mohos y levaduras: 16-42 %	Duarte-Molina et al. (2016)
Melón	Entero	0.3,0.6,0.9,1.2 J cm^{-2}	Inactivación de mohos y levaduras: >1.36 log Retención de propiedades fisicoquímicas y perdida de compuestos fenólicos	Koh et al. (2016)
Espinaca	Troceada	20-40 kJ m^{-2}	Inactivación de *E. coli*: 1.7 log, y de *L. innocua*: 1.9 log, Inactivación de mohos y levaduras: 0.4-2.2 log Aumento de la respiración, pérididdas en los compuestos fenólicos y de la capacidad antioxidante	Agüero et al. (2016)
Tomate	Laminado	4,6 y 8 J cm-2	Reducción de *E. coli*: 0.9 log, y *L. innocua*: 0.8 log Incremento de la respiración Cambios fisicoquímicos	Valdivia-Nájar et al. (2017

Tabla 4. Investigaciones recientes acerca de la aplicación de luz pulsada en alimentos de origen vegetal y animal (continuación).

Sustrato	Observaciones	Intensidad	Resultados	Referencia
Origen Animal				
Ternera Atún	Laminados	0.7-11.9 J cm^{-2}	Inactivación de *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhimurium* y *Vibrio parahaemolyticus*: 1 log Efectos negativo en color y atributos sensoriales	Hierro et al. (2012)
Leche Leche concentrada		14.9 J cm^{-2}	Inactivación de *E. coli*: >2.5 log	Miller et al. (2012)
Salchichon seco curado	Laminados	0.7-11.9 J cm^{-2}	Inactivación de *Listeria monocytogenes* y *Salmonella enterica serovar Typhimurium*: 1.5 - 1.8 log Cambios de color	Ganan et al. (2012)
Pescado Salmón Gambas	Laminados	0.0-17.2 J cm^{-2}	Inactivación de *Listeria monocytogenes*: 1.7 - 2.2 log Daño fisico de las paredes celulares de *L. monocitogenes*	Cheigh et al. (2013)
Huevo	Hervido	1-30 s / 5.5 y 9.5 cm distancia	Inactivación de *E. coli* K12 :>3.23 Log Incremento de la temperatura interna Conservacion de textura y color. Efecto fotofísico de los PL.	Macias-Rodriguez et al. (2014)
Queso	Laminado	0.7-11.9 J cm^{-2}	Incremento de la oxidación proteica	Fernández et al. (2014)
Queso	Troceado	1.02-12.29 J cm^{-2}	Reducción de *Pseudomonas fluorescens*, *Escherichia coli* ATCC 25922 y *Listeria innocua*: 3.8, 5.4 y 3.4 log, respectivamente	Proulx et al. (2015)
Leche cabra		13-100 J cm^{-2}	Reducción de *E. coli*: 6 log, cambios en el aroma, no se observaron diferencias fisicoquimicas	Kasahara et al. (2015)

1.4. Bibliografía

Ahmed, L., Martin-Diana, A. B., Rico, D., & Barry-Ryan, C. (2012). Extending the shelf life of fresh-cut tomato using by-product from cheese industry. *Journal of Food Processing and Preservation*, 36(2), 141-151.

Adalid, A. 2011. Mejora de la Calidad Nutritiva del Tomate: Búsqueda de Fuentes de Variabilidad, Estudio de la Influencia del Ambiente y Determinación del Control Genético. Tesis Doctoral. Universidad Politécnica de Valencia, Valencia, España.

Aguayo, E., Escalona, V., & Artés, F. (2004). Quality of fresh-cut tomato as affected by type of cut, packaging, temperature and storage time. *European Food Research and Technology*, 219(5), 492-499.

Aguayo, E., Escalona, V. H., & Artés, F. (2006). Effect of cyclic exposure to ozone gas on physicochemical, sensorial and microbial quality of whole and sliced tomatoes. *Postharvest Biology and Technology*, 39(2), 169-177.

Agüero, M. V., Jagus, R. J., Martín-Belloso, O., & Soliva-Fortuny, R. (2016). Surface decontamination of spinach by intense pulsed light treatments: Impact on quality attributes. *Postharvest Biology and Technology*, 121, 118-125.

Aguiló-Aguayo, I., Charles, F., Renard, C. M. G. C., Page, D., & Carlin, F. (2013). Pulsed light effects on surface decontamination, physical qualities and nutritional composition of tomato fruit. *Postharvest Biology and Technology*, 86, 29-36.

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

Allende, A., Tomás-Barberán, F. A., & Gil, M. I. (2006). Minimal processing for healthy traditional foods. *Trends in Food Science and Technology*, 17(9), 513-519.

Ames, B. M., Shigena, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants and the degerative diseases of aging. Proceeding of the National

Academy of Sciences of the United States of America, 90, 7915-7922.

Antunes, M. D. C., Rodrigues, D., Pantazis, V., Cavaco, A. M., Siomos, A. S., & Miguel, G. (2013). Nutritional quality changes of fresh-cut tomato during shelf life. *Food Science and Biotechnology*, 22(5), 1-8.

Ashie, I.N.A., Simpson, B. K. &Smith, J. P. (1996). *Critical Reviews in Food Science Nutrition* 36(1-2), 1-30.

Ayala-Zavala, J. F., Oms-Oliu, G., Odriozola-Serrano, I., González-Aguilar G. A., Álvarez-Parrilla, E., & Martín-Belloso, O. (2008). Bio-preservation of fresh-cut tomatoes using natural antimicrobials. *Eur. Food Res.Technol*. 226, 1047-1055.

Alasalvar, C., Al-Farsi, M., & Shahidi F. (2005). Compositional characteristics and antioxidant components of cherry laurel varieties and pekmez. J*. Food Sci*., 70 (1), 47 - 52.

Aron Maftei, N., Ramos-Villarroel, A. Y., Nicolau, A. I., Martín-Belloso, O., & Soliva-Fortuny, R. (2014). Pulsed light inactivation of naturally occurring moulds on wheat grain. *Journal of the Science of Food and Agriculture*, 94(4), 721-726.

Artés, F., & Allende, A. (2005). Processing lines and alternative preservation techniques to prolong the shelf-life of minimally processed leafy vegetables. *European Journal of Horticultural Science*, 70, 231-245.

Artés, F., Conesa, M. A., Hernández, S., & Gil, M. I. (1999). Keeping quality of fresh-cut tomato. *Postharvest Biology and Technology*, 17(3), 153-162.

Artés, F., Gómez, P., Aguayo, E., Escalona, V., & Artés- Hernández, F. (2009). Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharvest Biol Technol 51*, 287–296.

Artés, F., & Artés-Hernández, F. (2003). Etapas decisivas y diseño de instalaciones para la elaboración de productos procesados en fresco. En: Lobo M.G. y González, M. (Eds.). Productos hortofrutícolas

mínimamente procesados, pp. 57-78. Eds: Ed it. Gobierno de Canarias.

Artés-Hernández, F., Escalona, V. H., Robles, G. B. Martínez-Hernández, P. A., & Artés F. (2009). Effect f UV-C radiation on quality of minimally processed spinach leaves. *J. Sci. Food Agric*., 89 (3), 414–421.

Asplund, K., & Nurmi, E. (1991). The growth of salmonellae in tomatoes. International Journal of Food Microbiology*,* 13*,* 177-182.

Avalos Llano, K. R., Marsellés-Fontanet, A. R., Martín-Belloso, O., & Soliva-Fortuny, R. (2016). Impact of pulsed light treatments on antioxidant characteristics and quality attributes of fresh-cut apples*. Innovative Food Science and Emerging Technologies,* 33, 206-215*.*

Barbosa-Canovas, G. V., Pothakamury, U. R., Palou, E., & Swanson, B. G. (1998). *Nonthermal preservation of foods* pp. 139–161. New York: Marcel Dekker.

Barrett, D. M., Beaulieu, J. C., & Shewfelt, R. (2010). Color, Flavor, Texture and Nutitional Quality of Fresh-cut Fruits and Vegetbales: Desirable levels, Instrumental and Sensory Measurement, and the Effects of Processing. *Critical Reviews in Food Science and Nutrition*, 50, 369-389.

Beuchat, L. R. (1996a). Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection*, 59, 204-216.

Bialka, K. L., & Demirci, A. (2008). Efficacy of pulsed UV-light for the decontamination of *Escherichia coli* O157:H7 and *Salmonella spp*. On raspberries and strawberries. *Journal of Food Science*, 73(5) doi:10.1111/j.1750-3841.2008.00743.x

Brecht, P.E., Keng, L., Bisogni, C. A., & Vantonder, A. J. (1976). Effect of the fruit portion, stage of ripeness and growth habits on chemical composition of fresh tomatoes*.* Journal of Food Science, 41:945-948.

Bolton, J. R., & Linden, K. G. (2003). Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. *Journal of Environmental Engineering*, 129(3), 209-215.

Borguini, Galhardo, R. B., Markowicz, D. H., Moita-Neto, J. M., Capasso, S. F., & Torres, E. A. F. da S.. (2013). Antioxidant potential of tomatoes cultivated in organic and conventional systems. *Brazilian Archives of Biology and Technology*, 56(4), 521-529.

Caminiti, I. M., Noci, F., Muñoz, A., Whyte, P., Morgan, D. J., Cronin, D. A., & Lyng, J. G. (2011). Impact of selected combinations of non-thermal processing technologies on the quality of an apple and cranberry juice blend. *Food Chemistry*, 124(4), 1387-1392.

Can, F. O., Demirci, A., Puri, V. M., & Gourama, H. (2014). Decontamination of hard cheeses by pulsed UV light. *Journal of Food Protection*, 77(10), 1723-1731.

Charles, F., Vidal, V., Olive, F., Filgueiras, H., & Sallanon, H. (2013). Pulsed light treatment as new method to maintain physical and nutritional quality of fresh-cut mangoes. *Innovative Food Science and Emerging Technologies*, 18, 190-195.

Cheigh, C., Hwang, H., & Chung, M. (2013). Intense pulsed light (IPL) and UV-C treatments for inactivating *Listeria monocytogenes* on solid medium and seafoods. *Food Research International*, 54(1), 745-752.

Chen, J. (2002). Microbial enzymes associated with fresh-cut produce. O. Lamikanra (Ed.), Fresh-cut Fruits and Vegetables. Science, Technology, and Market, CRC Press, Boca Raton, FL, USA (2002), pp. 249–266.

Chien, R., Yang, S., Lin, L., & Mau, J. (2016). Anti-inflammatory and antioxidant properties o pulsed light irradiated *Lentinula edodes*. *Journal of Food Processing and Preservation*, doi:10.1111/jfpp.13045

Cisneros‐Zevallos, l., Saltveit, M. E., & Krochta, J. M. (1995). Mechanism of surface white discoloration of peeled (minimally processed) carrots during storage. *Journal of food Science*, 60(2), 320-323.

Cisneros-Zevallos, L., & Krochta, J. M. (2005). Internal modified atmospheres of coated fresh fruits and vegetables. Understanding relative humidity effects. *Innovations in Food Packaging*, 173-184.

Cocci, E., Rocculi, P., Romani, S., & Rosa, M. D. (2006). Changes in nutritional properties of minimally processed apples during storage. *Postharvest Biology and Technology*, 39,265-279.

Coronel, J., & Castillo, P. (2009). Alternativas de mejora en el manejo postcosecha de tomate riñón cultivado en la provincia de Santa Elena. Centro de Investigaciones Científicas y Tecnológicas. [Documento en línea]. Disponible: http:// www. dspace.espol.edu.ec/handle/123456789/11926. [Consulta: 21-03-17].

Day, B. (2000). Modified atmosphere packaging (MAP) of fresh fruits and vegetables-an overview. En 4th International Conference on Postharvest Science. Jerusalen, Israel.

D'Souza, M. C., Ingle, M., & Head, M. K. (1992). Quality of processed field-grown tomatoes as influenced by growth of plants under less than optimum soil moisture conditions. *Journal of Sustainable Agriculture*, 2(1), 41-48.

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

Dixon, R. A., &. Paiva, N. L (1995). Stress-induced phenylpropanoid metabolism. *The Plant Cell*, 7, 1085–1097.

Dorais, M., Papadopoulos, A. P., & Gosselin, A., (2001). Greenhouse tomato fruit quality. *Hort. Rev*. 26, 239-270.

Du, Y., Williams, B. A., Francis, L. F., & Mccormick, A. V. (2017). Pulsed irradiation for high-throughput curing applications. *Progress in Organic Coatings*, 104, 104-109.

Duarte-Molina, F., Gómez, P. L., Castro, M. A., & Alzamora, S. M. (2016). Storage quality of strawberry fruit treated by pulsed light: Fungal decay, water loss and mechanical properties. *Innovative Food Science and Emerging Technologies*, 34, 267-274.

Dunn, J. E., Clark, R. W., Asmus, J. F., Pearlman, J. S., Boyerr, K., & Painchaud, F. (1989). Methods for preservation of foodstuffs. US Patent 4,871,559.

Dunn, J., Ott, T., & Clarck, W. (1995). Pulsed light treatment of food and packaging. *Food Technologist*, 49(9), 95-98.

Durán, F. 2006. Manual del Ingeniero de Alimentos. Grupo Latino Ltda., Cartagena, Colombia.

FAO Food and Agricultural Organization. (2016). FAOSTAT, Programa de trabajo estadístico de la FAO. Http://fao.org/faostat/es*data/QC. (Accessed January 24 2017).

FAO Food and Agricultural Organization. (2007) CODEX STAN 293-2007. Normal para el tomate. http://www.fao.org/fao-who-codexalimentarius/shproxy/ru/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCODEX%2BSTAN%2B293-2008%252FCXS_293s.pdf

FEN Fundación Española de nutrición (2017). www.fen.org.es/mercadoFen/pdfs/tomate.pdf

FDA Food and Drug Administration. (2016). Department of Health and Human Services. Irradiation on the production, production and handling of food. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=179.41

FEPEX (Federación Española de Asociaciones de Productores Exportadores de Frutas, Hortalizas, Flores y Plantas vivas) (2016) http://www.fepex.es/noticias/detalle/Espa%C3%b1a-primer-exportador-mundo-frutas-hortalizas-frescas.

Fernández, M., Ganan, M., Guerra, C., & Hierro, E. (2014). Protein oxidation in processed cheese slices treated with pulsed light technology. *Food Chemistry*, 159, 388-390.

Fernández, M., Hospital, X. F., Arias, K., & Hierro, E. (2016). Application of pulsed light to sliced cheese: Effect on *Listeria* inactivation, sensory quality and volatile profile. *Food and Bioprocess Technology*, 9(8), 1335-1344.

Ferrario, M., Alzamora, S. M., & Guerrero, S. (2015). Study of the inactivation of spoilage microorganisms in apple juice by pulsed light and ultrasound. *Food Microbiology*, 46, 635-642.

Ferrario, M. & Guerrero, S. (2016). Effect of a continuous flow-through pulsed light system combined with ultrasound on microbial survivability, color and sensory shelf life of apple juice. *Innovative Food Science and Emerging Technologies*, 34, 214-224.

Food and Drug Administration. (2000). Kinetics of microbial inactivation for alternative food processing technologies: pulsed light technology. Available at: http://vm.cfsan.fda.gov/-comm/ift-puls.html. (Accessed January 22 2017).

Food Agricultural Organization of the Unites Nations (2003a). Code of hygienic practice dor fresh fruits and vegetables CAC/RCP 53. Available at http.www.codexalimentarius.net/web/standard-list.do. Accessed March 12, 2017).

Food Agricultural Organization of the Unites Nations (2003b). Recommended international code of practice. General principles of food hygiene. CAC/RCP 1-1969, Rev. 4-2003. Available at http.www.codexalimentarius.net/web/standard-list.do. Accessed March 12, 2017).

Ganan, M., Hierro, E., Hospital, X. F., Barroso, E., & Fernández, M. (2013). Use of pulsed light to increase the safety of ready-to-eat cured meat products. *Food Control*, 32(2), 512-517.

Garvey, M., Thokala, N., & Rowan, N. (2014). A comparative study on the pulsed UV and the low-pressure UV inactivation of a range of microbial species in water. *Water Environment Research*, 86(12), 2317-2324.

Giese, N., & Darby, J. (2000). Sensitivity of microorganisms to different wavelengths of UV light: implications on modeling of medium pressure UV systems. *Water Research*, 34, 4007- 4013.

Gil, M. I., Aguayo, E., & Kader, A. A. (2006). Quality and nutrient retention in fresh-cut versus whole fruit during storage. *Journal Agricultural and Food Chemistry*, 54, 4284-4296.

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

Gómez, P. L., García-Loredo, A., Nieto, A., Salvatori, D. M., Guerrero, S., & Alzamora, S. M. (2012). Effect of pulsed light combined with an antibrowning pretreatment on quality of fresh cut apple. *Innovative Food Science and Emerging Technologies*, 16, 102-112.

Gómez, P. L., Salvatori, D. M., García-Loredo, A., & Alzamora, S. M. (2012). Pulsed light treatment of cut apple: Dose effect on color, structure, and microbiological stability*. Food and Bioprocess Technology*, 5(6), 2311-2322.

Gómez-López, V. M., Devlieghere, F., Bonduelle, V. & Debevere, J. (2005). Intense light pulses decontamination of minimally processed vegetables and their shelf-life. International *Journal of Food Microbiology*, 103(1), 79-89. Doi:10.1016/j.ijfoodmicro.2004.11.028.

Gómez-López, V. M., Ragaert, P., Debevere, J., & Devlieghere, F. (2007). Pulsed light for food decontamination: A review. *Trends in Food Science and Technology*, 18(9), 464-473.

Gorinstein, S., Caspi, A., Libman, I., Katrich, E., Lerner, H. T., & Trakhtenberg, S. (2004). Fresh Israeli Jaffa sweetie juice consumption improves lipid metabolism and increases antioxidant capacity in hypercholesterolemic patients suffering from coronary artery disease: Studies in vitro and in humans and positive changes in albumin and fibrinogen fractions. *Journal of Agricultural and Food Chemistry*, 52(16), 5215-5222.

Gorny, J. R., Cifuentes, R. A., Hess-Pierce, B., & Kader, A. A. (2000). Quality changes in fresh-cut pear slices as affected by cultivar,

ripeness stage, fruit size, and storage regime. *Journal of Food Science*, 65(3), 541-544.

Guerrero-Beltrán, J. A., & Barbosa-Cánovas, G. V. (2004). Review: Advantages and limitations on processing foods by UV light. *Food Science and Technology International*, 10, 137–147.

Hakim, A., Austin, M., Batal, D., Gullo, S., & Khatoon, M. (2002). Effect of heat treatment and controlled atmosphere on quality of fresh-cut tomatoes. *Advances in Horticultural Science*, 16(2), 88-94.

Harbone, J. B., & Williams, C. (2000) Advances in Flavonoid Research Since 1992. *Phytochemistry*, 55, 481-504.

Heard, G. M. (2002). Microbiology of fresh-cut produce. O. Lamikanra (Ed.), Fresh-cut Fruits and Vegetables. Science, Technology, and Market, CRC Press, Boca Raton, FL, USA (2002), pp. 187–247.

Hierro, E., Barroso, E., La Hoz, L. D., Ordóñez, J. A., Manzano, S., & Fernández, M. (2011). Efficacy of pulsed light for shelf-life extension and inactivation of *Listeria monocytogenes* on ready-to-eat cooked meat products. *Innovative Food Science and Emerging Technologies*, 12(3), 275-281.

Hiramoto, T. (1984). Method of sterilization. US Patent 4, 464-336.

Hoornstra, E., de Jong, G. & Notermans, S. (2002). Preservation of vegetables by light. In conference frontiers in microbial fermentation and preservation (pp. 75-77). The Netherlads: Wageningen.

Huang, Y., & Chen, H. (2014). A novel water-assisted pulsed light processing for decontamination of blueberries. *Food Microbiology*, 40, 1-8.

Huang, Y., Sido, R., Huang, R., & Chen, H. (2015). Application of water-assisted pulsed light treatment to decontaminate raspberries and blueberries from *Salmonella*. *International Journal of Food Microbiology*, 208, 43-50.

IFPA International Fresh-cut Produce Association and Produce Marketing Association (PMA). (1999). Fresh-cut produce handling guidelines. Alexandria (VA), 3 rd. Edition. 17-26; 27-42.

Ignat, A., Manzocco, L., Maifreni, M., Bartolomeoli, I. & Nicoli, M. C. (2014). Surface decontamination of fresh-cut apple by pulsed light: Effects on structure, colour and sensory properties. *Postharvest Biology and Technology*, 91, 122-127.

IUPAC International Union of Pure and Applied Chemistry (1996). Glossary of terms used in photochemistry. *Pure and Applied Chemistry*,12, 2223 e2286.

Iyengar R,, & mcevil, A. J. (1992). Anti-browning agents alternatives to the use of sulfites in foods. *Trends of Food Sci. Technol.* 3, 60-64.

Izquier, A., & Gómez-López, V. M. (2011). Modeling the pulsed light inactivation of microorganisms naturally occurring on vegetable substrates. *Food Microbiology*, 28(6), 1170-1174.

Jay, J. (2002). Microbiología Moderna de los Alimentos. 4ª edición. Acribia, Zaragoza, España.

Jenkins, J. A. (1948). The Origin of the Cultivated Tomato. Economic Botany Vol. 2, No. 4, pp. 379-392.

Jeong, J., Brecht, J. K., Huber, D. J., & Sargent, S. A. (2004). 1-methylcyclopropene (1-MCP) for maintaining texture quality of fresh-cut tomato. *Hortscience*, 39(6), 1359-1362.

Kader, A. A. (2002). Quality parameters of fresh-cut fruit and vegetable products, in Fresh-Cut Fruit and Vegetables: Science, Technology and Market. Lamikanra, O., Ed., CRC Ress, Boca Raton,FL. P. 11.

Kader, A. A., Morris, L. L., Stevens, M. A. & Albright-Holton, M. (1978). Composition and flavor quality of fresh market tomatoes as influenced by some postharvest handling procedures. *J. Am. Soc. Hort. Sci*., 103, 6-13.

Kasahara, I., Carrasco, V., & Aguilar, L. (2015). Inactivation of *Escherichia coli* in goat milk using pulsed ultraviolet light. *Journal of Food Engineering*, 152, 43-49.

Ke, D., & Salveit, M. (1989). Wounded-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. *Physiology Plantarum* 76, 412-418.

Kim, H. S., Dhage, S. R., Shim, D. E. & Hahn, H. T. (2009) Intense pulsed light sintering of copper nanoink for printed electronics *Appl. Phys. A. Mater. Sci. Process*., 97, 791–798.

Kim, H., Fonseca, J. M., Kubota, C., & Choi, J. (2007). Effect of hydrogen peroxide on quality of fresh-cut tomato. *Journal of Food Science*, 72(7).

Klein, B. P. (1987). Nutritional consequences of minimal processing of fruits and vegetables. *J. Food Qual., 10, 795-798.*

Krishnamurthy, K., Tewari, J. C., Irundayaraj, J., & Demirci, A. (2008). Microscopic and spectroscopic evaluation of inactivation of Staphylococcus aureus *by pulsed UV light and infrared heating.* Food and Bioprocess Technology*. Doi:10.1007/s11947-008-0084-8.*

Koh, P. C., Noranizan, M. A., Karim, R., & Nur Hanani, Z. A. (2016a). Microbiological stability and quality of pulsed light treated cantaloupe (*Cucumis melo L. reticulatus* cv. *glamour*) based on cut type and light fluence. *Journal of Food Science and Technology*, 53(4), 1798-1810.

Koh, P. C., Noranizan, M. A., Karim, R., & Nur Hanani, Z. A. (2016b). Repetitive pulsed light treatment at certain interval on fresh-cut cantaloupe (*Cucumis melo L. reticulatus* cv. *glamour*). *Innovative Food Science and Emerging Technologies*, 36, 92-103.

Koutchma, T. (2014). Food Plan Safety. UV applications for Food and Non-Food Surfaces. Regulatory status. Academic Press, London (2014), pp. 45-46

Kovach, C. R., Taneli, Y., Neiman, T., Dyer, E. M., Arzaga, A. J. A. & Kelber, S. T. (2017). Evaluation of an ultraviolet room disinfection protocol to decrease nursing home microbial burden, infection and hospitalization rates. *BMC Infectious Diseases,* 17, 186. *Http://doi.org/10.1186/s12879-017-2275-2.*

Koyyalamudi, S. R., Jeong, S., Pang, G., Teal, A., & Biggs, T. (2011). Concentration of vitamin D2 in white button mushrooms (*Agaricus bisporus*) exposed to pulsed UV light. *Journal of Food Composition and Analysis*, 24(7), 976-979.

Kramer, B., Wunderlich, J., & Muranyi, P. (2015). Pulsed light decontamination of endive salad and mung bean sprouts and impact on color and respiration activity. *Journal of Food Protection*, 78(2), 340-348.

Lamikanra, O,. & Bett-Garber, K. (2005). Fresh-cut fruit moves into the fast line. *Agr. Res*. (http://www.ars.usda.gov./is/AR/archive/aug05/fruit0805).

Lana, M. M., & Tijskens, L. M. M. (2006) Effects of cutting and maturity on antioxidant activity of fresh-cut tomatoes. *Food Chemistry* 97, 203-211.

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

Laurila, E., & Ahvenainen, R. (2002). Minimal processing in practice: fresh fruits and vegetables, pp. 219-244. In: Minimal Processing Technologies in the Food Industry. OHLSSON, T.; BENGTSSON, N. (eds.). Woodhead. Publishing, Ltd.Cambridge, England.

Lee, S. K., & Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*, 20(3), 207-220.

Lewinsohn, E., Schalechet, F., Wilkinson, J., Matsui, K., Tadmor, Y., Nam, K.-H., …& Pichersky, E. (2001). Enhanced Levels of the Aroma and Flavor Compound S-Linalool by Metabolic Engineering of the Terpenoid Pathway in Tomato Fruits. Plant *Physiology*, 127(3), 1256–1265.

Lopes, M. M. A., Silva, E. O., Canuto, K. M., Silva, L. M. A., Gallão, M. I., Urban, L., . . . & Miranda, M. R. A. (2016). Low fluence pulsed light enhanced phytochemical content and antioxidant potential of 'tommy atkins' mango peel and pulp. *Innovative Food Science and Emerging Technologies*, 33, 216-224.

Macias-Rodríguez, B., Yang, W., Schneider, K., & Rock, C. (2014). Pulsed UV light as a post-processing intervention for decontamination of

hard-cooked peeled eggs. *International Journal of Food Science and Technology*, 49(11), 2472-2480.

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

Mahajan, P. V., Caleb, O. J., Singh, Z., Watkins, C. B., & Geyer, M. (2014). Postharvest treatments of fresh produce. Philosophical Transactions. Series A, Mathematical, Physical, and Engineering Sciences, 372(2017), 20130309. http://doi.org/10.1098/rsta.2013.0309

MAPAMA. (2017). (http://www.mapama.gob.es/es/agricultura/temas/producciones-agricolas/frutas-y-hortalizas/)

MAPAMA. (2015). Ministerio de agricultura, alimentación y medio ambiente. (España)http://www.mapama.gob.es/imagenes/es/informeconsumoalimentacion2015_tcm7-422016.pdf (Accessed March, 12 2017).

Markovic, K., Vahçic, I., Kovaçevic, K., & Banovic, M. (2007). Aroma volatiles of tomatoes and tomato products evaluated by solid-phase microextraction. *Flavorur Fragr. J*. 22, 395–400

Martín-Belloso, O., & Rojas-Graü, M. A. (2005). Factores que afectan la calidad. En nuevas tecnologías de conservación. Productos vegetales frescos cortados. Ed. González-Aguilar, G. A., Cuamea-Navarro, F. Centro de investigación en Alimentación y Desarrollo. México. Pp. 77-93.

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

Martín-Belloso, O., Soliva-Fortuny, R., & Oms-Oliu, G. (2012). Fresh-cut fruits. Handbook of fruits and fruit processing: Second edition (pp. 245-262).

Mcglasson, B. (1993), “Tomatoes”, in Macreae, R., Robinson, R.K. and Sandler, M.J. (Eds), Encyclopedia of Food Science, Food Technology, and Nutrition, Academic Press, New York, NY, pp. 4579-84.

*Miedes, E., & Lorences, E. P. (2004). Apple (*Malus domestica*) and tomato (*Lycopersicum*) fruits cell-wall hemicelluloses and xyloglucan degradation during penicillium expansum infection.* Journal of Agricultural and Food Chemistry*, 52, 7957–796.*

Miller, B. M., Sauer, A., & Moraru, C. I. (2012). Inactivation of *Escherichia coli* in milk and concentrated milk using pulsed-light treatment. *Journal of Dairy Science*, 95(10), 5597-5603.

Moreíra, M. R., Tomadoni, B., Martín-Belloso, O., & Fortuny, R. S. (2015). Preservation of fresh-cut apple quality attributes by pulsed light in combination with gellan gum-based prebiotic edible coatings. *LWT - Food Science and Technology*, 64(2), 1130-1137.

Murugesan, R., Orsat, V., & Lefsrud, M. (2012). Effect of pulsed ultraviolet light on the total phenol content of elderberry (*Sambucus nigra*) fruit *Food and Nutrition Sciences*, 3 (2012), pp. 774–783.

Nguyen-Yhe, C., & Carlin, F. (1994). The microbiology of minimally processed fresh fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, 34, 371-401.

Odriozola-Serrano, Isabel. (2009). Tesis doctoral. Obtención de zumos y frutos cortados con alto potencial antioxidante mediante tratamientos no térmicos. Dririgida por Olga Martín Belloso y Vicente Gimeno Añó. Universidad de Lleida. Lleida, España.

Odriozola-Serrano, I., Oms-Oliu, G., Soliva-Fortuny, R. & Martín-Belloso, O. (2009b). Effect of high-oxygen atmospheres on the antioxidant potential of fresh-cut tomatoes. *Journal of Agricultural and Food Chemistry*, 57(15), 6603-6610.

Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Effect of minimal processing on bioactive compounds and color attributes of fresh-cut tomatoes. *LWT - Food Science and Technology*, 41(2), 217-226.

Oms Oliu, Gemma (2009) Tesis doctoral: Alternativas de envasado de pera y melón frescos cortados en atmósfera modificada. Dirigida por: Olga

Martín Belloso y Robert Soliva Fortuny. Universidad de Lleida. Lleida, España.

Oms-Oliu, G., Aguiló-Aguayo, I., Martín-Belloso, O., & Soliva-Fortuny, R. (2010a). Effects of pulsed light treatments on quality and antioxidant properties of fresh-cut mushrooms (agaricus bisporus). *Postharvest Biology and Technology*, 56(3), 216-222.

Oms-Oliu, G., Martín-Belloso, O., & Soliva-Fortuny, R. (2010b). Pulsed light treatments for food preservation. A review. *Food and Bioprocess Technology*, 3(1), 13-23.

Ozer, N. P., & Demirci, A. (2006). Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* inoculated on raw salmon fillets by pulsed UV-light treatment. *International Journal of Food Science & Technology*, 41, 354–360.

Palacios, A. P., Marín, J. M., Quinto, E. J., & Wiper, M. P. (2014). Bayesian modeling of bacterial growth for multiple populations. *Annals of Applied Statistics*, 8(3), 1516-1537.

Palgan, I., Caminiti, I. M., Muñoz, A., Noci, F., Whyte, P., Morgan, D. J., & Lyng, J. G. (2011). Combined effect of selected non-thermal technologies on *Escherichia coli* and *Pichia fermentans* inactivation in an apple and cranberry juice blend and on product shelf life. *International Journal of Food Microbiology*, 151(1), 1-6.

Pangaribuam, D. H. (2005). Postharvest physiology of fresh-cut tomato slices. Doctoral thesis. University of Queensland, Australia.

Pangaribuan, D. H., Irving, D. E., & O'Hare, T. J. (2010). Effect of maturity and 1-MCP on physiology of tomato slices. Retrieved from www.scopus.com.

Paskeviciute, E., Buchovec, I., & Luksiene, Z. (2011). High-power pulsed light for decontamination of chicken from food pathogens: A study on antimicrobial efficiency and organoleptic properties. *Journal of Food Safety*, 31(1), 61-68.

Pataro, G., Muñoz, A., Palgan, I., Noci, F., Ferrari, G., & Lyng, J. G. (2011). Bacterial inactivation in fruit juices using a continuous flow pulsed light (PL) system. *Food Research International*, 44(6), 1642-1648.

Pataro, G., Sinik, M., Capitoli, M. M., Donsì, G., & Ferrari, G. (2015). The influence of post-harvest UV-C and pulsed light treatments on quality and antioxidant properties of tomato fruits during storage. *Innovative Food Science and Emerging Technologies*, 30, 103-111.

Perera, C. O., & Baldwin, E. A. (2001). Biochemistry of fruits and its implications on processing. In: D. Arthey and R.P. Ashurst. Fruit Processing. Nutrition, Products, and Quality Management, 2nd edition. Aspen Publishers, Inc., New York, NY, USA.

Prior, R. L., & Cao, G. (2000) Antioxidant Phytochemicals in Fruits and Vegetables: Diet and Health Implications.*HortScience*, 35:4, 588-592

Proulx, J., Hsu, L. C., Miller, B. M., Sullivan, G., Paradis, K., & Moraru, C. I. (2015). Pulsed-light inactivation of pathogenic and spoilage bacteria on cheese surface. *Journal of Dairy Science*, 98(9), 5890-5898.

Proulx, J., Sullivan, G., Marostegan, L. F., VanWees, S., Hsu, L. C., & Moraru, C. I. (2017). Pulsed light and antimicrobial combination treatments for surface decontamination of cheese: Favorable and antagonistic effects. *Journal of Dairy Science*, 100(3), 1664-1673.

Rajkovic, A., Tomasevic, I., Smigic, N., Uyttendaele, M., Radovanovic, R., & Devlieghere, F. (2010). Pulsed UV light as an intervention strategy against *Listeria monocytogenes* and *Escherichia coli* O157:H7 on the surface of a meat slicing knife. *Journal of Food Engineering*, 100(3), 446-451.

Rajkowski, K. T., & Baldwin, (2003). Concerns with minimal processing in apple, citrus, and vegetable products. J.S. Novak, G.M.

Ramos-Villarroel, A. Y., Martín-Belloso, O., & Soliva-Fortuny, R. (2011). Using antibrowning agents to enhance quality and safety of fresh-cut avocado treated with intense light pulses. *Journal of Food Science*, 76(9), S528-S534.

Ramos-Villarroel, A. Y., Marťn-Belloso, O., & Soliva-Fortuny, R. (2013). Intense light pulses: Microbial inactivation in fruits and vegetables. [Pulsos de luz intensa: inactivació n microbiana en frutas y hortalizas] *CYTA - Journal of Food*, 11(3), 234-242.

Ramos-Villarroel, A. Y., Aron-Maftei, N., Martín-Belloso, O., & Soliva-Fortuny, R. (2012). Influence of spectral distribution on bacterial inactivation and quality changes of fresh-cut watermelon treated with intense light pulses. *Postharvest Biology and Technology*, 69, 32-39.

Ramos-Villarroel, A.Y., Aron-Maftei, N., Martín-Belloso, O., & Soliva-Fortuny, R. 2012. The role of pulsed light spectral distribution in the inactivation of *Escherichia coli* and *Listeria innocua* on fresh-cut mushrooms. *Food Control*, 24, 206-213.

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

Rodov, V., Vinokur, Y., & Horev, B. (2012). Brief postharvest exposure to pulsed light stimulates coloration and anthocyanin accumulation in fig fruit (*Ficus carica* L.). Research note. Postharvest Biology and Technology, 68, 43–46.

Rojas-Graü, M. A., Oms-Oliu, G., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). The use of packaging techniques to maintain freshness in fresh-cut fruits and vegetables: A review. *International Journal of Food Science and Technology*, 44(5), 875-889.

Rosnes, J. T., Sivertsvik, M., & Skara, T. (2003). Combining MAP with other preservation techniques. R. Ahvenainen (Ed.), Novel food packaging techniques, Woodhead Publishing Limited/CRC Press LLC, Cambridge, UK/Boca Raton, FL, pp. 288-290.

Saladié, M., Matas, A. J., Isaacson, T., Jenks, M. A., Goodwin, S. M., Niklas, K. J., Xiaolin, R., Labavitch, J. M., Shackel, K. A., Fernie, A. R., Lytovchenko, A., O'Neill, M. A., Watkins, C. B., & Rose, J. K. C.

(2007). A reevaluation of the key factors that influence tomato fruit softening and integrity. *Plant Physiol*, 144, 1012-1028.

Salunkhe, D. K., Boun, H. R., & Reddy, N. R. (1991). Storage Processing and Nutritional Quality of Fruits and Vegetables, pp. 156–161, CRC Press Inc., Boston, MA.

Sánchez-Moreno, C., De Pascual-Teresa, S., De Ancos, B., & Cano, M. P. (2012). Nutritional quality of fruits. Handbook of fruits and fruit processing: Second edition (pp. 73-84).

Sapers, V.K. Juneja (Eds.). (2003). Microbial safety of minimally processed foods, CRC Press LLC, Boca Raton, FL, p. 39.

Seubert, C. M., & Nichols, M. E. (2004). Alternative curing methods of UV curable automotive clearcoats. *Progress in Organic Coatings*, 49(3), 218-224.

Shewfelt, R. L., Prussia, S. E. Resurreccion, A. V. A. Hurst, W. C., & Campbell, D. T. (1987). Quality changes of Vine-Ripened tomatoes within the postharvest handling system. *Journal of Food Science*, 52(3), 661-664.

Shewfelt, R. L., Thai, C. N., & Davis, J. W. (1992). Prediction of changes in color of tomatoes during ripening at different constant temperatures. *Journal of Food Science*, 53(5), 1433-1437.

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

Shuwaish, A., Figueroa, J. E., & Silva, J. L. (2000). Pulsed light treated prepackaged catfish fillets. IFT Annual Meeting, 10–14 June 2000, Dallas, USA.

Slieman, T. A., & Nicholson, W. L. (2000). Artificial and solar UV radiation induces strand breaks and cyclobutane dimers in Bacillus Subtilis spore DNA. *Applied and Environmental Microbiology*, 66, 199- 205.

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

Soliva-Fortuny, R. C., Alòs-Saiz, N., Espachs-Barroso, A., & Martín-Belloso, O. (2004). Influence of maturity at processing on quality attributes of fresh-cut conference pears. *Journal of Food Science*, 69(7) Retrieved from www.scopus.com

Solovchenko, A. E., & Merzlyak, M. N. (2008). Screening of visible and UV radiation as a photoprotective mechanism in plants Russ. J. *Plant Physiol*., 55, 719–737.

Takeshita, K., Shibato, J., Sameshima, T., Fukunaga, S., Isobe, S., & Arihara, K., (2003). Damage of yeasts induced by pulsed light irradiation. *International Journal of Food Microbiology*, 85,151- 158.

Tamer, C. E., & Çopur, Ö. U. (2010). Chitosan: An edible coating for fresh-cut fruits and vegetables Retrieved from www.scopus.com

Tatsumi, Y., Watada, A. E. & Wergin, W. P. (1991). Scanning electron microscopy of carrot stick surface to determine cause of white translucent appearance. *Journal of Food Science*, 56(5), 1357–1359.

Thumula, P. (2006). Studies on Storage Behaviour of Tomatoes Coated with Chitosan Lysozyme Films. McGill University Montreal, Quebec, Canada. MSc.Thesis.

Thompson, K. A., Marshall, M. R., Sims, C. A., Wei, C. I., Sargent, S. A., & Scott, J. W. (2000) Cultivar, maturity and heat treatment on lycopene content in tomatoes. *Journal of Food Science,* 65, 791-795.

Tijskens, L. M. M., & Evelo, R. G. (1994). Modelling colour of tomatoes during postharvest storage. *Postharvest Biology and Technology*, 4(1-2), 85-98.

Toivonen, P.M.A. (2003). Effects of storage conditions and postharvest procedures on oxidative stress in fruits and vegetables. In: Hodges, D.M. (Ed.),Postharvest Oxidative Stress in Horticultural Crops. The Haworth Press Inc.,Binghamton, New York, pp. 69–90.

Traber, M. G., & Stevens, J. F. (2011). Vitamins C and E: Beneficial effects from a mechanistic perspective. *Free Radical Biology & Medicine*, 51:5, 1000–1013.

USDA, FAS. 2017. WORLD MARKETS AND TRADE: Tomato & Tomato Products.

Valdivia-Nájar, C. G., Martín-Belloso, O., Giner-Seguí, J., & Soliva-Fortuny, R. (2017). Modeling the inactivation of *Listeria innocua* and *Escherichia coli* in fresh-cut tomato treated with pulsed light. *Food and Bioprocess Technology*, 10(2), 266-274.

Vallverdú-Queralt, A., Oms-Oliu, G., Odriozola-Serrano, I., Lamuela-Raventos, R. M., Martín-Belloso, O., & Elez-Martínez, P. (2012). Effects of pulsed electric fields on the bioactive compound content and antioxidant capacity of tomato fruit. *Journal of Agricultural and Food Chemistry*, 60(12), 3126-3134.

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

Waldron, K. W., Parker, M. L., & Smith, A. C. (2003). Plant cell walls and foodquality.*Comprehensive Reviews in Food Science and Food Safety*, 2, 101–119.

Wambura, P., & Verghese, M. (2011). Effect of pulsed ultraviolet light on quality of sliced ham. *LWT - Food Science and Technology*, 44(10), 2173-2179.

Watada, A. E. Aulenbach, B. B., & Worthington, J. T. (1976). Vitamins A and C in ripe tomatoes as affected by stage of ripeness at harvest and by supplementary ethylene. *Journal of Food Science*, 41(4), 856-858.

Watada, A. E., & Qi, L. (1999). Quality control of minimally-processed vegetables.

Wekhof, A. (2000). Disinfection with flash lamps. PDA *Journal of Pharmaceutical Science and Technology / PDA*, 54(3), 264-276.

Wekhof, A. (2001). Pulsed UV disintegration (PUVD): a new sterilization mechanism for packaging and broad medical-hospital applications. In The first International Conference on Ultraviolet Technologies. Washington D. C. USA.

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

Woodling, S. E. & Moraru, C. I. 2007. Effect of spectral range in surface inactivation of *Listeria innocua* using broad-spectrum pulsed light. Journal of Food Protection, 70, 909–916.

XENEX (Xenex German group)(2017). Https://www.xenex.com/research-and-results.

OBJETIVOS

OBJETIVO GENERAL

El objetivo general en esta tesis es estudiar el impacto de los tratamientos por pulsos de luz (PL) sobre la calidad del tomate fresco cortado y almacenado en refrigeración.

OBJETIVOS ESPECÍFICOS

- Establecer el efecto de los PL sobre la microbiota nativa e inoculada y determinar su impacto en la vida útil del tomate fresco cortado.

- Estudiar el efecto de los PL en los parámetros involucrados en la textura y propiedades físicas durante el almacenamiento del tomate fresco cortado.

- Examinar la influencia de los PL sobre la capacidad antioxidante y los compuestos bioactivos en tomate fresco cortado a lo largo de su almacenamiento.

- Evaluar el impacto de los PL sobre los atributos sensoriales del tomate fresco cortado y su relación con la aceptabilidad global.

PLAN DE TRABAJO

PLAN DE TRABAJO

Para lograr los objetivos planteados en esta tesis doctoral, se elaboró un plan de trabajo en el que se propuso la realización de los siguientes cuatro estudios:

1. Estudio de la inactivación de *Listeria innocua* y *Escherichia coli* durante el almacenamiento de tomate fresco cortado expuesto a pulsos de luz (PL).

- Determinar los cambios en los recuentos microbiológicos de *Listeria innocua* y *Escherichia coli* inoculados en el tomate fresco cortado.
- Estudiar los contenidos de O_2 y CO_2 en el interior de los envases con tomate fresco cortado.
- Evaluar las modificaciones en los parámetros de pH, acidez y sólidos solubles totales en el tomate fresco cortado.
- Establecer el impacto de la fluencia (Φ) aplicada y del tiempo de almacenamiento en los cambios observados en el tomate fresco cortado.

2. Estudio del impacto de los tratamientos de PL y el almacenamiento sobre el crecimiento de microorganismos y la textura del tomate fresco cortado durante el almacenamiento.

- Evaluar el impacto de los PL en la microbiota nativa (bacterias psicrófilas y mohos y levaduras) en el tomate cortado.
- Estudiar las modificaciones de la textura del tomate fresco cortado.
- Determinar la pérdida de peso del tomate fresco cortado.

- Investigar los cambios en las enzimas relacionadas a la textura: pectin-metilesterasa (PME) y poligalacturonasa (PG).
- Realización de evaluación sensorial de la textura.

3. Evaluación del efecto de los PL sobre los compuestos bioactivos en el tomate cortado durante el almacenamiento.

- Estudiar los cambios en la capacidad antioxidante del tomate fresco cortado.
- Evaluar los cambios en los contenidos de vitamina C, licopeno y compuestos fenólicos del tomate fresco cortado.
- Determinar del impacto de la Φ y del tiempo de almacenamiento sobre la capacidad antioxidante y los compuestos bioactivos del tomate cortado.

4. Establecer y relacionar los parámetros fisicoquímicos de calidad y la calidad sensorial del tomate cortado expuesto a pulsos de luz (PL).

- Evaluación de las modificaciones en los atributos sensoriales del tomate cortado.
- Definir los parámetros sensoriales asociados a la aceptación del tomate cortado.
- Estudiar el peso de la Φ aplicada y el tiempo de almacenamiento en los cambios de calidad del tomate fresco cortado.
- Establecer la relación entre los parámetros analíticos y la aceptabilidad sensorial del tomate cortado.

RESULTADOS

CAPITULO 1

Modeling the Inactivation of Listeria innocua and Escherichia coli in Fresh-Cut Tomato Treated with Pulsed Light

Abstract

The effectiveness of pulsed light (PL) treatments to inhibit microorganisms on fresh-cut tomatoes (*Lycopersicon esculentum* Mill., cv. Daniela) was investigated. Tomato slices inoculated with *Escherichia coli* or *Listeria innocua* were exposed to PL-treatments (4, 6 or 8 J cm^{-2} fluence) and kept cold at 4 ºC for 20 days. *L. innocua* and *E. coli* counts, gases in the headspace of the containers (O_2 and CO_2), pH, titratable acidity and soluble solids content were monitored throughout the cold storage. The PL-treatments reduced significantly ($p< 0.05$) initial loads of both microbes. The effect of the PL-fluence on the survival number of microorganisms was described by a log-linear model (R^2= 0.849-0.999). At any fixed time within the cold storing, the microbial counts for untreated samples were always higher than those cut tomatoes that had been previously PL-treated. The behaviour of *L. innocua* and *E. coli* during the storage were well adjusted ($R^2> 0.930$) by Gompertzian models; the studied microorganisms exhibited different patterns during the storage period. On the other hand, O_2 and CO_2 partial pressures in containers with fresh-cut tomatoes were also significantly affected by PL-treatments ($p< 0.05$). The highest PL-fluence caused the greatest changes of O_2 and CO_2 contents. In addition, the application of PL triggered an acceleration of the O_2 consumption during the cold stage. PL-treatments might be used to effectively extend the safety of fresh-cut tomatoes over 12 days of storage against *E. coli* and *L. innocua* growth.

Keywords: Fresh-cut tomato; pulsed light treatments; *Listeria innocua*; *Escherichia coli*; headspace gases

1. Introduction

Tomato is one of the most widely consumed vegetables either fresh or processed. Consumption of tomatoes is now considered as an indicator of good nutritional habits and healthy lifestyle mainly because of the presence of vitamins, phenolic compounds, flavonoids and, especially, carotenoids such as lycopene and β-carotene in the product (Odriozola-Serrano et al. 2008).

The growing demand from consumers for healthy, convenient and fresh-like products has motivated to study the effects of minimum processing on the quality of fruits and vegetables. Mechanical operations such as slicing, shredding or dicing bring about a rapid deterioration of vegetables involving physical and chemical changes (Francis and O'Beirne 2005). Moreover, fresh-cut fruits and vegetables are even more susceptible to spoilage due to the release of nutrient and cellular fluids by disruption of protective epidermal layers (Siddiqui et al. 2011). Indeed, fresh foods consumption remains as a major cause of outbreaks of foodborne diseases (Newell et al. 2010).

On the other hand, pathogenic microorganisms may contaminate the products and, thus, the risk of foodborne diseases increases (Beuchat 1996a). Foodborne pathogens, such as *Listeria monocytogenes* and *Escherichia coli*, may also be present on plant foods; a number of outbreaks associated with consumption of different vegetables contaminated with *L. monocytogenes* (Francis et al. 1999; Sagoo et al. 2003) and *E. coli* O157:H7 (Ethelberg et al. 2010; Friesema et al. 2008) have been reported. In fact, the occurrence of these microorganisms has been observed on the surface of fresh-cut tomatoes (Asplund and Nurmi 1991; Beuchat 1996b).

Nowadays, researchers have focused on the study of non-thermal technologies for microbial inactivation to minimize the negative impact on the physicochemical, nutritional and sensorial characteristics of fresh-cut products. Among these preservation treatments, pulsed light (PL) is emerging as an alternative to the disinfection of fruits and vegetables surfaces. This technology involves the application of light pulses using

intense broad-spectrum light for short periods and it is able to inactivate vegetative bacteria, spores, yeast and molds (Oms-Oliu et al. 2010). The germicidal effect of the PL-treatments has been mainly attributed to photochemical or photothermal actions (Gómez-López et al. 2005), as well as to the physicochemical composition of fresh-cut commodities (Ramos-Villarroel et al. 2012). Likewise, an important part of the microbial studies is the search of mathematical models with the aim of predicting microbial growth or depletion considering the process conditions and the storage time (Palacios et al. 2014).

Several studies have shown the ability of PL to inactivate spoilage and pathogenic microorganisms in processed and minimally processed fruits and vegetables using *L*. *innocua* and *E*. *coli* as surrogate strains for *L*. *monocytogenes* (Ramos-Villarroel et al. 2014) and *E*. *coli* O157:H7 (Palgan et al. 2011), respectively. Therefore, the choice of adequate surrogate strains in studies for validation of processes can avoid the difficulties (mainly, pathogenicity) that the target microorganisms could have (FDA/CFSAN 2001).

Hence, the objectives of the present work were to evaluate the impact of different PL-treatments on the growth of surrogate-pathogenic strains, *Escherichia coli* and *Listeria innocua* bacteria, inoculated on fresh-cut tomato as well as to model the changes on the microbial growth and the oxygen and carbon dioxide partial pressures in the package headspace throughout cold storage.

2. Materials and Methods

2.1. Raw materials and processing

Tomatoes (*Licopersicon esculentum* Mill. cv. Daniela) were purchased in a local supermaket (Lleida, Spain) at red stage, characterized by red color in more than 90% of the surface, as defined by the U.S. standards for grades of fresh tomatoes (CFR, 1991) and kept under refrigerated conditions (4 ºC) before use.

Fresh whole tomatoes were sanitized by immersion in chlorinated water (100 ppm) at 4 ºC for 2 min, rinsed with tap water and gently dried by hand. Tomato fruits were then cut into 5 mm-thick slices using an electric slicer (Food Slicer-6128: Toastmaster Corp, Elgin, U.S.A.). Batches of three tomato slices (50 g) were placed in polypropylene trays and immediately inoculated with strains of *Listeria innocua* 1.17 and *Escherichia coli* 1.107.

2.1.1. Preparation of inocula

Listeria innocua CLIP11262 isolated from cheese and *Escherichia coli* 1.107 isolated from human feces were used as surrogates for the pathogenic bacteria *L. monocytogenes* and *E. coli* O157:H7, respectively. The original strains were kept on tryptone soy agar, (TSA: Biokar Diagnostic; Beauvais, France) into inclined test tubes at 5 ºC until use. A stock culture from *L. innocua* was grown in tryptone soy broth (TSB) with 0.6% (w/v) yeast extract (Biokar Diagnostics; Beauvais, France) at 35 ºC for 15 h at 100 rpm, whereas a stock culture of *E. coli* was grown in TSB (Biokar Diagnostics; Beauvais, France) at 37 ºC during 11 h at 80 rpm. These conditions of incubation were performed to obtain cells near their stationary growth phase (10^8 CFU ml^{-1}). Then, the cells in the stock cultures were resuspended using saline peptone water (Sharlau Chemie, S. A.; Barcelona, Spain) in agar and broth for dilution assays, respectively, up to cell densities of approximately 10^6 CFU ml^{-1}, which were subsequently used as inoculum cultures.

2.1.2. Inoculation of tomato slices and packaging

Fresh-cut tomatoes (50 g) were inoculated by spreading 500 μL of *L. innocua* or *E. coli* stocks cultures (10^6 CFU ml^{-1}) over their entire surface with a sterile micropipette and extended with a sterile Digralsky spreader. Immediately after the inoculation, transparent polypropylene trays (350 cm^3, 5025 RM PTT-ATS Packaging S.r.l.; Venice, Italy) were filled up with the tomato slices and the trays were then thermally sealed with plastic film using an ILPRA Food Pack Basic V/6 packaging machine (ILPRA Systems, CP; Vigevono, Italy). According to previous studies (Avalos-Llano et al, 2016),

the transparency of the film was 97% of the UV-radiation and almost a 100% of the visible wavelengths. Furthermore 85% of the energy associated to the 200-320 nm range reached the surface of the samples. The permeabilities for oxygen and carbon dioxide through the transparent sealing film were 5.2419 x 10^{-13} mol O_2 m^{-2} s^{-1} Pa^{-1} and 2.3825 x 10^{-12} mol CO_2 m^{-2} s^{-1} Pa^{-1} at 23 ºC and 0% relative humidity, respectively (ILPRA Systems España, S.L.; Mataró, Spain). After the sealing, the trays with the slices of inoculated tomato were immediately subjected to PL-treatments.

2.2. Pulsed light treatments

PL-treatments were carried out using an automatic laboratory flash lamp system (Steribeam Xe-Matic-2L-A; Kehl, Germany). The emission spectrum of the light source ranged from 200 to 1100 nm. The duration of each pulse was 0.3 ms with 0.4 J cm^{-2} fluence per emitted pulse from two xenon lamps at 8.5 cm above and below the sample holder. To evaluate the effect of applying different treatment doses, inoculated samples were subjected to 10, 15 or 20 pulses. Hence, the applied fluences per each side were 4, 6 and 8 J cm^{-2}, respectively. Treatment intensities were selected on the basis of pre-trials among those not causing undesirable sensory changes to the product. Transparency of the film was determined by measuring the amount of energy received by a photodiode detector placed at the sample holder (Avalos-Llano et al., 2016). Energy calculations were carried out according to the calibration with a standard light source following the manufacturer's directions. A set of untreated samples was kept as control reference. To observe the initial effects of PL processing, a number of determinations were carried out just after sealing and PL processing (about 30 min). Finally, sample trays were stored at 4 °C for 20 days in darkness until random withdrawal for analysis.

2.3. Headspace gases analysis

The atmosphere of each package was analyzed using a gas chromatograph equipped with a thermal conductivity detector (Micro-GP CP

2002 gas analyzer; Chrompack International; Meddelburg, Netherlands), so a sample of 1.7 ml was automatically withdrawn from the headspace atmosphere and fed in the chromatograph. Portions of 0.25 and 0.33 ml were injected for O_2 and CO_2 determinations, respectively. A CP-Molsieve 5 Å packed column (4 m x 0.32 mm, d.f. = 10 mm) at 60 ºC and 100 kPa was used to determine the O_2 partial pressure; on the other side, to determine the CO_2 partial pressure, a Pora-PLOT Q column (10 m x 0.32 mm, d.f. = 10 mm) held at 70 ºC and 200 kPa was used (both columns by Chrompack International; Middelburg, Netherlands).

2.4. Listeria innocua and Escherichia coli counts

Tomato slices (10 g) were aseptically removed from each tray and transferred into sterile stomacher bags (Standard bags, circulator 400, Stomacher®; Sussex, United Kingdom) containing 90 ml of 0.1% (w/v) saline peptone water (Biokar Diagnostics, Beauvais, France) and 0.85% (w/v) of NaCl (Sharlau Chemie, S. A.; Barcelona, Spain) and homogenized for 3 min in a stomacher blender (IUL Instruments; Barcelona, Spain). *E. coli* and *L. innocua* counts were carried out by direct plating technique. Serial dilutions were made and poured at reason of 0.1 ml on MacConkey and Palcam agar-selective supplement (Biokar Diagnostic; Beauvais, France) plates in duplicate for *E. coli* and *L. innocua* counts, respectively. Plates were incubated for 24-48 h at 35-37 ºC. A 50971-Colony Counter (Bioblock Scientific; Taiwan) was used for enumeration of colonies and the results were expressed as log (CFU g^{-1}).

2.5. Soluble solids, pH and acidity determinations

On each sampling day, inoculated tomato slices (20 g) from each tray were homogenized in a blender. The juice was then filtered through a 2-mm diameter steel sieve to remove peel and seeds. An aliquot was used to determine total soluble solids (TTS) using a 2WAJ-ABBE Refractometer (Atago Company Ltd.; Tokyo, Japan) and expressed as Brix degrees (ºBx). The pH was measured using a pH-meter (Crison Instruments S. A.;

Barcelona, Spain). Total acidity (TA) was assessed by titration with NaOH (0.1 N) to pH 8.1 and the results were expressed as grams of anhydrous citric acid per 100 g of fruit.

2.6. Statistical analysis and mathematical modeling

Each processing condition was assayed in duplicate at each sampling time and three replicate analyses were carried out for each trail (every 4 days throughout 20 days storage) to obtain the mean value (n= 6). Significance of the results and statistical differences were analyzed using the Statgraphics plus v. 5.1 Windows package (Statistical graphics Co.; Rockville, MD, U.S.A.). Analysis of variance (ANOVA) was performed to compare sample mean values. Also non-linear regression procedures were used to fit the experimental data to the models. LSD multiple range tests were applied to determine differences among means. The significance level for statistical tests and confidence intervals for all estimated parameters was p= 0.05.

Log-linear relationships, which are expressed by Equation 1, were used to describe the effect of PL-treatments on the microorganisms before the cold storage of the trays of the tomato slices. In the Equation 1, N_0 is the initial count of the microorganisms (CFU g^{-1}); N denotes the number of survival microorganisms after different PL-fluences Φ (J cm^{-2}); and δ (cm^2 J^{-1}) is an inactivation constant that depends on the microorganism and the rest of experimental conditions.

$$\log N = \log N_0 - \delta \cdot \Phi \quad \textbf{(1)}$$

On the other hand, second order polynomials, Equation 2, were used to relate the partial pressures of oxygen (C_o) or carbon dioxide (C_{CO2}) in the headspace of the trays as a function of the applied fluence (Φ) throughout each PL-treatment. In this equation, C_G denotes the partial pressure (kPa) of either oxygen or carbon dioxide and a, b and c are the parameters of the mathematical model.

$$C_G = a\Phi^2 + b\Phi + c \quad \textbf{(2)}$$

A Gompertz´s model, Equation 3, was used to describe the changes on the microbial growth as well as the in-packages O_2 and CO_2 partial pressures as a function of the storage time. (Flores-Cervantes, et al 2013; Oms-Oliu et al., 2007; Lancioti et al., 1999).

$$y = A + C\exp\{-\exp[-B(t-M)]\} \quad \textbf{(3)}$$

In equation 3, *y* denotes the decimal logarithm of the microorganism counts [log (CFU g^{-1})] or, also, the oxygen or carbon dioxide partial pressures (kPa); *A* are the values of the anterior variable (*y*) just at the beginning of their storage period; depending on *y* meaning, *A* value was linked to the outputs given for this parameter by prior models (Equation 1 or Equation 2); *B* is the relative rate of change at time equal to *M*; *M* is the time (day) at which the absolute rates of change is maxima; *C* is the difference between the initial and final asymptotic values attained for each variable during the cold storage; and *t* is the storage time.

From the values of Gompertz´s model parameters, the maximum exponential rates of change (μ_{max}) and the time before the beginning of changes, lag time (λ), were calculated using the Equation 4 and Equation 5 in which A_e, are the experimental values of the considered variable at the beginning of the storage period; and *e* is the Euler's number.

$$\upsilon_{\max} = \frac{B \times C}{e} \quad \textbf{(4)}$$

$$\lambda = M - \left(\frac{1}{B}\right) + \frac{A_e - A}{\upsilon_{\max}} \quad \textbf{(5)}$$

3. Results and discussions

3.1. Headspace gases

Partial pressures of O_2 and CO_2 inside the packages of the tomato slices were significantly affected by PL processing (Figure 1.1). The oxygen partial pressure inside the packages (20.9 kPa) at the beginning of the cold storage decreased immediately after exposure to PL; the maximum depletion (up to 17.4 kPa O_2) took place at the highest applied PL-fluence (8 J cm^{-2}).

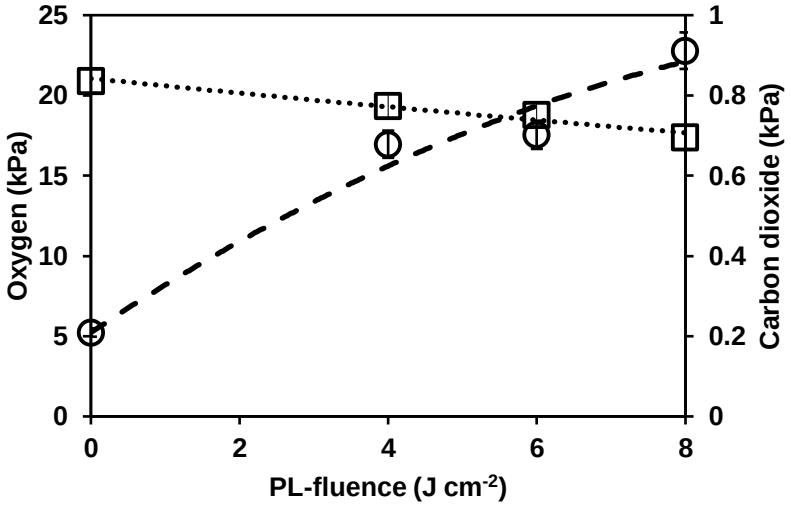

Figure 1.1. Changes on the oxygen (squares) and carbon dioxide (circles) partial pressures inside the packages of PL-treated tomato slices. Points are the means of three repetitions from two replicate packages ±SD. Lines are the fit of second-order polynomials to the experimental data.

Conversely, carbon dioxide partial pressure (0.2 kPa CO_2) at the start of this period of time increased significantly ($p< 0.05$) after the exposure to PL-treatments; indeed, CO_2 pressure reached 0.91 kPa at 8 J cm^{-2}. The fit of second-order polynomial functions to O_2 ($R^2= 0.967$) or CO_2 ($R^2= 0.998$) concentration as a function of the fluence of the PL-treatments led to Eqs. 6 and 7, respectively.

$$C_{O_2} = -0.0048\phi^2 + 0.123\phi + 0.209 \quad \textbf{(6)}$$

$$C_{CO_2} = 0.016\phi^2 - 0.627\phi + 20.8 \quad \textbf{(7)}$$

These mathematical models point that PL-treatments bring on a rapid consumption of O_2 that triggers an accelerated production of CO_2 from the tomato slices. To date, no report on the effect of PL on the respiration of tomato slices is known. However, the pattern observed for the headspace composition in the trays with tomato slices was in agreement with the one reported by Ramos-Villarroel et al. (2011a) who found that O_2 and CO_2 contents on PL-treated avocado slices were more affected when higher PL-fluences were applied. In this context, Gómez-López et al. (2005) reported that PL-treatments affect the plant cells causing DNA damages, disorders in tissues and photosynthetic apparatus and, thus altering the respiration of the vegetables.

During the cold storage period, oxygen and carbon dioxide partial pressures inside the packages decreased and increased, respectively, at any applied PL-fluence. Figure 1.2 and Figure 1.3 show that the higher applied PL-fluence the higher effect on the changes of partial pressures of O_2 and CO_2 during the cold storage. Indeed, a PL-fluence of 8 J cm^{-2} yielded lower O_2 and higher CO_2 contents (0.12 and 16.60 kPa, respectively) compared to untreated samples (0.91 and 17.40 kPa, respectively) at the end of the storage. Both the progress of O_2 and CO_2 partial pressures during the storage period were described by the Gompertz´s model (Equation 3). The estimates of the parameters of these models, determination coefficients (R^2) as well as other derived quantities are given in Table 1.1.

The values of the specific consumption rate of O_2 (μ_{max}) were negative for both PL-treated and untreated slices of tomato; this indicates that the prior processing had yet triggered the accelerated consumption of oxygen during the storage. Moreover, the highest lag time value (λ) was obtained for the assay without PL-treatment. In contrast, lag phase values were shorter

as the PL-fluence increased, as a quicker depletion in oxygen levels occurred.

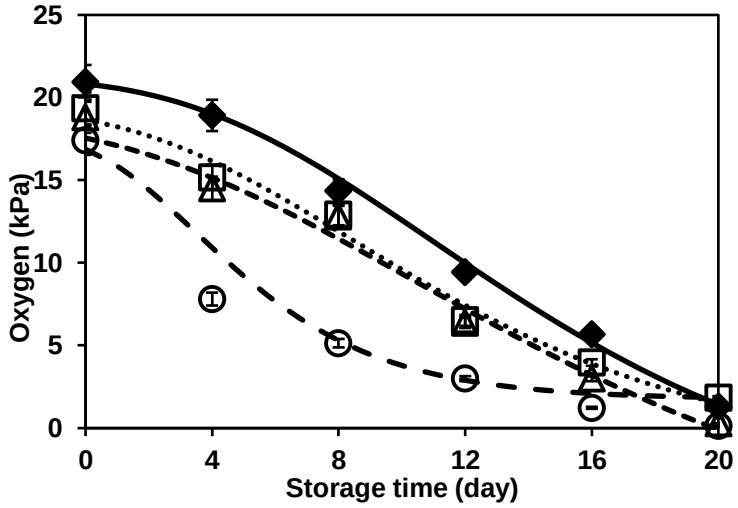

Figure 1.2. Effects of PL treatments on the in-package O_2 partial pressures through storage at 4 ºC. Points are the mean of three repetitions from two replicate packages ±SD. Lines are the fit to the Gompertz's modified model to the experimental data. PL-treatments: Untreated (diamonds); 4 J cm^{-2} (squares); 6 J cm^{-2} (triangles) and 8 J cm^{-2} (circles).

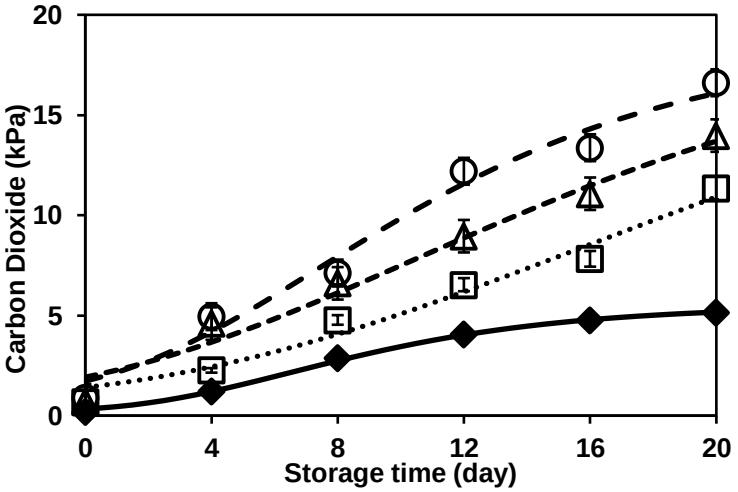

Figure 1.3. Effect of PL treatments (PL) on the in-package CO_2 partial pressures through storage at 4 ºC. Points are the mean of tree repetitions from two replicate packages ±SD. Lines are the fit of the Gompertz's modified model to the experimental data. PL-treatments: Untreated (diamonds); 4 J cm^{-2} (squares); 6 J cm^{-2} (triangles) and 8 J cm^{-2} (circles).

Table 1.1. Parameters of the Gompertz's modified model used to describe the evolution during 20 days of storage (4 °C) of the O_2 and CO_2 inside the packages with tomato slices that had been treated with pulsed light (PL).

Gas	PL-fluence (J cm^{-2})	*A*	*C*	*B*	*M*	μ_{max}	*λ*	R^2
O_2	0 (untreated)	20.9±2.65	-26.78±2.67	0.13±0.01	10.94±0.86	-1.30±0.04	3.29±0.17	0.996
	4	19.3±2.23	-21.35±2.33	0.15±0.02	8.35±0.92	-1.16±0.07	0.98±0.21	0.988
	6	18.8±7.02	-27.09±6.85	0.11±0.03	10.47±2.9	-1.06±0.04	-0.02±0.18	0.975
	8	17.4±1.05	-16.54±1.22	0.30±0.08	2.46±0.6	-1.85±0.11	-1.1±0.49	0.936
CO_2	0 (untreated)	0.21±0.02	5.28±0.25	0.20±0.03	6.50±0.41	0.39±0.06	1.3±0.28	0.994
	4	0.69±0.07	21.40±0.02	0.07±0.05	16.03±1.23	0.60±0.10	1.72±0.23	0.977
	6	0.71±0.05	19.26±0.69	0.09±0.04	10.43±4.25	0.69±0.02	-1.59±0.21	0.973
	8	0.91±0.12	17.63±2.57	0.15±0.04	7.44±1.32	0.97±0.06	1.65±0.27	0.978

A: content of the gases at the beginning of the storage (kPa); ***B***: relative rate of change at time equal to M (kPa day^{-1}); **C**: difference between the final and initial asymptotic values for the gas content (kPa); ***M***: time at the maximum rate of change (day); μ_{max}: specific change rate of O_2 consumption or CO_2 production over time (kPa day^{-1}); ***λ***: lag time (day). R^2: determination coefficient adjusted for degree or freedom, dimensionless. Values ± SD.

With regard to the carbon dioxide production, the assayed model fitted the experimental data with good accuracy (R^2= 0.994-0.973). The kinetic parameters μ_{max} (0.39-0.97 kPa^2 day^{-1}) and λ (< 1.65 day) values indicate an accelerated CO_2 production triggered after about 2 days and the greater the applied PL-fluence, the greater the production rate of CO_2 during the cold storage. Increases of the CO_2 rates after cutting of fresh tomatoes have been previously reported (Ahmed et al. 2012; Gil et al. 2002); these changes were related to an acceleration of respiration due to physical stress caused by processing. Indeed, mechanical processing (slicing) as well as PL-treatment could cause an initial damage of the fruit tissue, triggering an accelerated respiration of fresh-cut tomatoes through storage. Moreover, Ramos-Villarroel et al. (2014) indicated that changes on the headspace composition through storage could be well related to accelerated respiration

caused by modifications of the products physiology due to initial PL treatments.

3.2. Microbial inactivation

The *Listeria innocua* counts on tomato slices decreased significantly ($p< 0.05$) just after exposure to PL-treatments. The higher applied PL-fluence (Φ) the higher reduction of the *L. innocua* counts (Figure 1.4). The microbial inactivation obtained just after exposition to PL as a function of Φ could be described using a log-linear equation ($R^2= 0.849$) in which the inactivation constant was $\delta= 0.122$ cm^2 J^{-1}. Thus, the *L. innocua* counts (N) estimated by the log-linear model (Equation 1) before cold storage were 6.17, 5.76, 5.54 and 5.27 [log (CFU g^{-1})] for the PL-untreated tomato slices and for those submitted to 4, 6 and 8 J/cm^2, respectively.

Uesugi et al. (2013) could reduce up to 4-5 log units *L. innocua* in liquid substrate after exposure to a 5 J cm^{-2} PL-treatment. This higher microbial reduction might be due to the differences on the matrix where the bacteria were inoculated in. Indeed, physicochemical factors such as chemical composition, total soluble compounds, pH and light absorbance (especially due to compounds as carotenoids) could potentially protect the microorganisms from the PL-treatments and, thus, a different inactivation of the microorganisms is achieved.

During the cold storage, the counts of *L. innocua* on fresh-cut tomatoes that were subjected to PL-treatments did not significantly change through the first 12 days of storage, while progressive increases of *L. innocua* counts on the untreated tomato slices were observed after 4 days (Figure 1.5). Regardless the PL-treatment that was applied to the tomato slices, the *L. innocua* loads increased from day 12 to day 16 reaching a plateau by day 20. However, at day 12 differences in the microbial counts were evident. The lowest ($p<0.05$) *L. innocua* counts (5.9 log CFU g^{-1}) were found on fresh-cut tomatoes exposed to a fluence of 8 J cm^{-2} while the highest corresponded to the untreated samples (8.4 log CFU g^{-1}).

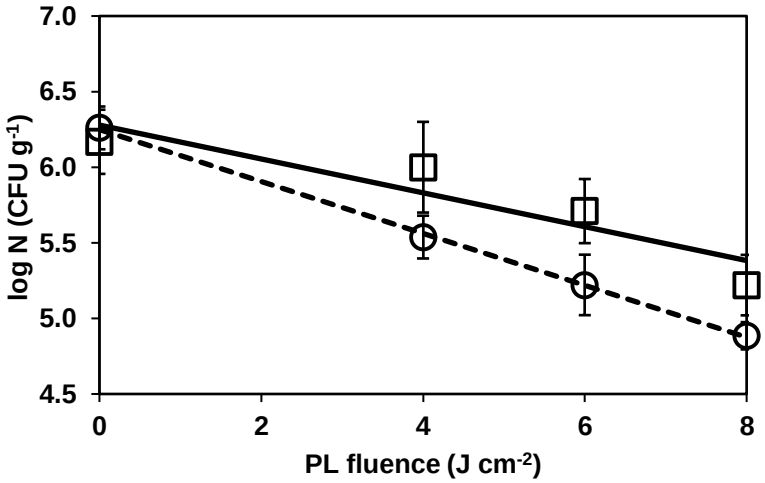

Figure 1.4. Fits of a log-linear decaying model to the counts of *Listeria innocua* (squares)) and *Escherichia coli* (circles) on PL-treated fresh-cut tomatoes.

The modified Gomperz´s model provided a good fit to the experimental data (R^2> 0.930). As can be seen in Table 2, the differences among the *C* values for *Listeria innocua* were not enough significant to assess the influence of PL-fluence on the microbiological counts. It is noticeable to point out that the lag phase period (λ), which corresponds to the period when de microorganism is adapting itself to the medium conditions and is unable to divide, became visibly lengthened when PL-treatments were applied; indeed, for untreated tomato slices, λ was 4.25 day, meanwhile, for PL-treated samples, λ ranged from 8.06 to 9.48 day.

Regarding *Escherichia coli* counts, significant decreases (p< 0.05) of about 0.8 and 1.1 log (CFU g^{-1}) were observed on fresh-cut tomato slices immediately after its exposure to 4 and 6 J cm^{-2} PL-fluence (Figure 1.4), respectively. Further reduction of initial load of *E. coli*, 1.4 log (CFU g^{-1}), was achieved applying 8 J cm^{-2} PL-fluence on the slices. The fit of a log-linear model (Equation 1) to the observed depletion of the microbial load as a function of the fluence (Φ) yielded a high agreement (R^2= 0.999) between the experimental data and the values predicted by the model. In this case, the inactivation constant was δ= 0.172 cm^2 J^{-1}, which was a clear sign of

less resistance to PL-treatments by *E. coli* in comparison with *L. innocua*. Hence, the decimal logarithm of the microorganism counts obtained from Equation 1 [6.34, 5.28, 5.18 and 4.72 log (CFU g^{-1})] for tomato slices untreated or exposed to 4, 6 and 8 J cm^{-2} fluence, respectively, was the estimated value for the microbial load on the tomato slices just at the beginning of their cold storage stage (parameter *A* in Equation 2).

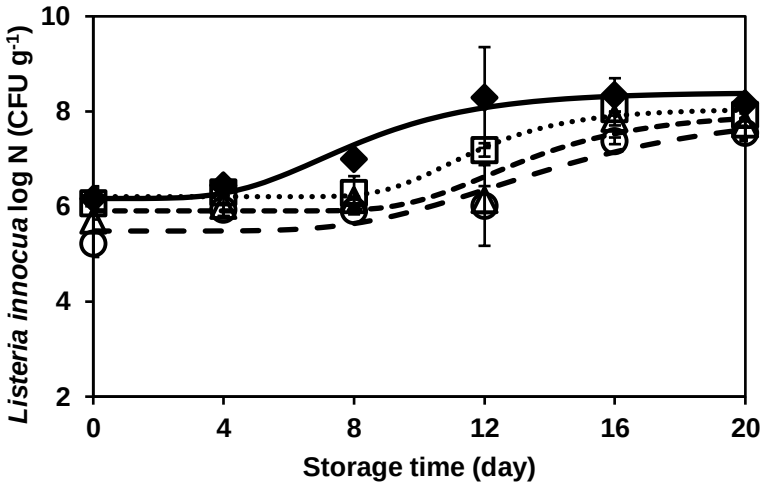

Figure 1.5. *L. innocua* counts on PL-treated fresh-cut tomatoes through storage at 4 °C. Points are the means of six determinations ± SD. Lines represent the fit of a modified Gompertz's model to the experimental data. PL-treatments: untreated (diamonds); 4 J cm^{-2} (squares); 6 J cm^{-2} (triangles) and 8 J cm^{-2} (circles).

Conversely to behavior of the *L. innocua* on the tomato slices during the cold storage, a significant inactivation of *E. coli* was observed during this period (Fig. 1.6). Moreover, at a fixed cold storage stage, the number of microorganisms in the fresh-cut tomatoes that had been subjected to PL-treatments was lower than in the untreated cut tomatoes, whichever of the PL-fluence applied to. So at the end of the cold stage, *E. coli* count on untreated fresh-cut tomato slices was 3.8 log (CFU g^{-1}) whereas for the slices that had been exposed to PL fluences of 4, 6 and 8 J cm^{-2}, the final counts were 2.8, 2.7 and 2.1. log (CFU g^{-1}), respectively. As was likewise reported for *L. innocua*, Gompertzian models fitted accurately the

experimental data obtained for the decline in the counts of *E*. coli on the tomato slices as a function of the cold storage time (Fig. 1.6); the determination coefficients (R^2), which ranged from 0.930 to 0.997, and the parameters of the model at the assayed PL-fluences are listed in Table 1.2. The highest values for *B* and μ_{max} parameters corresponded to the untreated cut tomatoes, while those ones that were exposed to PL-treatments exhibited lesser values for these parameters; this fact indicates that both relative death rates and maximum rates of death for *E*. *coli* were significantly ($p< 0.05$) lower for the PL-untreated tomato slices, which might be related to the lower initial microbial loads that the PL-processing had previously caused.

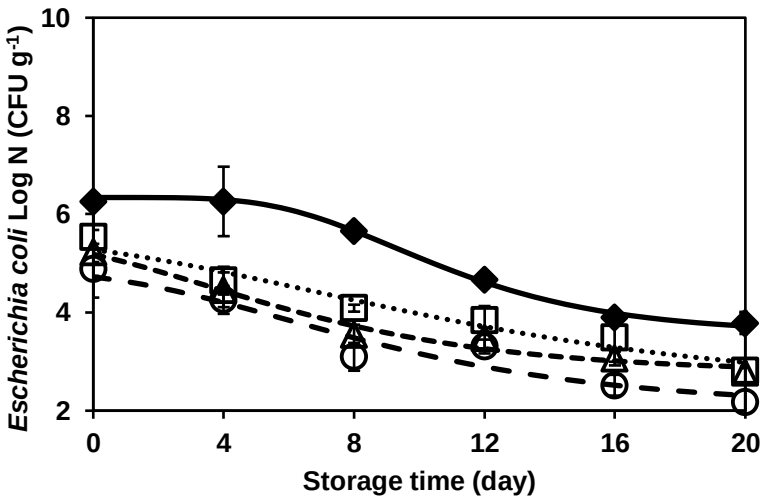

Figure 1.6. *E. coli* counts on PL-treated fresh-cut tomatoes through storage at 4 °C. Points are the means of six determinations ± SD. Lines are the fits of a modified Gompertz's model to the experimental data. PL treatments: untreated (diamonds); 4 J cm^{-2} (squares); 6 J cm^{-2} (triangles) and 8 J cm^{-2} (circles).

Moreover, this significant ($p< 0.05$) increment of λ is coupled with the fact that the higher fluence applied to samples, the higher values for time at which maximum growth rate (M) is reached (6.95 day and from 10.87 to 12.22 day for untreated and PL-treated tomato slices, respectively); besides, the relative growth rate (B) decreased from 0.49 to 0.24 log $(CFU \cdot g^{-1}) \cdot day^{-1}$

as PL-fluence increased from 4 to 8 J cm^{-2}; likewise, maximum exponential growth (μ_{max}) decreased from 0.33 to 0.22 log $(CFU \cdot g^{-1}) \cdot day^{-1}$ as PL-fluence increased. Thus, *L. innocua* exhibited a higher resistance to PL treatments but, at the same time, the growth is delayed throughout the storage period with respect to untreated fresh-cut tomatoes.

However, both the time at which death rates were maxima (*M*) and the lag time (λ), in which no growth of the microorganisms occurs; were significantly ($p < 0.05$) shortened when PL-treatments were applied regardless the fluence; these results pointed out the inactivation of *E. coli* become accelerated after PL-treatments and suggest that the microorganism was highly susceptible to them. Therefore, tomato slices exposed to higher fluences yielded lower microbial counts for a longer time than those untreated ones and, thus extending the safety of fresh-cut tomatoes up to 12 days after processing. In this respect, some studies have reported that *E. coli* was inactivated in solid and liquid substrates and fresh-cut products after exposure to similar PL-fluences and storage conditions (Uesugi et al. 2013; Kramer and Muranyi, 2014; Ramos-Villarroel et al. 2011a; Ramos-Villarroel et al. 2011b).

As it has been showed, a clear influence of PL-treatments on the evolution of *L. innocua* and *E. coli* during the cold storage stage was observed. Ramos-Villarroel et al. (2012) suggested that the variation in the reductions of *L. innocua* and *E. coli* counts on fresh-cut watermelon subjected to PL-treatments can be well explained by differences in the cell wall composition and structure of these microorganisms. Indeed, *E. coli* is a Gram-negative bacterium with less rigid and thinner cell wall than *L. innocua*, which is a Gram-positive one. Some authors (Rowan et al. 1999; Anderson et al. 2000) have demonstrated that Gram-negative bacteria are more susceptible to PL-treatments due to the environment where they grow. Sensibility of *Listeria* and *Escherichia* strains to PL-treatments is dependent of the environmental conditions and decontamination treatment applied (Rajkovic et al. 2010). In this context, Gram-negative bacteria are enteropathogens restricted to darkness in humans and animals tracts and do

not develop resistance to the presence of light; on the other hand, Gram-positive bacteria can be found in all types of environments and, hence, they are more light exposed, what made them more resistant to light damages (Hobbs and Robert 1987).

Table 1.2. Estimates of the parameters of the Gompertz's modified model used to describe the changes in *L. innocua* and *E. coli* counts in PL-treated tomato slices through chilled storage.

I	PL-fluence (J cm^{-2})	*A*	*C*	*B*	*M*	μ_{max}	λ	R^2
L. innocua	0 (Untreated)	6.17±0.21	2.23±0.15	0.37±0.09	6.95±0.48	0.30±0.02	4.25±0.61	0.980
	4	5.76±0.35	1.84±0.10	0.49±0.09	10.87±0.28	0.33±0.04	8.84±0.73	0.987
	6	5.54±0.21	2.04±0.32	0.38±0.16	12.11±0.75	0.28±0.01	9.48±0.77	0.938
	8	5.27±0.28	2.48±0.48	0.24±0.09	12.22±1.14	0.22±0.02	8.06±0.46	0.951
E. coli	0 (Untreated)	6.34±0.14	-2.76±0.13	0.27±0.03	9.24±0.32	-0.27±0.01	5.56±0.39	0.997
	4	5.28±0.14	-3.13±0.89	0.13±0.06	6.84±0.29	-0.15±0.03	-1.08±0.33	0.952
	6	5.18±0.92	-2.79±0.22	0.19±0.05	3.48±0.81	-0.19±0.03	-1.62±0.36	0.976
	8	4.72±0.14	-2.78±0.61	0.18±0.09	5.94±1.99	-0.18±0.01	0.34±0.49	0.930

I*:** inoculum; **A**: decimal logarithm of initial load, log (CFU g^{-1}); **C**: difference in value of the upper and the lower asymptotes, log (CFU g^{-1}); ***B: relative growth or death rate at time equal to M log (CFU g^{-1}) day^{-1}; ***M***: time at which growth or death rate (***B***) are maxima (day); μ_{max}**:** maximum exponential growth or death rate, log (CFU g^{-1}) day^{-1}; λ: lag time (day). R^2: determination coefficient adjusted for degree or freedom. Values ± SD.

The different pattern of behavior observed for the microorganisms studied in present work throughout the storage period, either with or without previous PL-processing, were also determined by the storage temperature and other environmental factors intrinsic to the product. Ramos-Villarroel et al. (2012) indicated that storage temperature could also impact on the microbial growth on fresh-cut vegetables. It has been demonstrated that *E. coli* is a mesophyll that can survive at temperatures between 5 and 45 °C. On the other hand, *L. innocua* belongs to the group of psychrophiles, which are able to grow between -15 and 20 ºC. Moreover, some inherent factors of tomatoes such as acidity and pH could influence the behavior observed on

L. innocua and *E. coli* counts through the storage period. *E. coli* generally grows within the pH range of 4.4-9.0, while *L. innocua* grows at pH of 4.0-9.6 (Riemann and Cliver 2006).

3.3. Soluble solids content (SSC), pH and titratable acidity (TA)

Table 1.3 shows the values of SSC, pH and TA of untreated and PL-treated fresh-cut tomato throughout the 20 days of chilled storage (4 ºC). Although SSC, pH and TA of fresh-cut tomatoes did not suffer changes just after PL-processing, significant differences (p< 0.05) with respect to the initial values were observed over storage.

Table 1.3. Changes along 20 days of storage at 4 °C in the content (SSC, °Bx), pH and titrable acidity (TA, g citric acid 100 g^{-1}) of fresh-cut tomato slices treated with pulsed light (PL).

Monitored variable	PL-fluence (J cm^{-2})	Storage time (day)					
		0	4	8	12	16	20
SSC	**0 (untreated)**	4.75 ^{aC}	4.83 ^{bB}	4.25 ^{bA}	4.67 ^{bB}	5.13 ^{cC}	5.08 ^{cC}
	4	4.25 ^{aA}	5.22 ^{bC}	4.33 ^{aA}	4.33 ^{aA}	4.92 ^{bC}	4.83 ^{bBC}
	6	4.25 ^{aA}	4.33 ^{aA}	4.92 ^{bB}	3.92 ^{aA}	4.83 ^{bA}	4.50 ^{aA}
	8	4.50 ^{aB}	4.83 ^{bB}	4.42 ^{aA}	4.25 ^{aA}	4.92 ^{bB}	4.75 ^{bB}
pH	**0 (untreated)**	4.59 ^{aA}	4.65 ^{aA}	4.63 ^{aA}	4.62 ^{aA}	4.71 ^{bB}	4.75 ^{bB}
	4	4.54 ^{aA}	4.67 ^{aA}	4.62 ^{aA}	4.66 ^{bA}	4.64 ^{bB}	4.60 ^{aA}
	6	4.45 ^{aA}	4.69 ^{bA}	4.59 ^{aA}	4.74 ^{bB}	4.62 ^{aB}	4.66 ^{aA}
	8	4.35 ^{aB}	4.71 ^{bB}	4.58 ^{aA}	4.81 ^{cC}	4.46 ^{aA}	4.62 ^{aA}
TA	**0 (untreated)**	0.31 ^{aB}	0.29 ^{aAB}	0.20 ^{aA}	0.34 ^{cB}	0.29 ^{b}	0.25 ^{aA}
	4	0.39 ^{aB}	0.29 ^{aA}	0.29 ^{bA}	0.29 ^{bA}	0.31 ^{bAB}	0.33 ^{bB}
	6	0.34 ^{aC}	0.28 ^{aB}	0.29 ^{aB}	0.26 ^{aA}	0.25 ^{aA}	0.27 ^{aA}
	8	0.33 ^{aBC}	0.34 ^{bC}	0.29 ^{aB}	0.30 ^{bcB}	0.35 ^{cC}	0.27 ^{aA}

Values are the mean of three independent determinations of two replicates (n=6). Different lower case letter in the same column denotes significant differences among treatments (p<0.05). Different capital letters in the same row for each treatment denotes significant differences with cold storage time (p<0.05).

Regarding SSC, fresh-cut tomatoes exposed to PL exhibited lower values (< 4.83 ºBx) with respect to untreated samples (5.08 ºBx) at the end

of storage. The slight differences in SSC on the tomato slices during the storage stage might be related to mild increases in transpiration rates, which had been triggered by unavoidable tissue breakdowns during the processing of the tomatoes. Actually, slight water exudates were observed visually in both untreated and PL-treated cut tomatoes through the cooling stage. Although changes on pH were observed in all samples throughout chilled storage, pH values of fresh-cut tomatoes exposed to PL were lower than those on untreated samples. Furthermore, slight decreases on the acidity values of untreated and PL-treated samples were observed during the storage period. Similarly, Ramos-Villarroel et al. (2011b) reported decreased pH and acidity on PL-treated avocado attributing this effect to *L. innocua* and *E. coli* inactivation by PL-treatments. In fact, microbial deterioration, as well as physiological activity of fresh-cut tomato, play an important role in the degradation of organic acids and, in turn, affecting the pH (Odriozola-Serrano et al. 2008; Gil et al. 2006).

4. Conclusions

PL-treatment could be a good alternative to reduce the growth of pathogenic microorganisms, thus improving safety of fresh-cut tomatoes. The effectiveness of the PL-treatments to reduce the microbial counts depends of the fluence applied and the sort of the microorganism itself. It has been proved that the higher PL-fluence applied, the higher achieved inactivation of the studied microbes. *L. innocua* exhibited a greater resistance to PL-treatments compared to *E. coli*. Indeed, the patterns that the microorganisms showed during the cold stage differed significantly. The behavior of both microbes on fresh-cut tomatoes slices during cold storing was adequately described by Gompertzian models regardless of PL-treatments. Moreover, this modeling was also a useful tool for describing the O_2 and CO_2 changes in the packages headspace. It is outstanding that the O_2 consumption was slightly accelerated by PL-treatments, which showed to trigger a rapid decrease of the O_2 concentrations during the storage time.

The results of the present study may contribute to the advancement of predictive models involving PL-treatments and microorganisms.

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5. References

Ahmed, L., Martin-Diana, A. B., Rico, D., & Barry-Ryan, C. (2012). Quality and nutritional status of fresh-cut tomato as affected by spraying of delactosed whey permeate compared to industrial washing treatment. *Food and Bioprocess Technology, 5*(8), 3103-3114.

Anderson, J. G., Rowan, N. J., MacGregor, S. J., Fouracre, R. A., & Parish, O. (2000). Inactivation of food-borne enteropathogenic bacteria and spoilage fungi using pulsed-light. *IEEE Transactions on Plasma Science, 28*(1), 83-88.

Asplund, K., & Nurmi, E. (1991). The growth of salmonellae in tomatoes. *International Journal of Food Microbiology*, *13*, 177-182.

Avalos Llano, K. R., Marsellés-Fontanet, A. R., Martín-Belloso, O., & Soliva-Fortuny, R. (2016). Impact of pulsed light treatments on antioxidant characteristics and quality attributes of fresh-cut apples. *Innovative Food Science and Emerging Technologies, 33*, 206-215.

Beuchat, L.R. (1996a). Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection*, *59*, 204-216.

Beuchat, L. R. (1996b). *Listeria monocytogenes*: Incidence on vegetables. *Food Control, 7*, 223-228.

Code of Federal Regulations. 1991. United Stated standards for grades of fresh tomatoes. USDA Agricultural Marketing Service, Washington, D.C.

Ethelberg, S., Lisby, M., Bottiger, B., Schultz, A. C., Villif, A., Jensen, T., & Muller, L. (2010). Outbreaks of gastroenteritis linked to lettuce. Denmark, January 2010. *Euro Surveillance, 15*(6), 19484.

Francis, G. A. & O'Beirne, D. (2005). Variation among strains of *Listeria monocytogenes*: Differences in survival on packaged vegetables and in response to heat and acid conditions. *Food Control, 16*, 687-694.

Francis, G. A., Thomas, C., & O'Beirne, D. (1999). The microbiological safety of minimumly processed vegetables. *International Journal of Food Science and Technology, 34*(1), 1-22

Friesema, I., Sigmundsdottir, G., van der Zwaluw, K., Heuvelink, A., Schimmer, B., de Jager, C. & Van Pelt, W. (2008). An international outbreak of shiga toxin-producing *Escherichia coli* O157 infection due to lettuce, september-october 2007. *Euro Surveillance,* 13(50).

Gil, M. I., Aguayo, E., & Kader, A. A. (2006). Quality changes and nutrient retention in fresh-cut versus whole fruits during storage. *Journal of Agricultural and Food Chemistry, 54* (12), 4284-4296.

Gil, M. I., Conesa, M. A., & Artés, F. (2002). Quality changes in fresh cut tomato as affected by modified atmosphere packaging. *Postharvest Biology and Technology, 25*(2), 199-207.

Gómez-López, V. M., Devlieghere, F., Bonduelle, V., & Debevere, J. (2005). Intense light pulses decontamination of minimumly processed vegetables and their shelf-life. *International Journal of Food Microbiology, 103*(1), 79-89.

Hobbs, B. C., & Roberts, D. (1987). Food poisoning and food-borne infection, in: Hobbs, B. C., & Roberts, D. (Eds.) *Food Poisoning and Food Hygiene*. E. Arnold, London, U. K. Pp. 3-120.

Kramer, B., & Muranyi, P. (2014), Effect of pulsed light on structural and physiological properties of *Listeria innocua* and *Escherichia coli*. *Journal of Applied Microbiology*, *116*, 596–611.

Newell, D. G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., & Kruse, H. (2010). Food-borne diseases - the challenges of 20years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology, 139*(1), S3-S15.

Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Effect of minimum processing on bioactive compounds and color attributes of fresh-cut tomatoes. *Lebensmittel Wissenschaft Techologie, 41*(2), 217-226.

Oms-Oliu, G., Aguiló-Aguayo, I., Martín-Belloso, O., & Soliva-Fortuny, R. (2010). Effects of pulsed light treatments on quality and antioxidant properties of fresh-cut mushrooms (*Agaricus bisporus*). *Postharvest Biology and Technology, 56*, 216-222.

Oms-Oliu, G., Soliva-Fortuny, R., & Martín-Belloso, O. (2007). Effect of ripeness on the shelf-life of fresh-cut melon preserved by modified atmosphere packaging. *European Food Research and Technology, 225*(3-4), 301-311.

Palacios, A. P., Marín, J. M., Quinto, E. J., & Wiper, M. P. (2014). Bayesian modeling of bacterial growth for multiple populations. *Annals of Applied Statistics, 8*(3), 1516-1537.

Palgan, I., Caminiti, I. M., Muñoz, A., Noci, F., Whyte, P., Morgan, D. J., & Lyng, J. G. (2011). Combined effect of selected non-thermal technologies on *Escherichia coli* and *Pichia fermentans* inactivation in an apple and cranberry juice blend and on product shelf life. *International Journal of Food Microbiology, 151*(1), 1-6.

Rajkovic, A., Tomasevic, I., Smigic, N., Uyttendaele, M., Radovanovic, R., & Devlieghere, F. (2010). Pulsed UV light as an intervention strategy against *Listeria monocytogenes* and *Escherichia coli* O157:H7 on the surface of a meat slicing knife. *Journal of Food Engineering, 100*(3), 446-451.

Ramos-Villarroel, A., Aron-Maftei, N., Martín-Belloso, O., & Soliva-Fortuny, R. (2014). Bacterial inactivation and quality changes of fresh-cut avocados as affected by intense light pulses of specific spectra.

International Journal of Food Science and Technology, 49(1), 128-136.

Ramos-Villarroel, A. Y., Aron-Maftei, N., Martín-Belloso, O., & Soliva-Fortuny, R. (2012). Influence of spectral distribution on bacterial inactivation and quality changes of fresh-cut watermelon treated with intense light pulses. *Postharvest Biology and Technology, 69*, 32-39.

Ramos-Villarroel, A. Y., Martín-Belloso, O., & Soliva-Fortuny, R. (2011a). Bacterial inactivation and quality changes in fresh-cut avocado treated with intense light pulses. *European Food Research and Technology, 233*(3), 395-402.

Ramos-Villarroel, A. Y., Martín-Belloso, O., & Soliva-Fortuny, R. (2011b). Using antibrowning agents to enhance quality and safety of fresh-cut avocado treated with intense light pulses. *Journal of Food Science*, *76*(9), S528-S534.

Siddiqui, M. W., Chakraborty, I., Ayala-Zavala, J. F., & Dhua, R. S. (2011). Advances in minimum processing of fruits and vegetbales: a review. *Journal of Scientific and Industrial Research*, *70*, 823-834.

Riemann, P. H., & Cliver, O. D. (2006). *Foodboorne Infections and Intoxications*. Academic Press publications. Elsevier Inc. (USA) 2105-207, 313-3118.

Rowan, N. J., MacGregor, S. J., Anderson, J. G., Fouracre, R. A., McIlvaney, L., & Farish, O. (1999). Pulsed-light inactivation of food-related microorganisms. *Applied and Environmental Microbiology, 65*(3), 1312-1315.

Sagoo, S. K., Little, C. L., Ward, L., Gillespie, I. A., & Mitchell, R. T. (2003). Microbiological study of ready-to-eat salad vegetables from retail establishments uncovers a national outbreak of salmonellosis. *Journal of Food Protection, 66*(3), 403-409.

Uesugi, A. R., Hsu, L., & Moraru, C. I. (2013). Effect of pulsed light treatments on the growth and resistance behavior of *Listeria monocytogenes* 10403S, *Listeria innocua* and *Escherichia coli* ATCC

25922 in a liquid substrate. *Journal of Food Protection, 76*(3), 435-439.

United States of America Food and Drug Administration/Center for Food Safety and Applied Nutrition (FDA/CFSAN) (2001). *Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial Hazards on Fresh and Fresh-Cut Produce*. CFSAN, College Park, MD.

CAPITULO 2

Impact of pulsed light treatments and storage time on the texture quality of fresh-cut tomatoes

Abstract

The effect of pulsed light (PL) treatments at fluences of 4, 6 or 8 J·cm^{-2} on microbial growth, weight loss, pectinmethyl esterase (PME) and polygalacturonase (PG) activities of fresh-cut tomatoes was evaluated through 20 days of storage at 5 ºC. Additionally, a pair-wise comparison test was assayed to determine whether potential consumers could detect differences between untreated and PL-treated samples.

Microbial counts of PL-treated tomato slices were up to 2 log CFU g lower than those on untreated samples over storage. Fresh-cut tomatoes exhibited slight firmness decrements, changes on the pectinolytic enzymes and increased weight losses over the storage. However, sensory evaluation did not reveal significant differences over at least 10 days. In summary, PL-treatments showed to be effective to reduce the microbial growth with a low impact on the physical quality of fresh-cut tomatoes.

Industrial relevance: PL-treatments are proposed as a non-thermal strategy to increase the safety of fresh-cut commodities. In spite of their non-thermal nature, these treatments may have a photothermal effect, which could be deleterious to the product quality and shelf-life. This study contributes to the understanding of PL and its impact on the physical quality of fresh-cut tomatoes, thus helping to identify the range of conditions that can be industrially applied without causing major texture damage on the treated product.

Keywords: Fresh-cut tomatoes; pulsed light; texture; pectin methylesterase; polygalacturonase; sensory evaluation.

1. Introduction

Tomato (*Lycopersicon esculentum*) is one of the most demanded vegetables worldwide. Tomato consumption is considered as an indicator of good nutritional habits and healthy lifestyle due to its remarkable contents in folates, vitamin C and vitamin E (Gahler et al., 2003). Moreover, it is a good source of other natural antioxidants especially carotenoids and phenolic compounds (Navia et al., 2006 and Odriozola-Serrano et al., 2008).

The increasing consumer's demand for fresh-cut tomatoes to be used in salads and other ready to eat products has promoted the development of technologies that contribute to undertake their industrial production. Because quality and marketability of fresh-cut tomatoes deteriorates rapidly after cutting (Artés et al., 1999), proper processing and packaging conditions are fundamental to avoid the deleterious consequences of processing (Aguiló-Aguayo et al., 2013; Francis and O'Bierne, 2005). Tomato ripening is usually accompanied by the degradation of the middle lamella and loss of cell adhesion. Ripening process in plants has been related to the depolymerization of pectic components and/or action of hydrolytic enzymes such as polygalacturonase (PG; EC 3.2.1.15) and pectin methylesterase (PME; EC 3.1.1.11) leading to the loss of integrity of the cell walls (Chisari et al., 2011). PG and PME are considered as the primary hydrolysis enzymes involved in tomato softening. However, softening of fresh-cut fruits and vegetables is not only influenced by enzymatic action. Mechanical processes such as slicing, shredding or dicing operations can also cause dramatic losses in firmness of fruit tissues (Soliva-Fortuny and Martín-Belloso, 2003) activating, accelerating or promoting physicochemical phenomena such as dehydration (Oms-Oliu et al., 2010), ethylene production (Rugkong at el., 2010), ripeness and changes in turgor and crispness (Toivonen and Brunell, 2008; Soliva-Fortuny et al., 2004).

Thermal food processing methods ensure microbiological safety. However, these treatments can also modify the sensory properties of fresh-cut produce. As a result, non-thermal technologies may be an alternative in order to preserve the nutritional content and quality of fresh-cut commodities

demanded by consumers. Pulsed light (PL) treatments have emerged as a non-thermal method for microbial decontamination of food surfaces. The main advantage of applying this technology to fresh-cut fruits concerns the reduction of microbial loads without significantly affecting the physical characteristics of the living tissues (Ramos-Villarroel et al., 2012). Microbial cell death may be attained through the generation of photochemical and photothermal effects. Photochemical damage is related to the induction of DNA strand breaks and formation of pyrimidine dimers by UV wavelengths. Nevertheless, several studies have described changes in the antioxidant properties, enzymatic browning and nutritional characteristics of fresh-cut produce subjected to PL treatments (Gómez-López et al., 2005; Oms-Oliu et al., 2010). As well, texture changes after exposure to PL may also occur, and this phenomenon has been attributed to disruption of cell wall membranes caused by PL-fluences, probably as a consequence of undesired thermal effects of the treatment (Aguiló-Aguayo et al., 2013; Charles et al., 2008). Therefore, although food researchers have investigated the possibility of exploiting pulsed light technology to inactivate enzymes in fruits and vegetables, still controversial results are available in literature (Manzocco, et al., 2009).

Hence, the aim of this work was to investigate the effects of PL treatments applied with decontamination purposes on the activity of pectinolytic enzymes (pectinmethyl esterase and polygalacturonase) and to evaluate their relationship with texture modifications occurring in fresh-cut tomato over chilled storage.

2 Materials and Methods

2.1. Raw materials and processing

Tomatoes (*Lycopersicon esculentum* Mill. cv. Daniela), were purchased in a local wholesale distributor (Lleida, Spain) at commercial maturity and refrigerated at 5 ± 1 ºC before processing.

Table 2.1. Changes on the physicochemical properties of fresh-cut tomatoes subjected to PL-treatments and stored at 5 ºC during 20 days.

Storage time (day)	PL- treatment (fluence)			
	Untreated	4 J cm^{-2}	6 J cm^{-2}	8 J cm^{-2}
Soluble solids				
0	4.00 ^{aA}	4.25 ^{aA}	4.25 ^{aA}	4.50 ^{aB}
5	4.71 ^{aA}	5.07 ^{aB}	4.46 ^{aA}	4.75 ^{aA}
10	4.81 ^{aA}	5.39 ^{bB}	4.92 ^{aB}	4.67 ^{aA}
15	5.40 ^{bA}	5.37 ^{bA}	5.61 ^{aA}	5.36 ^{bA}
20	5.58 ^{bA}	5.50 ^{bA}	5.75 ^{aB}	6.00 ^{bB}
pH				
0	4.59^{aA}	4.54 ^{aA}	4.45 ^{aB}	4.35 ^{aB}
5	4.73 ^{aA}	4.73 ^{bA}	4.73 ^{bA}	4.73 ^{bB}
10	4.65 ^{aA}	4.65 ^{bA}	4.65 ^{bA}	4.66 ^{bA}
15	4.68 ^{aA}	4.62 ^{bB}	4.78 ^{bB}	4.93 ^{cC}
20	4.75 ^{bB}	4.60 ^{aA}	4.66 ^{bB}	4.62 ^{bA}
Titratable acidity				
0	0.31 ^{aB}	0.39 ^{cC}	0.34 ^{bB}	0.33 ^{bAB}
5	0.33 ^{bB}	0.31 ^{aB}	0.29 ^{aA}	0.30 ^{bA}
10	0.34 ^{bC}	0.28 ^{aB}	0.29 ^{aB}	0.26 ^{aA}
15	0.35 ^{cB}	0.29 ^{aA}	0.27 ^{aA}	0.28 ^{aA}
20	0.25 ^{aA}	0.33 ^{bB}	0.27 ^{aA}	0.27 ^{aA}

Values are expressed as mean ± standard deviation.

^{abc} Different lower case letter in the same column for each sample indicate significant differences among storage time (p< 0.05).

^{ABC}Different capital letters in the same row for the each sample indicate significant differences among treatments.

Fresh whole tomatoes were sanitized for 2 min in chlorinated water (100 ppm free chlorine L^{-1}) at 5 ± 1 ºC, rinsed with tap water and gently dried by hand. Tomato fruits were then cut into 5 mm-thick slices using an electric slicer (Food Slicer-6128: Toastmaster Corp, Elgin, USA). Tomato slices (ca. 100g) were weighed in polypropylene trays (350 cm^3, 5025 RM PTT-ATS Packaging S.r.l. VE, Italia), which were thermo sealed using an ILPRA Food

Pack Basic V/6 packaging machine (ILPRA Systems, CP, Vigevono, Italia). The O_2 and CO_2 permeances of the sealing film were 5.2419 x 10^{-13} mol O_2 m^{-2} s^{-1} Pa^{-1} and 2.3825 x 10^{-12} mol CO_2 m^{-2} s^{-1} Pa^{-1} at 23ºC and 0 % RH, respectively (ILPRA Systems España, S.L. Mataró, Spain). The packages were stored at 5 °C in darkness prior to PL application.

A physico-chemical characterization of tomato slices was carried out before and after PL processing and through 20 days storage (Table 2.1). pH (Crison 2001 pH-meter; Crison instruments S. A., Barcelona, Spain), tritable acidity and soluble solids content (Atago R-X-1000 refractometer; Atago Company Ltd., Japan) were assayed.

2.2. Pulsed light treatments

Pulsed light (PL) treatments were performed using an automatic laboratory flash lamp system (Steribeam Xe-Matic-2L-A, Kehl, Germany). The emitted spectrum ranged from 200 to 1100 nm. The duration of each pulse was 0.3 ms with a fluence of 0.4 J cm^{-2} per pulse emitted from each one of two xenon lamps situated above and below the sample holder, respectively. Samples were treated with 10, 15 or 20 pulses to evaluate the effect of different treatment doses. Hence, the fluences applied were 4, 6 and 8 J cm^{-2} respectively. According to previous studies (Aron Maftei, et al., 2014) the film transparency was 97%. A set of untreated samples was kept as reference. Finally, samples were stored at 4 °C for 20 days in darkness until random withdrawal for analysis.

2.3. Microbiological stability

In order to evaluate the sanitizing effect of PL treatments, microbiological analyses were carried out prior to analytical and sensory evaluations. Psychrophilic bacteria and yeast and mold counts were carried out over 20 days of storage. Samples of 10 g tomato were homogenized for 2 min with 90 ml of 0.1% sterile peptone solution with a Stomacher Lab blender 400 (Seward Medical, London, UK). Serial dilutions of fruit homogenates were made and plated onto plate-count agar (PCA) (Biokar

Diagnostics, Beauvais, France) and incubated for 10 days at 5 ± 1 ºC for psychrophilic bacteria counts. For yeast and mold counts, serial dilutions of tomato homogenate were spread on agar plates with chloramphenicol glucose (CGA) (Biokar Diagnostics, Beauvais, France) and incubated in the dark at 25 ± 1 ºC for 3-5 days. Analyses were carried out every 5 days in randomly sampled pairs of trays. Three replicate counts were performed for each tray.

2.4. Texture evaluation

The texture of the samples was evaluated using a TA-XT2 texturometer (Stable Micro Systems Ltd., Surrey, England, UK) equipped with a 5 kg load cell. A resistance test was used to discriminate texture differences among cross sectional sliced tomato pieces. Uniaxial compression test was assayed to measure the tomato slices resistance to uniaxial single type forces using a 50 mm diameter aluminum cylindrical probe (P/50), with a test speed of 5 mm/s, a final target at 25% strain, plus 10 s holding time at the maximum strain.

Force-displacement-time data were recorded using Texture Exponent 32 software (Stable Micro Systems LTD. Surrey England). The firmness was measured as the maximum force required for shearing 5 mm-thick tomato slices. At least 4 repetitions from 2 replicate packages were evaluated at each sampling time and results were expressed as firmness in N.

2.5. Weight loss

Tomato slices are highly susceptible to weight loss, mainly attributed to transpiration, and then leading to softening (Mencarelli & Salveit, 1988). With the aim of not interrupting the storage conditions, containers were pierced (2 mm) and then juice was drained using a sterile micropipette at each sampling time. Drilled trays were sealed again with polypropylene film and stored. The last method was conducted in duplicate and three replicate analyses were carried out for each sample in order to obtain the mean value.

Weight losses were expressed as percentage (%) weight loss relative to the initial weight.

2.6. Enzymatic determinations

2.6.1 Sample preparation

To obtain the enzyme extracts for PME and PG activity determinations, 25 g of sliced tomatoes were ground with an Ultra Turrax T25 (IKA® WERKE, Germany) equipped with a S25N-G25G probe and filtered throughout a 2-mm diameter steel sieve Then, tomatoes juice was collected and immediately analyzed.

2.6.2 Determination of pectin methylesterase (PME) activity

PME activity was measured using the method described by Kimball (1991). Pectin from citrus fruit (67-71% esterified), sodium chloride and NaOH were purchased from Acros Organics (NJ, U.S.A.), Rectapur (Fontenay, France) and Panreac Química (Barcelona, Spain), respectively. Five milliliters of tomato juice sample were mixed with 20 ml of 1% citrus pectin solution in 2 N NaCl. After reaching a temperature of 30 ºC, the pH of the mixture was adjusted to 7.7 with 2 N NaOH using a pH-meter (C-2001; Crison Instruments S.A., Alella, Barcelona, Spain). When the pH was stabilized, 1 ml of 0.05 N NaOH was added and the time required for the pH to return to 7.7 was measured. PME activity (A) was calculated through Eq. 1 and expressed in pectin esterase units (PEU) per ml of juice. Then 1 unit of PME activity was defined as the amount of the enzyme that liberates 1.0 micro equivalent of acid per minute under the assay conditions.

$$A\left(\frac{PME}{mL}\right) = \frac{(NaOH \cdot V_{NaOH} \cdot 10^2}{V_{juice} \cdot t'} \quad \textbf{(1)}$$

where [NaOH] is the NaOH concentration (0.05 N), V_{NaOH} is the volume of NaOH solution (0.05 N) V_{juice} is the volume of juice (5 ml) and t' is the time in

minutes required for the solution to reach a pH of 7.7 after the addition of NaOH.

2.6.3 Determination of polygalacturonase (PG) activity

PG activity was determined using the method described by Aguiló-Aguayo et al. (2009). A sample of 2.5 g of tomato juice was transferred to a 5 ml centrifuge tube and centrifuged at 8,000 *g* for 15 min at 4 ºC. The supernatant was discarded and the pellet was dispersed into cold (4 ºC) distilled water at 1:1 ratio (w/v). Then, the mixture was adjusted at pH 3.0 with 0.1 M HCl. After that, the sample was centrifuged at 9,000 *g* for 15 min at 4 ºC. The supernatant was again discarded and the pellet was dissolved into a 1.2 M NaCl solution in a ratio of 1:1 (w/v). After stirring during one hour at 4 ºC, the mixture was centrifuged at 18,200 g for 10 min at the same temperature. The resulting supernatant was assayed for PG activity.

The PG activity assay was based on the release of reducing groups produced by PG and measured by spectrophotometry (Gross, 1982). Then, 100 μL of the extracted enzyme solution were mixed and incubated with 300 μL of 0.2% polygalacturonic acid at 35 ºC for 10 min. To stop the reaction, 2 ml of 0.1 M borate buffer (pH 9.0) and 400 μL of 1% cyanoacetamide solution were added and the homogenate was boiled for 10 min. After cooling down, the absorbance was measured at 276 nm at 22 ºC. A blank sample was determined in the same way without the addition of the enzymatic extract. A standard curve was built with α-D-galacturonic acid as reducing sugar. Hence, 1 unit (U) of PG activity was defined as the amount of enzyme that releases 1 μmol of galacturonic acid per min under the assay conditions.

2.6.4 Relative residual activity

Residual PME (RA_{PME}) and PG (RA_{PG}) were calculated through Eq. 2, were A_o denotes the enzyme activity of untreated tomato slices and A_t is the enzyme activity on the PL-treated samples.

$$RA(\%) = \frac{A_t}{A_0} \cdot 100 \quad \textbf{(2)}$$

2.7. Sensory testing

The texture-sensory evaluation was carried out in a sensory analysis room with individual booths. Pair-wise ranking tests were carried out just after processing and after 5 and 10 days of refrigerated storage. Sensory tests were not conducted beyond that point in view of the results of the microbial analysis. To evaluate sensory quality in fresh-cut tomato, samples were coded using random numbers (to avoid bias) and a set of six sample pairs were served on a white dish to a panel composed of twelve members (20-55 years of age). Judges were then instructed to select and record the inclination for a sample of every pair based on their own preference about the texture of tomato slices. Evaluations were performed immediately after the tomato slices were removed from refrigeration.

2.8. Statistical analysis

Statistical analysis was performed using Statgraphics plus v. 5.1 Windows package (Statistical graphics Co., Rockville, MD). Each processing condition was assayed in triplicate at each sampling time and three replicate analyses were carried out for each trail to obtain the mean value (n= 9). Analysis of variance (ANOVA) was carried out to compare sample mean values. Data were analyzed using multifactor analysis of variance and a LSD multiple range test was applied to determine differences among means with a significance level of 0.05.

The results of the sensory analysis were evaluated by a Friedman-type statistical analysis. The first step of this analysis was to obtain the rank sum by addition of preferences rank for each sample to twice the sum of the preferences frequencies. Then, the Friedman's test (T) (Eq. 3) was used to determine significant differences between samples.

$$T = \frac{4}{pt}\sum_{i-1}^{t} R^2 - \left(9p[t-1]^2\right) \quad \textbf{(3)}$$

where p is the number of times the design is repeated; t is the number of compared treatments; R_i is the rank sum for each treatment; and $\Sigma\ R^2$ is the sum of all *R's* squared from R_1 to R_t.

Hence, T values obtained to each tomato sample were compared to the critical value of X^2 with (t-1) degrees of freedom (7.81). Additionally, the HSD value (honestly significant difference) was determined to compare two rank sums (α=0.05).

Finally, Pearson correlation coefficients (r) were calculated to measure the strength and direction lineal relationship between firmness and related parameters (weight loss, PG and PME activities and the texture sensory rank-sums).

3. Results

3.1. Microbiological stability

The effects of pulsed light (PL) treatments on the inactivation of psychrophilic bacteria (PB) and moulds and yeasts (MY) growing on fresh-cut tomatoes are shown in Figure 2.1. The initial PB counts on tomato slices (3.1±0.1 log CFU g^{-1}) were not substantially affected by PL treatments (Figure 2.1-A). Conversely, initial MY loads (4.9±0.1 log CFU g^{-1}) on fresh-cut tomatoes were significantly reduced (p< 0.05) just after exposure to fluences of 6 and 8 J cm^{-2} (4.2 and 3.8 log CFU g^{-1}, respectively) (Figure 2.1-B).

Psychrophilic bacteria and molds and yeasts counts significantly increased (p< 0.05) on fresh-cut tomatoes through storage time. Regarding the effect of PL-treatments, tomato slices subjected to PL exhibited lower microbial counts than those on untreated at each sampling time (p< 0.05), being the higher the PL-fluence applied, the lower the increase in microbial counts over storage. At the end of storage, PB counts on untreated samples increase up to

7.0±0.2 Log (CFU g^{-1}), while those on samples subjected to PL-treatments were downgraded up to 0.7-1.8 Log (CFU g^{-1}), with respect those on untreated samples. On the other hand, the MY loads on fresh-cut tomatoes reached 8.0±0.2 Log (CFU g^{-1}), being the samples exposed to PL-fluences of 8 J cm^{-2} which exhibited the lower increases (up to 0.5 Log CFU g^{-1}) with respect to those on untreated tomato slices.

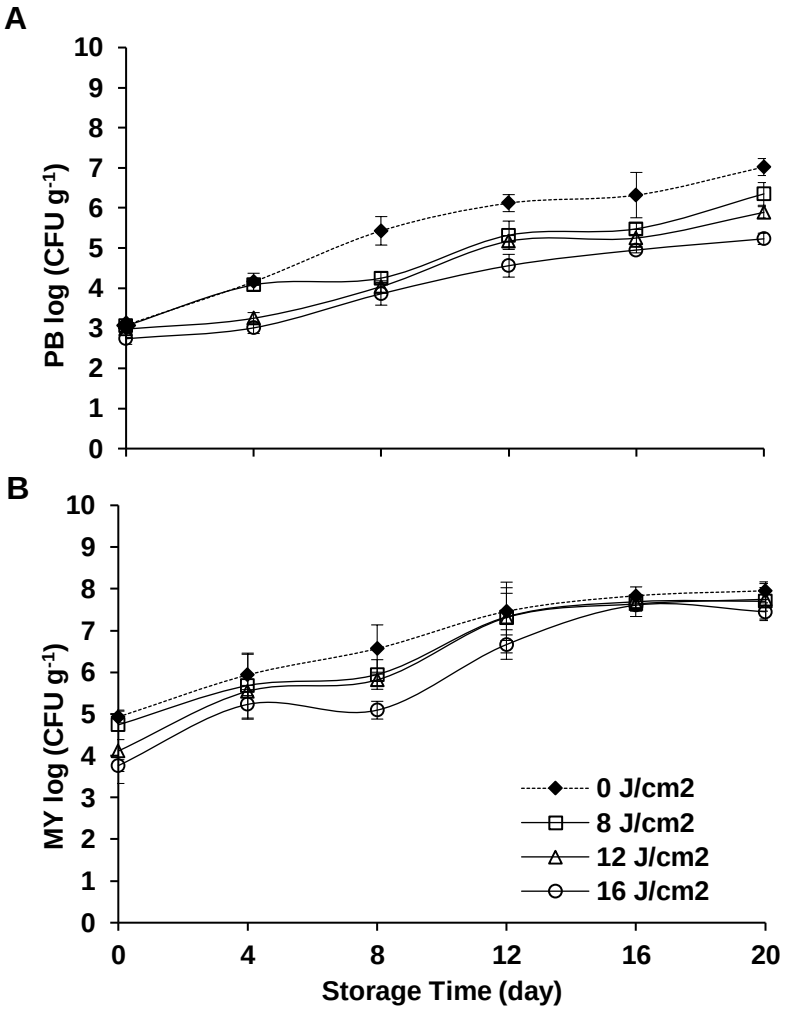

Figure 2.1. Effect of pulsed light (PL) treatments on microbial load: (A) psychrophilic bacteria (PB) and (B) moulds and yeast (MY) through 20 days of chilling storage (5 ºC) of fresh-cut tomatoes. PL-treatments: Untreated (♦); 4 J cm^{-2} (□); 6 J cm^{-2} (Δ) and 8 J cm^{-2} (○). Points are the means of three repetitions from two replicate packages ± SD.

3.2. Firmness

Changes in firmness of fresh-cut tomato over chilled storage as affected by PL treatments are presented in Table 2.2. PL treatments did not lead to firmness modifications of fresh-cut tomatoes immediately after processing (p< 0.05). However, firmness values significantly decreased (p< 0.05) throughout the storage period regardless the treatment applied, being the higher the PL-fluence applied, the faster the firmness decrements. Then, untreated tomato slices exhibited reductions of 63% of their initial firmness over 20 days of storage, whereas those subjected to fluences of 4 and 6 J cm^{-2} exhibited greater firmness losses (72 and 74%, respectively). In contrast, firmness of fresh-cut tomatoes exposed to fluences of 8 J cm^{-2} only lost a 55.7% of their initial value throughout storage.

Table 2.2. Firmness (N s^{-1}) of fresh-cut tomatoes subjected to pulsed light treatments and stored through 20 days at 5 ºC.

Storage time	PL-treatment (fluence)			
(day)	Untreated	4 J cm^{-2}	6 J cm^{-2}	8 J cm^{-2}
Firmness values				
0	20.7±10.9^{c}	17.8±10.1^{c}	18.5±10.3^{c}	19.9±8.9^{e}
2	16.9±2.1^{abc}	16.8±10.9^{bc}	16.1±7.9^{bc}	17.3±8.6^{cde}
4	15.0±10.5^{abc}	16.0±3.7^{bc}	15.6±5.3^{bc}	15.2±7.4^{bcde}
8	13.6±9.1^{abc}	12.5±4.3^{abc}	12.3±5.6^{abc}	10.0±2.0^{abc}
12	13.3±5.3^{abc}	10.9±3.3^{ab}	12.0±1.9^{abc}	9.9±3.8^{abc}
16	10.8±6.4^{ab}	8.7±1.7^{a}	11.2±5.5^{ab}	9.0±4.7^{ab}
20	7.7±2.4^{a}	7.9±1.5^{a}	7.9±3.9^{a}	7.3±1.0^{a}

^{abc} Different lower case letter in the same column for each sample indicate significant differences among storage time (p< 0.05).
Values ± SD.

3.3. Relative weight los

As shown in Figure 2.2, fresh-cut tomatoes underwent slight weight changes just after PL processing. Tomato slices exposed to 8 J cm^{-2} exhibited the

highest weight loss (1.6%), which was 4-fold and twice greater than that observed for fresh-cut tomatoes treated with 4 and 6 J cm^{-2}, respectively. At that point, weight loss values were found to be directly related with the amount of incident energy received by fruit samples.

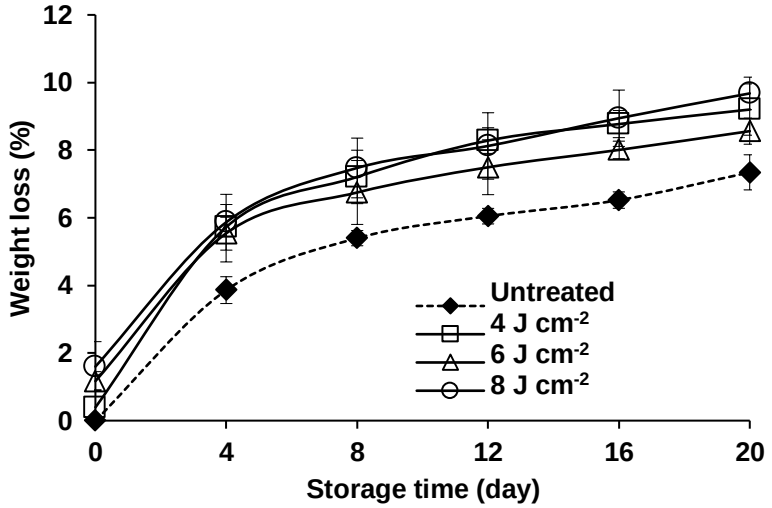

Figure 2.2. Weight loss of fresh-cut tomatoes throughout 20 days of storage at 5 ºC. PL-treatments: Untreated (diamonds); 4 J cm^{-2} (squares); 6 J cm^{-2} (triangles) and 8 J cm^{-2} (circles). Points are the means of three repetitions from two replicate packages ± SD.

Moreover, PL exposure of fresh-cut tomatoes led to consistently higher weight loss values through storage irrespective of the applied conditions (p< 0.05). For all untreated and PL-treated tomato slices, increased weight loss was especially evident during the days following processing (ca. day=4), whereas the rate of increase significantly dropped beyond the first week of storage. Then, differences between weight loss values for untreated and PL-treated tomato slices during the resting storage period were maintained over time (p< 0.05). Hence, weight loss values on fresh-cut tomatoes subjected to PL and stored for 20 days ranged from 9 to 10 %, while a lower but not negligible value was observed for untreated samples (7%).

3.4. Pectin methylesterase and polygalacturonase activities

Changes in pectin methylesterase (PME) and polygalacturonase (PG) activities in untreated and PL treated fresh-cut tomatoes as affected by PL fluence are shown in Figures 2.3 and 2.4, respectively.

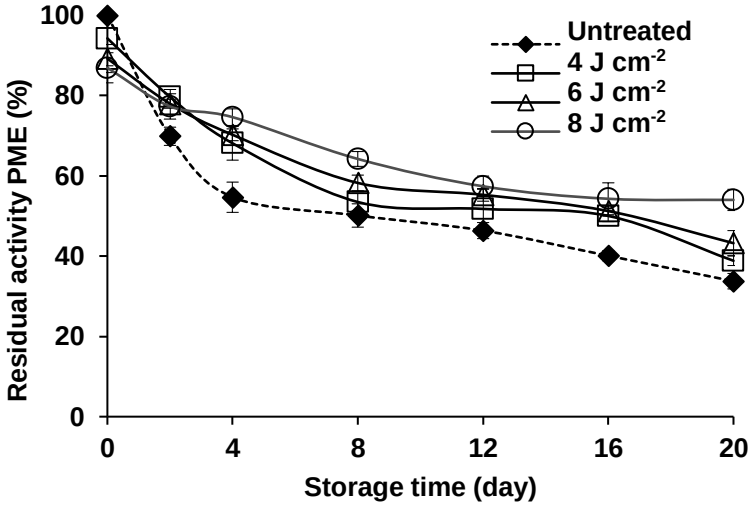

Figure 2.3. Pectin methylesterase activity (PME) on fresh-cut tomatoes throughout 20 days of storage at 5 ºC for. PL-treatments: Untreated (diaminds); 4 J cm^{-2} (squares); 6 J cm^{-2} (triangles) and 8 J cm^{-2} (circles). Points are the means of three repetitions from two replicate packages ±SD.

PL processing did not cause dramatic changes in the initial PME activity on fresh-cut tomatoes. The PME activity of untreated and PL-treated fresh-cut tomatoes significantly decreased ($p< 0.05$) over storage time. However, tomato slices subjected to PL treatments kept higher PME activity values than untreated samples over storage ($p< 0.05$). Indeed, tomato samples exposed to highest PL-treatments (8 J cm^{-2}) maintained greater PME activity than on those exposed to 4 and 6 J cm^{-2}.

Regarding PG, PL treatments did not appear to have a significant effect ($p< 0.05$) on the activity of this pectinolytic enzyme. PG activity dropped sharply (ca. 60%) during the few days following processing regardless the applied

treatment. Residual PG activity values were kept without substantial changes beyond the initial depletion (p<0.05).

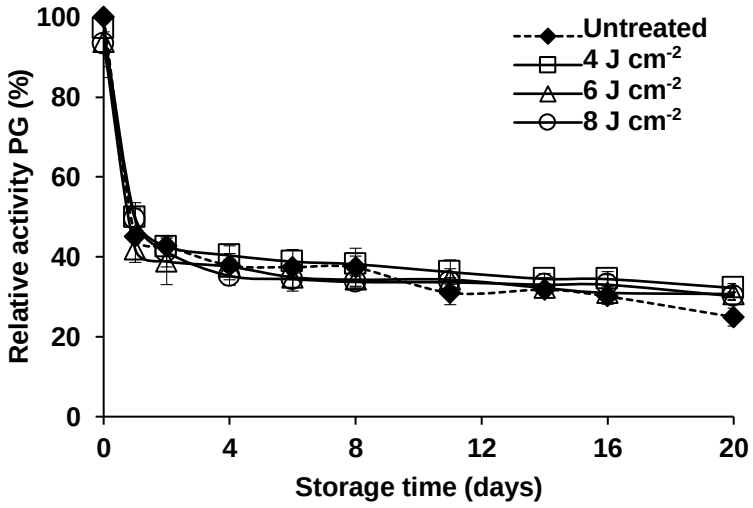

Figure 2.4. Polygalacturonase activity (PG) on fresh-cut tomatoes throughout 20 days of storage at 5 ºC. PL-treatments: Untreated (diamonds); 4 J cm^{-2} (squares); 6 J cm^{-2} (triangles) and 8 J cm^{-2} (circles). Points are the means of three repetitions from two replicate packages ±SD.

3.5. Sensory analysis

Texture acceptability scores for untreated and PL-treated fresh-cut tomatoes are shown in Table 2.3. Significant differences (p= 0.05) between untreated and PL-treated fresh-cut tomatoes were not immediately observed after processing.

After 5 days of storage, untreated tomato slices were preferred to those treated with fluencies of 6, 8 and 4 J cm^{-2}, following this order. . PL processing did not cause dramatic changes in the initial PME activity on fresh-cut tomatoes. However, the PME activity of untreated and PL-treated fresh-cut tomatoes significantly decreased (p<0.05) over storage time.

However, over a 10-day period differences in sensory scores among samples appeared to be less significant. Remarkably, at that point judges preferred tomato samples exposed to higher fluences, although differences in acceptability values for untreated and PL-treated tomato slices were

scarcely significant (α=0.05). Some judges reported certain undesirable quality attributes such as mealiness and dryness in several samples in random way.

Table 2.3. Rank sums obtained through the sensory evaluation of texture of fresh-cut tomatoes after PL treatments and stored at 5 ºC for 10 days.

PL-treatment (fluence)	Storage time (day)		
	0	5	10
Rank Sums			
Untreated	54	60	52
4 J cm^{-2}	56	48	54
6 J cm^{-2}	51	56	53
8 J cm^{-2}	55	52	57

Values ± SD.
Friedman's T= 10

3.6 Correlation analysis

Table 2.4 shows correlation coefficients between firmness, microbial growth, weight loss and enzymatic activities. Increases in microbial counts were inversely correlated with firmness values ($r \leq$-0.92).

Besides, a good correlation was observed between firmness and weight loss values ($r \leq$-0.91), meaning that weight loss values and firmness reduction were directly related. Regarding the enzymatic activities, correlation between PME activity and firmness was also high ($r \leq$-0.93). Although PME and PG activities exhibited different patterns over the storage, PG activity influenced on the firmness change over the first 2 days of storage. Eventually, significant correlations between firmness and PG activity were not observed regardless the applied treatment (p< 0.05).

Table 2.4. Correlation coefficients among the firmness values, microbial growth, weight loss and PG-PME activities on fresh-cut tomatoes subjected to different PL-fluences and stored at 5 ºC for 20 days.

	PL treatment (fluence)			
	Untreated	**4 J cm^{-2}**	**6 J cm^{-2}**	**8 J cm^{-2}**
Firmness	-	-	-	-
PB	-0.950	-0.956	-0.939	-0.981
MY	-0.937	-0.965	-0.918	-0.966
WL	-0.960	-0.906	-0.931	-0.915
PG	0.886	0.758	0.860	0.719
PME	-0.948	-0.929	-0.983	-0.967

PB: psychrophilic bacteria; **MY**: moulds and yeast; **WL**: weight loss (%); **PG**: polygalacturonase activity (%) and **PME**: pectinmethyl esterase activity (%).
p-value <0.05 at the 95 % confidence level.

4. Discussion

Firmness is a key component determining the fresh-like quality of tomato slices. However, microbial growth triggered by minimal processing can lead to texture modification of fresh-cut commodities (O'Beirne, 2006; Francis & O'Beirne, 1998; Barth et al., 2009). In this study, PL treatments have demonstrated to be an effective alternative to maintain lower microbial counts on fresh-cut tomatoes along the storage (figure 1). Microbial reductions were similar to those previously reported for microbial inactivation on tomato slices exposed to PL-fluences of 4, 6 and 8 J cm^{-2} (Valdivia-Nájar et al., 2017). Although the effect of PL treatments can be different depending on the structure and physicochemical characteristics on every commodity, some studies have related the effectiveness of PL on the microbial growth to the amount of PL-fluence applied, initial level of contamination and temperature conditions (Gleeson & O'Beirne, 2005; Ramos-Villarroel et al., 2013, 2012a, 2012b; Gómez, et al., 2011).

Previous reports indicate that the mode of action of PL is generally related to structural changes and cell wall alterations provoked by photochemical (Gómez-López et al., 2007 and Manzocco et al., 2009),

photophysical (Ramos-Villarroel et al., 2013) and photothermal (Oms-Oliu et al., 2010) effects, which may lead to quality changes of fresh-cut products. As a matter of fact, fresh-cut tomatoes did not evidence important physical alterations just after slicing or exposure to pulsed light treatments (Table 1). However, slight changes in firmness of fresh-cut tomatoes subjected to PL were detected during the storage period. In this way, the highest the PL-fluence applied (8 J cm^{-2}) the highest the observed changes (Table 2). Similarly, Aguiló-Aguayo et al. (2013) observed physical alterations on whole tomatoes subjected to lower PL-fluences (2.68-5.36 J cm^{-2}) and stored for 15 days at room temperature. Nevertheless, the extent of the changes was in the same order or magnitude with those occurring in the untreated fruits. Some authors reported that minimal processing (Ahmed et al., 2011; Gil et al., 2002; Sandhya et al., 2010) as well as PL treatments (Rico et al, (2007) are capable of increasing the stress and respiration rate and possibly induce a lignification-like process, causing structural changes as water-soaked areas, dehydration and cellular decompartmentalitzation, and thus leading to weight loss of the fresh-cut commodities. In fact, a linear dependency between PL-fluence and the increase in the respiration rate of fresh-cut tomatoes stored under chilled conditions was previously described (Valdivia-Nájar et al., 2017). Therefore, decreased firmness on tomato slices could be related to those triggered physicochemical changes. Similarly, Gómez et al. (2011) observed an increase in the weight loss of sliced apples after a treatment with UV-C light and related it to decreased rigidity and turgidity, as a consequence of membrane breakage and vacuole burst provoked by pulsed light treatments.

Moreover, physicochemical changes facilitate the contact between cell wall enzymes and their substrates trigger the enzymatic hydrolysis of cell wall pectic substances (Alandes et al., 2006), prompting water losses and thus altering the firmness. Some authors mentioned that changes on the PME and PG activities cause textural changes along the storage of tomato (Duvetter et al., 2009; Van Djik et al., 2006). In fact, the results of the present study show a rapid decrease in PG activity (Figure 4) during the first 2 days of storage, while PME activity decreased progressively over storage (Figure 3). These results coincide with those reported by Wei et al. (2011), who indicated that PME and

PG activities in sweet tomato cherry presented dramatic changes over 30 days of storage at low temperature. Moreover, imbalances of the PG and PME activities could be the cause of symptoms such as firmness decline and manifestation of undesirable characteristics. Mealiness development in fresh-cut commodities has been associated to imbalances in the pectinolytic enzyme activities. It is widely assumed that low PG activities, together with high PME activities, promote this disorder (Artés et al, 1996; Lurie et al, 2003; Brumell et al., 2004), which is in line with our results. Then, mealiness reported through sensory tests could be well related to the different trends of PME and PG activities observed along the storage. Furthermore, some authors have related mealiness and firmness decrease in fresh-cut tomatoes with the loss of turgor and subsequent development of water-soaked areas caused by chilling injury (Hong & Gross, 2000; Rugkong et al., 2010 and Natalini et al., 2011) with coincides with our findings.

From our results it can be stated that firmness changes may be triggered in tomato slices when PL treatment is applied. These effects are related to changes in cell wall integrity, thus allowing microbial growth and triggering weight losses and enzymatic activation over the storage. Remarkably, consumers could not detect differences among the untreated samples and those subjected to any PL-fluence, along 10 days of storage (Table 3). In general, PL-treatments of 8 J cm^{-2} allowed reducing microbial loads without drastically affecting the texture of fresh-cut tomatoes. The results of the present study contribute to the development of novel applications of PL technology, exploring its ability to reduce microbial loads and its effects on the physical and sensorial quality of tomato slices.

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5. References

Aguiló-Aguayo, I., Charles, F., Renard, C. M. G. C., Page, D., & Carlin, F. (2013). Pulsed light effects on surface decontamination, physical qualities and nutritional composition of tomato fruit. *Postharvest Biology and Technology, 86*, 29-36.

Aguiló-Aguayo, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Changes in viscosity and pectolytic enzymes of tomato and strawberry juices processed by high-intensity pulsed electric fields. *International Journal of Food Science & Technology*, 44(11), 2268-2277.

Ahmed, L., Rico, D., Martin-Diana, A. B. and Barry-Ryan, C. (2011). Optimization of application of delactosed whey permeate treatment to extend the shelf life of fresh-cut tomato using response surface methodology. *Journal of Agricultural and Food Chemistry*. 59(6), 2377-3285.

Alandes, L., Hernando, I., Quiles, A., Pérez-Munuera, I., & Lluch, M. A. (2006). Cell wall stability of fresh-cut Fuji apples treated with calcium lactate. *Journal of Food Science, 71*(9), S615-S620.

Aron Maftei, N., Ramos-Villarroel, A. Y., Nicolau, A. I., Martín-Belloso, O., & Soliva-Fortuny, R. (2014). Pulsed light inactivation of naturally occurring moulds on wheat grain. *Journal of the Science of Food and Agriculture, 94*(4), 721-726.

Artés, F., Cano, A., & Fernández-Trujillo, J. P. (1996). Pectolytic enzyme activity during intermittent warming storage of peaches. *Journal of Food Science, 61*(2), 311-321.

Artés, F., Conesa, M. A., Hernández, S., & Gil, M. I. (1999). Keeping quality of fresh-cut tomato. *Postharvest Biology and Technology*, 17, 153-162.

Barth, M., Hankinson, T.R., Zhuang, H., & Breidt, F. (2009) Microbiological Spoilage of Fruits and Vegetables. *In:* Sperber, W.H.; Doyle, M.P. (Eds).

Compendium of the Microbiological Spoilage of Foods and Beverages (Food Microbiology and Food Safety) (pp. 135-183). New York, USA.

Brummell, D. A., Dal Cin, V., Crisosto, C. H., & Labavitch, J. M. (2004). Cell wall metabolismo Turing maturation, ripening and senescente of peach fruit. *Journal of Experimental Botany, 55*(405), 2029-2039.

Charles, M. T., Makhlouf, J., & Arul, J. (2008). Physiological basis of UV-C induced resistance to *Botrytis cinerea* in tomato fruit. II. Modification of fruit surface and changes in fungal colonization. *Postharvest Biology and Technology*, 47(1), 21-26.

Chisari, M., Barbagallo, R. N., Spagna, G., & Artes, F. (2011). Improving the quality of fresh-cut melon through inactivation of degradative oxidase and pectinase enzymatic activities by UV-C treatment. *International Journal of Food Science and Technology, 46*(3), 463-468.

Duvetter, T., Sila, D. N., Van Buggenhout, S., Jolie, R., Van Loey, A., & Hendrickx, M. (2009). Pectins in processed fruit and vegetables: Part I - stability and catalytic activity of pectinases. *Comprehensive Reviews in Food Science and Food Safety, 8*(2), 75-85.

Francis, G. A., & O´Beirne, D. (1998). Effects of storage atmosphere on *Listeria monocytogenes* and competing microflora using a model system. Int. J. Food Sci. Tech. 33, 465-476.

Francis, G. A., & O´Beirne, D. (2005). Variation among strains of Listeria monocytogenes: Differences in survival on packaged vegetables and in response to heat and acid conditions. *Food Control*, 16 (8 SPEC. ISS.), 687-694.

Gahler, S., Otto, K., & Böhm, V. (2003). Alterations of vitamin C, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products. *Journal of Agricultural and Food Chemistry, 51*(27), 7962-7968.

Gil, M. I., Conesa, M. A., & Artés, F. (2002). Quality changes in fresh cut tomato as affected by modified atmosphere packaging. *Postharvest Biology and Technology*, 25(2), 199-207.

Gleeson, E., & O'Beirne, D. (2005). Effects of process severity on survival and growth of *Escherichia coli* and *Listeria innocua* on minimally processed vegetables. *Food Control, 16*(8 SPEC. ISS.), 677-685.

Gómez-López, V. M., Devlieghere, F., Bonduelle, V., & Debevere, J. (2005). Intense light pulses decontamination of minimally processed vegetables and their shelf-life. *International Journal of Food Microbiology, 103*(1), 79-89.

Gómez-López, V. M., Ragaert, P., Debevere, J., & Devlieghere, F. (2007). Pulsed light for food decontamination: A review. *Trends in Food Science and Technology, 18*(9), 464-473.

Gómez, P. L., García-Loredo, A., Salvatori, D. M., Guerrero, S., & Alzamora, S. M. (2011). Viscoelasticity, texture and ultrastructure of cut apple as affected by sequential anti-browning and ultraviolet-C light treatments. *Journal of Food Engineering*, 107, 214-225.

Guiavarc'h, Y., Sila, D., Duvetter, T., Van Loey, A., & Hendrickx, M. (2003). Influence of sugars and polyols on the thermal stability of purified tomato and cucumber pectinmethylesterases: A basis for TTI development. *Enzyme and Microbial Technology, 33*(5), 544-555.

Hong, J. H., & Gross, K. C. (2000). Involvement of ethylene in development of chilling injury in fresh-cut tomato slices during cold storage. *Journal of the American Society for Horticultural Science, 125*(6), 736-741.

Kimball, D.A. (1991). Citrus Processing: Quality Control and Technology. New York: Van Nostrand Reinhol, New York, USA.

Lurie, S., Zhou, H., Lers, A., Sonego, L., Alexandrov, S., & Shomer, I. (2003). Study of pectin esterase and changes in pectin methylation during normal and abnormal peach ripening. *Physiologia Plantarum, 119*(2), 287-294.

Manzocco, L., Dri, A., & Quarta, B. (2009). Inactivation of pectic lyases by light exposure in model systems and fresh-cut apple*. Innovative Food Science and Emerging Technologies*, 10(4), 500-505.

Navia, B., Perea, J.M., Faci, M., Montero A., & Plaza Fraile, L. Tablas de composición de frutas y hortalizas. www.5aldia.org/v_5aldia/apartados/apartado.asp?te=139.

Natalini, A., Guidi, L., Kiferle, C., Mensuali-Sodi, A., Degl'Innocenti, E., & Pardossi, A. (2011). Response of fresh-cut kiwifruits and tomatoes to cold storage. *Agrochimica, 55*(1), 54-64.

O'Beirne, D. (2006). Microbial safety of fresh-cut vegetables. *Acta Horticulturae,* 746, 159-172.

Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Effect of minimal processing on bioactive compounds and color attributes of fresh-cut tomatoes. *Lebensmittle Wissenschaft & Technologie,* 41 (2), 217-226.

Oms-Oliu, G., Aguiló-Aguayo, I., Martín-Belloso, O., & Soliva-Fortuny, R. (2010). Effects of pulsed light treatments on quality and antioxidant properties of fresh-cut mushrooms (*Agaricus bisporus*). *Postharvest Biology and Technology*, 56, 216-222.

Poole, N. J. (1993). Improved tomatoes produced by modification of polygalacturonase activity. *International Journal of Food Sciences and Nutrition*, 44 (Suppl. 1), 1618-1619.

Ramos-Villarroel, A. Y., Aron-Maftei, N., Martín-Belloso, O., & Soliva-Fortuny, R. (2012a). Influence of spectral distribution on bacterial inactivation and quality changes of fresh-cut watermelon treated with intense light pulses. *Postharvest Biology and Technology*, 69, 32-39.

Ramos-Villarroel, A. Y., Aron-Maftei, N., Martín-Belloso, O., & Soliva-Fortuny, R. (2012b). The role of pulsed light spectral distribution in the inactivation of escherichia coli and listeria innocua on fresh-cut mushrooms. *Food Control*, 24(1-2), 206-213.

Ramos-Villarroel, A. Y., Marťn-Belloso, O., & Soliva-Fortuny, R. (2013). Intense light pulses: Microbial inactivation in fruits and vegetables. [Pulsos de luz intensa: inactivació n microbiana en frutas y hortalizas] *CYTA - Journal of Food,* 11(3), 234-242.

Rico, D., Martín-Diana, A. B., Frías, J. M., Barat, J. M., Henehan, G. T. M., & Barry-Ryan, C. (2007). Improvement in texture using calcium lactate and heat-shock treatments for stored ready-to-eat carrots. *Journal of Food Engineering*, 79, 1196-1206.

Rugkong A., Rose, J. K. C., Lee, S. J., Giovannoni, J. J., O'Neill, M., & Watkins, C. B. (2010) Cell wall metabolism in cold-stored tomato fruit. *Postharvest Biology and Technology,* 57, 106-113.

Sandhya. (2010). Modified atmosphere packaging of fresh produce: Current status and future needs. *LWT - Food Science and Technology,* 43(3), 381-392.

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

Soliva-Fortuny, R. C., Alòs-Saiz, N., Espachs-Barroso, A., & Martín-Belloso, O. (2004). Influence of maturity at processing on quality attributes of fresh-cut conference pears. *Journal of Food Science*, 69, S290-S294.

Toivonen, P. M. A., & Brummell, D. A. (2008). Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. *Postharvest Biology* and *Technology*, 48 1-14.

Van Djik, C., Boeriu, C., Stolle-Smits, T., & Tijskens, L. M. M. (2006). The firmness of stored tomatoes (cv. tradiro). 2. Kinetics and near infrared models to describe pectin degrading enzymes and firmness loss. *Journal of Food Engineering*, 77(3), 589-593.

Wei, J., Qi, X., Guan, J., & Zhu X. (2011). Effect of cold storage and 1-MCP treatment on postharvest changes of fruit quality and cell wall metabolism in sweet cherry. *Journal of Food, Agriculture and Environment*, 9 (3-4), 118-122.

Young, R. (1995). Improved tomato products: Utilizing modern biotechnology. *Food Australia* 5, 203-204.

CAPITULO 3

Kinetics of the changes on the antioxidant potential of fresh-cut tomatoes as affected by pulsed light treatments and storage time

Abstract

The effects of pulsed light (PL) on the antioxidant capacity (AC) and bioactive compounds content of fresh-cut tomatoes (*Lycopersicon esculentum* Mill., cv. Daniela) were studied. Tomato slices were subjected to fluences of 4, 6 and 8 J cm^{-2} and stored over 18 days. Modified first-order and Gompertzian functions were used to explain the changes of the studied parameters as affected by PL-fluence and storage time.

PL processing led to an increase in the contents of lycopene and phenolic compounds, while vitamin C was notoriously depleted ($p< 0.05$). Those patterns were fitted by linear equations ($R^2 \geq 0.95$), while changes in the bioactive compounds contents through the storage were well described by Gompertzian functions ($R^2_{adj} \geq 0.94$ and $A_f \geq 0.99$). AC of tomato slices was strongly affected by PL-processing ($p< 0.05$) as well as by the storage period ($p< 0.05$). The influence of PL on the initial AC of tomato slices was expressed by linear model ($R^2 \geq 0.95$) while variations over storage were appropriately fitted by a first-order equation ($R^2_{adj} \geq 0.95$ and $A_f \geq 0.99$). The higher the fluence applied, the greater the change in the AC and bioactive compounds contents of tomato slices over the storage period. The high correlation coefficients and the estimated kinetic constants seem to suggest that AC of tomato slices was highly conditioned by changes on the vitamin C concentration along the storage as a function of the PL-fluence applied.

Industrial relevance: Since little information about the impact on physicochemical aspects is available, results obtained in this work may contribute to identify the optimal treatment conditions to achieve healthy and fresh-like cut tomatoes. In general, PL processing has demonstrated a significant reduction of the microbial loads, with a minimal impact on the physicochemical characteristics of fresh-cut tomatoes.

Keywords: Pulsed light, fresh-cut tomatoes, antioxidant capacity, phenolic compounds, lycopene, vitamin C

Highlights

- Antioxidant capacity of tomato slices was highly affected by PL-processing, being the higher the PL-fluence, the lower the antioxidant capacity.
- Tomato slices subjected to PL exhibited an enhancement of the lycopene and phenolic compounds contents.
- Minimal processing triggered the vitamin C depletion regardless the PL-fluence applied.

1. Introduction

Tomatoes are the most demanded horticultural crops worldwide. The increase in their consumption has been associated with a reduced risk of suffering from various degenerative diseases, including heart disease and cancer (Sánchez-Moreno et al., 2012). These protective effects have been mainly attributed to their bioactive components with antioxidant properties (Borguini & Torres, 2009). Tomatoes are a rich source of vitamins, such as ascorbic acid and tocopherols, phenolic compounds, and carotenoids, namely lycopene (Vallverdú-Queralt et al., 2011).

The increasing consumers demand for fresh-cut tomatoes has been largely influenced by their fresh-like characteristics and high nutritional value. However, postharvest processing can lead to undesirable alterations such as water loss and physical softening, promoting microbial contamination and changes in the sensorial attributes of fresh-cut commodities and thus negatively affecting their fresh-like quality (Soliva-Fortuny et al., 2003; Odriozola-Serrano et al., 2009). In addition, nutritional losses in tomato slices have been principally associated to increased tissular respiration and biochemical deterioration after minimal processing (Odriozola-Serrano et al., 2008, 2009 and Kim et al., 2008). In order to satisfy the consumer's demand for safe, nutritive and fresh-like tomato slices, food researchers have been exploring different preservation methods.

Pulsed light (PL) treatments are based on the application of short pulses of high intensity and broad spectrum. Although PL has been widely used for the disinfection of surfaces, some studies have demonstrated their efficacy to reduce microbial loads with a minimal impact on the quality of fresh-cut fruits and vegetables (Ramos-Villarroel et al., 2011; Valdivia-Nájar et al., 2016). The efficacy of PL on the microbial inactivation mainly depends of photothermal and photochemical effects generated by those treatments (Ramos-Villarroel et al., 2013). In addition, prior mentioned effects could also prompt changes of the physicochemical characteristics of fresh-cut vegetables (Rodov, et al., 2012; Aguiló-Aguayo et al., 2013). Indeed, some researchers (Pataro et al., 2015; Aguiló-Aguayo et al., 2017) indicated that stress generated by PL in vegetable tissues might provoke increases of nutritional and bioactive compounds.

Therefore, the use of PL to reduce the microbial population could contribute to the enhancement of the quality and shelf life of fresh-cut products (; Charles et al., 2013; Koh et al., 2016).

In a broader context, modeling the changes on the quality characteristics of fresh-cut commodities could contribute to process optimization and shelf-life prediction. The use of mathematical functions could play an important role in the development of PL treatments as they help to predicting their performance and impact in quality of fresh-cut commodities. First and second-order, logistic and Weibull functions have been commonly used to describe and predict the changes in bioactive compounds of fresh-cut fruits and vegetables during storage (Odriozola-Serrano et al., 2009; González-Centeno et al., 2015; Bialka et al., 2008). Several studies also report the use of Gompertzian equations for describing microbial activity as well as physicochemical, enzymatic and physiological processes as a function of time in vegetable commodities with good results (Çelekli et al., 2014; Farmani, 2015; Giuggioli et al., 2017; Tran et al., 2017; Valdivia-Nájar et al., 2017;). Anywise, few works report the use of mathematical models to describe the effects of PL-treatments on quality attributes of fresh-cut commodities.

Thus, the main objective of this research was to evaluate the effect of PL-treatments on the antioxidant capacity as well as on the lycopene, vitamin C and total phenolic compounds contents on fresh-cut tomatoes. Additionally, modified first-order and Gompertzian functions were used to describe the changes of the health-related compounds in fresh-cut tomatoes through storage.

2. Materials and methods

2.1. Minimal processing and pulsed light treatments

Tomatoes (*Lycopersicon esculentum* Mill cv. Long life) were purchased at a local market (Lleida, Spain) and maintained at 4±1 ºC prior to processing. Whole tomatoes were washed in water, dipped in a chlorinated solution (100 ppm free chlorine L^{-1} for 2 min), rinsed with tap water and gently dried. Tomato fruits were then cut into 5 mm-thick slices using an

electric slicer (Food Slicer-6128: Toastmaster Corp.; Elgin, USA) with tangential orientation. Tomato slices (ca. 100g) were packaged in polypropylene trays (350 cm^3, 5015 RM PTT-ATS Packaging S.r.l.; Venice, Italy) and thermo sealed using an ILPRA Food Pack Basic V/6 packaging machine (ILPRA Systems, CP.; Vigevono, Italy). Transparency of the film was 97 % of the UV radiation and almost a 100 % of the visible wavelengths (Avalos-Llano et al. 2016). Finally, the packages were randomly subjected to PL-treatments.

PL-treatments were performed using a flash lamp system (Steribeam Xe-Matic-2L-A, Kehl, Germany). Pulses were emitted from two xenon lamps situated at 8.5 cm above and below the sample holder. The emitted spectrum ranged from 180 to 1100 nm. According to manufacturer's directions, a standard light source was used to calibrate the equipment and the energy readings were collected by a photodiode placed into the PL chamber. Tomato slices were subjected to 10, 15 and 20 pulses with an emitted fluence of 0.4 J cm^{-2} per pulse (4, 6 and 8 J cm^{-2}, respectively). PL conditions were selected from previous works related to the microbial inactivation and physical impact of PL on fresh-cut tomatoes (Valdivia-Nájar et al. 2017). A set of untreated trays was kept as reference. All trays were stored at 5 ºC for 18 days in darkness until random withdrawal for analysis.

2.2. Quality parameters

The surface color of fresh-cut tomatoes was assessed by tristimulus reflectance colorimetry, using a Minolta Chroma Meter Model CR-400 (Minolta Sensing Inc.; Osaka, Japan) properly calibrated with a Hunterlab standard white plate (L^* 93.4; a^*= -067; b^*= 0.78). The equipment was set up for a D65 illuminant and 10° observer angle. CIE L^* (lightness), a^* (red-green) and b^* (yellow-blue) parameters were measured through reflectance values. On the other hand, tomato slices were homogenized in a blender and then filtered through a 2-mm diameter steel sieve to remove the peel and seeds. The juice was then used to determine total soluble solids (2WAJ-

ABBE Refractometer; Atago Company Ltd.; Tokyo, Japan) and pH (Crison 2001 pH-meter; Crison Instruments S.A.; Alella, Barcelona, Spain).

Table 3.1. Changes of the quality parameters in fresh-cut tomatoes exposed to pulsed light (PL) and stored at 5 ºC throughout 18 days.

Quality parameter	PL-treatment (J cm^{-2})	Storage time (days)			
		0	6	12	18
pH	**0 (untreated)**	4.6 ± 0.1	4.8 ± 0.0	4.7 ± 0.0	4.6 ± 0.01
	4	4.5 ± 0.1	4.8 ± 0.0	4.7 ± 0.0	4.6 ± 0.0
	6	4.4 ± 0.1	4.8 ± 0.0	4.7 ± 0.1	4.6 ± 0.0
	8	4.3 ± 0.1	4.7 ± 0.0	4.8 ± 0.0	4.5 ± 0.0
TSS	**0 (untreated)**	4.2 ± 0.3	4.8 ± 0.4	5.2 ± 0.1	5.5 ± 0.3
	4	4.3 ± 0.1	5.3 ± 0.0	5.5 ± 0.3	5.5 ± 0.4
	6	4.3 ± 0.1	4.4 ± 0.1	5.3 ± 0.4	5.6 ± 0.4
	8	4.5 ± 0.2	4.8 ± 0.1	5.1 ± 0.0	5.4 ± 0.4
L*	**0 (untreated)**	33.0 ± 1.0	32.0 ± 2.2	33.4 ± 3.0	38.6 ± 3.3
	4	33.0 ± 6.5	32.2 ± 0.7	32.4 ± 0.6	31.4 ± 2.9
	6	36.1 ± 3.9	31.4 ± 3.1	32.4 ± 1.2	30.7 ± 1.6
	8	34.3 ± 1.5	29.3 ± 2.3	33.4 ± 1.6	32.3 ± 4.2
a*	**0 (untreated)**	11.2 ± 0.8	15.1 ± 0.5	16.7 ± 1.3	18.8 ± 0.6
	4	14.1 ± 0.2	17.7 ± 1.4	18.3 ± 1.2	19.4 ± 1.2
	6	14.4 ± 2.6	16.3 ± 1.9	18.0 ± 0.9	18.4 ± 0.8
	8	16.1 ± 1.9	17.5 ± 1.0	19.1 ± 0.3	20.2 ± 0.5

TSS= Total soluble solids (ºBrix); L*= lightness and a*=green-red- colour. Data shown are mean ± standard deviation, *n*=6.

2.3. Lycopene content

Total lycopene was measured spectrophotometrically as described by Odriozola-Serrano et al. (2008). For this assay, 0.6 g of tomato juice was mixed with a solution containing 5 ml of 0.05 % (w/v) butylated hydroxytoluene (BHT) in acetone, 5 ml of 95 % USP grade ethanol and 10 ml of hexane and then centrifuged at 320 × g for 15 min on ice. Subsequently, 3 ml of deionized water were added and shaken during 5 min. Finally, mixture was left at room temperature to allow phase separation. The absorbance of the upper, hexane layer, was measured at 503 nm and

blanked with hexane. The lycopene content of each sample was then estimated according to the following equation (Equation 1):

$$Lycopene = \frac{\Delta_{503} \cdot MW \cdot DF \cdot 1000}{\varepsilon \cdot L} \quad \textbf{(1)}$$

where *MW* is the molecular weight of lycopene (536.9 g^{-1} mol), *DF* is the dilution factor, *L* is the pathlenght in cm, and *ε* is the molar extinction coefficient for lycopene (172,000 L mol cm^{-1}). Results were expressed as mg of lycopene per kg of fresh-cut tomato.

2.4. Vitamin C content

The extraction procedure was based on a previously validated method (Odriozola-Serrano et al., 2007). First, 25 g of fresh-cut tomatoes were crushed, filtered through an steel sieve (2 mm diameter) and the juice was mixed with 25 ml of 4.5 % metaphosforic solution with 7.2 g/L of DL-1,4-dithiotreitol as reducing agent. The mixture was centrifuged at 22100 × g for 15 min at 4 °C (Centrifuge Avanti™ J-25, Beckman Instruments Inc.; Fullerton, California, U.S.A). The supernatant was vacuum-filtered through Whatman No. 1 and subsequently filtered through a 0.45 µm Millipore membrane. Then, the Vitamin C content was analyzed by HPLC. An aliquot of 20 µL was injected onto the HPLC system (Waters 600E, Waters, Milford, MA.) via a manual injector. The flow rate was fixed at 1.0 ml/min at room temperature. A reverse-phase C18 Spherisorb® ODS (5 µm) stainless steel column (4.6 mm × 250 mm) and a 486 Absorbance Detector (Waters, Mildford, MA) were used. A 0.01 % solution of sulfuric acid adjusted to pH=2.6 was used as eluent. Detection was performed at 245 nm. A calibration curve with ascorbic acid (Scharlau Chemie, S.A., Barcelona, Spain) was built for vitamin C quantification, and results were expressed as mg· kg^{-1} (fw).

2.5. Total phenolic compounds

The content of total phenolic compounds in fresh-cut tomatoes was determined according to the Folin-Ciocalteu procedure (Singleton et al.

1999) with some modifications. Fresh-cut tomatoes were crushed and centrifuged at 6000 × g for 15 min at 4 ºC with an AVANTI™ J-25 centrifuge (Beckman Instruments Inc., Fullerton, CA, USA) and then passed through a Whatman No. 1 filter paper. An aliquot of 0.5 ml of the supernatant was added to 0.5 ml of Folin-Ciocalteu solution. After 3 min, 10 ml of saturated sodium carbonate solution were added and brought up to 25 ml with distilled water. The absorbance at 725 nm was measured after incubation at 20 ºC for 1 h in darkness conditions. Results were expressed as milligrams of gallic acid per kg of fresh-cut tomatoes.

2.6. Antioxidant capacity analysis

2.6.1. DPPH method

The antioxidant capacity (AC) of fresh-cut tomatoes was determined through the evaluation of the scavenging effect of the tomato extract on the 1,1-diphenil-2-picryhydrazil (DPPH) radical according to the method proposed by De Ancos et al. (2002). First, 25 g of tomato slices were crushed and the juice was centrifuged at 6000 × g for 15 min at 4º C (Centrifuge Avanti™ J-25, Beckman Instruments Inc., Fullerton, CA, USA) and aliquots of 0.01 ml of the supernatant were mixed with 3.9 ml of methanolic DPPH (0.025 g/L) and 0.09 ml of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. Absorption of the samples was measured with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at 515 nm against a blank of methanol without DPPH.

2.6.2. ABTS method

Antioxidant capacity was also measured using the trolox equivalent antioxidant potential assay. Determination was based on the method proposed by Re et al. (1999). The $ABTS^{++}$ solution was generated by dissolving 19.2 mg of 2,2-azino-bis (3-rthylbenzotriazoline-6-sulfonic acid) ($ABTS^{++}$) into 5 ml of HPLC-grade water and 88 µL of potassium persulfate ($K_2S_2O_8$) (0.0378 g^{-1} ml^{-1}). The mixture was then incubated in darkness at room temperature for 16 h. Finally, 1 ml of $ABTS^{++}$ activated radical was diluted with 88 ml of HPLC-grade ethanol, pH 7.4, to an absorbance of 0.7 (± 0.02) at 734 nm and equilibrated to

30 °C. The reaction was initialized by adding 2970 µL of the $ABTS^{++}$ solution to 30 µL of tomato juice or trolox standard solution in methanol and the absorbance at 734 nm was monitored at 1 and 6 min.

2.6.3. Antioxidant capacity determination

Antioxidant capacity was reported as percentage of $DPPH^{++}$ or $ABTS^{++}$ inhibition. Then, AC was calculated using the equation (2):

$$\%\ inhibition\ of\ DPPH^{++}\ or\ ABTS^{++} = ([A_i - A_f] / A_i) \times 100 \quad \textbf{(2)}$$

where A_i, is the absorbance before the reaction and A_f is the absorbance after reaction has taken place.

2.7. Statistical analysis

Analyses were conducted in duplicate at each sampling time and three replicates were carried out for each sample (n= 6). Statistical analysis was performed using the Statgraphics plus v. 5.1 Windows package (Statistical graphics Co., Rockville, MD). Analysis of variance (ANOVA) was employed to compare sample mean values. Data were analyzed through multifactor analysis of variance and a LSD multiple range test was applied to determine differences among means with a significance level of 0.05. Moreover, correlation among experimental values obtained for antioxidant capacity and bioactive compounds was evaluated using a Spearman rank correlation analysis.

2.7.1. Curve fitting

Subsequently, integrated equations were performed to fit processing variables to changes of AC and bioactive compounds contents of tomato slices. First, linear functions (Equation 3) were used to fit the initial values of antioxidant capacity or concentrations of a bioactive compound in fresh-cut tomatoes as affected by the applied PL-fluence.

$$X_0 = (k_1 \cdot \Phi) - b \quad \textbf{(3)}$$

where X_0 is the value of antioxidant capacity (%) or the bioactive compound content (mg kg^{-1}) as affected by the PL-fluence (Φ, J cm^{-2}); k_i is a the rate of change constant (cm^{-2} J^{-1}) and b is the value of antioxidant capacity (%) or bioactive compound (mg kg^{-1}) in the untreated tomato slices.

Then, a modified Gompertz function (Equation 4) was used to predict the changes in the contents of vitamin C, lycopene and phenolic compounds in fresh-cut tomatoes through storage.

$$BC_{(x)} = X_0 \pm k \cdot \exp\left\{-\exp\left[\frac{u_{max} \cdot e}{k}\right] \cdot (\lambda - t) + 1\right\} \quad \textbf{(4)}$$

where $BC_{(X=LYC,\ VC,\ TPC)}$ is the content of bioactive compound (mg kg^{-1}); X_0 is the value expressed by Equation 2 (X_0) for the initial content of each bioactive compound (mg kg^{-1}); k is the difference among the highest and the lowest values attained for each variable during the cold storage (mg kg^{-1} day^{-1}); μ_{max} is the maximum change rate (mg kg^{-1} day^{-1}); λ, is the lag time (day) and t, is the storage time (day).

On the other hand, a first-order kinetic (Equation 5) was used to describe the changes in the antioxidant capacity on fresh-cut tomatoes as a function of the PL-fluence applied and throughout the storage period.

$$AC = X_0 \exp^{-\delta \cdot y} \quad \textbf{(5)}$$

where AC denotes the antioxidant capacity (%) as affected by the applied fluence (Φ) and the storage time (t), X_0 are the values of antioxidant capacity (X_0) given by Equation 2 (%), δ is the rate of change constant (day^{-1}) and t is the storage time (day).

Mathematical validation of the methods was performed through the analysis of the adjusted determination coefficient (R^2_{adj}) (Equation 6) and the accuracy factor (A_f) (Equation 7). The closer the R^2 and A_f value to 1, the better the adequacy of the models to describe the observed changes.

$$R^2_{adj} = 1\frac{n-1}{n-p} \cdot (1-R^2) \quad \textbf{(6)}$$

$$Af = \frac{10^{\sum \log\left(\frac{V_{predicted}}{V_{observed}}\right)}}{n} \quad \textbf{(7)}$$

where n is the sample size; p is the number of parameters and R^2 is equal to 1- (residual sum of squares / total sum of squares).

3. Results and discussions

3.1. Lycopene

Lycopene content of tomato slices (46.76 mg kg^{-1}) significantly increased just after exposure to the PL-treatments (52.94 – 58.97 mg kg^{-1}). Changes in the lycopene content as a function of the PL-fluence applied were well fitted by a linear equation with determination coefficient (R^2) of 0.99 (Equation 3). In this case, estimated of the kinetic constant was k_i= 0.15 × 10^{-1} cm^{-2} J, which is a clear sign of the positive effect of PL on the enhancement of lycopene content.

Furthermore, the lycopene contents of untreated and PL-treated sliced tomatoes increased along the storage period (Figure 3.1). At the end of storage, the lycopene content in untreated tomato slices was 52.37 mg kg^{-1}, while the contents of tomato slices subjected to fluences of 4, 6 and 8 J cm^{-2} increased up to 77.76, 81.31 and 88.83 mg kg^{-1}, respectively.

Changes in the lycopene contents over cold storage were well fitted by a Gompertzian model (Equation 4). The high R^2_{adj} and A_f values (≥ 0.92 and ≥ 1.0, respectively) indicated the great suitability of this function to describe the effect of the PL-fluence (Φ) and the storage period (t) on the lycopene content of tomato slices. Estimates of the parameters of the Gompertzian model used to describe lycopene concentrations as a function of the storage time are displayed in table 3.2. Tomato slices exhibited increments of the lycopene content over the storage (k) regardless the PL treatment applied.

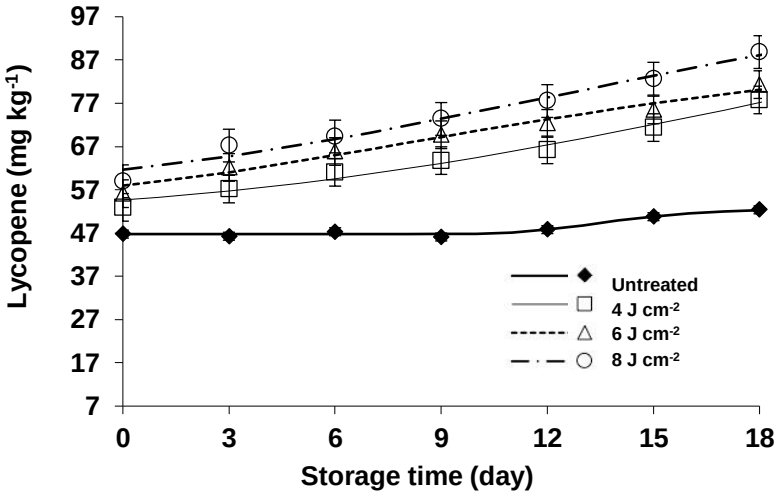

Figure 3.1. Lycopene content of fresh-cut tomatoes exposed to pulsed light (PL) and stored throughout 18 days at 5 ºC. Lines show the models fitted by Gompertz function and grids display the experimental values (mean ± SD).

However increases of lycopene content on tomato slices subjected to PL ($k \geq 3.62 \times 10^{-1}$ mg kg^{-1}) was higher than those on untreated ($k = 0.62 \times 10^{-1}$ mg kg^{-1}) and, thus indicating that PL enhanced the lycopene content along the storage period. Indeed, increments of the lycopene content on PL-treated tomato slices started before (λ=2.29 and 0.13 day^{-1}) than those on untreated (λ=13.24 day^{-1}). Thereby, PL processing seems to induce a physiological stress on the vegetable tissues that accelerates the production of lycopene production.

Table 3.2. Results of the statistical analysis on the kinetic changes of lycopene in fresh-cut tomatoes subjected to pulsed light (PL) and stored for 18 days at 5 ºC.

	PL-fluence (J cm^{-2})	Kinetic constants				Validation	
		BC	***k***	μ_{max}	λ	R^2_{adj}	A_f
Lycopene	**0 (untreated)**	46.76	0.62×10^{-1}	1.16	13.24	0.96	46.76
	4	51.39	9.09×10^{-1}	1.80	2.29	0.97	51.39
	6	54.46	3.62×10^{-1}	1.43	0.81	0.96	54.46
	8	57.25	5.90×10^{-1}	1.81	0.13	0.96	57.25

BC is the content of bioactive compound (mg kg^{-1}); ***k***, is the difference among the highest and the lowest values attained for each variable during the cold storage; μ_{max}, maximum change rate over time (mg kg^{-1} day^{-1}) and λ, is the time before the beginning of the changes or lag time (day). R^2_{adj} is the adjusted determination coefficient (%); and A_f is the accuracy factor (%).

On the other hand, lycopene concentration on tomato slices exhibited higher increases (μ_{max}= 1.43 - 1.81 mg kg day^{-1}) than those on untreated (1.1 mg kg day^{-1}) over the storage and suggesting that the positive effect over the lycopene production remain during the days following the PL processing. In fact, the higher the Φ applied, the lower the λ and the higher the μ_{max} values. Then, PL seems to play an important role on the content and production rate of lycopene in fresh-cut tomatoes. Similarly, Solovchenko and Merzlyak, (2008) and Rodov et al. (2012) reported increases in the carotenoid content of different commodities after PL exposure. Pigments can absorb the incident radiation, acting as photoprotectors, thus avoiding the degradation of other compounds (Falguera et al. 2011). Similarly, Bravo, et al. (2012) and Castagna, et al. (2013) observed an enhancement of lycopene content in tomatoes subjected to PL. The latter authors attributed this effect to the activation of lycopene pathway synthesis as a protective defense response to an oxidative stress caused by PL-fluences and presence of oxygen into the packages headspace.

3.2. Vitamin C

The initial vitamin C content in untreated tomato slices was 114.71 mg kg^{-1}, which is consistent with the values reported in the literature (69.6-212-3 mg kg^{-1} fw) (Odriozola-Serrano et al., 2008a and 2008b). The concentration of vitamin C in fresh-cut tomatoes significantly decreased just after exposure to fluences of 4, 6 and 8 J cm^{-2} (104.30 92.16 and 90.61 mg kg^{-1}, respectively). Changes in the content of vitamin C as a function of the PL-fluence (Φ) applied were fitted by a primary linear equation (R^2= 0.95) (Equation 3) in which the estimate of the kinetic constant was k_i= -0.32 × 10^{-1} cm^{-2} J. Hence, vitamin C degradation seemed to be proportional to the Φ applied, following a linear trend. In this context, oxidative processes stimulated by presence of light, oxygen, heat peroxides and oxidative enzymes can lead to vitamin C degradation (Davey et al., 2000). In fact, Falguera et al. (2014) reported decreased vitamin C contents in fruit juices subjected to PL (UV-Vis), attributing this effect to the light absorptivity of vitamin C. Ascorbic acid is highly susceptible to degradation when it is exposed to wavelengths near to 250 nm.

Furthermore, decreases on the contents of vitamin C of untreated and PL-treated tomato slices were observed along the storage period (Figure 3.2). A modified Gompertz function predicted well the vitamin C degradation as a function of storage time (Equation 4) with high determination coefficients (R^2_{adj}= 0.96 - 0.98) and accuracy factor above 0.99. Estimates of the parameters of this model are shown in Table 3.3. Relative change rate (k) was 0.20 × 10^{-2} kg mg day^{-1} for untreated tomato slices while values between 0.24 × 10^{-2} and 0.43 × 10^{-2} kg mg day^{-1} were observed on PL-treated samples, being the higher the Φ applied, the higher the change rate. Thereby, increasing PL influenced degradation of the vitamin C of tomato slices along the storage period.

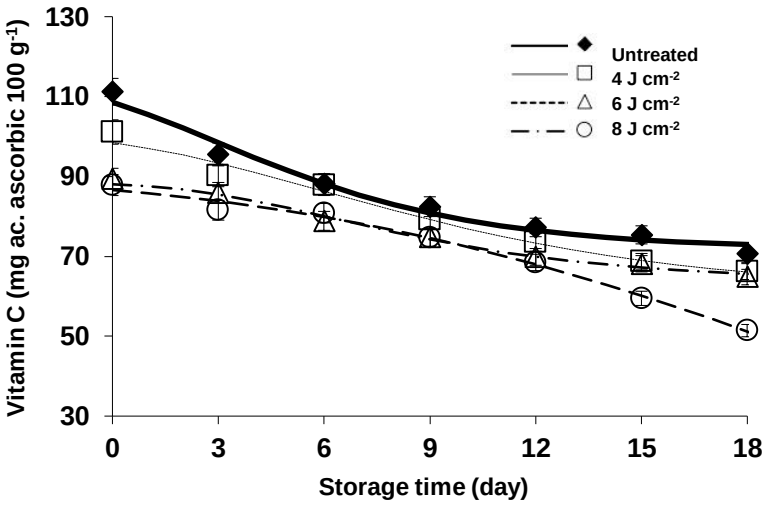

Figure 3.2. Effect of pulsed light (PL) on the vitamin C content on fresh-cut tomatoes stored at 4 °C for 18 days. Lines show the models fitted by Gompertz function and grids display the experimental values (mean ± SD, n= 6).

Those declines of vitamin C content on untreated tomato slices began just after processing (λ=0). Indeed, the lag phase was extended as consequence of the increasing Φ applied (λ= 0 - 6.87 day^{-1}). Conversely, the μ_{max} on PL-treated tomato slices were lower (μ_{max}= 2.03 - 3.64 mg kg^{-1} day^{-1}) than those on untreated tomato slices (μ_{max}= 3.68 mg kg^{-1} day^{-1}).

Table 3.3. Kinetic constants estimated by modified Gompertz model in order to describe the changes in the vitamin C content in fresh-cut tomatoes subjected to pulsed light (PL) and stored for 18 days at 4 ºC.

	PL-fluence (J cm^{-2})	Kinetic constants				Validation	
		BC	***k***	μ_{max}	λ	R^2_{adj}	A_f
Vitamin C	**0 (untreated)**	111.21	2.04 ×10^{-1}	3.68	-2.38	0.97	100
	4	101.13	2.44 ×10^{-1}	2.47	-0.01	0.97	100
	6	88.42	3.99 ×10^{-1}	2.03	2.35	0.98	99
	8	90.61	4.31 ×10^{-1}	3.64	6.87	0.99	99

BC is the content of bioactive compound (mg kg^{-1}); ***k***, is the difference among the highest and the lowest values attained for each variable during the cold storage; μ_{max}, maximum change rate over time (mg kg^{-1} day^{-1}) and λ, is the time before the beginning of the changes or lag time (day). R^2_{adj} is the adjusted determination coefficient (%); and A_f is the accuracy factor (%).

Then, increasing Φ seems to provoke a strong impact on the initial vitamin C content of tomato slices and in turn induces a delayed evolution of their content along the storage period. Although information about the effects of PL on the vitamin C is scarce, some studies reported vitamin C degradation after exposition to non-ionizing radiation (Oms-Oliu et al., 2012). Some authors have reported that the deleterious effect of UV light treatments on vitamin C is similar to that of thermal treatments (Tran and Farid, 2004). In this context, photothermal effects provoked by PL-treatments seem to induce an initial physiological stress, which triggers the synthesis of natural antioxidant on tissue cells. Thereby, vitamin C reacts rapidly with radical species to protect the cell integrity upon redox imbalances and the consequent production of reactive oxygen species (ROS) over the storage period. (Surjadinata et al., 2017)

3.3. Changes in total phenolic compounds

Fresh-cut tomatoes exhibited an initial total phenolic content (TPC) of 231.26 mg kg^{-1}. That value is within the range observed by Odriozola-Serrano et al. (2008a) and Martínez-Valverde et al. (2002) in other tomato cultivars (187.4 - 498.6 mg kg^{-1}). PL significantly affected the initial TPC of tomato slices. In fact, the TPC content increased as PL-fluence increased. Thus, tomato slices exposed to a fluence of 8 J cm^{-2} exhibited the highest phenolic content (241.25 mg kg^{-1}). Changes in TPC as a function of the PL fluence (Φ) applied were well

fitted by a primary linear equation (Equation 3) (R^2= 0.96). The estimated kinetic constant (k_i= 0.14 × 10^{-1} cm^{-2} J) is a clear sign of the positive effect of the increasing PL-fluence on the increases of TPC content.

Similarly, Liu et al, (2011) reported an enhancement of the amount of phenolic compounds in tomato fruits immediately after exposure to fluences of 2 and 4 J cm^{-2}. Bravo et al. (2013) also observed significant increases on the phenolic content of tomato fruits treated with fluences of 0.3 and 1.22 J cm^{-2}. In this context, some authors (Luthria et al., 2006 and Jagadeesh et al., 2011) mentioned that some PL-fluences appear to induce the accumulation of TPC in tomato fruit as a mechanism of adaptation against light stress. Moreover, stress caused by PL-treatments can also stimulate the synthesis of the enzyme phenylalanine ammonia-lyase, triggering the phenylpropanoids production and thus leading to an increase in phenol phytoalexins and lignins, which thereby explains the increases on the TPC (Ryalls et al., 1996).

A slight increase of TPC on untreated and PL-treated fresh-cut tomatoes was observed throughout the storage period (Figure 3.3). Significant differences (p< 0.05) among the TPC contents of untreated (238.5 mg kg^{-1}) and PL-treated (241.7 - 252.56 mg kg^{-1}) fresh-cut tomatoes were observed after prolonged storage. Modified Gompertz model (Equation, 4) fitted the experimental data as a function of applied PL-fluence as well as the storage time, with high determination coefficients (R^2_{adj}> 0.94) and accuracy factor (A_f= 1.0). Those changes in the patterns of TPC were well expressed by the estimates of the kinetic parameters of the model (Table 3.4).

Differences in TPC among different treatments over the storage period were explained by the relative change (X) values, while the lag time (λ) and maximum change rate (μ_{max}) values represent the changes on the TPC contents along the storage. Untreated tomato slices exhibited increases of the TPC from the beginning of the storage (λ= 0.03 day^{-1}). Those increases were maintained thorough the storage period (0.92 mg kg day^{-1}).

Conversely, PL processing lead to a higher lag phase values ($\lambda \geq$ 1.96 day^{-1}) and lower values of change rates ($\mu_{max} \leq$ 0.47 mg kg day^{-1}) and thus suggesting an accelerated synthesis of phenolic compounds induced by PL

processing which consequently reduced the increment of the TPC in fresh-cut tomatoes over the storage.

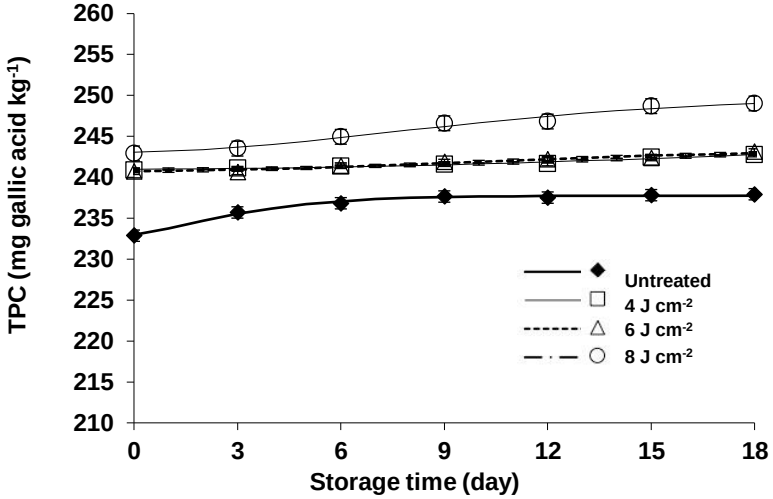

Figure 3.3. Total phenolic compounds (TPC) contents of fresh-cut tomatoes exposed to PL treatments and stored throughout 18 days at 5 ºC. Lines show the models fitted by Gompertz function and grids display the experimental values (mean ± SD).

Table 3.4. Kinetic constants estimated by modified Gompertz model in order to describe the changes in the total phenolic compounds on fresh-cut tomatoes subjected to pulsed light (PL) and stored for 18 days at 4 ºC.

	PL-fluence (J cm^{-2})	Kinetic constants				Validation	
		BC	***k***	μ_{max}	λ	R^2_{adj}	A_f
Phenolic content	**0 (untreated)**	232.26	0.55 ×10^{-1}	0.92	0.03	1	1
	4	240.88	0.75 ×10^{-1}	0.18	7.54	0.95	1
	6	240.68	0.29 ×10^{-1}	0.16	2.67	0.94	1
	8	242.92	0.71 ×10^{-1}	0.47	1.96	0.97	1

BC is the content of bioactive compound (mg kg^{-1}); ***k***, is the difference among the highest and the lowest values attained for each variable during the cold storage; μ_{max}, maximum change rate over time (mg kg^{-1} day^{-1}) and λ, is the time before the beginning of the changes or lag time (day). R^2_{adj} is the adjusted determination coefficient (%); and A_f is the accuracy factor (%).

Results obtained in this study are in agreement with those published by some authors (Ayala-Zavala et al., 2008; Antunes et al., 2013) who reported increases on the phenolic contents of different fresh-cut vegetables throughout the chill storage (16-20 days). The latter authors also attributed the TPC increases to an accelerated physiological activity in response to ripening and initial wounding. Costa et al. (2006) reported the enhancement of the phenolic content on fresh-cut broccoli exposed to PL (UV-C). These authors relate the TPC increases to some disorders on the cells caused by PL, which liberate phenolic compounds from pigment-protein complexes and thus facilitating the formation of enzymatic reactions.

3.4. Antioxidant capacity assays

Figure 3.5 displays the changes in the antioxidant capacity (AC) of fresh-cut tomatoes determined through the DPPH and ABTS methods. AC of untreated tomato slices determined with the DPPH and ABTS assays was 13.16 % and 16.75 %, respectively. Those values significantly decreased (p< 0.05) just after exposure to different PL-treatments. Thus, the AC of PL-treated tomato slices initially ranged between 11.90 and 12.56 % for the DPPH assay and between 15.18 to 16.08 % for the ABTS assay. In fact, the higher PL-fluence applied, the lower the AC of fresh-cut tomatoes. Those changes in the AC were satisfactorily fitted to the values obtained with the DPPH and ABTS methods by primary linear equations (Equation 3) with determination coefficients (R^2) of 0.99 and 0.95, respectively. The AC of tomato slices exposed to PL exhibited a linear decaying tendency with negative constants of k_i= -0.01 and -0.02 cm^{-2} J for DPPH and ABTS methods, respectively. Slight differences in the antioxidant capacity determined by different methods can be attributed to the experimental conditions and specificity of radical scavenger capacity used on each assay (Cao et al., 1993). However, significant differences (p< 0.05) between AC assayed by both DPPH and ABTS assays were not observed. Indeed, values obtained by both DPPH and ABTS assays exhibited a strong correlation (R^2= 0.93)

On the other hand, a significant decrease (p<0.05) in the antioxidant capacity of fresh-cut tomatoes was observed through storage regardless the PL-fluence applied (Figure 3.4).

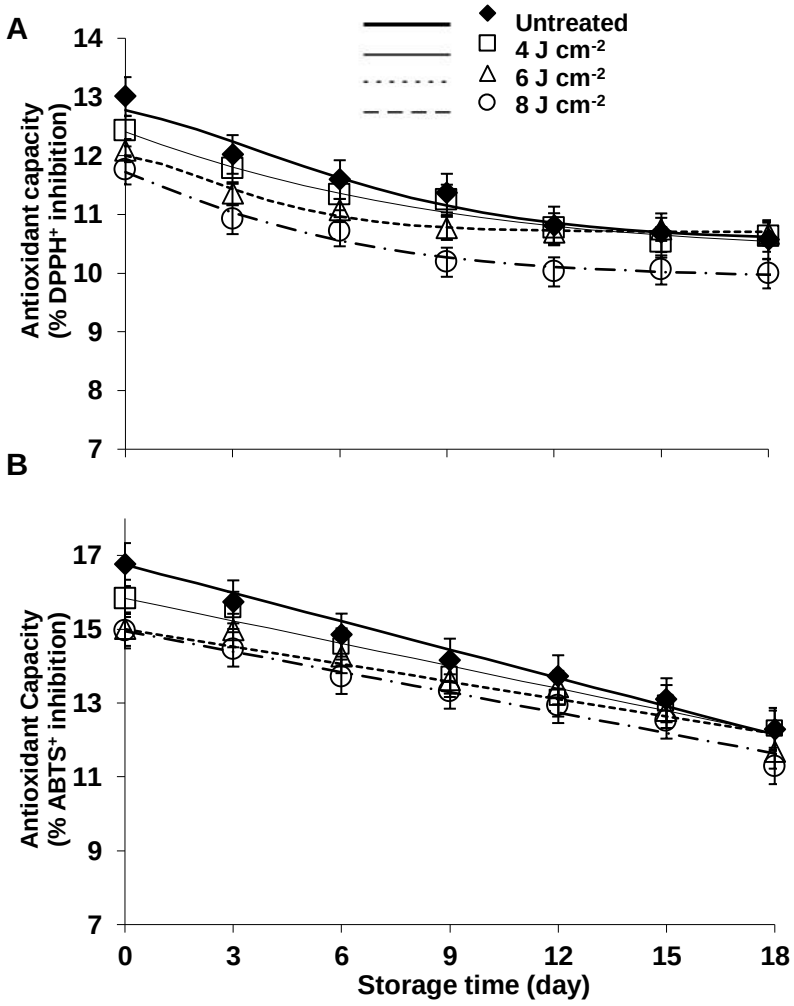

Figure 3.4. Changes in the antioxidant capacity of fresh-cut tomatoes exposed to pulsed light treatments and stored throughout 18 days at 5 ºC. Lines represent the fit of first-order functions to de experimental data and grids are the experimental values. Data shown are mean ± standard deviation, n=6.

Untreated tomato slices and those subjected to PL exhibited similar AC values (10.10 − 12.48 %, regardless the AC assay) at the end of storage period. A first-order model (Equation 5) fitted the experimental data obtained through both DPPH and ABTS assays with good determination coefficients (R^2_{adj}≥ 0.95)

and accuracy factors ($A_f \geq 0.99$). Estimates of the kinetic parameters, R^2_{adj} and A_f of the models used to describe the changes of the antioxidant capacity along the storage are given in Table 3.5. The rates of change (δ) were lower than -1.37 and 2.56×10^{-2} day^{-1} for DPPH and ABTS, respectively. In addition a slight inverse dependency of δ values against fluence was observed. Differences on the δ values can be associated to the different sensibility between the DPPH and ABTS methods to estimate the antioxidant capacity of fresh-cut tomatoes.

Table 3.5. Kinetic constants estimated by the first-order model to describe the changes in the antioxidant capacity of fresh-cut tomatoes subjected to PL and stored for 18 days at 5 ºC.

PL-fluence (J cm^{-2})	DPPH				ABTS			
	AC	***δ***	R^2_{adj}	A_f	***AC***	***δ***	R^2_{adj}	A_f
0 (Untreated)	13.01	1.4×10^{-2}	0.95	0.99	16.75	2.6×10^{-2}	0.98	1
4	12.42	1.1×10^{-2}	0.98	0.99	15.83	2.0×10^{-2}	0.97	1
6	12.09	0.8×10^{-2}	0.97	1	15.00	1.6×10^{-2}	0.93	0.99
8	11.76	1.0×10^{-2}	0.97	1	14.95	1.8×10^{-2}	0.97	0.99

DPPH, 1,1-diphenyl-2-picrylhydrazil radical; **ABTS**, 2,2-azinobis(3-ethylbenzothiazoline)-6-sulfonate radical; ***AC*,** is the antioxidant capacity (%); δ, first-order rate constant. R^2_{adj} is the adjusted determination coefficient; and A_f is the accuracy factor.

Our findings are in agreement with the observations of Odriozola-Serrano et al. (2009) and Antunes et al. (2010), who reported similar patterns in the antioxidant capacities of fresh-cut tomato and kiwi fruits determined by the DPPH and ABTS assays. Similarly, Agüero et al., 2016; Oms-Oliu et al., 2010; Costa et al., 2006) reported similar decreasing trend on the AC of fresh-cut spinach, mushroom and broccoli exposed to PL and stored under low temperatures (4-5 ºC). Those authors mentioned that PL provoke a deleterious effect of the tissue integrity, which cause membrane damages and alter the composition and content of antioxidant compounds and in turn prompt decreases of the AC. In this context, a strong correlation between the AC and bioactive compounds contents were observed (Table 3.6). Changes on AC values were well correlated to vitamin C decrements (r= 0.93), while correlations between AC and lycopene and phenolic compounds (r= -0.59 − -

0.76) suggest the low involvement of these compounds on the content of AC in fresh-cut tomatoes. Remarkably, the high correlation (r= 0.91) between the increments of lycopene and phenolic compounds contents is a clear sign of the effect of PL processing on the synthesis of these compounds, and thus minimizing the AC depletion along the storage period.

Table 3.6. Correlation coefficients between the antioxidant capacity (ABTS and DPPH methods), lycopene, vitamin C and phenolic compounds on untreated and PL-treated tomato slices stored at 5 ºC for 18 days.

	Antioxidant capacity		Bioactive compounds		
	DPPH	**ABTS**	**Lyc**	**Vit C**	**TPC**
DPPH	1				
ABTS	0.93	1			
Lyc	-0.76	-0.70	1		
Vit C	0.93	0.97	-0.81	1	
TPC	-0.69	-0.59	0.91	-0.67	1

% inh. DPPH, 1,1-diphenyl-2-picrylhydrazil radical; % inh. ABTS, 2,2-azinobis(3-ethylbenzothiazoline)-6-sulfonate radical; Lyc, Lycopene; Vit C, vitamin C and TPC, total phenolic compounds.
Estimated correlations are statistically significant at p< 0.01.

4. Conclusions

PL treatments could be a good alternative to increase the amounts of antioxidant compounds of fresh-cut tomatoes and in turn, enhancing their nutritive value. Antioxidant capacity and bioactive compounds contents of fresh-cut tomatoes were substantially affected by the exposure to PL and those changes were more evident along the storage of tomato slices. AC decreases were mostly attributed to losses of vitamin C, which were attributed to PL processing. PL are capable to trigger the synthesis of secondary metabolites as lycopene and total phenolic compounds in fresh-cut tomatoes, hence increasing their relative content. Trends of AC and bioactive compounds on fresh-cut tomatoes along storage period can be described with good accuracy by the first-order and Gompertzian models. Results obtained in this work contribute to

the development of mathematical models that can be used as a predictive tool to evaluate and optimize PL treatments in order to better retain the antioxidant capacity and bioactive compounds content of tomato slices.

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5. References

Aguiló-Aguayo, I., Charles, F., Renard, C. M. G. C., Page, D., & Carlin, F. (2013). Pulsed light effects on surface decontamination, physical qualities and nutritional composition of tomato fruit. *Postharvest Biology and Technology, 86*, 29–36

Agüero, M. V., Jagus, R. J., Martín-Belloso, O., & Soliva-Fortuny, R. (2016). Surface decontamination of spinach by intense pulsed light treatments: Impact on quality attributes. *Postharvest Biology and Technology, 121*, 118–125

Antunes, M. D. C., Dandlen, S., Cavaco, A. M., & Miguel, G. (2010). Effects of postharvest application of 1-MCP and postcutting dip treatment on the quality and nutritional properties of fresh-cut kiwifruit. *Journal of Agricultural and Food Chemistry, 58*(10), 6173–6181

Antunes, M. D. C., Rodrigues, D., Pantazis, V., Cavaco, A. M., Siomos, A. S., & Miguel, G. (2013). Nutritional quality changes of fresh-cut tomato during shelf life. *Food Science and Biotechnology, 22*(5), 1–8

Avalos Llano, K. R., Marsellés-Fontanet, A. R., Martín-Belloso, O., & Soliva-Fortuny, R. (2016). Impact of pulsed light treatments on antioxidant characteristics and quality attributes of fresh-cut apples. *Innovative Food Science and Emerging Technologies, 33*, 206–215

Ayala-Zavala J. F., Oms-Oliu G., Odriozola-Serrano I., González-Aguilar G.A., Álvarez-Parrilla E. & Martín-Belloso O. (2008). Bio-preservation of fresh-cut tomatoes using natural antimicrobials. *European Food Research and Technology,* 226: 1047–1055

Ayhan, Z., Yeom, H. W., Howard Zhang, Q., & Min, D. B. (2001). Flavor, color, and vitamin C retention of pulsed electric field processed orange juice in different packaging materials. *Journal of Agricultural and Food Chemistry, 49*(2), 669–674

Bravo, S., García-Alonso, J., Martín-Pozuelo, G., Gómez, V., Santaella, M., Navarro-González, I., & Periago, M. J. (2012). The influence of post-harvest UV-C hormesis on lycopene, β-carotene, and phenolic content and antioxidant activity of breaker tomatoes. *Food Research International, 49*(1), 296–302

Bravo, S., García-Alonso, J., Martín-Pozuelo, G., Gómez, V., García-Valverde, V., Navarro-González, I. & Periago, M. J. (2013). Effects of postharvest UV-C treatment on carotenoids and phenolic compounds of vine-ripe tomatoes. *International Journal of Food Science & Technology*, 48: 1744–1749

Bialka, K. L., Demirci, A., & Puri, V. M. (2008). Modeling the inactivation of escherichia coli O157:H7 and salmonella enterica on raspberries and strawberries resulting from exposure to ozone or pulsed UV-light. *Journal of Food Engineering, 85*(3), 444–449

Castagna, A., Chiavaro, E., Dall'Asta, C., Rinaldi, M., Galaverna, G., & Ranieri, A. (2013). Effect of postharvest UV-B irradiation on nutraceutical quality and physical properties of tomato fruits. *Food Chemistry, 137*(1-4), 151–158

Cao, G., Alessio, H. M. & Cutler, R. G. (1993). Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology and Medicine*, 14, 303–311

Costa, L., Vicente, A. R., Civello, P. M., Chaves, A.R. & Martínez, G. A. (2006). UV-C treatment delays postharvest senescence in broccoli florets. *Postharvest Biol. Technol*., 39 (2) (2006), pp. 204–210

Çelekli, A., Bozkurt, H., & Dönmez, G. (2014). Predictive modeling of β-carotene accumulation by dunaliella salina as a function of pH, NaCl, and irradiance. *Russian Journal of Plant Physiology, 61*(2), 215–223

Charles, F., Vidal, V., Olive, F., Filgueiras, H., & Sallanon, H. (2013). Pulsed light treatment as new method to maintain physical and nutritional quality of fresh-cut mangoes. *Innovative Food Science and Emerging Technologies, 18*, 190–195

Davey, M. W., Montagu, M. V., Inzé, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I. J., Strain, J. J., Favell, D. and Fletcher, J. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Sciences of Food and Agriulture*, 80: 825–860

De Ancos, B., Sgroppo, S., Plaza, L., & Pilar Cano, M. (2002). Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment. *Journal of the Science of Food and Agriculture, 82*(8), 790–796

Falguera, V., Pagán, J., & Ibarz, A. (2011). Effect of UV irradiation on enzymatic activities and physicochemical properties of apple juices from different varieties. *LWT - Food Science and Technology, 44*(1), 115–119

Falguera, V., Garvín, A., Garza, S., Pagán, J., & Ibarz, A. (2014). Effect of UV-vis photochemical processing on pear juices from six different varieties. *Food and Bioprocess Technology, 7*(1), 84–92

Farmani, J. (2015). Modeling of solid fat content of chemically interesterified fully hydrogenated soybean oil and canola oil blends as a function of temperature and saturated fatty acids. *Journal of Food Measurement and Characterization, 9*(3), 281–289

Giuggioli, N. R., Girgenti, V., Baudino, C., Giacalone, G., & Chiabrando, V. (2017). Prediction models for the softening of new commercial actinidia cultivars. *Journal of Food Processing and Preservation,* doi:10.1111/jfpp.13245

González-Centeno, M. R., Comas-Serra, F., Femenia, A., Rosselló, C., & Simal, S. (2015). Effect of power ultrasound application on aqueous extraction

of phenolic compounds and antioxidant capacity from grape pomace (vitis vinifera L.): Experimental kinetics and modeling. *Ultrasonics Sonochemistry, 22*, 506–514

Jagadeesh, S. L., Charles, M. T., Gariepy, Y., Goyette, B., Raghavan, G. S. V., & Vigneault, C. (2011). Influence of postharvest UV-C hormesis on the bioactive components of tomato during post-treatment handling. *Food and Bioprocess Technology, 4*(8), 1463–1472

Kim, H., Fonseca, J. M., Kubota, C., Kroggel, M., & Choi, J. (2008). Quality of fresh-cut tomatoes as affected by salt content in irrigation water and post-processing ultraviolet-C treatment. *Journal of the Science of Food and Agriculture, 88*(11), 1969–1974

Koh, P. C., Noranizan, M. A., Karim, R. & Hanani, Zan. (2016). Microbiological stability and quality of pulsed light treated cantaloupe (*Cucumis melo* L. Reticulatus cv. Glamour) based on cut type and light fluence. *Journal of Food Sciences Technology*, *53*(4), 1798–1810

Liu, C., Han, X., Cai, L., Lu, X., Ying, T., & Jiang, Z. (2011). Postharvest UV-B irradiation maintains sensory qualities and enhances antioxidant capacity in tomato fruit during storage. *Postharvest Biology and Technology, 59*(3), 232–237

Luthria, D. L., Mukhopadhyay, S., & Krizek, D. T. (2006). Content of total phenolics and phenolic acids in tomato (lycopersicon esculentum mill.) fruits as influenced by cultivar and solar UV radiation. *Journal of Food Composition and Analysis, 19*(8), 771–777

Martínez-Valverde, I., Periago, M. J., Provan, G. & Chesson, A. (2002). Phenolic compounds, lycopene and antioxidant capacity in commercial varieties of tomato (Lycopersicum esculentum). *Journal of the Science of Food and Agriculture,* 82: 323–330

Odriozola-Serrano, I., Aguiló-Aguayo, I., Soliva-Fortuny, R., Gimeno-Añó, V., & Martín-Belloso, O. (2007). Lycopene, vitamin C, and antioxidant capacity of tomato juice as affected by high-intensity pulsed electric fields critical parameters. *Journal of Agricultural and Food Chemistry, 55*(22), 9036–9042

Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Antioxidant properties and shelf-life extension of fresh-cut tomatoes stored at different temperatures. *Journal of the Science of Food and Agriculture, 88*(15), 2606-–2614

Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Effect of minimal processing on bioactive compounds and color attributes of fresh-cut tomatoes. *LWT - Food Science and Technology, 41*(2), 217–226

Odriozola-Serrano, I., Oms-Oliu, G., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Effect of high-oxygen atmospheres on the antioxidant potential of fresh-cut tomatoes. *Journal of Agricultural and Food Chemistry, 57*(15), 6603–6610

Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2010). Changes in bioactive composition of fresh-cut strawberries stored under superatmospheric oxygen, low-oxygen or passive atmospheres. *Journal of Food Composition and Analysis, 23*(1), 37–43

Oms-Oliu, G., Aguiló-Aguayo, I., Martín-Belloso, O. & Soliva-Fortuny, R. (2010). Effects of pulsed light treatments on quality and antioxidant properties of fresh-cut mushrooms (Agaricus bisporus). *Postharvest Biol. Technol*., 56 (3) (2010), pp. 216–222

Pataro, G., Sinik, M., Capitoli, M. M., Donsì, G. & Ferrari, G. (2015). The influence of post-harvest UV-C and pulsed light treatments on quality and antioxidant properties of tomato fruits during storage. *Innovative Food Science and Emerging Technologies, 30*, 103–111

Ramos-Villarroel, A. Y., Martín-Belloso, O., & Soliva-Fortuny, R. (2011). Using antibrowning agents to enhance quality and safety of fresh-cut avocado treated with intense light pulses. *Journal of Food Science, 76*(9), S528–S534

Ramos-Villarroel, A. Y., Martín-Belloso, O. & Soliva-fortuny, R. (2013) Pulsos de luz intensa: Inactivación microbiana en frutas y hortalizas. CyTA-Journal of Food, 11, 234–242

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation

decolorization assay. *Free Radical Biology and Medicine, 26*(9-10), 1231–1237

Rodov, V., Vinokur, Y., & Horev, B. (2012). Brief postharvest exposure to pulsed light stimulates coloration and anthocyanin accumulation in fig fruit (ficus carica L.). *Postharvest Biology and Technology, 68*, 43–46

Ryalls, J., Neuenschwander, U., Willits, M., Molina, A., Steiner, H. Y., & Hunt, M. (1996). Systemic acquired resistance. *The Plant Cell*, *8*, 1809–1819

Sánchez-Moreno, C., De Pascual-Teresa, S., De Ancos, B. & Cano, M. P. (2012). Nutritional Quality of Fruits, in Handbook of Fruits and Fruit Processing, Second Edition (eds N. K. Sinha, J. S. Sidhu, J. Barta, J. S. B. Wu and M. P. Cano), Wiley-Blackwell, Oxford, UK

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

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

Solovchenko, A. E., & Merzlyak, M. N. (2008). Screening of visible and UV radiation as a photoprotective mechanism in plants. *Russian Journal of Plant Physiology, 55*(6), 719–737

Surjadinata, B. B., Jacobo-Velázquez, D. A. & Cisneros-Cevallos, L. (2017). UVA, UVB and UVC light enhances the biosynthesis of phenolic antioxidants in fresh-cut carrot through a synergistic effect with wounding. *Molecules 22*(4), 268

Tran, D. T., Hertog, M. L. A. T. M., Tran, T. L. H., Quyen, N. T., Van De Poel, B., Mata, C. I., & Nicolaï, B. M. (2017). Population modeling approach to optimize crop harvest strategy: the case of field tomato. *Frontiers in Plant Science, 8* doi:10.3389/fpls.2017.00608

Tran, M. T. T., & Farid, M. (2004). Ultraviolet treatment of orange juice. *Innovative Food Science and Emerging Technologies, 5*, 849–855

Valdivia-Nájar, C. G., Martín-Belloso, O., Giner-Seguí, J., & Soliva-Fortuny, R. (2017). Modeling the inactivation of *Listeria innocua* and *Escherichia coli*

in fresh-cut tomato treated with pulsed light. *Food and Bioprocess Technology, 10*(2), 266–274

Vallverdú-Queralt, A., Medina-Remón, A., Andres-Lacueva, C., & Lamuela-Raventos, R. M. (2011). Changes in phenolic profile and antioxidant activity during production of diced tomatoes. *Food Chemistry, 126*(4), 1700–1707

Yan-Hwa, C., Chang, C.-L., & Hsu, H. F. (2000). Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture, 80*(5), 561–566

CAPITULO 4

Modeling the Shelf life and Sensorial Acceptability of Fresh-Cut Tomatoes Subjected to Pulsed Light Treatments

Abstract

Fresh-cut tomatoes deteriorate quickly due to damage caused by minimal processing, triggering negative symptoms that affect the quality and thus shortening their shelf life. In the present work, the effectiveness of pulsed light (PL) treatments to extend the shelf life and sensorial acceptability of fresh-cut tomatoes (*Lycopersicon esculentum* Mill., cv. Daniela) was investigated. Tomato slices were exposed to PL-fluences of Φ= 4, 6 or 8 J cm^{-2} and kept under shilled storage (4 ºC) for 15 days. Microbial growth, physicochemical parameters and sensorial attributes were monitored over storage period. Quantitative structure-property relationship model (QSPR) was used to relate the sensorial attributes with the consumer's acceptability. A second QSPR model was use to develops a unique model to explore the relation among the acceptability and quality parameters. Model was validated by a leave-one-out cross validation (LOO-CV).

PL was capable to inflict damage on the microbial replication and thus extending the shelf-life of tomato slices, which was described by a Gompertzian function (R^2_{adj}= 0.92). Fresh-cut tomatoes subjected to PL exhibited higher SL than those untreated, achieving the maximum values when were exposed to the highest PL-fluences. Thus, PL enlarged up to 4 days the shelf-life of tomato slices. Physicochemical parameters were affected as consequence of PL processing. However, some of those changes influenced positivitely the consumer´s preference of tomato slices. Fresh-cut tomatoes subjected to a fluence of 8 J cm^{-2} exhibited the highest acceptability values after 10 days of storage. QSPR model described well (R^2_{adj}= 99.48) the relationship among the sensorial attributes and pointing out the relevance of color and flavor on the acceptability. A second QSPR model represented well (R^2_{adj}= 99.68) the correlations between acceptability and the physicochemical parameters. QSPR model can be a useful intermediary on the recognizing of variables with alleged influence on the sensorial perceptions. Validation of the model (Q^2_{LOO}≥ 0.93) indicated the great adequacy of the model to express the sensorial acceptability through the analytical variables. In conclusion, PL extended the shelf-life and improved the sensorial attributes, which resulted in the highest acceptability of tomato slices.

Keywords: Fresh-cut tomato; pulsed light; physicochemical characteristics; consumers acceptability

1. Introduction

Tomato fruit (*Lycopersicum esculentum*) is one of the most consumed vegetables worldwide either fresh or processed. Freshness and sensorial characteristics as well as their high nutritional content make tomatoes greatly appreciated by consumers. Quality of fresh-cut tomatoes is associated to visual appearance (Lund and Snowdon, 2000) as color (Amiot et al., 1997) as well as texture (Karakurt and Huber, 2003; Agar et al., 1999), flavor (Baldwin, 2004; Meilgaard et al., 1991) and safety aspects (Martín-Belloso et al., 2006; Francis et al., 2012).

In the last years, fresh-cut fruits and vegetables demand has experienced a rapid growing due to their convenience and quality characteristics. However, fresh-cut commodities exhibit shorter shelf life than whole fruits. Minimal processing usually induces damages on vegetable tissues, triggering deleterious phenomena such as nutritional losses, physicochemical changes presence of off-flavor and microbial spoilage (Ma et al., 2017; Soliva-Fortuny et al., 2003). Shelf life of minimally processed products is conditioned by safety, physicochemical and biochemical characteristics, but is majority limited by their sensorial quality (Putnik et al., 2017). For this reason, food researchers have been exploring conservative methods capable to reduce the microbial charges and retain the overall quality of fresh-cut products.

Pulsed light (PL) is a non-thermal technology that have demonstrated good results on the microbial inactivation, retaining bioactive compounds and even maintaining the physicochemical quality of fruits and vegetables (Ramos-Villarroel et al., 2014; Agüero et al., 2016; Aguiló-Aguayo et al., 2015; Salinas-Roca et al., 2016). In fact, effectiveness of PL to reduce the microbial growth keeping some physicochemical characteristics of fresh-cut tomatoes has been previously reported (Valdivia-Nájar et al., 2017a and 2017b). However, information associated to the effects of PL on fresh-cut tomatoes quality is still limited.

The study of the effects of PL in the shelf life and sensory quality of fresh-cut tomatoes is necessary to the completely understanding of this technology. However, an extensive compilation of instrumental data and statistical analysis are required to develops mathematical models capable to

relate those trends (Putnik et al., 2017). Some mathematical functions as those based on Quantitative Structure-Property Relationships (QSPR) are applied to determine the independent variables most influential on a dependent variable (Cao, et al., 2014; Pomilio et al., 2010). Therefore, relationships among sensorial attributes and physicochemical characteristics can be accomplished.
Hence, the main objective of the present study was to model the shelf life of fresh-cut tomatoes exposed to PL. The specific research objectives were: (i) evaluate the impact of PL treatments on the microbial, physicochemical and sensorial quality of tomato slices through chilled storage; (ii) determine the shelf-life of tomato slices as afected by PL and storage time; and (iii) descibe the influential attributes related to the sensorial acceptability.

2. Materials and Methods

2.1. Plant materials and processing

Tomatoes (*Lycopersicon esculentum* Mill. cv. Daniela), were purchased in a local supermarket (Lleida, Spain) at uniform size, commercial maturity and freedom from defects and blemishes. Tomatoes remained under refrigerated conditions (at 5 $\pm$ 1 ºC) prior processing (12 h). Fresh whole tomatoes were sanitized for 2 min in chlorinated water (200 μL L^{-1}) at 5 $\pm$ 1 ºC, rinsed with tap water and gently dried by hand. Fruits were then cut into 5 mm-thick slices using an electric slicer (Food Slicer-6128: Toastmaster Corp, Elgin, USA). Tomato slices (ca. 100 g) were packaged in polypropylene trays (350 cm^3, 5025 RM PTT-ATS Packaging S.r.l. VE, Italia) and thermo sealed using an ILPRA Food Pack Basic V/6 packaging machine (ILPRA Systems, CP, Vigevono, Italia). The transparency of the film was determined by calculating the amount of energy detected by a photodiode placed into the chamber of PL equipment (Avalos-Llanos et al., 2016). Hence, transparency of the film was 97 % of the UV radiation and almost a 100 % of the visible wavelengths (Aron Maftei, et al., 2013). The O_2 and CO_2 permeances of the sealing film were 5.2419 x 10^{-13} mol O_2 m^{-2} s^{-1} Pa^{-1} and 2.3825 x 10^{-12} mol CO_2 m^{-2} s^{-1} Pa^{-1} at 23ºC and 0 % RH, respectively

(ILPRA Systems España, S.L. Mataró, Spain). The packages were stored at 5 °C in darkness prior to PL application.

2.2. Pulsed light treatments and storage conditions

Pulsed light (PL) treatments were carried out with a flash lamp system (Steribeam Xe-Matic-2L-A, Kehl, Germany) equipped with two Xenon flash lamps situated at 8.5 cm above and below the sample holder. The lamps emitted spectrum ranged from 180 to 1100 nm with a maximum emitted energy of 700 J, those values were calculated through calibration with a standard light source following the manufacture's directions. The duration of each pulse was 0.3 ms with fluence of 0.4 J/cm^2. The Food and Drug Administration (FDA) established a maximu.m fluence of 12 J cm^{-2} when food commodiites are exposed to PL. So, tomato slices were subjected to 4, 6 and 8 J cm^{-2}, respectively according to prior microbial and physicochemical studies (Valdivia-Nájar et al. 2017a, 2017b) . A set of untreated trays was kept as reference. All trays were stored at 5ºC for 15 days in darkness until random withdrawal for analysis.

2.3. Evaluation of tomato slices quality

2.3.1. Microbiological stability

Microbial growth was monitored throughout chilling storage. Psychrophilic bacteria and molds and yeast counts were carried out during 15 days of storage. Tomato slices (ca. 10 g) were homogenized for 2 min with 90 mL of 0.1% sterile peptone solution with a Stomacher Lab blender 400 (Seward Medical, London, UK). Then, 10-fold dilutions were carried out from the homogenates and1 mL of each serial dilution was plated onto plate-count agar (PCA) (Biokar Diagnostics, Beauvais, France). Plates were incubated for 10 days at 5 ºC ± 1 ºC for psychrophilic bacteria counts. For molds and yeast counts, 1 mL of those serial dilutions was spread on plates with chloramphenicol glucose Agar (CGA) (Biokar Diagnostics, Beauvais, France). The plates were incubated at 25 ºC ± 1 for 3-5 days. Analyses were carried out every 5 days in randomly sampled pairs of trays. Three replicate

counts were performed for each tray and results were calculated as log (CFU g^{-1}).

2.3.2. Headspace gases analysis

The oxygen (O) and carbon dioxide (CO_2) contents on the packages headspace was analyzed using a gas chromatograph equipped with a thermal conductivity detector (Micro-GP CP 2002 gas analyzer; Chrompack International, Middelburg, Netherlands). A sample of 1.7 mL was automatically withdrawn from the headspace atmosphere. Portions of 0.25 and 0.33 mL were injected for O_2 and CO_2 determination, respectively. A CP-Molsieve 5 Å packed column (4 m x 0.32 mm, d.f.= 10 mm) (Chrompack International, Middelburg, Netherlands) at 60 ºC and 100 kPa, was used to determine the O_2 concentration. For CO_2 quantification, a Pora-PLOT Q column (10 m x 0.32 mm, d.f.= 10 mm) (Chrompack International, Middelburg, Netherlands) held at 70 ºC and 200 kPa was used.

2.3.3. TSS, titratable acidity and pH

Tomato slices (ca. 20 g) were crushed using a blender and the juice was filtered through a 2-mm diameter steel sieve to remove peel and seeds. Total soluble solids (SSC) were determined using a 2WAJ-ABBE Refractometer (Atago Company Ltd., Tokyo, Japon) and expressed as º Brix. The pH was measured using a pH-meter (Crison Instruments S. A., Barcelona, Spain). Total acidity (TA) was assessed by titration with NaOH (0.1 N) to pH 8.1 and the results were expressed as grams of anhydrous citric acid per 100 g of fresh-cut tomatoes. Values of SSC and TA were used to determine the patterns of sugar-acid ratio for all samples during the storage period. All measurements were carried out according to AOAC procedures (AOAC, 1990).

2.3.4. Color

Color of fresh-cut tomatoes surfaces was assessed by tristimulus reflectance, using a colorimeter (Minolta Chroma Meter Model CR-400,

Minolta Sensing Inc., Osaka, Japan) appropriately calibrated with a Hunterlab standard white plate (*L** 93.4; *a**= -067; *b**= 0.78). The equipment was set up for a D65 illuminant and 10° observer angle. CIE L^* (lightness), a^* (red-green) and b^* (yellow-blue) parameters were measured through reflectance values. The obtained values were used to calculate the total color differences (ΔE^*), Chroma (*C*) and Hue angle (º *Hue*). At least 4 samples from 2 replicate packages were evaluated for each treatment and sampling time.

2.3.5. Lycopene and total phenolic compounds

Total lycopene was measured spectrophotometrically using a modification of the method proposed by Davis et al. (2003) as described by Odriozola-Serrano et al. (2008). Results were expressed as mg of lycopene per kg of fresh-cut tomato.

The content of total phenolic compounds in fresh-cut tomatoes was determined based on the Folin-Ciocalteu procedure (Singleton et al. 1999) with some modifications as described by Valdivia-Nájar et al. 2017. Results were expressed as milligrams of gallic acid per kg of fresh-cut tomatoes.

2.3.6. Texture and juice loss

Shearing strength (SS) of the tomato slices was determinated using a TA-XT2 texturometer (Stable Micro Systems Ltd., Surrey, England, UK) equipped with a 5 kg load cell. SS tests were carried out using a 7 cm length knife-edge with slotted insert (HDP/BPS) at 15 mm penetration depth and test speed of 5 mm/s. Then, SS was measured as the force needed to cause a significant break in the curve of hardness. Force values were expressed as N g^{-1}. Force-displacement-time data were recorded using Texture Exponent 32 software (Stable Micro Systems LTD. Surrey England). At least 4 replicate measurements from 2 replicate packages were evaluated at each sampling time.

Juice loss of fresh-cut tomatoes was also studied through 15 days of storage at 5 ºC. Packages with tomato slices were weighed just after

minimal processing (W_0) and PL-treatment (W_s). Containers with fresh-cut tomatoes were drained using a sterile micropipette and juices were weighted at each sampling time through storage (W_1). Juice loss was expressed as relative juice loss (%) (Equation 1).

$$JL(\%) = \left(\frac{W_0 - W_1}{W_s} \right) \times 100 \quad \textbf{(1)}$$

2.3.7. Sensory test

Sensory discriminant tests were performed immediately after the tomato slices processing and after 5 and 10 days of refrigerated storage. The evaluation was carried out in a sensory evaluation room with separate booths at room temperature (23 ± 2 ºC) (Department of Food Technology, University of Lleida, Spain). Pair-wise ranking tests were carried out to evaluate sensory quality in fresh-cut tomato. Samples were coded using random numbers (to avoid bias) and a set of six sample pairs were served on a white dish to a panel composed of forty-eight members (20-55 years of age). Judges were then instructed to select and record their preferences by 4 attributes (color, aroma, texture and flavor) as well as the general "acceptability". Obtained scores were processed and reported as rank sums.

2.4. Data analyses

Statistical analysis was performed using Statgraphics plus v. 5.1 Windows package (Statistical graphics Co., Rockville, MD). Analysis of variance (ANOVA) was carried out to compare sample mean values. Data were analyzed using multifactor analysis of variance and Duncan multiple range tests was applied to determine differences among means with a significance level of 0.05, while results of the sensory analysis were evaluated by a Friedman-type statistical analysis and the HSD value (honestly significant difference) was determined to compare two rank sums (p= 0.05).

In order to locate the parameters into the same coordinate systems (PC's components) through their variance values a PCA analysis was used and then determine the parameters related to compressive quality of fresh-cut tomatoes.

2.4.1. Microbial growth and shelf-life

Log-linear function (Equation 2) was used to fit microbial growth as a function of the PL-fluences (Valdivia-Nájar et al., 2016), while shelf-life of fresh-cut tomatoes was calculated through Equation 3 (Corbo et al., 2006).

$$A = \log N_o - \delta \cdot \Phi \quad \textbf{(2)}$$

were A is the decimal logarithm of the microorganism loads [log (CFU g^{-1})]; N_0 is the initial load of microorganisms after exposure to PL-fluences (Φ) (J cm^{-2}) and δ is the inactivation constant (cm^2 J^{-1}).

$$\log(CFU) = \log(10^6) - A\exp\left\{-\exp\left\{(\upsilon_{\max} \cdot e)\cdot\left[\frac{\lambda - SL}{A}\right]+1\right\}\right\} + A\exp\left\{-\exp\left\{(\upsilon_{\max} \cdot e)\cdot\left[\frac{\lambda - t}{A}\right]+1\right\}\right\} \quad \textbf{(3)}$$

were log (5 x 10^7), is the limit of acceptability for psychrophilic and molds and yeast microorganisms populations [log (CFU g^{-1})], respectively (IFPA, 2003); A, is the initial value estimated by the model [log (CFU g^{-1})] (Equation 1); t, is the storage time; μ_{max}, is the maximum changing rates [log (CFU g^{-1}) day^{-1}]; λ or lag time is the time before the beginning of changes (day); SL is the shelf life estimated by the model and e is the Euler's number.

2.4.2. Sensory model

Sensory rank sums obtained from sensory evaluations were converted into proportions and lastly into normal deviates which created an interval scale values for the stimulus. Therefore, a scale was build through the Thurstone's model of comparative judgments, which is capable to

provide a continuous dimensionless variable scale of preferences based on the real perception to the stimulus (Equation 4).

$$R_i - R_j = z_{ij}\sqrt{\sigma_i^2 + \sigma_j^2 - 2r_{ij}\sigma_i\sigma_j} \quad \textbf{(4)}$$

were R_i and R_j represent the scale values of stimuli *I* and *j*, σ_i and σ_j, are the standard deviations of the respective discriminant dispersions, r_{ij}, is the correlation between the two discriminant processes, and z_{ij}, is the normal desviation (*z-score*) corresponding to the proportion of times (*n*) the stimulus *j* is judge greater than stimulus *i*

Moreover, multi-linear (MLR) and regression and stepwise regressions (STR) was proposed to select the most useful sensorial descriptors related to the acceptability of fresh-cut tomatoes as a function of the PL-fluence applied and the storage period (Equation 5):

$$A_p = k + \beta_0 + \beta_{1x1} + \beta_{2x2} + \ldots + \beta_{pxp} + \varepsilon \quad \textbf{(5)}$$

where A_p, is the estimated value and represents the independent variable "acceptability", k, is the constant rate as a function of the fluence applied (Φ); $x_1x_2x_p$ were measures of without correlated variables that may help to the estimation of *y*. The coefficient β_0 was the estimated constant, β_1, β_2, …, β_p are the regression coefficients and ε is the random error. MLR was performed to discriminate the most influential variables over the acceptability and describes the influent properties using a step-wise procedure.

2.4.3. Acceptability model

Quantitative structure-property relationship model (QSPR) based on correlation analysis and multilineal function was used to describe the most appropriate physicochemical descriptors on the sensorial acceptability. Correlation analysis and MLR-STR were employed for variable selection and model development, respectively. Principal component analysis (PCA) is

usually carried out to reduce the variables and thus explaining the total variability of original magnitudes (Vainionpaa et al., 2004). Hence, PCA was employed to determine the most appropriate physicochemical descriptors on the sensorial "acceptability", while MLR-STR function described the relationship among "acceptability" and multi-independent variables (Equation 5).

Moreover, leave-one-out cross validation (LOO-CV) method was used to describe correlations between sensory acceptability and instrumental measurements of physic-chemical characteristics. Then, predicted and observed values were compared by error sum squares method. Finally, internal validation of procedure was developed by the statistical parameter Q^2_{L00} (Equation 6).

$$Q^2_{LOO} = 1 - \frac{\sum_{i-1}(y_i - \hat{y}_i)^2}{\sum_{i-1}(y_i - \ddot{y}_i)^2} \quad \textbf{(6)}$$

where y_i is the observed acceptability value; $\hat{y}_i$ is the predicted value calculated by leave-one-out cross validation and $\ddot{y}_i$ is the averaged value of the entire data set.

3. Results and discussion

3.1. Microbial growth and shelf life estimation

Initial psychrophilic bacteria (PB) count on fresh-cut tomatoes was 2.96 log (CFU g^{-1}). PB loads were significantly decreased ($p<0.05$) just after exposure to PL, being the higher PL-fluence applied (Φ), the higher the PB counts reduction. PB counts reduction obtained after PL processing as a function of Φ was accurately fitted by primary log-linear model (Equation 2) (R^2 =0.99). Estimated of the inactivation constant was δ=-0.22 cm^{-2} J^{-1}, indicating a reduction of the PB counts as a function of the increasing PL-fluence (Φ). Then, the PB counts estimated by the model were 2.57, 2.38 and 2.18 log (CFU g^{-1})

for tomato slices subjected to fluences of 4, 6 and 8 J cm^{-2}, respectively. A proliferation of PB throughout the storage period was observed on all untreated and PL-treated tomato slices. However, PB counts on PL-treated tomato slices remained lower than those on untreated. A Gompertzian model fitted well the experimental data with high R^2_{adj} (≥ 0.98) (Equation 3).

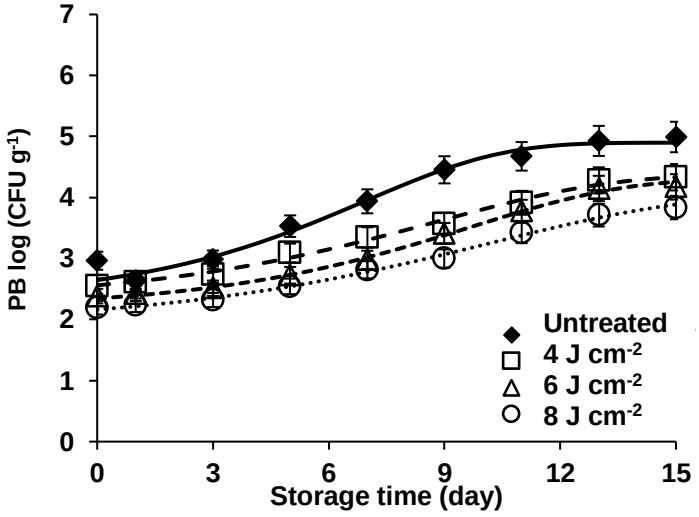

Figure 4.1. Fits of gompertzian model to the psychrophilic bacterias (PB) on fresh-cut tomatoes exposed to pulsed light and stored for 15 days at 4 ºC. Grids represent the experimental counts and lines the predicted model. The values are mean of 6 determinations ± standard deviation.

Estimates of the model that describe the patterns of PB along the storage period are listed in Table 1. Maximal growth rate values were lower (μ_{max}≤ 0.53) with respect those untreated (μ_{max}= 0.73); these results pointed out that reduction of the PB counts was triggered after PL. Moreover, the lag phase period (λ) became slightly lengthened after PL and thus suggesting the high impact of those treatments on the replication mechanism of PB's. Fresh-cut tomatoes subjected to PL exhibited significant lower (p< 0.05) λ values (λ≤ 2.96) than those on untreated (λ= 3.52). Therefore, tomato slices subjected to PL exhibited lower PB counts for a longer time than those

untreated ones and thus extending the shelf life (*SL*= 13.3-15.3 days) with respect to untreated tomato slices (*SL*= 10.5 days).

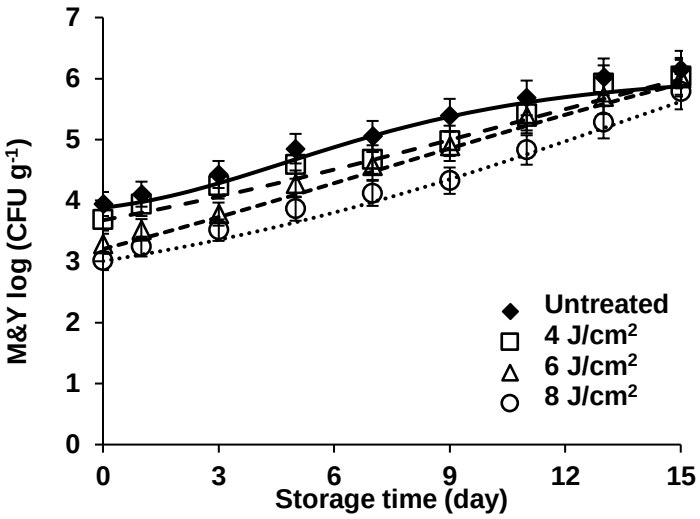

Figure 4.2. Fits of gompertzian model to the molds and yeast (M&Y) on fresh-cut tomatoes exposed to pulsed light and stored for 15 days at 4 ºC. Grids represent the experimental counts and lines the predicted model. The values are mean of 6 determinations ± standard deviation.

Initial molds and yeast (M&Y) count on fresh-cut tomatoes was 3.9 log (CFU g^{-1}). PL influenced the reduction ($p< 0.05$) of the M&Y loads on fresh-cut tomatoes. Then, tomato slices subjected to fluences of 4, 6 and 8 J/cm^2 exhibited decreases of about 0.3, 0.6 and 0.9 log (CFU g^{-1}), respectively. The fit of the model (Equation 2) to the reduction of M&Y counts as a function of the fluence applied (Φ) provided a good adjustment (R^2= 0.92) between experimental data and values predicted by the model. The inactivation constant was δ= -0.25 cm^{-2} J^{-1}, thus suggesting a lower resistance to PL in comparison with PB. M&Y counts increased through storage regardless the PL treatment applied. However, tomato slices subjected to PL exhibited decreased M&Y counts with respect those untreated for a longer time over the storage period. The behavior of M&Y in fresh-cut tomatoes was satisfactorily fitted by Gompertzian model (Equation

2) ($R^2_{adj} \geq 0.98$). Kinetic parameters of the model, determination coefficients and validation values are given in table 1.

Table 4.1. Kinetic constants estimated by the modified Gompertz model in order to predict the microbial (psychrophilic bacteria and molds and yeast) shelf life on untreated and PL-treated fresh-cut tomatoes through the chilling storage (5 ºC).

Treatment	*A*	*μmax*	*λ*	*SL*	R^2_{adj}	A_f	B_f	*RMSE*
Psychrophilic bacteria								
Untreated	2.65 ± 0.14	0.73 ± 0.03	3.52 ± 3.43	10.48 ± 0.22	99.15	0.99	1.00	0.17
4 J/cm^2	2.56 ± 0.10	0.46 ± 0.02	2.96 ± 0.89	13.29 ± 0.29	98.99	1.00	1.00	0.17
6 J/cm^2	2.34 ± 0.32	0.53 ± 0.02	1.32 ± 0.81	13.68 ± 0.18	99.43	0.99	1.00	0.16
8 J/cm^2	2.16 ± 0.48	0.42 ± 0.02	0.33 ± 0.30	15.32 ± 0.27	99.27	1.00	1.00	0.17
Molds and yeast								
Untreated	3.88 ± 0.13	0.48 ± 0.06	1.19 ± 0.11	18.81 ± 2.37	99.61	0.98	1.00	0.16
4 J/cm^2	3.68 ± 0.89	0.45 ± 0.04	1.82 ± 0.08	21.92 ± 0.64	98.04	0.99	1.00	0.16
6 J/cm^2	3.20 ± 0.22	0.51 ± 0.01	1.89 ± 0.05	21.99 ± 0.29	99.69	0.99	1.00	0.16
8 J/cm^2	3.00 ± 0.61	0.59 ± 0.05	0.85 ± 0.06	21.85 ± 0.56	99.07	0.98	1.00	0.16

A is the initial value estimated by the model [log(CFU g^{-1})]; $\boldsymbol{\mu_{max}}$ is the maximum changing rates; ***λ*** or lag time(days); ***SL*** :estimated shelf life (days). Validation parameters: $\boldsymbol{R^2_{adj}}$ is the adjusted determination coefficient; $\boldsymbol{A_f}$, the accuracy factor; $\boldsymbol{B_f}$ is the bias factor and ***RMSE*** is the root mean square error.

PL affected substantially the values of maximal growth rate (μ_{max}) and lag time (λ). The μ_{max} on samples subjected to PL was slightly extended ($\geq$ 0.48 log (CFU g^{-1}) day^{-1}) with respect to untreated samples (0.45 log (CFU g^{-1}) day^{-1}), while the lag time value on untreated tomato slices was $\lambda \geq$ 1.19 day^{-1} and increased as the PL-fluence increased ($\lambda \geq$ 1.82 day^{-1}). Consequently, tomato slices subjected to PL exhibited lower M&Y loads over the storage period and prolonging the shelf-life ($SL \geq$ 20.99 day^{-1}) with respect to those untreated (18.78 day^{-1}).

3.2. Physicochemical characteristics

Minimal processing provokes physiological modifications inducing to metabolic activation and thus, altering the quality of fresh-cut tomatoes. Figure 4.3 shows the evolution of the oxygen (O_2) and carbon dioxide (CO_2)

contents inside the packages of fresh-cut tomatoes. Pulsed light treatments induced to a rapid consumption of O_2, causing an accelerated CO_2 production inside the packages. Initial O_2 content inside the packages (17.9kPa) decreased up to 16.4-14.8 kPa, while the initial CO_2 content (0.02 kPa) was quickly enlarged after PL treatments (0.20 - 0.29 kPa). Besides that, the higher PL fluence applied the higher O_2 and CO_2 changes along the storage period. At the end of storage, O_2 contents on samples exposed to PL (3.77-1.49 kPa) were significantly lower ($p \geq 0.05$) than it on untreated samples (5.33 kPa). Conversely to O_2, the CO_2 contents on untreated samples exhibited moderated increases (3.93 kPa) compared to those on samples exposed to PL (6.43-11.16 kPa). Changes on the headspace of trays with tomato slices subjected to PL treatments can be well related to increased respiration triggered by stress and physiological modifications caused by slicing processing and PL treatments. Some studies have reported similar patterns in O_2 and CO_2 contents inside the headspace of packages with fresh-cut tomatoes, watermelons and avocados subjected to PL treatments (Hong and Gross, 1998; Ramos-Villarroel et al. 2011 and 2012).

The initial pH on tomato slices was 4.59 and changes were almost nulls after PL. However, slight increases of the pH values were observed through storage regardless the PL-treatment (Figure 4.4-A). At the end of storage, samples subjected to PL exhibited pH values of 4.67-4.69. In fact, microbial growth as well as physiological activity of fresh-cut tomato plays an important role in the degradation of organic acids, in turn affecting the pH (Odriozola-Serrano et al., 2008; Gil et al., 2006). Moreover, increases of soluble solids content (SSC) in fresh-cut tomatoes (4.00 °Bx) were observed after PL-treatments (4.25-4.50 °Bx). A constant increment in the SSC of fresh-cut tomatoes was observed throughout the storage period regardless the PL-treatment applied (Figure 4.4-B), thus reaching pH values of 4.33-4.67 °Bx at the end of storage period.

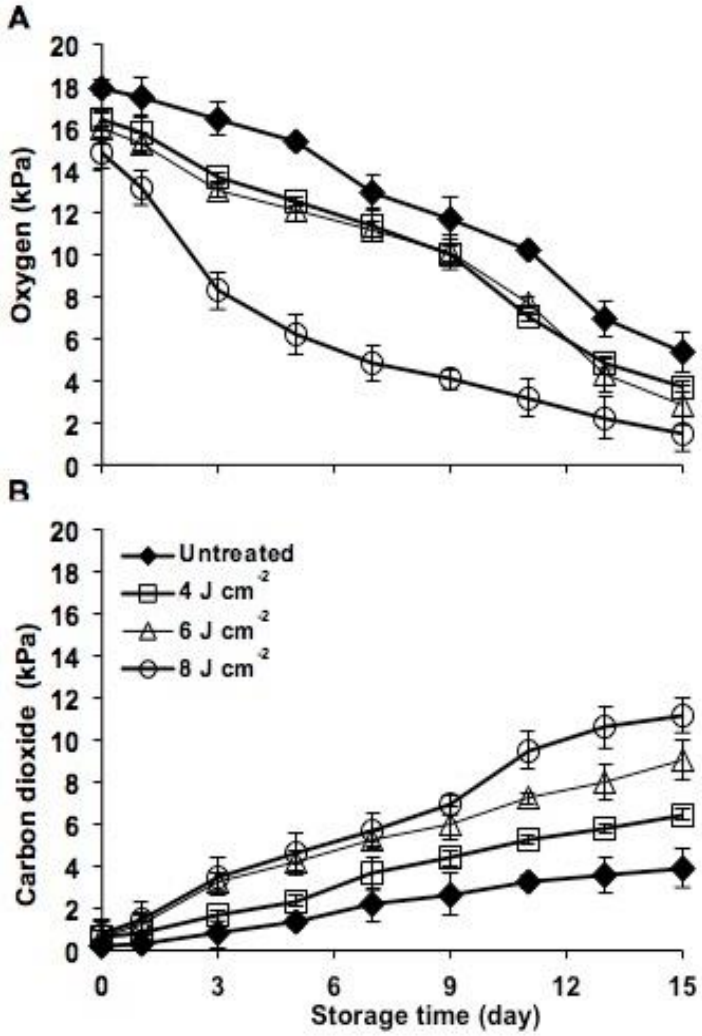

Figure 4.3. Changes on the oxygen (A) and carbon dioxide (B) partial pressures inside the packages with untreated fresh-cut tomatoes and those subjected to pulsed light and stored along 15 days at 5 ºC.

On the other hand, PL influenced an increase in the tritratable acidity (TA) of tomato slices (0.33-0.39 g ac. citric 100 g^{-1}) with respect untreated samples (0.31 g ac. citric 100 g^{-1}). However, TA values decreased over the storage regardless the treatment applied. Tomato slices subjected to PL exhibited lower TA values (0.27-0.28 g ac. citric 100 g^{-1}) than those on untreated samples (0.36 g ac. citric 100 g^{-1}) at the end of storage (Figure 4.4-C). Hence, TSS-TA ratio of fresh-cut tomatoes was 13.02 and minimally

decreased after PL. TSS-TA values increased over storage regardless the PL-treatment applied (Figure 4.4-D). As a matter of fact, tomato slices subjected to PL exhibited higher TSS-TA values (16.73-20.83) with respect to untreated samples (16.91). In this context, tomatoes quality can be well related to pH, SSC and TA values. pH which is primarily determined by the acid content of the fruit (acidity) as well as the SSC related to the amount of soluble solids (predominantly sugars) are the highest contributors to the flavor of tomatoes (Anthon et al., 2011). Then, the ratio TSS-TA play the most important role on the tomatoes flavor (Hobson and Grierson, 1993). Kader et al., (1978) reported that high quality of tomato is characterized by a ratio TSS:TA upper to 10.

On the other hand, the color of tomatoes is an important quality attribute that influences strongly the consumer's acceptability. Color of tomato slices was highly affected by PL treatments. Tomato slices subjected to fluences of 4, 6 and 8 J/cm^2 suffered changes of the red color (a*= 2.94, 3.26 and 4.88, respectively) with respect to untreated tomato slices (a*= 11.22). Moreover, an improvement on the redness (a*), Hue angle (º Hue) and chroma (C) values of fresh-cut tomatoes were observed throughout the storage period (Figure 4.4 E, F, G) being more noticeable in tomato slices exposed to higher PL-fluences. Increases in the red color can be principally related to modifications of the lycopene contents on fresh-cut tomatoes. Changes in the lycopene contents of fresh-cut tomatoes through the storage period are shown in Figure 4.4 H.

The initial lycopene content in tomato slices (45.2 mg kg^{-1}) was enhanced after PL treatments (51.9-59.4 mg kg^{-1} FW), been the higher PL-fluence applied, the higher lycopene content. Untreated tomato slices maintained a similar value through the storage (49.1 mg kg^{-1}); while samples subjected to pulsed light reached values above 69.1-80.1 mg kg^{-1} (Figure 4.4-H). Besides, the initial amount of phenolic compounds (196.58 mg kg^{-1}) in fresh-cut tomatoes suffered slight changes just after PL processing (203.35-207.61 mg kg^{-1}), being the higher the PL fluence applied, the higher the TPC content. Slight increases on the TPC contents were observed along

the fresh-cut tomato storage (Figure 4.4 I). Only tomato slices subjected to PL-fluences of 8 J/cm^2 exhibited increased TPC contents (210.79 mg kg^{-1}) with respect to untreated samples (200.76 mg kg^{-1}) at the end of storage.

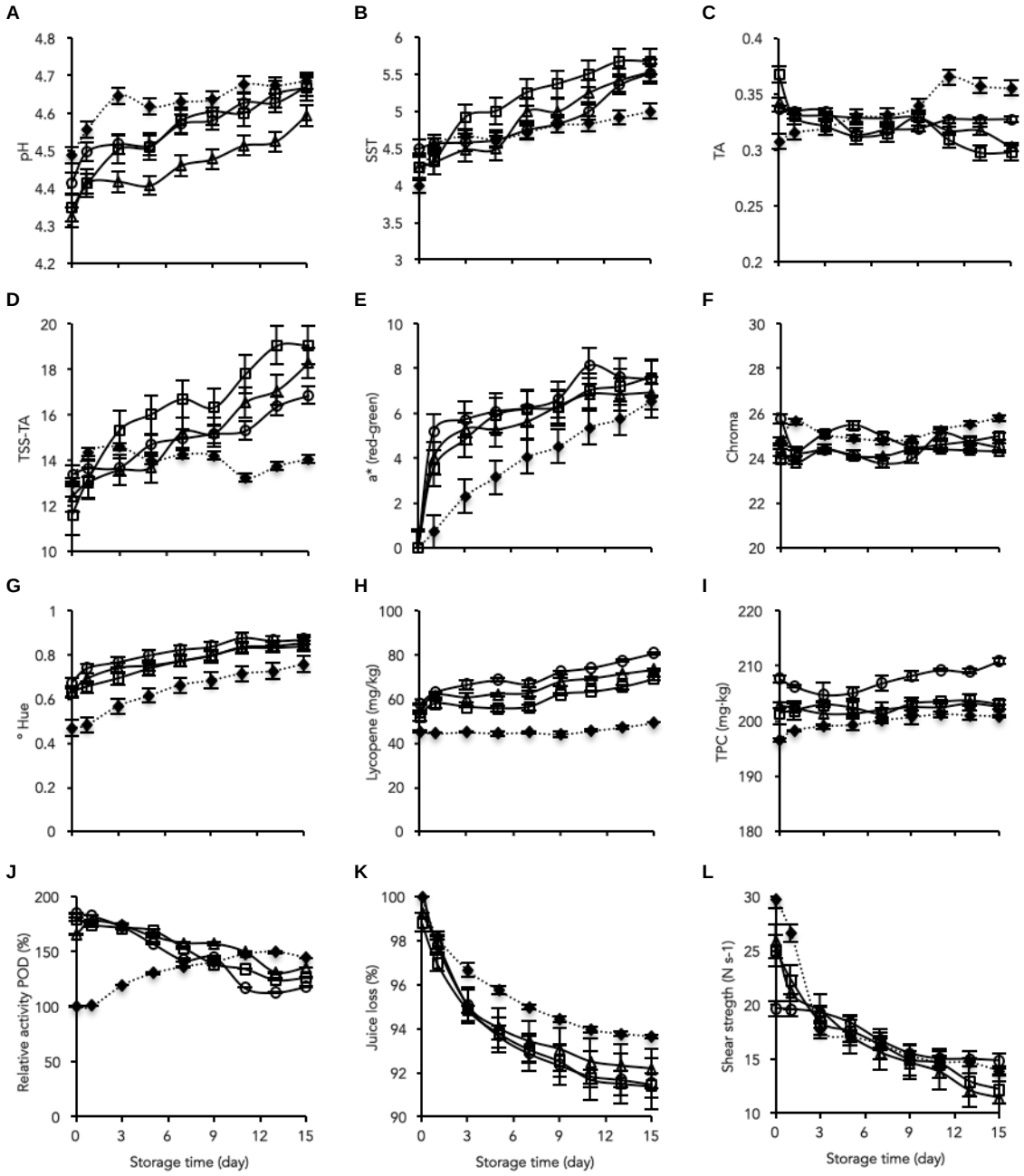

Figure 4.4. Changes on the physicochemical parameters of fresh-cut tomatoes untreated and PL-treated and stored over 15 days at 5 ºC. Untreated tomato slices are represente by ◆, while those subjected to PL-fluences of 4 Jcm^{-2}, 6 Jcm^{-2} and 8 J cm^{-2} are represented by ■, ▲ and •, respectively.

Slight juice losses of fresh-cut tomatoes were observed just after PL processing. Juice loss of fresh-cut tomatoes was significantly increased (p< 0.05) through storage period (Fig. 4.4-K). Untreated tomato slices exhibited juice losses of 6.3 %, while PL-treated samples were decreased up to 7.8-8.6 (p< 0.05) after 15 days of storage. Shear strength values of tomato slices were also affected by PL processing. Initial shear strength of fresh-cut tomatoes (29.8 N s^{-1}) decreased immediately after exposure to 4, 6 and 8 J/cm^2 (25.0, 25.9 and 19.6 N s^{-1}, respectively). Furthermore, a clear decrement pattern was observed along the tomato slices storage (Figure 4.4 L). However, differences among the shear strength of untreated and PL treated tomato slices were decreased at the end of storage. Increased respiration and membrane deterioration are the first consequences of minimal processing.

3.3. Sensory evaluation

Sensory rank sums for the general acceptability of fresh-cut tomatoes are shown in Figure 4.5. Significant differences among the attributes scores of untreated and PL-treated samples were not observed along 0, 5 and 10 days of storage. However, slight variations on the aroma scores were detected at day 5. Interestingly, judges were not capable to detect differences among treatments and neither along the storage period. Indeed, the acceptability values analyzed by Friedman´s T did not exhibit significant differences (p= 0.05) among untreated and PL-treated tomato slices.

On the other hand, rank sums were scaled through Thurstone's model (Thurstone's degrees; TD) (Figure 4.5). Based on the estimates of the model, untreated tomato slices were more accepted (0.83 TD) than those subjected to PL (0.81 TD) just after processing. However, those values were increased along the storage, revealing an increasing preference for PL-treated tomato slices over the 10 days of storage (Figure 4.6). In fact, the higher the PL-fluence applied, the higher the acceptability. After 10 days of storage, acceptability of tomato slices subjected to a fluence of 8 J cm^{-2} was evidently higher (4.63 TD) than for untreated tomato slices (3.78 TD).

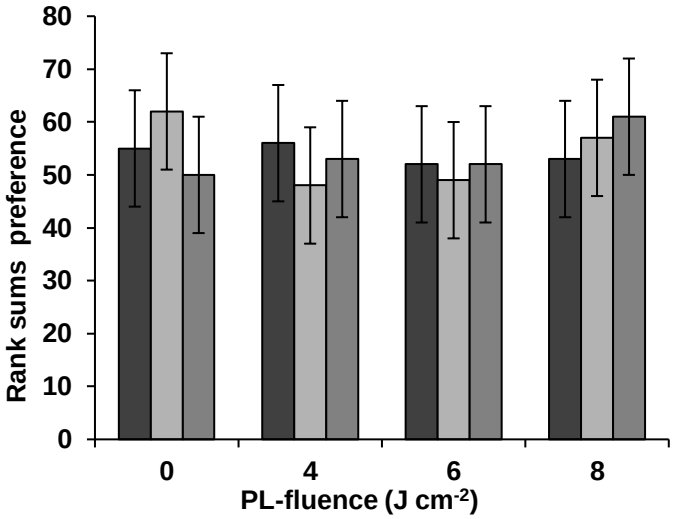

Figure 4.5. Rank sum from data obtained through the sensory evaluation of fresh-cut tomatoes untreated subjected to pulsed light (PL) treatments and stored throughout 10 days at 5 ºC.

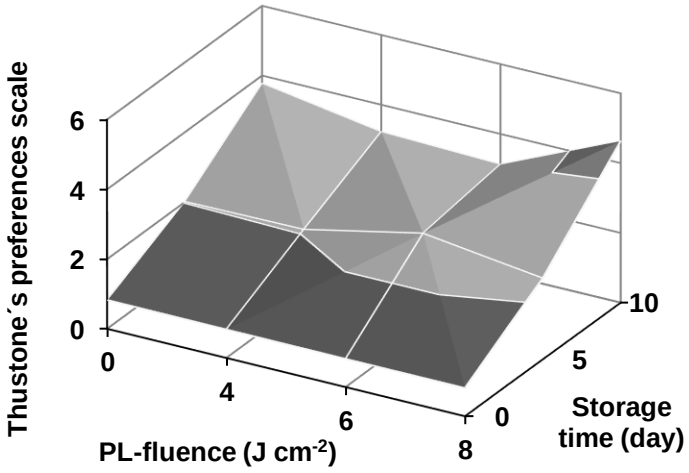

Figure 4.6. Scale of preferences obtained through the Thurstone's model. The scale represent the preferences obtained for fresh-cut tomatoes subjected to PL and stored along 10 days at 5 ºC.

3.4. Principal components analysis (PCA)

Principal components analysis (PCA) was performed to explore the correlation of the original variables. Two PCs explained the 73.56 % of the variability of original data with eigenvalue (EV) greater than 1 (Figure 4.7). First principal component (PC1) with EV= 7.43 accounted a 57.15 % of the original variables, while the second one (PC2) with EV= 2.13 explained a 16.40 %. PC1 was positively related to TA, SS and JL, but negatively associated to *ΔE**, *a**, º Hue, SSC, pH, SSC-TA and lycopene (Lyc). On the other hand, PC2 was positively mainly correlated to Chroma while a negative correlation with POD and Lyc was observed. Summarizing, the quality of fresh-cut tomatoes is highly related to most of attributes assayed during sensory testing (color, flavor, aroma and texture).

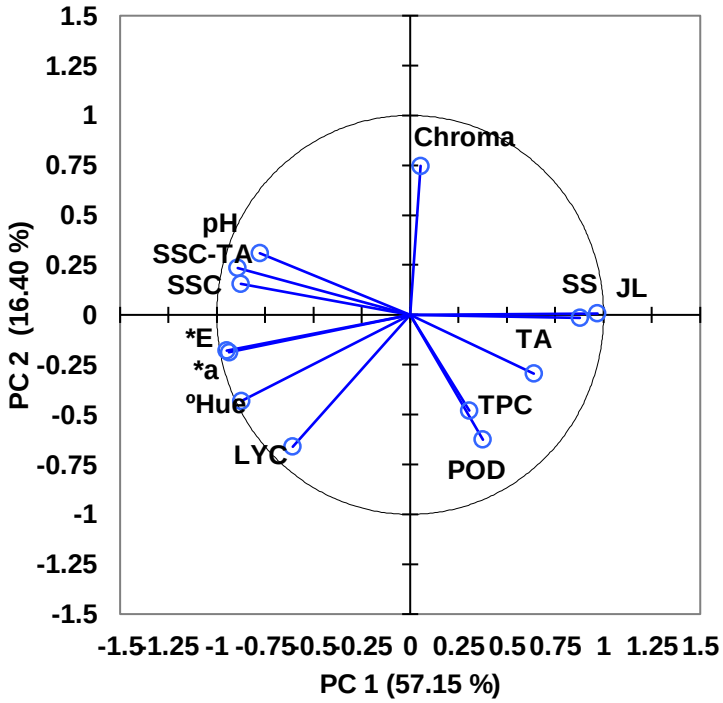

Figure 4.7. Plot of the principal components of untreated and PL-treated fresh-cut tomatoes and stored at 5 ºC for 15 days.

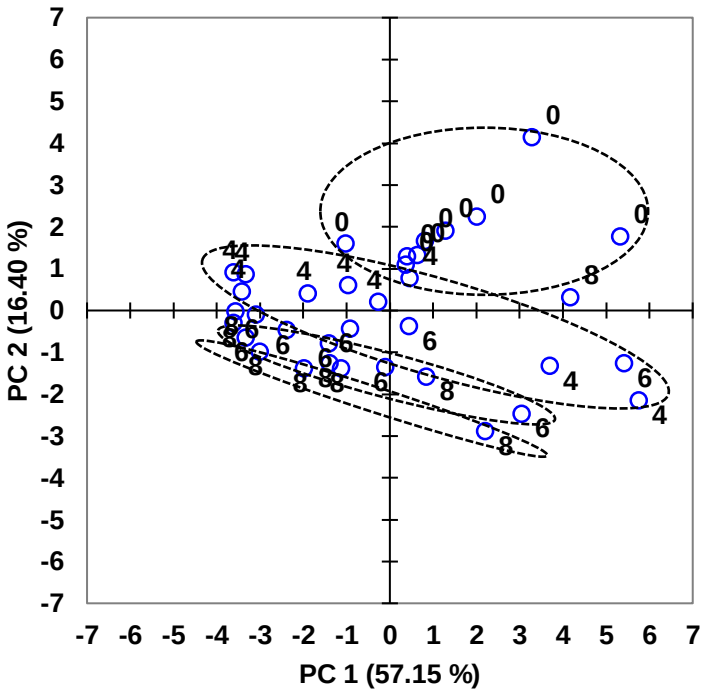

Figure 4.8. Score plot of PC1 vs. PC2 of the non-treated and PL-treated tomato slices stored for 15 days at 5 ºC.

Interestingly, score plot of PC1 against PC2 (Figure 4.8) seems to discriminate the results based on the PL-treatment applied. It can be observed that most of the untreated samples are located at the top and right-hand side of the plot, while tomato slices exposed to PL mostly appear on the bottom and left-hand side of the plot. Hence, changes on the physicochemical parameters of tomato slices seem to be directly influenced by the intensity of the PL-treatment as well as by the storage period.

3.5. Acceptability modeling

In this study, were proposed two quantitative structure-property relationship models (QSPR). These models are based on multi-linear (MLR) and stepwise regressions (STR). The first one model was used to explore the correlation between sensory preference and the four sensory attributes (color, flavor, aroma and texture) evaluated on fresh-cut tomatoes. QSPR

model as a function of sensory attributes predicted well the general acceptability for fresh-cut tomatoes subjected to PL treatments and stored throughout 15 days at 5 ºC (Equation 7). Curve fitting shows a good determination coefficient (R^2_{adj}= 0.995).

$$Acceptability = k_0 - k_1 \cdot color + k_2 \cdot aroma - k_3 \cdot texture + k_4 \cdot flavor \quad \textbf{(7)}$$

Then, “acceptability” of tomato slices exhibited an estimated constant rate (k_0) of 0.515 cm^{-2} J^{-1}, thus suggesting that PL influenced the changes of the 4 sensorial attributes evaluated along the storage period. Color, aroma and flavor attributes were statistically significant at 90% ($p<0.05$) with association constants of k_1= -0.41, k_2= 0.70 and k_4= 0.17, respectively. However, the texture association constant (k_3= 0.003) could be expendable of the model because their low statistical significance (<90%).

Therefore, the acidity reduction and the increased content of TSS lycopene and phenolic compounds contents were positively correlated to the increased acceptability of tomato slices. Although judges reported some undesirable alterations as dehydration, astringency and mealiness, the final acceptability seems not to be influenced by these phenomena.

Finally, a second QSPR model was used to explore the correlation between general preference and the physicochemical parameters. Principal variables accounted by PC’s were used to develop the model. Then, selection of determining parameters was performed by STR, which enlarged the R^2 between the observed number and experimental value by insertion of variables one by one. Then, stepwise regression revealed that color (*a,*E, ºHue) and texture (JL) parameters were crucial in determining the sensory preference. Those central parameters with high contribution to acceptability of fresh-cut tomatoes were used to construct the model by MLR. Sensory acceptability value was the response variable and predictor variables were those explanatory parameters selected by the STR. Then, mathematical model established by MLR-STR (R^2_{adj}= 0.99) is listed below (Equation 8):

$$Acceptability = -k_E \cdot \Delta E + k_{a^*} \cdot a^* - k_h \cdot {}^{o}hue + k_{JL} \cdot JL \quad \textbf{(8)}$$

Then, ΔE, a*, º hue and juice loss were statistically significant at 90% (p< 0.05) with association constants of -k_E= 0.391 x10^{-2}, k_{a^*}= 0.011 x 10^{-2}, k_h= 0.139 x 10^{-2} and k_{JL}= 0.072 x 10^{-2}, respectively. Therefore, it can be demonstrated that acceptability of tomato slices is mostly influenced by visual appearance and flavor characteristics. In this context, juice loss can be related to both visual impact and increases of SSC, which enhanced the flavor of tomato slices.

Moreover, regression models were validated by a leave-one-out cross validation (LOO-CV). In this process, one sample was kept out (leave-one-out) of the calibration and used for prediction. Predicted values were compared to observed values using the prediction error sum of squares. Therefore, the high Q^2_{LOO} coefficient (≥ 0.93) indicated the great adequacy of the model to describe the acceptability data obtained through analytical characteristics.

4. Conclusions

PL-treatments are capable to reduce microbial loads and thus enlarging the shelf-life of fresh-cut tomatoes. Some desirable changes in the physicochemical attributes are triggered after PL processing, which leads to major preference by consumers. PC's study reveals that analytical parameters related to contents of acidity, sweetness, physical changes and color influenced strongly the acceptability of tomato slices by consumers. Thereby, results disclose by QSPR model, which suggest a strong impact of color and flavor attributes on the acceptability of tomato slices seems to be reasonable. On the other hand, a great relationship among physicochemical parameters and the acceptability were achieved through the integration of all the studied variables done by a second one QSPR function. Hence, the model is capable to bind the physicochemical patterns with the acceptability

of tomato slices along 10 days of storage and finally confirming that color and JL were the more crucial factors on the acceptability of fresh-cut tomatoes. Accuracy and validation of the model indicate that it could be used to predict the consumer's preferences based on the physicochemical attributes. Incorporation of mathematical models to the quality control on industry could allow a fast access to the consumer's preferences and thus increasing the marketability of ready to use tomato slices.

Acknowledgements

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5. References

Aguiló-Aguayo, I., Charles, F., Renard, C. M. G. C., Page, D., & Carlin, F. (2013). Pulsed light effects on surface decontamination, physical qualities and nutritional composition of tomato fruit. *Postharvest Biology and Technology,* 86, 29-36.

Agüero, M. V., Jagus, R. J., Martín-Belloso, O., & Soliva-Fortuny, R. (2016). Surface decontamination of spinach by intense pulsed light treatments: Impact on quality attributes. *Postharvest Biology and Technology,* 121, 118-125.

Anthon, G. E., Lestrange, M., & Barrett, D. M. (2011). Changes in pH, acids, sugars and other quality parameters during extended vine holding of ripe processing tomatoes.*Journal of the Science of Food and Agriculture*, 91(7), 1175-1181.

Antunes, M. D. C., Rodrigues, D., Pantazis, V., Cavaco, A. M., Siomos, A. S., & Miguel, G. (2013). Nutritional quality changes of fresh-cut tomato during shelf life. *Food Science and Biotechnology,* 22(5), 1-8

Corbo, M. R., Del Nobile, M. A., & Sinigaglia, M. (2006). A novel approach for calculating shelf life of minimally processed vegetables. *International Journal of Food Microbiology,* 106(1), 69-73.

FDA (1982). Food and Drug Administration. http://www.fda.gov.ph/attachments /article/29016/FDA%20Regulation%20001%20s.%201982.pdf.

Garg, N., & Cheema, D. S. (2011). Assessment of fruit quality attributes of tomato hybrids involving ripening mutants under high temperature conditions. *Scientia Horticulturae,* 131(1), 29-38.

Hobson, G. E. & Grierson, D. (1993). Tomato. In: Seymour G, Taylor JE, Tucker G, eds. Biochemistry of Fruit Ripening, London. Chapman & Hall, 405, 42.

Ibitoye, D. O., Akin-Idowu, P. E. & Ademoyegu, O. T. 2009. Agronomic and lycopene evaluation in tomato (Lycopersiconlycopersicum Mill.) as a function of genotype. World Journal of Agricultural Sciences, 5 (S), 892-895.

Kader, A. A., Morris, L. L., Stevens, M. A., & Albright-Holton, M. (1978). Compostion and flavor quality of fresh market tomatoes as influenced by some postharvest handling procedures. *Journal of Am. Soc. Hort. Sci*., 103, 6-13.

Lenucci, M. S., Leucci, M. R., Piro, G., & Dalessandro, G. (2008). Variability in the content of soluble sugars and cell wall polysaccharides in red-ripe cherry and high-pigment tomato cultivars. *Journal of the Science of Food and Agriculture,* 88(10), 1837-1844.

Ma, L., Zhang, M., Bhandari, B., & Gao, Z. (2017). Recent developments in novel shelf life extension technologies of fresh-cut fruits and vegetables. *Trends in Food Science and Technology, 64*, 23-38.

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

Odriozola Serrano, Isabel. (2009). Tesis doctoral. Obtención de zumos y frutos cortados con alto potencial antioxidante mediante tratamientos no térmicos. Drirrigida por Olga Martín Belloso y Vicente Gimeno Añó. Universidad de Lleida. Lleida, España.

Rajkowski, K. T., & Baldwin, E. A. (2003). Concerns with minimal processing in apple, citrus, and vegetable products. J.S. Novak, G.M.

Salinas-Roca, B., Soliva-Fortuny, R., Welti-Chanes, J., & Martín-Belloso, O. (2016). Combined effect of pulsed light, edible coating and malic acid dipping to improve fresh-cut mango safety and quality. *Food Control,* 66, 190-197.

Valdivia-Nájar, C. G., Martín-Belloso, O., Giner-Seguí, J., & Soliva-Fortuny, R. (2017). Modeling the inactivation of listeria innocua and escherichia coli in fresh-cut tomato treated with pulsed light. *Food and Bioprocess Technology,* 10(2), 266-274.

DISCUSIÓN GENERAL

5. DISCUSIÓN GENERAL

Los tratamientos de pulsos de luz (PL) son una tecnología no-térmica, económica y respetuosa con el medio ambiente que generalmente es utilizada para desinfección de superficies. Por ello, ha surgido un gran interés en su aplicación en la conservación de alimentos. Sin embargo, la información relativa a los efectos de los PL sobre la calidad de los productos tratados es aún escasa. En este trabajo se evaluaron los efectos de los PL sobre la calidad del tomate fresco cortado. Se estudió el impacto sobre la microbiota y sobre las características fisicoquímicas más relevantes asociadas con la calidad y aceptabilidad del tomate cortado. Se utilizaron modelos matemáticos para describir, analizar y predecir las evoluciones de los diferentes parámetros relacionados en función de las condiciones de tratamiento y el tiempo de almacenamiento. Este trabajo se completó analizando la influencia de los PL sobre la aceptabilidad sensorial de los tomates frescos cortados por los consumidores.

5.1. *Efectos de los PL sobre la estabilidad microbiológica del tomate fresco cortado.*

La proliferación de microorganismos es el mayor limitante de la calidad del tomate fresco cortado. Aunque algunos estudios han evaluado el efecto de los PL en los microorganismos presentes en algunas frutas y verduras frescas cortadas, no existen datos acerca del efecto de esta tecnología sobre tomate mínimamente procesado (Ramos-Villarroel et al., 2011a; Ramos-Villarroel et al., 2011b). Por este motivo, el estudio de la microbiota nativa y de los microorganismos potencialmente patógenos presentes en el tomate fresco cortado es de suma importancia.

5.1.1. Microbiota nativa: bacterias psicrófilas (BP) y mohos y levaduras (MyL)

Se observaron ligeros descensos ($p< 0.05$) de los recuentos iniciales de bacterias psicrófilas (BP) (2.9 log (UFC g^{-1})) y mohos y levaduras (MyL)

(3.9 log (UFC g^{-1})) justo después de los tratamientos de PL. Cuánto más alta fue la Φ de tratamiento (8 J cm^{-2}) mayor la reducción de los recuentos de BP y MyL (0.7 y 0.9 log (UFC g^{-1}), respectivamente) en los tomates cortados. La influencia de los PL sobre la carga microbiana fue bien descrita por una función de tipo log-lineal (R^2> 0.85) (Ecuación 1). Esta función define A_0 como el número de microorganismos sobrevivientes después de la exposición a diferentes Φ (*log N*), el recuento inicial de microorganismos (N_0) y la constante de inactivación dependiente de cada microorganismo (δ).

$$A_0 = log\ N_0 - \delta \cdot \Phi \quad \textbf{(1)}$$

Las contantes positivas de inactivación microbiana (δ) para PB (δ= 0.22 cm^{-2} J) y MyL (δ= 0.25 cm^{-2} J), evidenciaron la incidencia del incremento de la Φ de tratamiento en la reducción microbiana en el tomate fresco cortado. De esta manera, los valores de A_0 para los recuentos de PB en el tomate fresco cortado expuestos a Φ= 4, 6 y 8 J cm^{-2} PL fueron de 2.57, 2.38 y 2.18 log (CFU g^{-1}), respectivamente, mientras que para los recuentos de MyL fueron de 3.94, 3.69, 3.29 y 3.01 log (UFC g^{-1}), respectivamente. Algunos estudios acerca de los efectos de los PL sobre la carga microbiana han reportado patrones similares de inactivación de microorganismos a los expuestos en este trabajo (Oms-Oliu et al., 2010; Izquier y Gómez-López, 2011; Agüero et al., 2016). El mecanismo de inactivación microbiana ejercido por los PL puede ser atribuido a efectos fotoquímicos, fototérmicos y fotofísicos (Elmnasser et al., 2007; Krishnamurthy et al., 2007; Oms-Oliu et al., 2009: Wuytack et al., 2003). La efectividad de los PL sobre la inactivación microbiana está relacionada con la cantidad de energía que incide en el alimento, con las características fisicoquímicas del alimento y con el tipo de microorganismo (Oms-Oliu et al., 2010; Ramos-Villarroel et al., 2013).

Durante el almacenamiento del tomate cortado se observaron incrementos de los recuentos de BP y MyL. Sin embargo, los tomates

frescos cortados expuestos a PL exhibieron menores recuentos microbianos que los tomates no tratados. Se empleó una modificación del modelo gompertziano (Ecuación 2) para describir y determinar la vida útil (*SL*) del tomate fresco cortado (R^2_{adj}= 0.98). En esta ecuación, *log (5 x 10^7)*, es el límite aceptable de microorganismos (log UFC g^{-1}); A_0 es el valor inicial estimado por el modelo (Ecuación 1) [log (UFC g^{-1})]; *t*, es el tiempo de almacenamiento; μ_{max}, es la velocidad máxima de cambio [log (UFC g^{-1}) day^{-1}]; λ, es el tiempo anterior al inicio de los cambios (día); *SL* es el tiempo de vida útil estimado por el modelo y *e* es la contante de Euler.

$$\log(UFC) = (10^7) - Aexp\left\{-exp\left\{(\upsilon_{max} \cdot e) \cdot \left[\frac{\lambda - SL}{A}\right] + 1\right\}\right\} + Aexp\left\{-exp\left\{(\upsilon_{max} \cdot e) \cdot \left[\frac{\lambda - t}{A}\right] + 1\right\}\right\} \quad \textbf{(2)}$$

Los valores de μ_{max} y λ en los tomates frescos cortados expuestos a PL (μ_{max}≤ 0.53 log (UFC g^{-1}) día^{-1} y λ≤ 2.96 día^{-1}) fueron significativamente menores (p<0.05) que en los tomates sin tratar (μ_{max}= 0.73 log (UFC g^{-1}) día^{-1} y λ= 0.3.52 día^{-1}). Estas constantes facilitaron la cuantificación del impacto del incremento de la Φ del tratamiento inicial, sobre la ralentización del crecimiento microbiano y sobre la reducción de los recuentos finales de PB. Las características fisicoquímicas del tomate cortado, tales como pH, contenido de sólidos solubles y algunos compuestos fotoprotectores contra la luz (carotenoides) presentes en el tomate parecen tener suma relevancia para el desarrollo de microorganismos en el tomate cortado y expuesto a PL. En este sentido, la alteración de la integridad celular causada por los PL puede desencadenar la liberación de agua y nutrientes al exterior, promoviendo y facilitando el crecimiento de microorganismos (Oms-Oliu et al., 2010).

La vida útil de los tomates frescos cortados no tratados fue de 10.5 día^{-1}, mientras que en las rodajas expuestas a PL fue de 13.1-13.3 día^{-1}. De este modo, se determinó que los PL permitieron prolongar la vida útil del tomate cortado durante poco más de 3 días adicionales con respecto a los no tratados por PL, tomando como referencia los valores máximos de

microorganismos aceptados en alimentos cortados (5 x10^7 log UFC) (IFPA, 2003). Ramos-Villarroel et al. (2011) sugirieron que los PL son capaces lesionar las membranas celulares de los microorganismos, alterando el mecanismo de replicación de ADN y evitando su reproducción.

5.1.2. Microorganismos sustitutivos de patógenos de relevancia: *Listeria innocua* y *Escherichia coli*

Los recuentos de microorganismos substitutivos de los patógenos de referencia *(L. innocua* y *E. coli*) inicialmente inoculados en los tomates frescos cortados fueron 6.17 y 6.34 log (UFC g^{-1}), respectivamente, y estos valores experimentaron descensos significativos ($p< 0.05$) inmediatamente después de los tratamientos por PL. El incremento de la densidad de energía (Φ) resultó en mayores reducciones de los recuentos de estos microorganismos.

Los patrones de inactivación de *L. innocua* y *E. coli* fueron descritos por ecuaciones de tipo log-linear (R^2= 0.85 y 0.99, respectivamente) (Ecuación 1). Las constantes de inactivación (δ) para *L. innocua* (δ= 0.12 cm^{-2} J) y *E. coli* (δ= 0.17 cm^{-2} J) reflejaron el impacto positivo del incremento de la Φ sobre la reducción de los recuentos de *L. innocua* y *E. coli*. Así, los valores de A_0 para los recuentos de *L. innocua* en el tomate fresco cortado expuestos a Φ= 4, 6 y 8 J cm^{-2} PL fueron de 5.76, 5.54 y 5.27 log (UFC g^{-1}), respectivamente, mientras que para los recuentos de *E. coli* fueron de 5.28, 5.18 y 4.72 log (UFC g^{-1}), respectivamente. De esta manera, la reducción de los conteos de estos microorganismos en el tomate fresco cortado y expuesto a Φ de 8 J cm^{-2} fue de casi 1 log (UFC g^{-1}) con respecto al tomate fresco cortado sin tratamiento de PL.

Aunque estos microorganismos presentaron diferentes patrones de evolución durante el almacenamiento, los tomates cortados sujetos a PL exhibieron cargas microbianas más bajas que los no tratados. La ecuación modificada de Gompertz (Ecuación 3) definió los parámetros cinéticos relacionados a la inactivación de *L. innocua* y *E. coli* en el tomate fresco

cortado en función del incremento de la Φ aplicada y del tiempo de almacenamiento (t) ($R^2_{adj} \geq 0.93$).

$$\log UFC = A_0 + C\exp\{-\exp[-B(t-M)]\} \quad \textbf{(3)}$$

donde A_0, es el valor inicial de recuentos microbianos estimado por la ecuación 1 [log (UFC g^{-1})]; B, es la constante de cambio en relación al tiempo igual a M; M, es el tiempo (días) en el que se observa el máximo cambio; C, es la diferencia entre la asíntota inicial y final; y t es el tiempo de almacenamiento.

Los estimados para las constantes cinéticas facilitaron la obtención de los valores de la velocidad máxima de cambio (μ_{max}) (Ecuación 4) y del periodo precedente al inicio de los cambios (λ) (Ecuación 5).

$$\upsilon_{\max} = \frac{B \times C}{e} \quad \textbf{(4)}$$

$$\lambda = M - \left(\frac{1}{B}\right) + \frac{A_e - A}{\upsilon_{\max}} \quad \textbf{(5)}$$

En el caso de *L. innocua* se observó que el incremento de la Φ de tratamiento influyó en la reducción de los valores de μ_{max} (0.33 a 0.22 log (UFC g^{-1}) $día^{-1}$) y en el incremento de los valores de λ (8.06 a 9.48 day^{-1}) en relación a los tomates no tratados (μ_{max}= 0.30 log (UFC g^{-1}) $día^{-1}$ y λ= 4.25 $día^{-1}$). En el caso de *E. coli*, los tomates frescos cortados y expuestos a PL presentaron valores más bajos de μ_{max} (0.15 - 0.19 $día^{-1}$) y λ (-1.08 - 0.34 $día^{-1}$) que los no tratados (μ_{max}= 0.27 log (UFC g^{-1}) $día^{-1}$, y λ= 5.56 $día^{-1}$). Al final del almacenamiento, los recuentos de *L. innocua* fueron de 8.4 log (UFC g^{-1}), mientras que los de *E. coli* fueron de 6.3 log (UFC g^{-1}) en el tomate cortado sin tratamientos de PL. Sin embargo, el incremento de la Φ influyó en la reducción de los recuentos microbianos de *L. innocua* y *E. coli*.

Así, los tomates cortados sujetos a mayores Φ mostraron disminuciones de hasta 2.5 y 1.6 log (UFC g^{-1}) en los recuentos de *L. innocua* y *E. coli*, respectivamente, con respecto a los tomates sin tratar.

E. coli pareció tener una mayor susceptibilidad a los tratamientos de PL que *L. innocua*. Ramos-Villarroel et al. (2012) sugirieron que la variación en la reducción entre *L. innocua* y *E. coli* podría ser explicada por las diferencias en la complejidad y composición de sus paredes celulares. *E. coli* es una bacteria Gram negativa que tiene una pared celular más delgada y rígida que *L. innocua*, la cual es una bacteria Gram positiva. De hecho, algunos autores han demostrado que las bacterias Gram-negativas son más susceptibles a los tratamientos por PL que las Gram-negativas (Rowan et al., 1999; Anderson et al., 2000). Además, la susceptibilidad de las cepas de *Listeria* and *Escherichia* a los PL es dependiente de las condiciones del medio crecimiento y del tipo de tratamiento aplicado (Rajkovic 2010). La resistencia de algunos microorganismos a la inactivación mediante PL se ha asociado tanto a la complejidad de sus paredes celulares como a la tolerancia a la luz (Hobbs y Robert 1987).

5.2. Efectos de los PL sobre la calidad fisicoquímica del tomate fresco cortado.

Las operaciones de procesado tienen un alto impacto en la integridad de los productos frescos, provocando un estrés fisiológico que desencadena cambios en su estructura física y química. Por ello, mantener las características fisicoquímicas en el tomate fresco cortado es importante para preservar la calidad y la aceptación del producto por parte de los consumidores. Generalmente, la aceptabilidad de un alimento fresco está ligada a factores externos e internos que están relacionados con los atributos sensoriales de color, textura, gusto y aroma. Los cambios en estos atributos generalmente se asocian con el incremento de la actividad metabólica y senescencia del fruto cortado.

5.2.1 Concentración de oxígeno (O_2) y dióxido de carbono (CO_2) en el espacio de cabeza de los envases.

Los resultados obtenidos en este trabajo, mostraron grandes cambios en los contenidos de O_2 y CO_2 dentro de los envases de tomate fresco cortado. El contenido inicial de O_2 (20.9 kPa) se redujo justo después de los tratamientos de PL. Concretamente, los tomates cortados sometidos a Φ= 8 J cm^{-2} mostraron la mayor reducción de O_2 (17.4 kPa), y el mayor incremento de CO_2 (0.91 kPa) en el interior de los envases. Se usó una ecuación polinomial de segundo-orden (R^2≥ 0.97) para determinar la influencia de los PL (Φ) sobre el contenido inicial de O_2 (Ecuación 6) y CO_2 (Ecuación 7).

$$C_{O_2} = -0.0048\phi^2 + 0.123\phi + 0.209 \quad \textbf{(6)}$$

$$C_{CO_2} = 0.016\phi^2 - 0.627\phi + 20.8 \quad \textbf{(7)}$$

Mediante los estimados de las constantes de cambio se pudo cuantificar la influencia del incremento de la Φ sobre la disminución del contenido de O_2 y el incremento de CO_2 en el interior de los envases de las rodajas de tomate. El cambio en las concentraciones de O_2 y CO_2 en el espacio de cabeza de los envases se atribuyó a una aceleración en la tasa respiratoria del tomate fresco cortado.

Así mismo, se utilizó una función Gompertziana (R^2≥ 0.94) para estudiar los cambios en la evolución de las presiones parciales O_2 y CO_2 en el interior de los envases con tomate cortado durante el almacenamiento (Ecuación 8).

$$C_{(G)} = C_{G_0} + k \cdot \exp\left\{-\exp\left\{\left[\left(\upsilon_{\max} \cdot \frac{\lambda - t}{k}\right)\right] + 1\right\}\right\} \quad \textbf{(8)}$$

donde $C_{(G)}$ es el contenido estimado de oxígeno (G= O_2) o dióxido de carbono (G= CO_2) en el interior de los envases de tomate cortado (kPa); C_{G_0}, es el valor inicial de O_2 o CO_2 estimado por la ecuaciones 6 y 7, respectivamente (kPa); *k*, es la constante de cambio de cada uno de los gases durante el almacenamiento; *t,* es el tiempo de almacenamiento; μ_{max}, es la velocidad máxima de cambio (kPa día^{-1}) y λ, es el periodo precedente al inicio de los cambios (día).

Los valores de μ_{max} para el contenido de O_2 ($\mu_{max} \leq$ 1.06 kPa día^{-1}) y CO_2 (μ_{max}= 0.60 a 0.97 kPa día^{-1}) sugirieron que tanto el consumo de oxígeno como la producción de CO_2 fueron acelerados tras la exposición del tomate cortado a los tratamientos de PL. Por otro lado, el incremento de la Φ provocó una disminución en el tiempo anterior a los cambios (λ) en el contenido de O_2 ($\lambda \leq$ 0.98 día), con respecto a los que no fueron expuestos a PL (λ= 3.29 día), mientras que el cambio en el contenido de CO_2 se inició en tiempos muy similares (λ= 1.3 - 1.7 día) para todas las muestras de tomates cortados. De esta manera, los cambios en la concentración de gases fueron atribuidos al incremento de la actividad metabólica desencadenada durante el procesado y exposición de los tomates cortados a los PL. El incremento de la respiración se puede atribuir al estrés metabólico producido por el corte y procesado de los frutos frescos (Soliva-Fortuny et al., 2003).

Al final del almacenamiento, los tomates frescos cortados sometidos a la máxima Φ aplicada (8 J cm$^{-2)}$ presentaron el menor contenido de O_2 y el mayor de CO_2 (0.12 y 16.6 kPa, respectivamente) comparado con los tomates cortados sin tratamiento de PL (0.91 y 17.40 kPa, respectivamente). Oms-Oliu et al. (2010) atribuyeron el aumento de la tasa respiratoria al efecto fototérmico de los PL. En este sentido, el incremento de la temperatura dentro de la cámara de tratamiento puede provocar un estrés fisiológico que promueve una aceleración de la respiración del producto (Ahmed et al., 2012; Gil et al., 2002). De esta manera, el cambio en los contenidos de O_2 y CO_2 dentro de los envases podría ser una evidencia importante de la aceleración de la respiración causada por los PL.

5.2.2 Características Fisicoquímicas

Tanto el crecimiento de microorganismos como el aumento de la tasa respiratoria son aspectos clave que condicionan los cambios en las características fisicoquímicas.

El color es uno de los factores de calidad que impactan más profundamente en la apariencia del tomate (Brand et al., 2006). El color rojo en el tomate se atribuye ampliamente a su contenido de carotenoides pigmentados. El *color* del tomate fresco cortado se vio afectado por la exposición a los PL. Los valores de los parámetros (CIELab) *L**, *a** y *b** fueron utilizados para calcular tono, croma y diferencia de color (ΔE) en los tomates frescos cortados. Los valores iniciales para el parámetro *a** (verde-rojo) (11.2) en el tomate fresco cortado se incrementaron significativamente ($p< 0.05$) después de la exposición a los PL (*a**= 14.1-16.1). Además, los valores de tono (^{o}h) se aproximaron a valores cercanos a 0, lo que indica un color rojo más intenso y oscuro. Los cambios en los parámetros *a**, ^{o}h y ΔE se intensificaron durante el almacenamiento de los tomates cortados, siendo aún mayores en los tomates cortados y expuestos a los PL. Así, el incremento en la Φ aplicada dio como resultado tomates con un color rojo más intenso. Algunos autores mencionan que una mayor aceptabilidad del tomate está relacionada con valores bajos de *L**, valores altos de *a** y a valores de ^{o}h por debajo de los 36º (Shewfelt et al., 1987).

Así mismo, los cambios de color y pérdida de luminosidad del tomate cortado podrían ser debidos a fenómenos oxidativos catalizados por enzimas. Los tomates sin tratamiento de PL presentaron un incremento contante de la actividad enzimática peroxidasa (POD) durante el almacenamiento, alcanzando un valor máximo relativo de 44 % después de 18 días en refrigeración. En cambio, los tomates cortados expuestos a PL mostraron un rápido incremento de la actividad de la enzima POD (60-80 %) tras el tratamiento y un posterior descenso paulatino a lo largo del almacenamiento, alcanzando una actividad relativa de entre 19 y 35% de los valores de actividad inicial. La POD es una enzima comúnmente

encontrada en los vegetales y que lleva a cabo reacciones de oxido-reducción utilizando peróxido como oxidante. La inducción de la POD durante la senescencia de los frutos, es estimulada por la presencia de etileno, sobre todo en frutos de maduración climatérica (Abeles et al., 1989; Vamos-Vigyazo, 1981) y es capaz de oxidar compuestos donantes de hidrógenos como los polifenoles (Richard-Forget y Gauillard, 1997). De hecho, la exposición de los frutos a UV promueve las reacciones de oxido-reducción produciendo especies reactivas de oxígeno como peróxido de hidrogeno y radicales hidroxilo, e induciendo la oxidación por POD (Barka, 2001). Así, tanto el incremento de la Φ como el *t* de almacenamiento parecen tener un papel importante en el incremento del contenido de este tipo de compuestos pigmentados y de la acción de la POD que intensifican el color rojo de los tomates cortados durante el almacenamiento.

La *textura* también es un factor clave en la determinación de la calidad del tomate cortado. Jackman y Stanley (1995), definen la textura como "la suma de todas las características percibidas por el sentido del tacto y que su deformación está relacionada con una fuerza". Aunque la percepción de la textura por los consumidores se relaciona con el tacto, también se ve influenciada por la sensación generada al comer un alimento (Ranganathan et al. 2016). La textura del tomate se puede relacionar con la firmeza, la resistencia al corte, la pérdida de agua y con la homogeneidad de su superficie. Los pulsos de luz no causaron cambios importantes en los valores iniciales de firmeza (20.73 N s^{-1}). La firmeza del tomate cortado descendió paulatinamente durante todo el periodo de almacenamiento y manteniendo solamente un 40% de los valores iniciales, independientemente de si fueron expuestos o no a los PL. La resistencia al corte (RC) del tomate fresco cortado se vio afectada negativamente por los PL. El incremento de la Φ aplicada provocó una disminución de la RC (19.6 - 25.9 N s^{-1}) en los tomates frescos cortados con respecto a los no tratados (29.8 N s^{-1}). Al igual que los valores de firmeza, la RC se redujo durante todo el almacenamiento independientemente del tratamiento aplicado. Después de 20 días de almacenamiento, los tomates cortados sin PL y los

expuestos a Φ = 4 y 6 J cm^{-2} mostraron mantener solamente un 30% de su firmeza inicial. Los tomates cortados y sujetos a Φ= 8 J cm^{-2} exhibieron mayor resistencia al corte durante el periodo de almacenamiento y mantuvieron hasta un 67% de su valor inicial. Además, el incremento en la Φ aplicada se relacionó positivamente algunas alteraciones físicas en los tomates cortados. De esta manera, la *pérdida de peso* (> 10%), la *pérdida de agua* (> 7%) así como la inactivación de las enzimas pectinolíticas *pectin-metil-esterasa* (*PME)* y *poli-galacturonasa PG* durante el almacenado de los tomates cortados pueden relacionarse con la pérdida de textura. Los cambios físicos observados durante el almacenamiento del tomate cortado pueden estar relacionados con la ruptura de la integridad celular causada por el procesado mínimo.

Durante el procesado se pueden desencadenar una serie de desequilibrios fisicoquímicos en el tomate, ocasionando ablandamiento (Aguiló-Aguayo et al., 2013), deshidratación (Sandhya et a., 2010), pérdida de peso (Manurakchinakorn et al., 2012) y cambios en las actividades enzimáticas PME y PG (Chisari et al., 2011). Por otro lado, la presencia de microorganismos, el tipo procesado, el empaque y las condiciones de almacenamiento también están estrechamente relacionados con efectos negativos del procesado sobre la textura de los frutos frescos cortados (Soliva-Fortuny and Martín-Belloso, 2003). De este modo, la pérdida de textura observada durante el almacenamiento del tomate cortado puede ser básicamente atribuida a las alteraciones fisicoquímicas desencadenadas por el estrés inducido durante el procesado mínimo y los PL.

Por otra parte, el contenido y composición química de un alimento, están íntimamente relacionados con su sabor. Los tratamientos de PL no indujeron cambios mínimos sobre los valores iniciales de sólidos solubles totales (SST), pH y acidez del tomate cortado. Sin embargo, los tomates sujetos a PL exhibieron mayores cambios en estos parámetros durante el almacenamiento. Así, los tomates cortados exhibieron un ligero incremento del contenido de SST (4.33-4.37 ºBrix) al final de almacenamiento con respecto a su contenido inicial (4.00 ºBrix). La pérdida de agua causada por

el incremento en la traspiración durante el almacenamiento puede ser la principal causa del aumento de los SST en el tomate cortado. Por otro lado, los valores iniciales de pH (4.59) y acidez (0.31 mg kg^{-1} (equivalentes de ácido cítrico) sufrieron modificaciones durante el almacenamiento del tomate cortado, independientemente del tratamiento aplicado. De esta manera, el cociente entre SST y acidez, utilizado comúnmente para determinar la calidad del tomate, se incrementó de 16.91 en el tomate cortado sin PL a 16.73 - 20.83 en los que fueron expuestos a PL. Ramos-Villarroel et al. (2011) reportaron resultados similares, señalando una correlación positiva entre el incremento del contenido de SST, la reducción de pH, y el aumento de la carga microbiana en aguacates frescos cortados y expuestos a PL. De hecho, tanto el desarrollo de microorganismos como la actividad fisiológica del tomate fresco cortado juegan un papel importante en la degradación de ácidos orgánicos (Odriozola-Serrano et al., 2008; Gil et al., 2006).

5.3. *Efectos de los PL sobre la capacidad antioxidante y compuestos bioactivos del tomate fresco cortado*.

La estabilidad de los compuestos bioactivos durante el almacenamiento es esencial para asegurar la calidad del tomate fresco cortado. El efecto del incremento de la Φ de tratamiento sobre la capacidad antioxidante y el contenido de vitamina C, licopeno y compuestos fenólicos en el tomate cortado se evaluó mediante ecuaciones lineales (Ecuación 9).

$$X_0 = (k_i \cdot \Phi) + b \quad \textbf{(9)}$$

donde X_0, representa la capacidad antioxidante (%) o el contenido compuestos bioactivos (mg kg^{-1}) en el tomate fresco cortado después de la exposición a diferentes Φ (J cm^{-2}); k_i es la constante de cambio dependiente para cada uno de los parámetros y b, es la capacidad antioxidante (%) o el

contenido inicial de compuestos bioactivos (mg kg^{-1}) en el tomate fresco cortado.

Además, se utilizaron funciones de tipo gompertziano (Ecuación 10) y de primer orden (Ecuación 11) para describir los cambios en el contenido de los compuestos bioactivos y capacidad antioxidante, respectivamente, en el tomate cortado durante el almacenamiento.

$$CB_{(x)} = X_0 \pm k \cdot \exp\left\{-\exp\left[\frac{\upsilon_{\max} \cdot e}{x}\right] \cdot (\lambda - t) + 1\right\} \quad \textbf{(10)}$$

donde $CB_{(X=VC,LIC,CF)}$ es el contenido de compuestos bioactivos (mg kg^{-1}); X_0 es el valor expresado por la ecuación 9 para el contenido de cada uno de los compuestos bioactivos (mg kg^{-1}); *k*, es la diferencia entre los valores máximo y mínimo de cada uno de los compuestos durante el almacenamiento (mg kg^{-1} $día^{-1}$), μ_{max} es la constante máxima de cambio (mg kg^{-1} $día^{-1}$); λ es el tiempo anterior a los cambios (día) y *t* es el tiempo de almacenamiento (día).

$$CA = X0 \cdot \exp^{-\delta \cdot t} \quad \textbf{(11)}$$

donde *CA* representa la capacidad antioxidante (%) en función de la Φ y el tiempo almacenamiento (*t*); X_0, es el valor expresado por la ecuación 9 para la capacidad antioxidante (%) del tomate fresco cortado; δ, es la constante de cambio ($día^{-1}$) y *t* es el tiempo de almacenamiento (día).

Los compuestos bioactivos que contribuyen a la CA del tomate son la vitamina C, los compuestos fenólicos y los carotenoides, principalmente licopeno (Takeota et al., 2001). En esta tesis doctoral se evaluó el impacto de los tratamientos de PL sobre cada uno de ellos.

5.3.1. Licopeno

El licopeno es el principal carotenoide implicado en la pigmentación roja del tomate y tiene un alto poder antioxidante. Por ello, una alta concentración de este compuesto se relaciona con la calidad del tomate (Shi et al., 2008). Los tomates frescos cortados expuestos a PL exhibieron mayores contenidos de licopeno que los no tratados. El impacto del incremento de la Φ aplicada sobre los cambios iniciales del contenido de licopeno pudo ser descrito por una ecuación lineal (R^2 = 0.99) (Ecuación 9). La constante cinética (k_i) relacionó positivamente (k_i = 1.5 cm^{-2} J) el incremento del contenido de licopeno con el aumento de la Φ de tratamiento. De hecho, el incremento en la concentración de licopeno ha sido positivamente relacionado con el aumento de la Φ de tratamiento (Pataro et al., 2015).

Independientemente del tratamiento, todos los tomates cortados presentaron aumentos en el contenido de licopeno durante el almacenamiento, sin embargo, estos incrementos fueron más evidentes en las muestras expuestas a PL. Al final del almacenamiento, el contenido de licopeno en el tomate cortado no tratado por PL fue de 50.8 mg kg^{-1}, mientras que en los expuestos a Φ = 4, 6 y 8 J cm^{-2} incrementaron hasta los 75.5, 78.9 y 86.2 mg kg^{-1}, respectivamente.

Los cambios en el contenido de licopeno en el tomate cortado fueron descritos por una función gompertziana ($R^2_{adj} \geq 0.92$) (Ecuación 11) donde los estimados de las constantes cinéticas (k, λ, y μ_{max}) explicaron el efecto positivo de los PL sobre el incremento en los contenidos de licopeno durante el almacenamiento. La constante máxima de velocidad (k) en el tomate cortado se incremento justo después del tratamiento de PL (k = 0.36 $\times 10^{-2}$ – 0.91 $\times 10^{-2}$ mg kg^{-1}) con respecto a los no tratados (6.2 mg kg^{-1}), lo que sugirió que el tratamiento de PL influyó en el incremento del contenido de licopeno en el tomate cortado a lo largo del almacenamiento. Los tomates cortados sin tratamiento de PL mostraron un valor de λ de 11.2 $día^{-1}$ mientras que los que fueron expuestos a Φ = 4, 6 y 8 J cm^{-2} exhibieron

valores de 4.6, 0.4 y 0 día^{-1}, respectivamente. Además, el incremento de la Φ de tratamiento indujo mayores cambios en los valores de μ_{max}, los cuales variaron entre 1 y 1.8 mg kg^{-1} día^{-1}. Así, los tomates cortados expuestos a mayores Φ presentaron mayores concentraciones de licopeno a lo largo del almacenamiento. Algunos autores han reportado patrones similares en el incremento del contenido de licopeno en diferentes variedades de tomates e higos expuestos a PL (Solovchenko y Merzlyak, 2008; Rodov et al., 2012; Bravo et al., 2012; Castagna, et al., 2013). La síntesis de licopeno puede ser una respuesta al estrés metabólico causado por los efectos fototérmicos y fotoquímicos de los PL y, mantenerse activo durante todo el almacenamiento del tomate fresco cortado. El licopeno representa el 95% de todos los carotenoides coloridos en el tomate y por ello tiene una gran capacidad de absorción de la radiación incidente, actuando como fotoprotector y evitando la degradación de otros compuestos presentes en el tomate. De esta manera, el incremento del contenido de licopeno fue negativamente correlacionado (-0.70 – -0.76) con la disminución de la capacidad antioxidante del tomate fresco cortado.

5.3.2. Vitamina C

La concentración inicial de vitamina C en el tomate cortado (114.7 mg kg^{-1}) se vio negativamente afectada por los PL. Los tomates tratados registraron una pérdida de un 11 - 24 % de su contenido inicial como consecuencia directa de los tratamientos. Esta pérdida del contenido de vitamina C fue descrita por una función lineal (R^2 = 0.95) (Ecuación 9). El estimado de la constante de cambio (k_i = -3.2 cm^{-2} J) sugirió mayores pérdidas en el contenido de vitamina C conforme se incrementó la Φ de tratamiento. La vitamina C es un compuesto altamente sensible cuando es expuesto a la luz, oxígeno y calor (Davey et al., 2000). Así, los efectos fototérmico y fotoquímico atribuidos a los PL parecen ser las principales causas de las pérdidas de vitamina C en el tomate cortado. Tran y Farid (2004), reportaron que el mecanismo de degradación de la vitamina C provocada por algunas longitudes de onda de PL es similar a la de los

tratamientos térmicos, alcanzando una máxima fotodegradación cuando es expuesta a longitudes de onda cercanas a 245 nm (Bradshaw et al., 2001).

Además, se observaron pérdidas generalizadas de vitamina C en todas las muestras de tomate cortado durante el almacenamiento. La disminución del contenido de vitamina C en función de la Φ de tratamiento y del tiempo de almacenamiento (t) fueron descritos por un modelo Gompertziano ($R^2_{adj} \geq 0.96$) (Ecuación 11). El incremento de la Φ provocó mayores pérdidas de vitamina C (k = -0.24 $\times$ 10^{-2} – -0.43 $\times$ 10^{-2} mg kg^{-1} $día^{-1}$) con respecto a los tomates cortados no expuestos a PL (k = -0.20 $\times$ 10 mg kg^{-1} $día^{-1}$). Así mismo, el incremento de la Φ influyó en la reducción de los valores de μ_{max} (2.03 - 2.64 mg kg^{-1} $día^{-1}$) y extendiendo los valores de λ ($\lambda \geq$ 1 $día^{-1}$) en relación a los tomates cortados no expuestos a PL (μ_{max} = 3.68 mg kg^{-1} $día^{-1}$ y λ = 0 $día^{-1}$, respectivamente). Los valores de la constante de cambio relativa (k), el tiempo de retraso (λ) y la velocidad máxima de cambio (μ_{max}) indicaron el impacto negativo del incremento de la Φ y su trascendencia en el contenido de vitamina C a lo largo del almacenamiento. El contenido final de vitamina C en los tomates cortados sin tratamiento de PL fue de 69.99 mg kg^{-1}, mientras que en los expuestos a Φ = 4, 6 y 8 J cm^{-2} fueron de 67.1, 62.5 y 48.9 mg kg^{-1}, respectivamente. En este sentido, la pérdida de vitamina C puede ser incrementada debido a periodos largos de almacenamiento, exposición a altas temperaturas, daño físico del producto y daños por frio durante el almacenamiento (Lee y Kader, 2000).

Incluso, se ha demostrado que la presencia de vitamina C está asociada al control de las especies reactivas de oxígeno (ROS), lo que supone un descenso de su contenido conforme se incrementa la respiración (Woluckaa et al., 2005). Este efecto coincide con el rápido consumo de O_2 y producción de CO_2 a lo largo del almacenamiento de los tomates cortados. Eitenmiller y Laden (1999), señalaron que la degradación de la vitamina C puede ser vinculada a una alta concentración de oxígeno. Además, el incremento de la actividad enzimática de la POD como respuesta a los PL puede ser relacionado con la pérdida de vitamina C. Una alta concentración

de esta enzima se ha relacionado con pérdidas de la vitamina C (Mehlorn, 1990). Finalmente, la disminución del contenido de vitamina C fue positivamente correlacionada (0.93 – 0.97) con la pérdida de capacidad antioxidante del tomate fresco cortado.

5.3.3. Compuestos fenólicos

Los compuestos fenólicos (CF) también contribuyen de un modo determinante a la calidad del tomate. Estos compuestos están relacionados con algunos atributos relacionados con el sabor, formado por el gusto y aroma. El contenido inicial de compuestos fenólicos en los tomates frescos cortados fue de 231.3 mg kg^{-1}. Los tratamientos de PL influyeron en los incrementos del contenido fenólico de los tomates frescos cortados, siendo los expuestos a Φ = 8 J cm^{-2} los que exhibieron el mayor incremento de compuestos fenólicos (244.2 mg kg^{-1}). Los cambios en el contenido de CF en el tomate cortado fueron descritos mediante una ecuación lineal (R^2 = 0.96) (Ecuación 9), que reveló la influencia positiva del incremento de la Φ aplicada sobre el contenido del contenido de CF (k_i = 1.4 cm^{-2} J).

Durante el almacenamiento, el incremento del contenido fenólico en los tomates cortados sujetos a PL fue mayor que en las no tratadas. La Evolución del contenido fenólico en el tomate fresco cortado durante el almacenamiento fue descrito por una ecuación modificada de Gompertz (Ecuación 11) (R^2 ≥ 0.94). Los valores de μ_{max} de los tomates cortados sujetos a PL (0.18 – 0.47 mg kg^{-1} $día^{-1}$) fueron menores que en los no tratados (0.92 mg kg^{-1} $día^{-1}$), lo que sugirió que el incremento de la Φ promovió una menor liberación de compuestos fenólicos durante el almacenamiento. Además, los tomates frescos cortados sin tratamientos de PL mostraron cambios en el contenido fenólico desde el inicio del tratamiento (λ= 0.03 $día^{-1}$) mientras que los expuestos a PL mostraron una disminución de los λ conforme se incrementó la Φ (λ= 7.54 – 1.96 $día^{-1}$). De esta manera, los tomates cortados expuestos a PL alcanzaron un mayor contenido de compuestos fenólicos (242.8 - 249.0 mg kg^{-1}) que los observados en los no tratados por PL (237.9 mg kg^{-1}), al final del

almacenamiento. Este fenómeno podría relacionarse con incremento inicial de compuestos fenólicos provocado por los PL, lo que favoreció una liberación más lenta a lo largo del almacenamiento del tomate cortado. Así, los valores estimados de las constantes fueron capaces de expresar la influencia positiva de los PL sobre la evolución de los CF en el tomate cortado durante el almacenamiento. Liu et al, (2011) y Bravo et al, (2013) también han observado estos efectos positivos sobre el contenido fenólico en los frutos de tomate fresco expuesto a PL (Φ= 0.3 - 4 J cm^{-2}). Algunos autores señalan que incremento de la Φ, influye en la acumulación de compuestos fenólicos como mecanismo de adaptación de los vegetales frente al estrés provocado por estos tratamientos (Luthria et al, 2006 and Jagadeesh et al, 2011).

El estrés físico causado en los frutos frescos cortados estimula la producción de metabolitos secundarios como mecanismo de defensa a la pérdida de integridad (Kiem et al., 2008). De este modo, los PL pueden actuar como un catalizador de la síntesis metabólica, estimulando una acelerada producción inicial e incrementado los contenidos fenólicos en los tomates cortados durante el almacenamiento. Además, el estrés provocado por los PL podría estimular la síntesis de la enzima fenilalanina liasa (PAL), lo que desencadena la producción de fenilpropanoides e incrementa los niveles de fenol-fitoalexinas y ligninas (Ryalls et al., 1996). El aumento de la síntesis de la enzima PAL se ha relacionado con altos niveles de CO_2 (Ke y Salveit, 1989). Así, el procesado mecánico, los PL y el tiempo de almacenamiento parecen estar relacionados con el incremento del contenido fenólico en los tomates frescos cortados.

5.3.1. Capacidad antioxidante

Los PL influyeron en la pérdida de capacidad antioxidante (CA) del tomate fresco cortado. La CA del tomate descendió conforme se incrementó la Φ de tratamiento, perdiendo entre un 5 y un 11 % de la CA inicial. La ecuación lineal ($R^2 \geq 0.95$) (Ecuación 9) describió los descensos de la CA en base a la Φ aplicada. Los valores estimados para la constante de cambio

(k_i = -0.16 para DPPH y -0.25 cm^{-2} J para ABTS) indicaron que el incremento de la Φ causó una disminución de la CA. Estos cambios pueden estar relacionados tanto con el procesado inicial como con los tratamientos de PL aplicados al tomate cortado.

Estos resultados son distintos a los reportados para espinacas, champiñones y brócoli cortados y expuestos a PL, que muestran un incremento inicial de la CA tras la exposición a los PL (Agüero et al. 2016; Oms-Oliu et al. 2012; Costa et al. 2006). Sin embargo, estos mismos autores también señalan que después de los incrementos iniciales se observaron pérdidas durante todo el almacenamiento de estos productos. En este estudio, los tomates cortados exhibieron pérdidas significativas de la CA a lo largo del almacenamiento, independientemente del tratamiento de PL aplicado. La evolución de la CA fue descrita por una función exponencial de primer orden ($R^2_{adj} \geq 0.95$) (Ecuación 10). La disminución de los valores de la constante de cambio ($\delta \leq 0.14 \times 10^{-3}$ día^{-1} para DPPH y $\delta \leq 0.26 \times 10^{-3}$ día^{-1} para ABTS) permitió interpretar la relación entre incremento de la Φ aplicada y el descenso de la CA del tomate cortado durante el almacenamiento (t). La CA del tomate cortado fue de entre un 9.9 y un 12.3% al final del almacenamiento, independientemente del tratamiento aplicado. La aceleración en el estrés fisiológico, que desencadena cambios en el metabolismo oxidativo del tomate cortado, podría ser la principal causa de la oxidación y modificación de las concentraciones de las sustancias antioxidantes y, por tanto, de la variación de la capacidad antioxidante. De esta manera, el procesado del tomate cortado pudo causar un estrés fisiológico que desencadenó cambios en los contenidos de compuestos bioactivos y la consiguiente pérdida de la CA, lo cual ha sido reportado previamente en otros porductos frescos cortados (Lindley, 1998).

5.4 *Aceptabilidad y calidad del tomate cortado*

La calidad del tomate cortado está estrechamente ligada a sus características físicas, químicas y nutrimentales. Sin embargo, el procesado

de los frutos frescos puede llegar a alterar algunas de estas propiedades e influir en la aceptabilidad por parte de los consumidores. Por ello, el estudio del impacto de los pulsos de luz sobre los atributos sensoriales del producto es de suma importancia. La identificación de los parámetros fisicoquímicos que intervienen en la modificación de dichos atributos es necesaria para la optimización de los tratamientos por PL.

El estudio sensorial del tomate fresco cortado se realizo mediante pruebas de preferencia de comparación múltiple. Mediante esta técnica se determinó la preferencia de los consumidores por los atributos sensoriales que determinan la aceptabilidad del tomate cortado. No se detectaron diferencias significativas ($p< 0.05$) en la calidad sensorial entre los tomates frescos cortados expuestos a PL y los no tratados. Sin embargo, los valores de preferencia de estos atributos incrementaron ligeramente durante el almacenamiento. Después de 10 días, los valores de preferencia de la textura se mantuvieron al nivel del valor inicial, mientras que en los atributos de color, olor y sabor del tomate cortado fueron muy superiores a los iniciales, independientemente del tratamiento aplicado. Interesantemente, los tomates cortados expuestos a una mayor Φ fueron ligeramente mejor aceptadas por los consumidores.

Puesto que las pruebas de preferencia de comparación multiple son adimensionales, se utilizó el modelo de juicios comparativos de Thurstone para dimensionar los resultados (sumas de rangos) mediante una escala de preferencias basada en la percepción real del estimulo (Ecuación 12).

$$R_i - R_j = z_{ij}\sqrt{\sigma_i^2 + \sigma_j^2 - 2r_{ij}\sigma_i\sigma_j} \quad \textbf{(12)}$$

donde R_i y R_j , son los valores de la escala de estimulo *i* y *j,* σ_i y σ_j, son las desviaciones estándar de las respectivas dispersiones discriminantes, r_{ij}, es la correlación entre los dos procesos discriminantes y z_{ij}, es la desviación normal (puntuación z) correspondiente a la proporción de las veces que la muestra (*j*) es juzgada por encima que el estimulo psicológico (*i*).

De esta manera, se obtuvieron valores de preferencia sensorial dentro de una escala Thurnstoniana (ET). Inmediatamente tras el procesado, los tomates frescos cortados mostraron valores de aceptabilidad ligeramente más altos (ET= 0.83) que los que fueron expuestos a PL (ET= 0.81). Los valores de aceptabilidad del tomate cortado mantuvieron un incremento constante a lo largo de todo almacenamiento, independientemente de si fueron expuestos a PL o no. Sin embargo, los tomates cortados y expuestos a PL mostraron mayores valores que los no tratados ($p< 0.05$). Así, la aceptabilidad de los tomates frescos cortados expuestos a Φ= 8 J cm^{-2} fue mayor (ET= 4.63) que en los no tratados (ET= 3.78), después de 10 días de almacenamiento.

Para la identificación de los atributos relacionados con la "aceptabilidad" se usaron dos modelos QSPR (Quantitative Structure-Property Relationship) (Ecuación 13).

$$A= \beta_0 + \beta_{1X1} + \beta_{2X2} + \cdots\cdots + \beta_{pXp} + \varepsilon \quad \textbf{(13)}$$

donde A es el valor estimado y representa la variable independiente (aceptabilidad), $x_1x_2x_p$ representan los valores de las variables dependientes, β_0 es una constante de cambio, β_1, β_2, …, β_p son los coeficientes de regresión y ε es el error aleatorio

Este modelo describió la influencia de los atributos sensoriales color (*c*), aroma (*a*), textura (*tx*) y sabor (*s*) en la aceptabilidad general del producto (R^2_{adj}= 0.99) (Ecuación 14). El modelo identificó que los atributos sensoriales que influyeron en la aceptabilidad del tomate cortado fueron el "color", el "aroma" y el "sabor" con un 90 % de significancia ($p< 0.05$). Por el contrario, se observó una baja significancia estadística del atributo "textura" en la "aceptabilidad" por lo que podría ser retirado del modelo.

$$Aceptabilidad = k_0 - color\ (k_c) + aroma\ (k_a) - textura\ (k_{tx}) + sabor\ (k_s) \quad \textbf{(14)}$$

El valor estimado para la constante de cambio (k_o= 0.515) indicó la influencia positiva del incremento de la Φ aplicada sobre los atributos sensoriales del tomate cortado, mientras que los valores para las constantes de asociación (k_C= 0.41, k_a= 0.70 y k_s= 0.17) sugirieron que estos cambios se intensificaron durante el almacenado del tomate cortado.

Estos resultados sugieren que el incremento de la Φ influyó positivamente en los atributos sensoriales y así, aumentando el nivel de aceptabilidad del tomate cortado. De este modo, el incremento del contenido de SST, la disminución de la acidez, el incremento de licopeno y de compuestos fenólicos, atribuidos al incremento de la Φ aplicada, fueron positivamente relacionados con el incremento de la aceptabilidad visual, el sabor y el aroma del tomate cortado.

Algunos jueces reportaron la presencia de astringencia y harinosidad (granulosidad) durante la evaluación sensorial del tomate cortado. La astringencia fue asociada al incremento de compuestos fenólicos, mientras que la harinosidad pudo ser causada por los desequilibrios en las actividades enzimas pectinolítcas (PME y PG) observados durante el almacenamiento. Algunos autores señalan que una baja actividad enzimática PG junto a una alta actividad de la PME es la causa principal de manifestación de harinosidad en los frutos (Artés et al., 1996; Lurie et al, 2003; Brumell et al., 2004). Aunque durante el almacenamiento de los tomates fresco cortados se observo un importante deterioro de las características físicas asociadas a la textura de los tomates frescos cortados, estos cambios no fueron percibidos por los panelistas y por tanto no afectaron de forma directa en la aceptabilidad del tomate fresco cortado.

Por otro lado, la calidad del tomate cortado es una representación compleja entre seguridad alimentaria, características fisicoquímicas, nutricionales y sensoriales. Los PL influyeron positivamente en la reducción de los recuentos microbiológicos, lo que alargó su vida útil y con ello, asegurando la calidad del tomate cortado por más tiempo.

Además, los tomates cortados expuestos a PL mostraron cambios en la mayoría de los parámetros relacionados con la calidad. El análisis de

componentes principales (PCA) permitió explorar la inconexa correlación entre todas las variables relacionadas con la calidad del tomate. El modelo mostró dos componentes que explicaban el 73.6 % de la variabilidad de los datos (PC1:57.2% y PC2:16.4 %) y con un valor inherente superior a 1. La extrapolación de los valores de correlación para cada una de las variables que influyeron en los dos componentes principales permitió vincular los cambios en las variables con el incremento de la Φ aplicada y el tiempo de almacenamiento. Además, se estableció la estrecha relación entre estas variables medidas instrumentalmente y los atributos evaluados sensorialmente.

Estas correlaciones fueron usadas para desarrollar un modelo QSPR (Ecuación 15) ($R^2_{adj} \geq 99.68$ y $Q^2_{LOO} \geq 0.93$), que ayudó a analizar la interacción entre los parámetros analizados instrumentalmente y la preferencia sensorial del tomate fresco cortado. Este modelo permitió calcular la relación entre los parámetros analíticos (pH, acidez (AT), SST, SST-AT, a*, ºh, ΔE, croma, POD, pérdida de agua, resistencia al corte, CF y licopeno) la aceptabilidad del tomate cortado. Así, los parámetros analíticos que describieron los patrones de "aceptabilidad" fueron ΔE^*, a^*, ^oh, y JL (pérdida de agua), con un 90% de significancia ($p<0.05$) dentro del modelo.

$$Aceptabilidad = k_{\Delta E} \cdot \Delta E^* + k_{a^*} \cdot a^* - k_{^oh} \cdot {}^oh + k_{JL} \cdot JL \quad \textbf{(15)}$$

Básicamente el modelo atribuye la "aceptabilidad" a la apariencia del tomate cortado. Los valores estimados para las constantes de asociación (k_{a^*}= 1.09, $k_{\Delta E}$= -3.91 x 10^{-2}, $k_{^oh}$= -13.84, k_{JL}= 7.18 x 10^{-2}) indican la influencia del color y la pérdida de agua en la aceptabilidad. Aunque el modelo definió que la "pérdida de agua" parece influir de manera significativa en la aceptabilidad, su expresión dentro del modelo se podría atribuir tanto al impacto visual como al de gusto, debido a que este fenómeno promovió un incremento del contenido de SST y favoreciendo su preferencia. De este modo, los resultados de los parámetros analíticos parecen tener una relación bastante ajustada a la percepción sensorial.

Por tanto, los tratamientos de PL al tomate fueron capaces de reducir las cargas microbianas, además de producir y promover una serie de cambios fisicoquímicos que son deseables por los consumidores. Estos resultados son contrarios a los reportados para algunos frutos frescos cortados, en donde se atribuye un efecto negativo de los PL sobre los atributos sensoriales de estos productos Oms-Oliu et al., 2010; Ramos-Villarroel et al., 2011; Ramos-Villarroel et al., 2012 a y b; Kramer et al., 2015). Los cambios en los parámetros relacionados con la acidez, dulzura y alteraciones físicas tales como el color y la pérdida de agua, son en esencia responsables de la aceptación del tomate cortado.

5.5. *Bibliografía*

Abeles, F. B., Hershberger, W. L., & Dunn, L. J. (1989). Hormonal regulation, and intracellular localization of a 33-kD cationic peroxidase in excised cucumber cotyledons, *Plant Physiology*, 89, 664-668.

Aguiló-Aguayo, I., Charles, F., Renard, C. M. G. C., Page, D., & Carlin, F. (2013). Pulsed light effects on surface decontamination, physical qualities and nutritional composition of tomato fruit. *Postharvest Biology and Technology*, 86, 29-36.

Ahmed, L., Martin-Diana, A. B., Rico, D., & Barry-Ryan, C. (2012). Quality and nutritional status of fresh-cut tomato as affected by spraying of delactosed whey permeate compared to industrial washing treatment. *Food and Bioprocess Technology*, 5(8), 3103-3114.

Anderson, J. G., Rowan, N. J., MacGregor, S. J., Fouracre, R. A., & Parish, O. (2000). Inactivation of food-borne enteropathogenic bacteria and spoilage fungi using pulsed-light. *IEEE Transactions on Plasma Science*, 28(1), 83–88.

Barka, E.A., (2001) Protective enzymes against reactive oxygen species during ripening of tomato (*Lycopersicum esculentum*) fruits in

response to low amounts of UV5C. *Australian Journal of Plant Physilogy*, 28 (8), 785–791.

Bravo, S., García-Alonso, J., Martín-Pozuelo, G., Gómez, V., Santaella, M., Navarro-González, I., & Periago, M. J. (2012). The influence of post-harvest UV-C hormesis on lycopene, β-carotene, and phenolic content and antioxidant activity of breaker tomatoes. *Food Research International*, 49(1), 296-302.

Bravo, S., García-Alonso, J., Martín-Pozuelo, G., Gómez, V., García-Valverde, V., Navarro-González, I. & Periago, M. J. (2013). Effects of postharvest UV-C treatment on carotenoids and phenolic compounds of vine-ripe tomatoes. International Journal of Food Science & Technology, 48, 1744–1749.

Castagna, A., Chiavaro, E., Dall'Asta, C., Rinaldi, M., Galaverna, G., & Ranieri, A. (2013). Effect of postharvest UV-B irradiation on nutraceutical quality and physical properties of tomato fruits. *Food Chemistry*, 137(1-4), 151-158.

Chisari, M., Barbagallo, R. N., Spagna, G., & Artes, F. (2011). Improving the quality of fresh-cut melon through inactivation of degradative oxidase and pectinase enzymatic activities by UV-C treatment. *International Journal of Food Science and Technology*, 46(3), 463-468.

Costa, L., Vicente, A. R., Civello, P. M., Chaves, A. R. & Martínez, G. A. (2006). UV-C treatment delays postharvest senescence in broccoli florets. Postharvest Biol. Technol., 39 (2), 204–210.

Davey, M. W., Montagu, M. V., Inzé, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I. J., Strain, J. J., Favell, D. & Fletcher, J. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Sciences of Food and Agriulture*, 80, 825-860.

Eitenmiller, R. R., & Lande, W. O. (1999). Vitamin analysis for the health and food Sciences. CRC Press, Florida.

Elmnasser, N., Guillou, S., Leroi, F., Orange, N., Bakhrouf, A. & Federighi, M. 2007. Pulsed-light system as a novel food decontamination technology: A review. Journal of Microbiology, 53, 813-821.

Gil, M. I., Conesa, M. A., & Artés, F. (2002). Quality changes in fresh cut tomato as affected by modified atmosphere packaging. *Postharvest Biology and Technology*, 25(2), 199-207.

Gil, M. I. Aguayo, E., & Kader, A. A. (2006). Quality and nutrient retention in fresh-cut versus whole fruit during storage. *Journal Agricultural and food Chemistry*, 54, 4284-4296.

Jackman, R. L., & Stanley, D. W. (1995). Perspectives in the textural evaluation of plant foods. *Trends Food Sci. Technol*. 6, 187–194.

Jagadeesh, S. L., Charles, M. T., Gariepy, Y., Goyette, B., Raghavan, G. S. V., & Vigneault, C. (2011). Influence of postharvest UV-C hormesis on the bioactive components of tomato during post-treatment handling. *Food and Bioprocess Technology*, 4(8), 1463-1472.

Kader, A. A., Morris, L. L., Stevens, M. A., & Albright-Holton, M. (1978). Composition and flavor quality of fresh market tomatoes as influenced by some postharvest handling procedures. *J. Am. Soc. Hort. Sci*., 103, 6-13.

Ke, D., & Salveit, M. E. (1989). Carbon dioxide-induced brown stain development as related to phenolic metabolism in iceberg lettuce. *Journal of the American society for Horticultural Science*, 114, 789-794.

Krishnamurthy, K., Demirci, A., & Irudayaraj, J.M. 2007. Inactivation of *Staphylococcus aureus* in milk using flow-through pulsed UV-light treatment system. *Journal of Food Science*, 72, M233–M239.

Laing, B. M., Schlueter, D. L., & Labuza, T. P. (1978). Degradation kinetics of ascorbic acid at high temperature and water activity. *Journal of food science*, 43(5), 1440-1443.

Lee, S. K., & Kader, A. A. (2000). Preharvest and postharvet factors influencing vitamin C content of horticultural crops. *Postharvest Biology & Technology*, 20(3), 207–220

Lindley, M. G. 1998. The impact of food processing on antioxidants in vegetable oils, fruits and vegetables. *Trends Food Sci. Technol*. 9, 336-340.

Liu, C., Han, X., Cai, L., Lu, X., Ying, T., & Jiang, Z. (2011). Postharvest UV-B irradiation maintains sensory qualities and enhances antioxidant capacity in tomato fruit during storage. *Postharvest Biology and Technology*, 59(3), 232-237.

Luthria, D. L., Mukhopadhyay, S., & Krizek, D. T. (2006). Content of total phenolics and phenolic acids in tomato (*lycopersicon esculentum mill*.) fruits as influenced by cultivar and solar UV radiation. *Journal of Food Composition and Analysis*, 19(8), 771-777.

Manurakchinakorn, S., Chamnan, U., & Mahakarnchanakul, W. (2012). Appearance and textural changes of modified atmosphere-packed fresh-cut mangosteen after preservative dips. *J. Food Prosc. Preserv*. 36(6), 504-511.

Mehlhorn, H. (1990). Ethylene-promoted ascorbate peroxidase activity protects plants against hydrogen peroxide, ozone and paraquat. *Plant Cell Environ*, 13, 971-976.

Morkunas, I., & Gmerek, J. (2007). The possible involvement of peroxidase in defense of yellow lupine embryo axes against *Fusarium oxysporum*. *Journal of Plant Physiology,* 164,185-194.

Oms-Oliu, G., Martín-Belloso, O., & Soliva-Fortuny, R. (2009). Pulsed light treatments for food preservation. A review*. Food and Bioprocess Technology,* 3(1), 13–23.

Oms-Oliu, G., Aguiló-Aguayo, I., Martín-Belloso, O., & Soliva-Fortuny, R. (2010). Effects of pulsed light treatments on quality and antioxidant properties of fresh-cut mushrooms (*Agaricus bisporus*). *Postharvest Biol. Tech*. 56, 216-222.

Pataro, G., Donsi, G., & Ferrari, G. (2015). Post-harvest UV-C and PL irradiation of fruits and vegetables doi:10.3303/CET1544006.

Rajkovic, A., Tomasevic, I., Smigic, N., Uyttendaele, M., Radovanovic, R., & Devlieghere, F. (2010). Pulsed UV light as an intervention strategy against *Listeria monocytogenes* and *Escherichia coli* O157:H7 on the surface of a meat slicing knife. *Journal of Food Engineering*, 100(3), 446-451.

Ramos-Villarroel, A. Y., Martín-Belloso, O., & Soliva-Fortuny, R. (2011a). Bacterial inactivation and quality changes in fresh-cut avocado treated with intense light pulses. *European Food Research and Technology*, 233(3), 395–402.

Ramos-Villarroel, A. Y., Martín-Belloso, O., & Soliva-Fortuny, R. (2011b). Using antibrowning agents to enhance quality and safety of fresh-cut avocado treated with intense light pulses. *Journal of Food Science*, 76(9), S528–S534.

Ramos-Villarroel, A. Y., Marťn-Belloso, O., & Soliva-Fortuny, R. (2013). Intense light pulses: Microbial inactivation in fruits and vegetables. [Pulsos de luz intensa: inactivació n microbiana en frutas y hortalizas] *CYTA - Journal of Food*, 11(3), 234-242.

Richard-Forget, F. C., & Gauillard, F. A. (1997). Oxidation of chlorogenic acid, catechins, and 4-methylcatechol in model solutions by combinations of pear (*Pyrus communis cv. Williams*) polyphenol oxidase and peroxidase: A possible involvement of peroxidase in enzymatic browning. *Journal of Agricultural and Food Chemistry*, 45, 2472–2476.

Rodov, V., Vinokur, Y., & Horev, B. (2012). Brief postharvest exposure to pulsed light stimulates coloration and anthocyanin accumulation in fig fruit (*Ficus carica* L.). *Postharvest Biology and Technology*, 68, 43-46.

Rowan, N. J., MacGregor, S. J., Anderson, J. G., Fouracre, R. A., McIlvaney, L., & Farish, O. (1999). Pulsed-light inactivation of food-related microorganisms. *Applied and Environmental Microbiology*,

65(3), 1312–1315.

Ryalls, J., Neuenschwander, U., Willits, M., Molina, A., Steiner, H. Y., & Hunt, M. (1996). Systemic acquired resistance. *The Plant Cell*, 8, 1809–1819.

Sandhya. (2010). Modified atmosphere packaging of fresh produce: Current status and future needs. *LWT - Food Science and Technology*, 43(3), 381-392.

Shewfelt, R. L., Prussia, S. E. Resurreccion, A. V. A. Hurst, W. C. & Campbell, D. T. (1987). Quality changes of Vine-Ripened tomatoes within the postharvest handling system. *Journal of Food Science*, 52(3), 661-664.

Shi, J., Dai, Y., Kakuda, Y., Mittal, G., & Xue, S. J. (2008). Effect of heating and exposure to light on the stability of lycopene in tomato puree. *Food control*, 19(5), 514-520.

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

Solovchenko, A. E., & Merzlyak, M. N. (2008). Screening of visible and UV radiation as a photoprotective mechanism in plants. *Russian Journal of Plant Physiology*, 55(6), 719-737.

Takeoka, G. R., Dao, L. Flessa, S., Gillespie, D M., Jewel, W. T., Huebner, B., Bertow, D., & Ebeler, S. E. (2001). Processing Effects on Lycopene Content and Antioxidant Activity of Tomatoes. *Journal of Agricultural and Food Chemistry,* 2001 49(8), 3713-3717.

Vamos-Vigyazo, L. 1981. Polyphenol oxidase and peroxidase in fruits and vegetables. CRC *Crit. Rev. Food Sci. Nutr*. 15, 49-127.

Watada, A., Ko, N., & Minott, D. 1996. Factors affecting quality of fresh-cut horticultural products. *Postharvest Biology and Technology,* 9(2), 115-125.

Wuytack, E.Y., Phuong, L.T.D., Aertsen, A., Reyns, K.M., Marquenie, D. & De Ketelaere, B. (2003). Comparison of sub-lethal injury induced

in *Salmonella enterica serovar Typhimurium* by heat and by different nonthermal treatments. *Journal of Food Protection*, 66, 1-37.

CONCLUSIONES GENERALES

CONCLUSIONES

De manera general los tratamientos de pulsos de luz (PL) son capaces de alargar la vida útil del tomate fresco cortado, mejorando algunas características fisicoquímicas e influyendo positivamente en su aceptabilidad. De manera particular se deducen las siguientes conclusiones.

- Los PL indujeron reducciones en las cargas tanto de flora nativa como de microorganismos sustitutivos de patógenos de referencia en el tomate fresco cortado. El incremento en la fluencia aplicada (Φ) dio lugar a conteos microbiológicos más bajos a lo largo del almacenamiento, reduciendo hasta en 2 log (UFC g^{-1}) los recuentos PB y MYL, al final del almacenamiento del tomate fresco cortado. También, los PL influyeron en la disminución de las cargas de *L. innocua* y *E. coli.* Además, *L. innocua* presentó una mayor resistencia inicial a los tratamientos de PL con respecto a *E. coli*. Al final del almacenamiento, se redujo hasta en 2.5 log (UFC g^{-1}) los recuentos de *L. innocua* y *E. coli* en el tomate fresco cortado sujeto a la máxima densidad de energía (Φ= 8 J cm^{-2}). Finalmente, la vida útil del tomate cortado sujeto a PL fue de casi 4 días más con respecto a los no tratados.

- Los PL no tuvieron un impacto importante sobre la textura inicial del tomate fresco cortado. Sin embargo, los tomates cortados sujetos a PL exhibieron importantes pérdidas de peso y de agua (>7 %), disminución de la firmeza y resistencia al corte (>40 %) así como cambios en la actividades de las enzimas pectinmetilesterasa (PME) y poligalacturonasa (PG) durante el almacenamiento. Estos cambios fueron más pronunciados cuando las condiciones de tratamiento fueron más intensas.

- Algunas modificaciones fisicoquímicas causadas por los PL podrían ser responsables del cambio en el contenido de fitonutrientes del tomate cortado. Los PL parecen influir negativamente sobre el contenido inicial de vitamina C, lo que influyo en la pérdida de casi el 10% de la

capacidad antioxidante inicial. Aunque la CA descendió durante el almacenamiento, los tomates cortados expuestos a PL mostraron valores similares a la de los no tratados al final del almacenamiento (9-12%).

- Se observó un incremento en los contenidos de licopeno y de compuestos fenólicos en el tomate cortado expuesto a PL. Los cambios fueron intensificados como consecuencia del incremento de la Φ aplicada y por el tiempo de almacenamiento (t). Al final del almacenamiento, la CA de los tomates expuestos a PL fue muy similar a la de aquéllos no tratados, probablemente a causa del incremento en los contenidos de licopeno y de CF durante el almacenamiento.

- Los PL causaron algunos cambios en las características fisicoquímicas del tomate fresco cortado. Sin embargo, algunas de estas alteraciones parecen mejoraron la calidad sensorial. Los atributos sensoriales más valorados en la calidad del tomate cortado fueron el color, el aroma y el sabor. La aceptabilidad sensorial del tomate cortado no mostró grandes diferencias durante los primeros 5 días de almacenamiento. Sin embargo, los consumidores mostraron una clara preferencia por los tomates cortados sujetos a Φ= 8 J cm^{-2}, después de 10 días de almacenado.

- El modelo de tipo QSPR ayudó a calcular la influencia del incremento de la Φ sobre los atributos sensoriales relacionados con la aceptabilidad del tomate cortado (R^2_{adj}= 0.99). Además este modelo relacionar los cambios en los parámetros analíticos con la aceptabilidad del tomate cortado (R^2_{adj}= 0.99).

- De este modo, se determinó que los tratamientos de PL son una buena alternativa para alargar la vida útil del tomate fresco cortado, manteniendo una buena calidad sensorial y un alto contenido de compuestos bioactivos durante 10 días de almacenamiento a 4 ºC.

ANEXOS

EFFICACY OF PULSED LIGHT TREATMENTS ON THE INACTIVATION OF *Listeria innocua* AND *Escherichia coli* ON FRESH-CUT TOMATO

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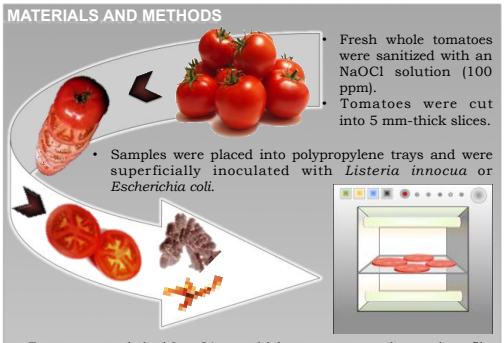
Tomato is one of the most widely consumed vegetables and its consumption is associated wit a healthy lifestyle because of its high content of vitamins, carotenoids and phenolic compounds.
An increasing consumers' demand for healthier and more convenient fresh-like products has urged industrial manufacturers to look for alternative ways of processing. Minimal processing techniques (cutting, washing, packaging) are emerging as an alternative to minimize quality changes caused by physical and chemical modifications in processed produce. However, fresh-cut products are more susceptive to microbial spoilage and may pose a safety issue due to the growth of potentially hazardous microorganisms. In fact, well-documented outbreaks caused by pathogenic microorganisms have been reported for this kind of produce during the last decade.
Pulsed light (PL) is a non-thermal emerging technology with good prospects for decontaminating minimally processed products. Using broad spectrum light at high frequency for short periods of time, PL-treatments are capable of inactivating vegetative bacteria, spores, yeast and molds.

The objective of the present study was to evaluate the efficacy of pulsed light treatments on the inactivation of *Listeria innocua* and *Escherichia coli* inoculated on fresh-cut tomato stored for 20 days at 5 °C.

MATERIALS AND METHODS



- Fresh whole tomatoes were sanitized with an NaOCl solution (100 ppm).
- Tomatoes were cut into 5 mm-thick slices.
- Samples were placed into polypropylene trays and were superficially inoculated with *Listeria innocua* or *Escherichia coli*.
- Trays were sealed with a 64 μm-thick transparent polypropylene film and then subjected to 8, 12 or 16 J/cm^2 fluences. **PL equipment**: double flash xenon lamp system (Steribeam Xe-Matic-2L-A, Kehl, Germany.
- *L. innocua* and *E. coli* counts were carried out during storage period. Headspace composition on each tray was also evaluated at each sampling time.
- Statistical analysis: ANOVA's and LSD ($p<0.05$) test were performed for each determination.

PL-treated tomato slices exhibited slightly lower *E. coli* and *L. innocua* counts than untreated samples just after being treated and throughout the whole storage period .
Fresh-cut tomatoes irradiated with an overall fluence of 16 J/cm^2 exhibited the lowest microbial counts just after processing, thus resulting into reductions of 1.4 and 1 log CFU/g for *E. coli* and *L. innocua* loads, respectively, with respect to untreated samples.

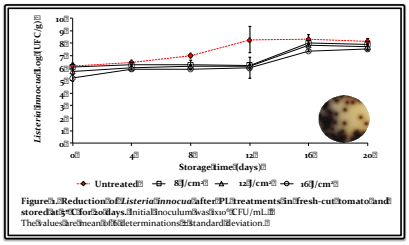


Figure 2. Reduction of *Listeria innocua* after PL treatments in fresh-cut tomato and stored at 5 °C for 20 days. Initial inoculum was 1x10^8 CFU/mL. The values are mean of 6 determinations ± standard deviation.

E. coli (Figure 2) was found to be more sensitive than *L. innocua* to the PL treatments and subsequent refrigerated storage. *E. coli* exhibited lower counts throughout refrigerated storage irrespectively of the applied fluence. Hence, after 20 days of chilled storage, *E. coli* and *L. innocua* counts were, respectively, up to 1.6 and 0.6 log CFU/g lower than those found on untreated fresh-cut tomatoes.

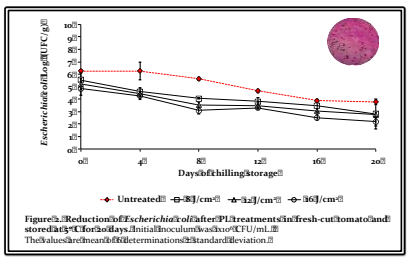


Figure 3. Reduction of *Escherichia coli* after PL treatments in fresh-cut tomato and stored at 5 °C for 20 days. Initial inoculum was 1x10^8 CFU/mL. The values are mean of 6 determinations ± standard deviation.

The effects of processing and chilled storage in the respiration rate of tomato slices are shown in Figure 3. Oxygen content was decreased during the chilled storage of tomato slices (Figure 3-A). Fresh-cut tomato exposed at higher PL fluence showed higher O_2 consumption than untreated fresh-cut tomatoes. However, the oxygen contents on untreated and PL-treated samples were not significantly different ($p<0.05$) after 20 days of chilled storage.

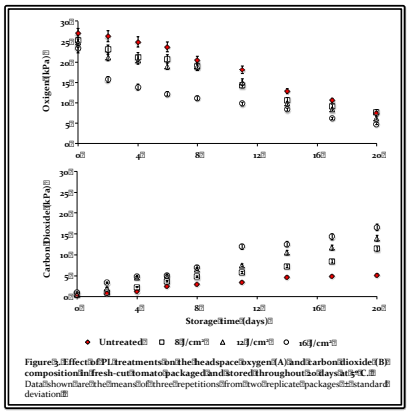


Figure 3. Effect of PL treatments on the headspace oxygen (A) and carbon dioxide (B) composition in fresh-cut tomato packaged and stored throughout 20 days at 5 °C. Data shown are the means of three repetitions from two replicate packages ± standard deviation.

On the other side, carbon dioxide content into the packages steadily increased along the storage time (Figure 3-B). Tomato slices exposed to pulsed light showed higher CO_2 production than those not exposed to PL. At the end of storage, the carbon dioxide production of samples treated with pulsed light was significantly higher than that of untreated samples.
The respiration rate is widely associated to shelf life potential, but also to microbial growth. Nevertheless, in this study, the O_2 consumption and the CO_2 production on fresh-cut tomatoes seems to be related to increases of respiration in response to cutting and PL-treatments.

Results obtained in this work suggest that PL-treatments can be used as an effective process to inactivate E. coli and L. innocua on fresh-cut tomatoes. However, further research regarding the effect of PL treatments over nutritional and quality parameters of fresh-cut tomato should be completed prior to industrial application.

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EFFECTS OF PULSED LIGHT TREATMENTS ON TEXTURE AND RELATED ENZYMES OF FRESH-CUT TOMATO

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Motivations

Tomato (*Lycopersicum esculentum*) is one of the most consumed vegetables either fresh or processed. Consumption of tomatoes is considered as an indicator of good nutritional habits because of its high content in vitamins, carotenoids and fenolic compounds.

The growing consumers demand for safe and fresh-like processed products has contributed to a rise in the production of fresh-cut fruits and vegetables. However, minimal processing operations may promote physical and chemical changes that reduce the product shelf life. Progressive softening is a consequence of the decomposition of cell wall components and structure. Namely, polygalacturonase (PG; EC3.2.1.15) and pectin methylesterase (PME; EC 3.1.1.11) are considered as the primary hydrolysis enzymes involved in the softening process.

Pulsed light (PL) is a non-thermal emerging technology with good prospect for decontaminating minimally processed products. Although just a few studies have been focused on the effects of PL on physiology and quality aspects of fruits and vegetables, knowledge regarding the effect on enzymes is yet required.

Hence, the aim of this work was to investigate the effect of pulsed light (PL) treatments on the texture quality of fresh-cut tomato stored under chill conditions, through the determination of their impact on the pectinolytic enzymes and their role in texture modifications.

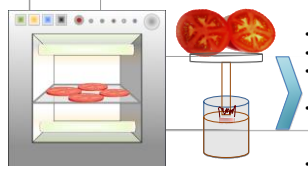
Processing and Methods

1. Fresh whole tomatoes were sanitized with chlorinated water and cut into 5 mm-thick slices using an electric slicer.

2.Samples were placed into polypropylene trays and then subjected to PL with fluences of 4 and 8 J/cm² per both sides.

PL equipment: a double flash lamp system (Steribeam Xe-Matic-2L-A, Kehl, Germany) was used, with an emitted spectrum ranging from 200 to 1100 nm and with 15–20% of the light in the UV region. The pulse duration was 0.3 ms and the fluence per pulse was 0.4 J cm^{-2}.

3. Trays with untreated and PL-treated tomato slices were stored throughout 15 days at 4°C and analyzed periodically for:



- Microbial growth (plate-count agar PCA/CGA)
- Weight loss
- Texture
- Pectinmethyl esterase (PME) (Kimball, 1991)
- Polygalacturonase (PG) (Aguiló-Aguayo, et al., 2009)
- Pairwise ranking tests

Results

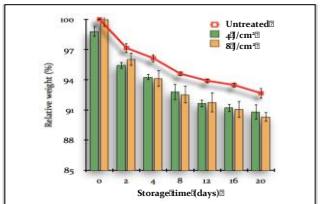


Figure 1. Changes in the relative weight of fresh-cut tomatoes subjected to PL treatments.
Data shown are the means of three repetitions from two replicate packages ± standard deviation

Weight loss of fresh-cut tomatoes significantly increased through refrigerated storage for either untreated or PL-treated samples (**Figure 1**). Untreated tomato slices exhibited weight losses of ca. 7% at the end of storage. In contrast fresh-cut tomatoes exposed to PL treatments exhibited slightly but significantly greater weight losses, thus losing a 10-12% of their initial weight. Heating of the fruit surface was not observed during the treatments. However, an increase in the respiratory and transpiration rates could be behind the observed differences.

Shear strength of fresh-cut tomatoes subjected to PL-treatments decreased significantly with respect to untreated samples just after processing and continued to decrease through chill storage (**Figure 2**). Nevertheless, significant differences between treated and untreated samples were not noticeable beyond the first days of refrigerated storage.

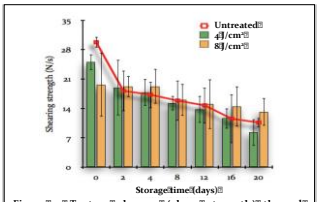


Figure 2. Texture changes (shear strength) through refrigerated storage of fresh-cut tomatoes subjected to PL treatments.
Data shown are the means of three repetitions from two replicate packages ± standard deviation

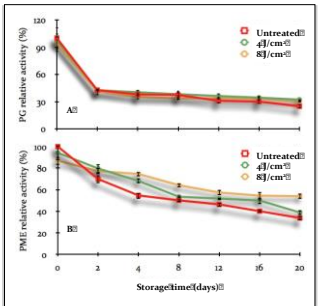


Figure 3. Changes in polygalacturonase (A) and pectin metylesterase (B) activities through refrigerated storage of fresh-cut tomatoes.
Data shown are the means of three repetitions from two replicate packages ± standard deviation

PG and PME activities on fresh-cut tomatoes decreased remarkably throughout storage (**Figure 3**). PG activity dramatically decreased just after processing irrespective of the treatment applied; hence, insignificant differences (p<0.05) were observed between PG activities of untreated and PL-treated tomato slices through refrigerated storage (**Figure 3-A**). Changes with respect to the initial PG activity values ranged between 70-75% at the end of the storage period.

On the other side, PL-treated fresh-cut tomatoes exhibited a significant decrease of their initial PME activity immediately after processing (6 and 13% for PL fluences of 4 and 8 J/cm², respectively) (**Figure 3-B**). However, the PME residual activities in PL-treated tomatoes remained higher than in untreated samples through the subsequent storage period. PME residual activity at the end of the storage period was a 67% (untreated), a 61% (4 J/cm²) and a 46% (8 J/cm²) lower than the initial values at the end of storage.

Microbial stability of fresh-cut tomato was evaluated throughout storage to ensure the safety of samples to be subjected to sensory evaluation tests (**Table 1**). Sensory judges did not observe significant differences among untreated and PL-treated samples (**Figure 4**). PL-treated tomatoes stored for 10 days scored higher in texture perception than untreated samples, although differences were not found to be significant.

	Storage time (days)	
	0	20
Psychrotrophic Bacteria		
0 J/cm²	3.1±0.1	7.0±0.2
4 J/cm²	3.1±0.1	6.4±0.3
8 J/cm²	2.7±0.1	5.2±0.1
Mould and yeast		
0 J/cm²	4.9±0.1	8.0±0.2
4 J/cm²	4.7±0.4	7.7±0.4
8 J/cm²	3.8±0.1	7.5±0.2

Table 1. Microbial counts (log CFU/g) of fresh-cut tomatoes subjected to PL treatments.
Data shown are the means of three repetitions from two replicate packages ± standard deviation

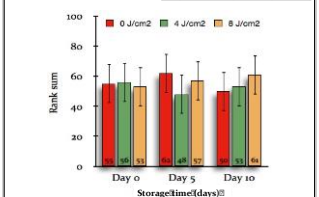


Figure 4. Sensory evaluation of texture on fresh-cut tomatoes after pulsed light (PL) treatments and stored at 5 °C for 10 days.
Data shown are the means of three repetitions from two replicate packages ± standard deviation

Conclusion

In conclusion, the application of pulsed light treatments did not led to negative changes on texture of fresh-cut tomato. Even when changes on the textural, physical and enzymatic properties were observed, sensory testers were not able to discriminate among untreated and PL-treated tomato slices. The PG and PME activities of PL-treated samples remained at levels comparable to those of untreated tomato slices, suggesting that the slight texture differences between treated samples during chilled storage were not influence by enzymatic factors and may be caused by other factors such as pH changes, weight loss and microbial growth.

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References:

- Kimball, D. A. (1991). Citrus Processing: Quality Control and Technology. New York: Van Nostrand Reinhol.
- Aguiló-Aguayo, I., Soliva-Fortny, R., & Martín-Belloso, O. (2009). Changes in viscosity and pectolytic enzymes of tomato and strawberry juices processed by high-intensity pulsed electric fie ds. Int. J. Food Sci. Tech. 44(11) 2268-2277.