**UNIVERSIDAD POLITÉCNICA DE VALENCIA**

Departamento de Tecnología de Alimentos

# Estudio comparativo de la calidad y seguridad de un puré de kiwi pasteurizado por calentamiento convencional o por microondas

**TESIS DOCTORAL**

Presentada por:

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UNIVERSITAT POLITÈCNICA DE VALÈNCIA

# DEPARTAMENTO DE TECNOLOGÍA DE ALIMENTOS

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***Estudio comparativo de la calidad y seguridad de un puré de kiwi pasteurizado por calentamiento convencional o por microondas***

Presentada por:

MARÍA BENLLOCH TINOCO

Para optar al título de DOCTORA por la Universidad Politécnica de Valencia.

Directoras:

Dra. Nuria Martínez Navarrete

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Valencia, 2015

Dña. Nuria Martínez Navarrete, Doctora en Ciencias Biológicas, Catedrática de la Universidad Politécnica de Valencia, y Dña. Mª Dolores Rodrigo Aliaga, Doctora Ingeniera Agrónomo por la Universidad Politécnica de Valencia, Científico Titular del CSIC (Consejo Superior de Investigaciones Científicas), en el Instituto de Agroquímica y Tecnología de Alimentos de Valencia,

CERTIFICAN que

Dña. María Benlloch Tinoco, Tecnóloga de Alimentos, ha realizado bajo nuestra dirección el trabajo que con el título "Estudio comparativo de la calidad y seguridad de un puré de kiwi pasteurizado por calentamiento convencional o por microondas", presenta para optar al grado de Doctora por la Universidad Politécnica de Valencia.

Para que así conste a los efectos oportunos, firman el presente certificado en Valencia, a 24 de Abril de 2015.

Fdo. Nuria Martínez Navarrete
Directora de Tesis

Fdo. Mª Dolores Rodrigo Aliaga
Directora de Tesis

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## RESUMEN

En la presente Tesis se evalúa la idoneidad del uso de las microondas como tecnología alternativa a la pasteurización convencional, para preservar un puré de kiwi desde el punto de vista de la seguridad y la calidad del mismo. Para ello, se ha estudiado el impacto de esta tecnología sobre diversas enzimas, microorganismos patógenos y alterantes y distintas propiedades fisicoquímicas, sensoriales, nutricionales y funcionales del puré, tras el procesado y durante el almacenamiento. Se ha establecido y validado un tratamiento de pasteurización por microondas y se ha comparado la efectividad de esta tecnología frente al calentamiento convencional a la hora de conservar el producto en base a diversos criterios.

Aunque la energía microondas dio lugar a un calentamiento heterogéneo del puré, detectándose el punto más frío en la zona central del producto y el punto más caliente en la zona superior de sus laterales, éste resultó efectivo frente a la inactivación tanto de enzimas como de microorganismos patógenos y alterantes, sin causar un excesivo deterioro de su calidad. Se obtuvieron diversos modelos cinéticos que permitieron predecir la inactivación microbiológica del puré de kiwi durante el calentamiento por microondas. Se empleó un diseño de experimentos para determinar las condiciones de proceso más adecuadas para pasteurizar el producto mediante esta tecnología, en base a la inactivación enzimática y al deterioro de sus propiedades funcionales. El tratamiento por microondas seleccionado dio lugar a un puré de kiwi tanto estable (90% de inactivación de la enzima peroxidasa) como inocuo (> 5 reducciones logarítmicas de *L. monocytogenes*) a un 99,9% de probabilidad. Asimismo, se estableció un tratamiento de pasteurización convencional equivalente con fines comparativos. A raíz de la comparación establecida, quedó patente la superioridad de las microondas para inactivar tanto enzimas como microorganismos, ya que, por un lado, se requirió de una menor carga térmica (menos unidades de pasteurización) para alcanzar un nivel equivalente de inactivación de peroxidasa y, por el otro, el tiempo de reducción decimal (valor de D) del microorganismo patógeno estudiado *L. monocytogenes* resultó ser menor, cuando el puré de kiwi se procesó mediante la aplicación de microondas que cuando éste se sometió al calentamiento

convencional. En consecuencia, la pasteurización por microondas, aunque causó un nivel de inactivación de *L. monocytogenes* semejante y afectó de forma similar a la consistencia y al contenido en carotenoides del mismo que el tratamiento térmico convencional, permitió alcanzar una mayor inactivación de la flora alterante del producto, así como, de las enzimas polifenoloxidasa y pectinmetilesterasa. Además, el tratamiento de pasteurización por microondas preservó en mayor medida el contenido en compuestos bioactivos, la actividad antioxidante y el contenido en clorofilas del producto, dando lugar a un puré de kiwi con un color más semejante al propio de la fruta fresca, que presentó además, una mayor aceptabilidad sensorial, una vida útil más larga (123 días a 4 ºC) y una mayor estabilidad de sus propiedades durante el almacenamiento.

En base a todo lo anterior, se recomienda la aplicación de la tecnología microondas como una alternativa interesante al procesado térmico convencional a la hora de pasteurizar un puré de kiwi, así como de otras frutas de características similares, con el fin de obtener productos procesados a base de fruta de mayor calidad sin que la innocuidad de los mismos se vea comprometida.

## ABSTRACT

In the present Doctoral Thesis, the suitability of the use of microwave energy as an alternative to conventional heating to safely pasteurise and efficiently preserve the quality of a kiwifruit puree was investigated. To this end, the impact of microwave heating on the enzymatic activity, microorganisms, pathogenic or spoilage, physicochemical, sensory, nutritional and functional properties of the product was studied, following the processing step and during successive storage. On this basis, a pasteurisation microwave treatment was designed and validated, and effectiveness of microwave technology and conventional heating to preserve the safety and quality of the product were compared based on several criteria.

Although microwave processing led to a non-uniform heating of the kiwifruit puree, with the coldest and the hottest spots being located at its central region and its edges, respectively, this technology allowed an effective inactivation of enzymes and microorganisms (pathogenic or spoilage) without severely affecting the quality of the product. On the basis of the data provided by the inactivation kinetics of *L. monocytogenes* under microwave heating, along with the outputs of an experimental design, which was used to study the effect of microwave power and process time on the enzymatic inactivation and the functional properties of the product, the best processing conditions were chosen. These treatment conditions permitted to reach the target level of microbial inactivation as well as minimise the enzymatic activity and maximise the preservation of the functional value of the product were selected. The optimum microwave treatment was found to cause a 90% of peroxidase inactivation and reduce more than 5-$\log_{10}$ cycles of *L. monocytogenes*, with a 99.9% of probability. An equivalent conventional pasteurisation treatment was designed with comparative purposes. From the comparison established, superiority of microwaves over conventional heating to inactivate enzymes and microorganisms was pointed out, given that, on the one hand, lower thermal load (lower value of pasteurisation units) was needed in order to achieve the same level of peroxidase inactivation and, on the other hand, a shorter decimal reduction time (lower D-value) of *L. monocytogenes* was obtained when the kiwifruit puree was processed by means of microwave technology. Accordingly, although microwave pasteurisation

led to an analogous inactivation of *L. monocytogenes* and similarly affected the consistency and carotenoids content of the puree, this treatment gave rise to a superior preservation of the bioactive compounds and antioxidant activity, as well as, the chlorophylls content of the product. Additionally, the microwave pasteurised kiwifruit puree showed a colour more similar to that of the fresh fruit, a greater sensory acceptability, a longer shelf-life (123 days at 4 ºC) and greater stability during storage.

In conclusion, more than conventional heating, microwave technology was found to be an appropriate means of processing a kiwifruit puree, as well as any other fruit puree with similar characteristics, so as to obtain high-quality and safe pasteurised fruit-based products.

## RESUM

En la present Tesi s'avalua la idoneïtat de l'ús de les microones com a tecnologia alternativa a la pasteurització convencional, per a preservar un puré de kiwi des del punt de vista de la seguretat i la qualitat del mateix. Per a això, s'ha estudiat l'impacte d'esta tecnologia sobre diversos enzims, microorganismes patògens i alterants i distintes propietats fisicoquímiques, sensorials, nutricionals i funcionals del puré, després del processat i durant l'emmagatzemament. S'ha establit i validat un tractament de pasteurització per microones i s'ha comparat l'efectivitat d'esta tecnologia enfront del calfament convencional a l'hora de conservar el producte basant-se en diversos criteris.

Encara que l'energia microones va donar lloc a un calfament heterogeni del puré, detectant-se el punt més fred en la zona central del producte i el punt més calent en la zona superior dels seus laterals, aquest va resultar efectiu per a inactivar tant enzims com microorganismes patògens i alterants, sense causar un excessiu deteriorament de la seua qualitat. Es van obtindre diversos models cinètics que van permetre predir la inactivació microbiològica del puré de kiwi durant el calfament per microones i es va utilitzar un disseny d'experiments per a determinar les condicions de procés més adequades per a pasteuritzar el producte per mitjà d'esta tecnologia, basant-se en la inactivació enzimàtica i el deteriorament de les seues propietats funcionals. El tractament per microones seleccionat va donar lloc a un puré de kiwi tant estable (90% d'inactivació de l'enzim peroxidasa) com innocu (> 5 reduccions logarítmiques de *L. monocytogenes*) a un 99,9% de probabilitat. Així mateix, es va establir un tractament de pasteurització convencional equivalent amb fins comparatius. Arran de la comparació establida, va quedar patent la superioritat de les microones per a inactivar tant enzims com microorganismes, ja que, d'una banda, es va requerir d'una menor càrrega tèrmica (menys unitats de pasteurització) per a aconseguir un nivell equivalent d'inactivació de peroxidasa i, per l'altre, el temps de reducció decimal (valor de D) del microorganisme patogen *L. monocytogenes* va resultar ser menor, quan el puré de kiwi es va processar per mitjà de l'aplicació de microones que quan este es va sotmetre al calfament convencional. En conseqüència, la pasteurització per microones, encara que va

causar un nivell d'inactivació de *L. monocytogenes* semblant i va afectar de forma anàloga a la consistència i al contingut en carotenoides del mateix que el tractament tèrmic convencional, va permetre aconseguir una major inactivació de la flora alterant del producte, així com, dels enzims polifenoloxidasa i pectinmetilesterasa. A més, el tractament de pasteurització per microones va preservar en major grau el contingut en compostos bioactius i activitat antioxidant i el contingut en clorofil·les del producte, donant lloc a un puré de kiwi amb un color més semblant al propi de la fruita fresca, que va presentar a més, una major acceptabilitat sensorial, una vida útil més llarga (123 dies a 4 ºC) i una major estabilitat de les seues propietats durant l'emmagatzemament.

Basant-se en tot l'anterior, es recomana l'aplicació de la tecnologia microones com una alternativa interessant al processat tèrmic convencional a l'hora de pasteuritzar un puré de kiwi, així com d'altres fruites de característiques semblants, a fi d'obtindre productes processats a base de fruita de major qualitat sense que la innocuïtat dels mateixos es veja compromesa.

# ÍNDICE DE CONTENIDOS

# Justificación del estudio

## JUSTIFICACIÓN DEL ESTUDIO

A causa del acelerado ritmo de vida actual, gran parte de las prácticas gastronómicas tradicionales han quedado obsoletas, abriendo paso a nuevas tendencias de consumo que se orientan principalmente hacia la ingesta de comida rápida y de fácil consumo y la adquisición de alimentos de larga vida útil. Mientras que la ingesta de carnes y otros compuestos de alto contenido calórico aumenta, la de alimentos de origen vegetal resulta insuficiente y se produce, en su mayoría, en forma de productos procesados. Si bien es cierto que los consumidores, cada vez más, remplazan los alimentos frescos por otros procesados, también muestran un creciente interés por la calidad de los mismos, al ser cada día más conscientes de en qué medida sus hábitos alimentarios repercuten sobre su salud. Es por ello, que actualmente la demanda de los consumidores se centra en productos de conveniencia y listos para consumir, que se ajusten a sus nuevas necesidades, pero que al mismo tiempo sean seguros, saludables, libres de aditivos y semejantes al producto fresco.

La industria alimentaria se encuentra, por tanto, frente a un nuevo reto. Asegurar el cumplimiento de los criterios microbiológicos estipulados para elaborar alimentos innocuos que cuenten con una extensa vida útil, ya no es suficiente. Los procesos de conservación comúnmente aplicados deben adaptarse y optimizarse para poder ofrecer a los consumidores productos que cumplan con sus más recientes expectativas. Una forma muy factible de afrontar tales cambios es apostar por las tecnologías de conservación alternativas a los procesos térmicos convencionales, pudiendo éstas aplicarse tanto para reemplazar parcial o totalmente a las técnicas de calentamiento convencional, con el fin de mejorar la calidad de los productos que a día de hoy se encuentran en el mercado, como para llevar a cabo el desarrollo de nuevos productos.

La energía microondas ofrece innumerables ventajas a la hora de procesar los alimentos que pueden contribuir a mejorar los tratamientos empleados tradicionalmente por la industria alimentaria, ya que, si se aplica en condiciones idóneas, esta tecnología puede dar lugar a procesos de alto rendimiento, que permitan transformar y/o preservar los alimentos en un tiempo considerablemente menor que el requerido por los procesos térmicos convencionales y que conlleven

pérdidas de calidad menores. Esta capacidad de las microondas para preservar en mayor medida las propiedades de los productos, hace que su aplicación en el ámbito de la preservación de las frutas sea particularmente interesante, al residir su principal atractivo para los consumidores en su amplia gama de sabores, colores y aromas y su gran valor nutricional y funcional, principalmente asociado al aporte de micronutrientes y otros compuestos bioactivos. Tanto las propiedades sensoriales, como las nutricionales y funcionales de las frutas tienden a verse seriamente afectadas durante los procesos térmicos convencionales a los que a menudo se ven sometidas, con el fin de obtener productos derivados de larga vida útil. En muchos casos, estos productos presentan características muy diferentes a las propias del alimento fresco.

Por tanto, el diseño de procesos basados en la aplicación de microondas, que permitan obtener fruta procesada de mejor calidad que los tratamientos convencionales, podría contribuir de forma significativa a ampliar la gama de productos a base de fruta de gran calidad disponibles en el mercado, que sean acordes con las nuevas tendencias de consumo. No obstante, pese a las innumerables ventajas que las microondas parecen ofrecer desde el punto de vista de la calidad del alimento, es imprescindible tener en cuenta los posibles riesgos microbiológicos asociados a su aplicación. Por ello, previamente a la implantación a nivel industrial de esta tecnología, debe corroborarse que los procesos basados en la aplicación de microondas no sólo aseguren una mejor preservación de las propiedades nutritivas y sensoriales de los alimentos, sino también, que los niveles de seguridad microbiológica que se obtienen son equivalentes a los alcanzados con el procesado térmico convencional al que pretenden sustituir. Por este motivo, resulta tanto interesante, como necesario, llevar a cabo estudios que contemplen el impacto de la energía microondas en la calidad y seguridad de los alimentos.

En base a lo expuesto anteriormente, la presente Tesis contribuye al estudio de la viabilidad de las microondas aplicadas a los procesos de conservación de alimentos. Se evalúa el uso de dicha tecnología para conservar un puré de kiwi, en comparación con el calentamiento convencional, desde el punto de vista de la seguridad alimentaria y de la calidad nutricional, funcional y sensorial del producto.

# Introducción

# I. INTRODUCCIÓN

## I.1. PRODUCCIÓN Y CONSUMO DE PRODUCTOS A BASE DE FRUTA

Existen evidencias científicas, cada vez más sólidas, que señalan la estrecha relación que hay entre los términos nutrición y salud. La alimentación desempeña un reconocido papel en el estado de salud de las personas, presentándose como un elemento clave para garantizar un óptimo funcionamiento del organismo y disminuir la incidencia de diversas patologías (Gil, 2010).

Llevar a cabo una alimentación saludable implica consumir alimentos variados y combinados en proporciones adecuadas, asegurando una ingesta suficiente de frutas y verduras. La importancia de consumir este tipo de alimentos reside mayoritariamente en su aporte de micronutrientes y otras sustancias bioactivas, los fitoquímicos, que sin tener una función nutricional clásicamente definida son indispensables a largo plazo para nuestra salud (Kalt, 2001). De hecho, numerosos estudios avalan los efectos beneficiosos derivados de una ingesta regular de frutas y verduras, entre los que destaca la reducción del riesgo de padecer enfermedades crónicas y degenerativas tales como cáncer, afecciones cardiovasculares, diabetes tipo 2, Alzheimer y/o desórdenes del sistema inmunitario (Antunes et al., 2011; Du et al., 2009; Park et al., 2008; Schieber et al., 2001).

Las frutas son alimentos que han formado parte de la dieta del ser humano desde tiempos inmemoriales, no tan sólo por su calidad nutritiva, sino también porque éstas ofrecen una amplia gama de colores, sabores y aromas que resultan atractivos para los consumidores y posibilitan que éstos las perciban como productos apetecibles (Khoo et al., 2011; Tavarini et al., 2008). Su consumo no sólo despierta interés desde un punto de vista nutricional sino también económico, ya que la producción de frutas supone una importante y emergente industria. En lo que a España respecta, el sector hortofrutícola es el principal sector de la producción de la rama agraria, siendo su contribución siempre superior al 30% (MAGRAMA, 2013). Más concretamente, por ejemplo en el año 2009 el sector hortofrutícola facturó 15.028 millones de euros es decir más del 18% del total de la facturación de la industria agroalimentaria en dicho año (MAGRAMA, 2011).

Los hábitos alimentarios de la población han cambiado recientemente. Mientras que por un lado, los consumidores, cada vez más informados, muestran una mayor conciencia por su salud a la hora de seleccionar los alimentos que ingieren, por el otro, se observa una desviación de los modelos y hábitos alimentarios más saludables, principalmente orientada hacia un mayor consumo de carnes y otros compuestos de alto contenido calórico y una reducción de la ingesta de productos vegetales y de cereales.

Los gustos y preferencias de los consumidores cambian y evolucionan con rapidez. La concentración demográfica, el ritmo de vida cada vez más acelerado, la mayor renta disponible y la globalización del comercio han dejado extintas gran parte de las prácticas gastronómicas tradicionales, al mismo tiempo que han favorecido la aparición de nuevas tendencias de consumo, principalmente basadas en la ingesta de comida rápida y de fácil consumo, así como también en la adquisición de alimentos con una vida útil relativamente larga (Elez-Martínez et al., 2006; O'Donnell et al., 2010). En la actualidad, por tanto, los consumidores manifiestan su preferencia por los alimentos procesados, pero se muestran muy exigentes al respecto y solicitan productos estables, saludables, inocuos y cuyas características sensoriales sean muy similares a las del producto fresco ("freshlike"), valorando especialmente atributos como su color, textura, olor y sabor a la hora de realizar sus elecciones (Rémésy, 2004). Teniendo en cuenta todos estos aspectos, ampliar la gama de productos a base de fruta disponibles en el mercado que cubran las expectativas del consumidor e incentiven la ingesta de fruta por parte de la población, se plantea como un gran reto para la industria alimentaria (Fellows, 2009; Señorans et al., 2003). Esta industria, en respuesta a tales demandas, apuesta por la comercialización de productos a base de fruta mínimamente procesados y listos para consumir, tales como frutas cortadas, zumos de fruta pasteurizados, "smoothies", purés de frutas, etc. (Elez-Martínez et al., 2006). La comercialización de esta nueva gama de productos, además de ofrecer alimentos que se adapten a las necesidades de los consumidores, se presenta como una oportunidad para aprovechar los excedentes de producción de aquellas frutas, que pese a sus excelentes propiedades nutricionales y sensoriales, anteriormente se habían visto relegadas al consumo en fresco. De hecho, las estadísticas indican que la

comercialización de frutas procesadas se encuentra en plena fase de crecimiento en España, alcanzando cifras tales como 1,5 millones de kilos de frutas preparadas en el año 2010, es decir, un 9,5% más respecto al año anterior (MAGRAMA, 2011).

## I.2 ASPECTOS GENERALES DEL KIWI

El kiwi (*Actinidia deliciosa*) es una fruta originaria del sur de China que se obtiene a partir de una planta trepadora cuyo cultivo con fines comerciales tuvo su origen en Nueva Zelanda en 1930, extendiéndose 40 años más tarde a otros países de zona templada (Childers et al., 1996; Ferguson et al., 1996; Nishiyama et al., 2005; Soufleros, 2001). Esta fruta presenta forma de elipse y su epidermis, recubierta de vellosidades, es de color pardo-verdoso. La pulpa está repleta de pequeñas semillas de color negro dispuestas en forma de círculo. En el centro se encuentra la columela, también comestible, de color blanco crema, con forma alargada en el sentido de la máxima longitud del fruto (Morley-Bunker y Lyford, 1999) (Figura I.1).


**Figura I.1.** Corte transversal del fruto kiwi (variedad Hayward)

Dentro de las distintas variedades cultivadas en todo el mundo, la variedad Hayward es la más difundida, en parte por sus adecuadas características agronómicas, tales como vigor, productividad, así como también por el adecuado tamaño y forma de los frutos. El fruto procedente de dicha variedad se caracteriza por una vellosidad suave, fácilmente eliminable y una pulpa que en la fase de madurez, además de mantenerse consistente presenta un color verde brillante y un equilibrio entre los sabores dulce y ácido (Gil, 2000).

En términos de producción y comercialización, el cultivo de kiwi ha mostrado un gran crecimiento a nivel mundial en los últimos tiempos (Fang et al., 2008). Tanto es así que su producción puede llegar a considerarse excedentaria. Nueva Zelanda es el principal productor de kiwi, aunque esta fruta también se cultiva en muchas otras zonas tales como Australia, Canadá, Estados Unidos, Chile, Japón y países mediterráneos (Fisk et al., 2006). Concretamente España dispone de unas condiciones climáticas favorables para su cultivo (Fúster et al., 1994) y la producción de kiwi es elevada, ascendiendo en el año 2013 a un total de 19.800 T (www.faostat.fao.org, 2015). A pesar de ello, España se ha convertido en uno de los principales importadores europeos de kiwi, dado su creciente consumo, que asciende ya aproximadamente a 2 kg/habitante y año (MAGRAMA, 2015).

El kiwi es una fruta que despierta el interés de los consumidores gracias a sus características sensoriales, valor nutricional y bajo aporte calórico (53-61 kcal/100g) (Tabla I.1). De hecho, el atractivo color y la equilibrada combinación de compuestos aromáticos y de sabores ácido y dulce propios de esta fruta, son algunos de los aspectos organolépticos más apreciados (García et al., 2012). Concretamente la variedad Hayward se distingue por presentar un color verde brillante que surge de la combinación de pigmentos que ésta contiene, es decir, de una mezcla de clorofilas (a y b) y carotenoides, principalmente 9'-*cis*-neoxanthina, violaxanthina, luteína y β-caroteno (Nishiyama et al., 2005). Otro aspecto destacable es su valor nutricional, ya que el kiwi se considera una fuente de vitaminas (C y E), minerales (calcio, hierro, potasio y fósforo), azúcares (glucosa, fructosa y sacarosa), ácidos orgánicos (cítrico, quínico, málico, galacturónico, succínico, oxálico, etc.), fibra y compuestos fenólicos (Beirão-da-Costa et al., 2006; Cassano et al., 2006; Du et al., 2009; Fang et al., 2008; Guldas, 2003; Kaya et al., 2008; Fúster et al., 1994; Jaeger et al., 2003; Soufleros et al., 2001).

**Tabla I.1.** Composición del kiwi por cada 100 g de parte comestible cruda.

| | | | |
|---|---|---|---|
| Agua (g) | 83,07 | Maltosa (g) | 0,19 |
| Proteínas (g) | 1,00-1,14 | Sacarosa (g) | 0,15-1,46 |
| Lípidos (g) | 0,44-0,52 | Vitamina A (Eq. Retinol µm) | 3 |
| Carbohidratos (g) | 12,10-14,66 | Tiamina (mg) | 0,027 |
| Pectina (g) | 0,30-1,10 | Riboflavina (mg) | 0,06 |
| Cloro (mg) | 65,00 | Niacina (mg) | 0,60 |
| Calcio (mg) | 34,00 | Ácido Pantoténico (mg) | 0,18 |
| Cobre (mg) | 0,13 | Piridoxina (mg) | 0,13 |
| Fósforo (mg) | 34,00 | Fenoles totales (g ácido gálico) | 0,4 |
| Hierro (mg) | 0,60-0,31 | Folatos totales (µm) | 29,30 |
| Magnesio (mg) | 17,00-27,00 | Vitamina C (mg) | 92,70-94,00 |
| Manganeso (mg) | 0,098-0,100 | Vitamina E (mg) | 1,46 |
| Potasio (mg) | 312,00 | Vitamina K (µm) | 40,30 |
| Sodio (mg) | 3,00-4,50 | Ácido cítrico (mg) | 990,00 |
| Zinc (mg) | 0,14 | Ácido málico (mg) | 500,00 |
| Galactosa (g) | 0,17 | Ácido oxálico (mg) | 0,18-1,63 |
| Glucosa (g) | 4,11-5,32 | Ácido quínico (mg) | 585,10 |
| Fructosa (g) | 4,35-4,92 | | |

(Adaptado de MAGRAMA, 2014)

Diversos autores señalan especialmente su aporte en vitamina C, muy superior al de otras frutas, vitamina E, fibra y ácido fólico, su elevada capacidad antioxidante (Antunes et al., 2010; Du et al., 2009; Park et al., 2011), así como sus niveles excepcionales de proteína soluble en comparación con otras frutas (Cassano et al., 2008). De hecho, se considera que la ingesta de tan sólo un kiwi aporta el 1,3% de la cantidad diaria recomendada de vitamina E, así como el 7% de ácido fólico, el 10% de fibra y el 100% de vitamina C (Cassano et al., 2008; Fiorentino et al., 2009; Hunter et al., 2010; Hunter et al., 2011). Sin embargo, su contenido en fenoles totales parece ser relativamente bajo en comparación con el encontrado en frutas como la manzana, fresa, uva o pomelo (Park et al., 2011). En relación a su contenido en micronutrientes y fitoquímicos se atribuyen diversos efectos beneficiosos al consumo regular de kiwi, tales como su contribución en la reducción del nivel de triglicéridos en sangre (15%) y la agregación plaquetaría (18%) (Park et al., 2011), su papel preventivo frente a enfermedades degenerativas (cáncer) y cardiovasculares (Du et al., 2009), su efecto positivo sobre el sistema digestivo y su

contribución al fortalecimiento del sistema inmunitario (Hunter et al., 2011). Incluso, existen evidencias de su uso con fines medicinales como parte de las tradiciones asiáticas (Hunter et al., 2010).

Además de todo lo mencionado hasta el momento, otro aspecto interesante a tener en cuenta sobre esta fruta, es su gran potencial para el procesado (Barboni et al., 2010). Aparte de su consumo en fresco, que asegura el máximo aprovechamiento de sus propiedades nutricionales y funcionales, la obtención de productos derivados, ofrece la posibilidad de aprovechar los destríos que, a causa de su aspecto y/o calibre inadecuados, no se consideran aptos para destinarse al consumo en fresco (Fúster et al., 1994), al mismo tiempo que permite poner a la disposición de los consumidores productos a base de kiwi que estén en consonancia con las tendencias de consumo actuales. Sin embargo, a día de hoy en España, el aprovechamiento de kiwi por parte de la industria alimentaria para la obtención de productos derivados, es muy limitado en comparación con el de otras frutas y su consumo en fresco sigue siendo la forma de ingesta habitual (www.faostat.org, 2013). A diferencia de España, otros países como China, comercializan una amplia gama de productos derivados de kiwi, destinando entre un 20 y un 35% del total de la producción de esta fruta a la obtención de zumos naturales y/o clarificados, zumos procedentes de concentrados, mermeladas, rodajas de kiwi en conserva, kiwi en almíbar, kiwi deshidratado y refrescos a base de kiwi, entre otros (Huang & Ferguson 2001).

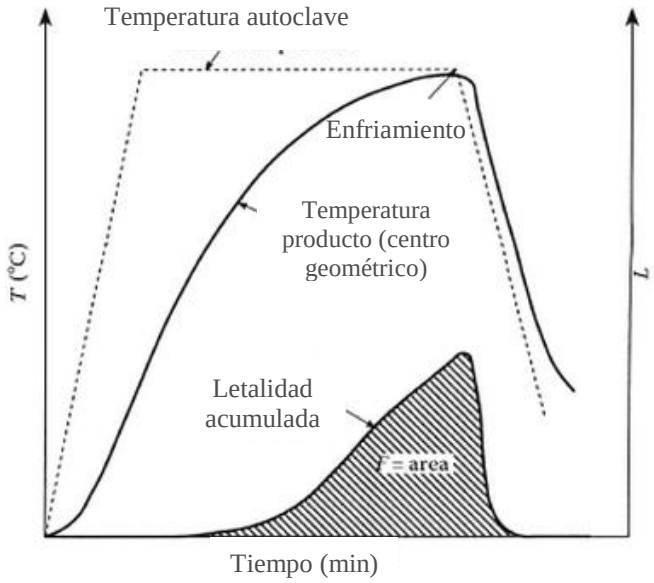
## I.3 PROCESADO TÉRMICO DE ALIMENTOS.PASTEURIZACIÓN

La aplicación de calor es probablemente la forma más antigua de preservar alimentos y, aún en la actualidad, se sigue considerando el pilar de los procesos de conservación y transformación empleados por la industria alimentaria (Awuah et al., 2007; Hernández & Sastre, 1999; Sun, 2012). El tratamiento térmico se basa en exponer el producto a una temperatura controlada durante un periodo de tiempo determinado, con el objetivo de preservar su vida útil, mediante la inactivación de microorganismos patógenos, que puedan suponer un riesgo potencial para la salud

del consumidor, microorganismos alterantes y/o enzimas que causen pérdidas de calidad (Awuah et al., 2007; Sun, 2012) y mejorar su palatabilidad (Richardson, 2001). Inevitablemente, este tipo de proceso, además de causar ciertos cambios deseables, siempre conlleva una pérdida de calidad del alimento, principalmente una degradación de nutrientes y alteración de las características organolépticas, que normalmente es proporcional a la carga térmica recibida por el mismo. Por tanto, seleccionar correctamente las condiciones de proceso es un aspecto primordial, ya que de ello no sólo depende poder asegurar la inocuidad del alimento a lo largo de su vida útil, sino también, la magnitud de las pérdidas de calidad, la regularidad de las características del alimento procesado, así como el consumo energético y los costes económicos asociados a dicho proceso (Moure et al., 1997).

Para diseñar un tratamiento térmico efectivo desde el punto de vista de la seguridad del alimento, resulta imprescindible conocer la evolución de la temperatura del producto durante el proceso y el impacto del mismo en el microorganismo patógeno de mayor relevancia (Awuah et al., 2007). El objetivo primordial de cualquier tratamiento térmico de conservación es asegurar una inactivación suficiente del microorganismo seleccionado en el punto del alimento más desfavorable de forma que la inocuidad del producto quede garantizada. De hecho, las condiciones de proceso de los tratamientos térmicos de conservación se establecen en base a dos premisas que permiten determinar la severidad o letalidad de los mismos: (i) la resistencia del microorganismo seleccionado para cada alimento y (ii) la carga térmica recibida por el producto en el punto más desfavorable. Además, siempre debe tenerse en cuenta que las características físicas propias de cada alimento así como del microorganismo pueden afectar marcadamente a la letalidad del proceso (Awuah et al., 2007, Wang & Sun, 2012). Por tanto, el perfil térmico del alimento y la cinética de inactivación del microorganismo objetivo son herramientas clave a la hora de obtener la información necesaria para establecer las condiciones de cada tratamiento. A modo de ejemplo, la Figura I.2 muestra la evolución de la temperatura de un alimento sometido a un proceso de esterilización en autoclave, así como la letalidad asociada al mismo. En dicha figura puede verse claramente como el tratamiento consiste en un ciclo

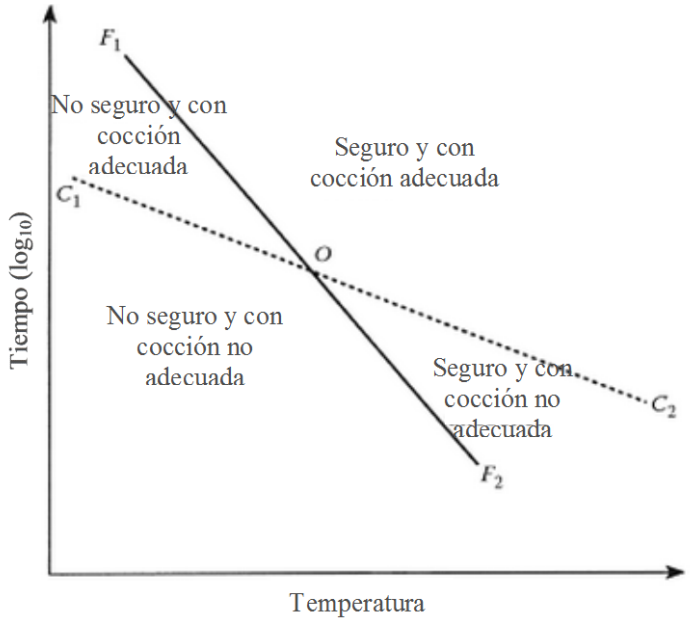
controlado de calentamiento, mantenimiento y enfriamiento que permite alcanzar la letalidad requerida.




Fuente: Adaptado de Holdsworth, 2009

**Figura I.2**. Perfil térmico del autoclave (.....) y del producto (—) durante un tratamiento de esterilización y la letalidad asociada al proceso (▨)

Tal y como se ha mencionado anteriormente, la letalidad es un factor clave a la hora de diseñar un proceso térmico, sin embargo, también deben valorarse los atributos de calidad del producto, ya que se puede alcanzar una letalidad equivalente mediante múltiples combinaciones de tiempo y temperatura, sin embargo, cada una de ellas puede tener un impacto muy diferente sobre el valor nutricional y funcional y la calidad sensorial del alimento (Figura I.3). Por este motivo, es habitual recurrir a la modelización matemática con el fin de optimizar los tratamientos térmicos, asegurando la máxima inactivación de microorganismos y enzimas pero la mínima pérdida de calidad (Hernandez & Sastre, 1999; Wang & Sun, 2012). Esta optimización principalmente se basa en aplicar el concepto HTST (high-temperature short-time), dada la menor termorresistencia de los microorganismos que de los compuestos que se utilizan como indicadores de calidad de los alimentos, tales como vitaminas, pigmentos, etc. (Awuah et al., 2007).




Fuente: Adaptado de Holdsworth, 2009

**Figura I.3**. Diagrama de optimización de un tratamiento térmico. Evolución de la letalidad (—) versus grado de cocción (.....) del producto en función de la temperatura y tiempo de tratamiento.

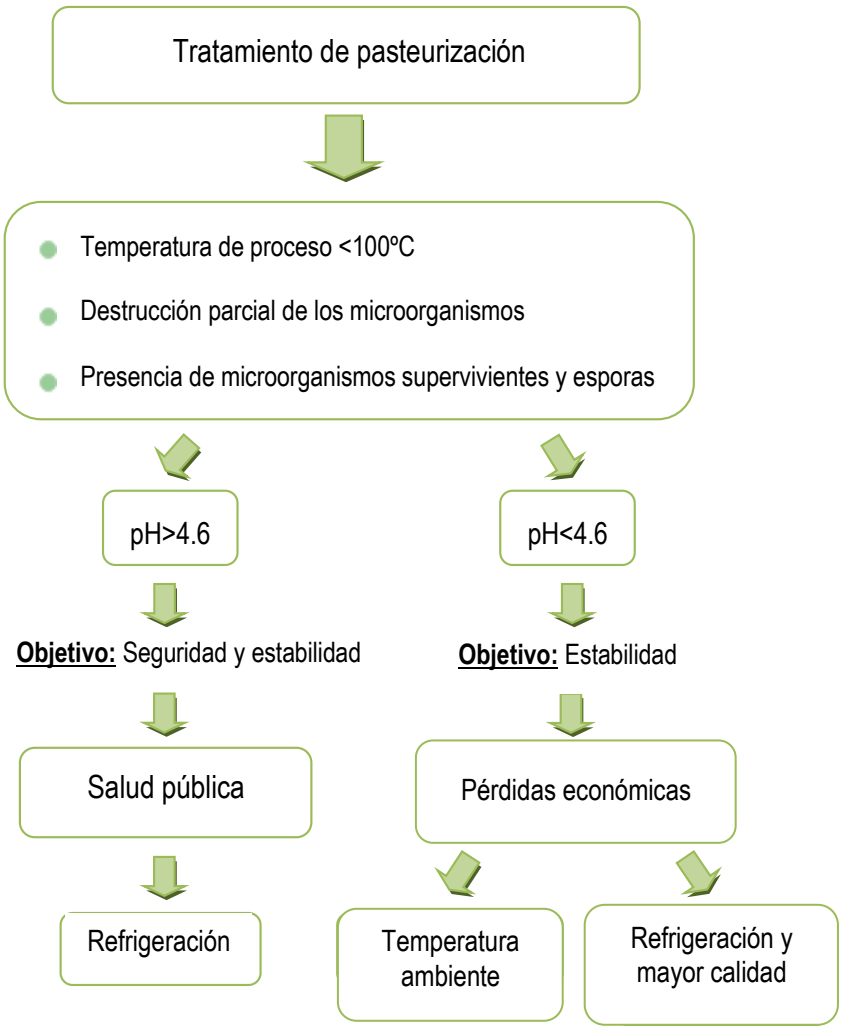
Además de todos estos aspectos, cabe tener en cuenta que en la actualidad, el procesado térmico de los alimentos no debe considerarse como una mera herramienta de conservación, ya que también juega un papel muy importante en la comercialización de productos alimenticios acordes a las necesidades y exigencias de los consumidores en cada momento (Deliza et al., 2005; Richardson, 2001). Las tendencias de consumo cambian a medida que lo hacen las necesidades de los consumidores y la industria agroalimentaria debe adaptarse a tales cambios, apostando por la innovación y el desarrollo de nuevos procesos de conservación y transformación de alimentos, que permitan ofrecer productos que se ajusten a las expectativas de los consumidores (Jaeger et al., 2003), es decir, alimentos seguros y con una vida útil relativamente larga, pero cuyas características sean muy similares a las del producto fresco (Richardson, 2001; Sun, 2012).

La pasteurización es uno de los distintos procesos térmicos de conservación comúnmente utilizados por la industria alimentaria y puede definirse como un tratamiento que permite alargar la vida útil de los alimentos mediante la inactivación de las células vegetativas de microorganismos patógenos y alterantes que éstos puedan contener. Tradicionalmente, la pasteurización se ha catalogado como un tratamiento térmico de carácter suave (50-90ºC) que reduce de forma parcial la flora microbiana del alimento, dando lugar a un producto estable pero con una vida útil relativamente corta, en comparación con otros tratamientos (Marques da Silva & Gibbs, 2009; Awuah, 2007). Recientemente, el término pasteurización ha sido redefinido a fin de englobar también aquellos procesos de conservación basados en el uso de tecnologías alternativas, dado el creciente interés que ha despertado la posibilidad de incorporar estas nuevas tecnologías al procesado de alimentos en las últimas décadas. Es por ello, que en la actualidad, se entiende por pasteurización cualquier tratamiento o proceso al que se someta un alimento que permita reducir el contenido del microorganismo patógeno de mayor relevancia, hasta un nivel tal que no represente un riesgo potencial para la salud del consumidor (NACMCF, 2006).

Pese a que la vida útil de los productos pasteurizados puede considerarse relativamente corta, en los últimos tiempos la pasteurización ha adquirido un gran protagonismo dentro de los tratamientos de conservación empleados por la industria alimentaria, gracias a que permite una mejor preservación de las propiedades del alimento que otros procesos de mayor intensidad (Awuah et al., 2007). Dadas las nuevas tendencias de consumo y la creciente demanda de productos mínimamente procesados, a día de hoy, la pasteurización se utiliza para preservar una amplia gama de alimentos, tales como leche y productos lácteos en general (queso, nata, etc.), zumos de fruta, cerveza, productos cárnicos (jamón cocido), productos derivados de pescado (salmón ahumado), salsas y encurtidos, entre otros (Hernandez & Sastre, 1999; Jay et al., 2005).

Los criterios a seguir a la hora de diseñar un tratamiento de pasteurización pueden variar con cada producto. Antes de seleccionar las condiciones de tratamiento más adecuadas, la industria alimentaria debe plantearse ciertos aspectos como, por ejemplo, si el alimento será comercializado en refrigeración o si

irá destinado a grupos específicos de la población, tales como ancianos o niños. Además, el proceso siempre debe establecerse en base a las características propias de cada alimento, para poder garantizar la obtención de productos seguros y estables pero de gran calidad y con una vida útil determinada (Marques da Silva & Gibbs, 2009). Tal y como se puede observar en la Figura I.4, el pH se considera un factor clave que determina en gran medida la vida útil de los alimentos pasteurizados. A diferencia de otros tratamientos de mayor intensidad, como por ejemplo la esterilización, la pasteurización tan sólo reduce una parte de los microorganismos presentes en el alimento, de forma que la flora microbiana restante y las esporas que éste pudiera contener, quedan activas y pueden crecer y desarrollarse durante la vida útil del producto (Hernandez & Sastre, 1999). En alimentos de baja acidez (pH>4,6), las esporas y microorganismos que sobreviven al tratamiento pueden germinar y desarrollarse con relativa facilidad, por ello, deben ser almacenados en refrigeración y/o a vacío y por lo general su vida útil tiende a ser corta (días o semanas), exceptuando aquellos casos en los que la propia composición del alimento no favorece el crecimiento microbiano, como por ejemplo productos con un elevado contenido de azúcar o sal (Awuah et al., 2007). Por el contrario, los alimentos de alta acidez (pH<4,6), en general se consideran estables a temperatura ambiente tras la pasteurización, precisamente gracias a su bajo pH, presentando una vida útil considerablemente larga (meses). No obstante, en ciertos casos, se puede optar por someter a este tipo de alimentos a un tratamiento más suave, aunque posteriormente se requiera de su almacenamiento en refrigeración, con el fin de preservar en mayor medida la calidad del producto (Hernandez & Sastre, 1999).




Fuente: Adaptado de Marques da Silva & Gibbs (2009).

**Figura I.4.** Tratamiento térmico de pasteurización.

Una vez se ha reunido toda la información necesaria referente al alimento, el diseño del proceso debe comenzar por la identificación de los microorganismos (patógenos o alterantes) capaces de crecer en el mismo y las enzimas que puedan alterar sus características durante el almacenamiento. Posteriormente, se debe recabar información relativa a la resistencia de dichos microorganismos y enzimas en el producto en cuestión, con el fin de seleccionar a uno de ellos como indicador de la efectividad del tratamiento. Con toda esta información, ya es posible establecer un proceso que permita alcanzar la letalidad requerida, que en última instancia, siempre deberá ser validado de forma experimental (Marques da Silva & Gibbs, 2009).

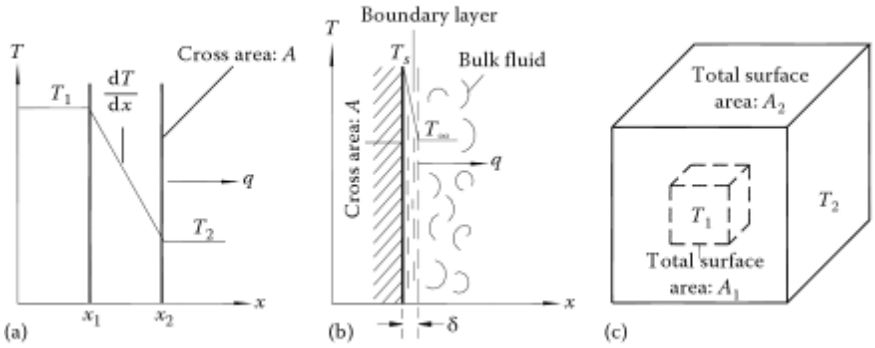
## I.4. TECNOLOGÍAS DE PASTEURIZACIÓN TÉRMICA

Durante el siglo pasado, las tecnologías térmicas de conservación han evolucionado de manera asombrosa, llegando a desarrollarse tecnologías alternativas que ofrecen diversas ventajas respecto a los métodos de calentamiento convencional y que presentan un gran potencial para el procesado de alimentos, tales como el calentamiento óhmico, el calentamiento dieléctrico (microondas y radiofrecuencia) y el calentamiento inductivo o por infrarrojos (Vicente & Castro, 2007). A continuación se comentan con más detalle algunos aspectos de las tecnologías térmicas utilizadas en el desarrollo de la presente tesis doctoral, como son el calentamiento convencional y el calentamiento por microondas ambos con el fin de conseguir la pasteurización del alimento procesado.

### I.4.1. Calentamiento convencional

#### *I.4.1.1.Fundamentos*

Cuando un alimento entra en contacto con una fuente de calor externa o medio calefactor, ya sea de forma directa o indirecta, se produce una transferencia de calor, es decir, un intercambio de energía térmica desde el medio calefactor al alimento, a causa de una diferencia de temperaturas (Holdsworth, 2009; Toledo, 2007). Dicha transferencia de calor puede tener lugar a través de tres mecanismos: (i) conducción, (ii) convección y (iii) radiación (Figura I.5). En términos generales se considera que cualquier tratamiento térmico implica una combinación de estos tres mecanismos, no obstante, uno de ellos siempre tiende a predominar sobre el resto.




Fuente: Wang and Sun (2012).

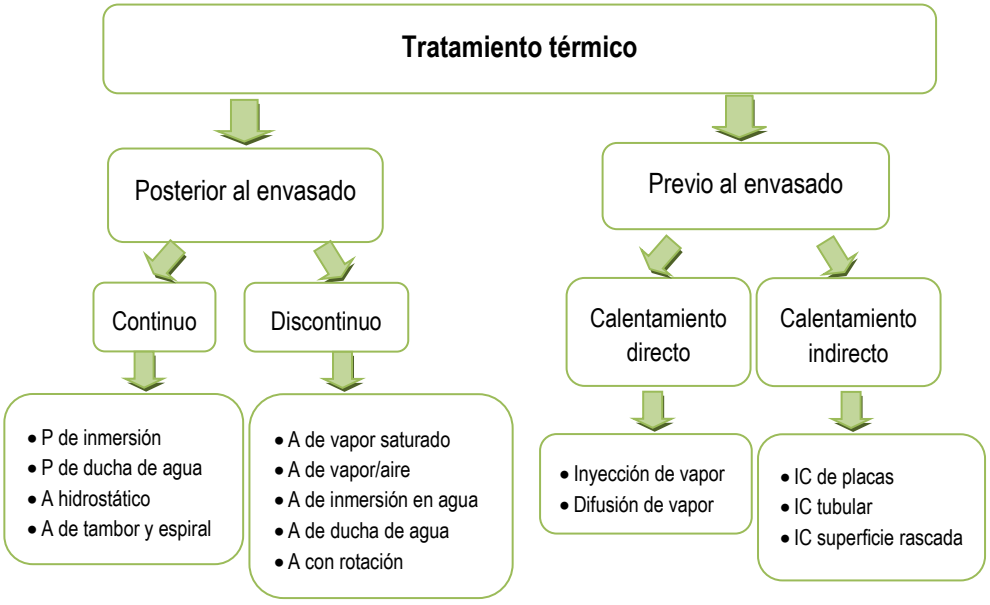
**Figura I.5.** Esquema de los tres mecanismos mediante los que se lleva a cabo la transferencia de calor: (a) conducción a través de una placa uniforme, (b) convección a través de una pared vertical, (c) radiación entre la superficie de un cuerpo (1) y sus alrededores (2).

Por conducción se entiende la transferencia de calor que tiene lugar entre moléculas adyacentes de elementos sólidos o fluidos estacionarios, debido a la vibración y/o colisión de sus partículas. En aquellos procesos en los que el calor se transmite mayoritariamente por conducción, es frecuente observar un gradiente de temperaturas entre el interior y el exterior del alimento (Toledo, 2007; Wang & Sun, 2012). Por otro lado, se habla de convección cuando la transferencia de calor ocurre entre moléculas que no son adyacentes, mecanismo que se observa fundamentalmente en fluidos, pudiendo darse de forma natural o forzada (Toledo, 2007). Por último, a diferencia de la conducción y la convección, la transferencia de calor por radiación no requiere de un medio que actúe como transmisor, ya que la fuente de calor emite ondas electromagnéticas que hacen posible dicha transmisión directamente al alimento (Wang & Sun, 2012).

### *I.4.1.2. Sistemas de tratamiento por calentamiento convencional*

El calentamiento convencional sigue siendo, aún a día de hoy, la forma más común de procesar los alimentos (Fang et al., 2008). Prueba de ello, es la gran diversidad de equipos y sistemas industriales que han sido diseñados para llevar a cabo procesos basados en la aplicación de esta tecnología (Holdsworth, 2009).

La Figura I.6 muestra una clasificación de los distintos equipos utilizados en la actualidad por la industria alimentaria para pasteurizar y/o esterilizar los alimentos mediante la aplicación de calentamiento convencional. Dentro de los distintos sistemas de procesado, es posible apreciar que ciertos aspectos tales como si el alimento a tratar está o no envasado, si el proceso se llevará a cabo en continuo o discontinuo, o si el calentamiento tendrá lugar de forma directa o indirecta, pueden marcar grandes diferencias entre las características de los distintos equipos (Richardson, 2001).




P: pasteurizador; A: autoclave; IC: intercambiador de calor.

Fuente: Adaptado de Richardson (2001).

**Figura I.6.** Sistemas de procesado y equipos industriales basados en la aplicación de calentamiento convencional para preservar los alimentos.

Con el paso del tiempo, el diseño de los diferentes equipos así como las técnicas de procesado han evolucionado considerablemente, claro ejemplo de ello son los autoclaves rotativos y el concepto de envasado aséptico. En comparación con los pasteurizadores y/o esterilizadores más convencionales, ambos han permitido reducir el impacto negativo del calentamiento en la calidad de los alimentos al mismo tiempo que mejorar la eficiencia de los tratamientos. A continuación se describen brevemente algunas de sus principales características.

- *Autoclaves con sistema de agitación*

A diferencia de los autoclaves convencionales, estos equipos posibilitan la agitación mecánica del alimento durante el procesado (Figura I.7), pudiendo ser ésta de tipo axial o tapa-fondo-tapa. Gracias a dicha agitación la transferencia de calor entre el medio calefactor y el alimento tiene lugar a mayor velocidad y además el calentamiento resulta más uniforme. En consecuencia, los tiempos de proceso se reducen, al igual que también lo hace la pérdida de calidad del producto a causa del tratamiento (Lespinard et al., 2012; Richardson, 2001).

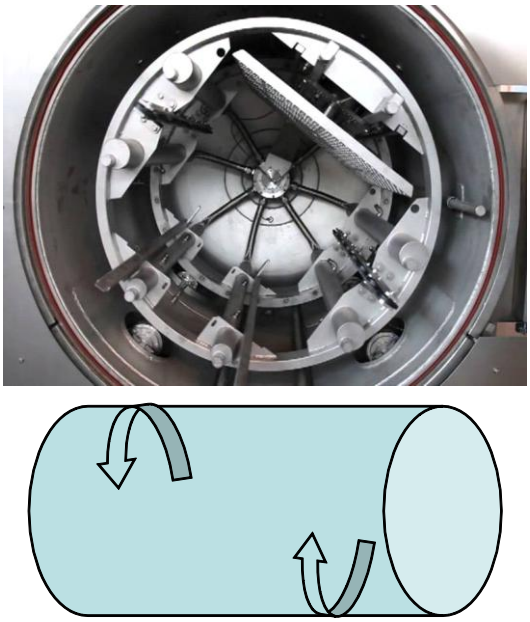


**Figura I.7.** Ejemplo de autoclave rotativo.

Este tipo de autoclaves se han utilizado en la producción comercial de diversos tipos de alimentos tales como conservas de guisantes, maíz, espárragos, champiñones, etc. Sin embargo, esta técnica puede no resultar muy provechosa en determinados casos, como por ejemplo cuando se trata de alimentos sólidos, como una conserva de atún o salmón, o por el contrario, productos líquidos, en los que fácilmente se producen corrientes de convección (Awuah et al., 2007).

- *Envasado aséptico*

Esta técnica de procesado se basa en aplicar el concepto *high-temperature short-time* (HTST) y/o *ultra-high-temperature* (UHT), es decir, llevar a cabo un tratamiento térmico en un intercambiador de calor (Figura I.8) durante un periodo de tiempo considerablemente más corto al requerido en aquellos procesos en los que el tratamiento se aplica sobre alimento envasado, seguido de una fase de enfriamiento rápido que minimiza el impacto negativo del calentamiento y en última instancia, el envasado aséptico del producto procesado (Awuah et al., 2007; Lespinard et al., 2012).

La principal ventaja de esta técnica, en comparación con los procesos convencionales, es la mayor velocidad a la que tiene lugar la transferencia de calor y la mayor eficiencia energética. A diferencia de los procesos de autoclavado en los que la letalidad requerida se alcanza entre el final de la etapa de calentamiento y el principio de la etapa de enfriamiento, en los procesos HTST y UHT, los objetivos del tratamiento se alcanzan mientras que el alimento se encuentra en el interior del intercambiador de calor a una temperatura constante y en cuestión de segundos (Awuah et al., 2007; Lespinard et al., 2012).

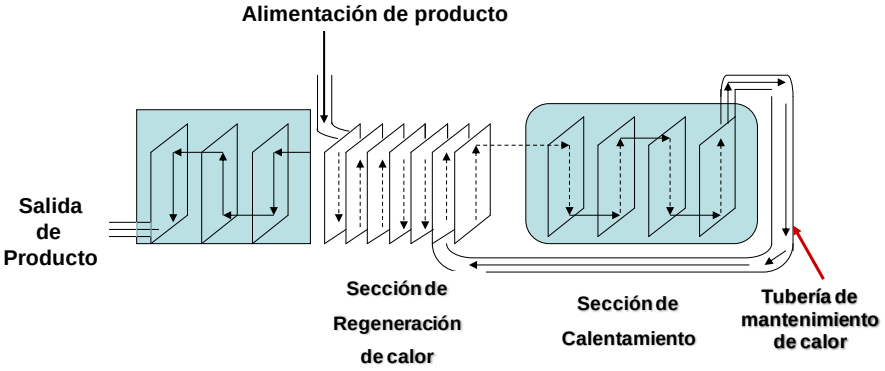



**Figura I.8.** Esquema de un intercambiador de calor de placas.

El envasado aséptico se considera toda una revelación a la hora de abordar el procesado de una gran variedad de alimentos líquidos, permitiendo una mejor preservación de la calidad de los mismos sin comprometer la seguridad. De hecho, en la actualidad, se utiliza para procesar diversos productos tales como leche, zumos de fruta, yogur, huevo, helado, queso y alimentos infantiles. Sin embargo,

esta técnica también presenta ciertas limitaciones, como por ejemplo, la dificultad para inactivar enzimas de elevada termorresistencia o para procesar alimentos que contengan partículas de gran tamaño (Awuah et al., 2007).

### *I.4.1.3. Parámetros de influencia en el proceso*

Diversos factores relacionados con las características del alimento, el sistema de procesado y las condiciones en las que se lleve a cabo el proceso en sí mismo, influyen considerablemente en la velocidad a la que tiene lugar la transferencia de calor durante el tratamiento, y por tanto también lo hacen sobre el tiempo de proceso, la eficiencia energética del mismo, la homogeneidad de calentamiento y en consecuencia, sobre la calidad del producto resultante (Holdsworth, 2009; Wang & Sun, 2012).

En primer lugar, deben considerarse aquellos factores relativos al propio proceso como: (i) la temperatura inicial del producto, (ii) si se trata de un proceso en continuo o discontinuo, (iii) si se lleva a cabo previa o posteriormente al envasado del producto y (iv) el tiempo de tratamiento (Richardson, 2001). Además, tal y como se ha mencionado en la sección anterior (Figura I.6), dada la diversidad de sistemas industriales disponibles en la actualidad para tratar térmicamente los alimentos, deben tenerse en cuenta aspectos relativos al equipo como: (i) el tipo de medio calefactor (agua o vapor), (ii) si es posible aplicar agitación mecánica durante el tratamiento y (iii) si el calentamiento se llevará a cabo de forma directa o indirecta. Por último, también es importante tener en cuenta las características propias de cada alimento tales como: (i) composición, (ii) propiedades térmicas (calor específico, entalpía asociada a las transiciones de fase, difusividad, conductividad), (iii) propiedades geométricas (densidad, porosidad y dimensiones), y (iv) propiedades reológicas (consistencia y viscosidad) (Wang & Sun, 2012).

### *I.4.1.4. Mecanismos de acción*

La inactivación tanto microbiológica como enzimática que tiene lugar durante el calentamiento convencional se debe única y exclusivamente a efectos térmicos, es decir, a la exposición de los alimentos a las elevadas temperaturas. La energía térmica puede causar cambios estructurales en las células, así como afectar la estabilidad y funcionalidad de las macromoléculas, alterando los procesos biológicos y causando la desnaturalización de proteínas y ácidos nucleicos, lo que induce la inactivación de microorganismos y enzimas (Cleary & Janes, 1977).

### *I.4.1.5. Aplicaciones y tendencias actuales*

El tratamiento térmico convencional es la base de los procesos de conservación empleados para preservar y transformar los alimentos, tanto a nivel industrial como doméstico (Fang et al., 2008). En la actualidad, la industria alimentaria aplica esta tecnología en múltiples procesos tales como cocción o pre-cocción, fritura, descongelado, horneado, deshidratación, escaldado, tostado, pasteurización o esterilización.

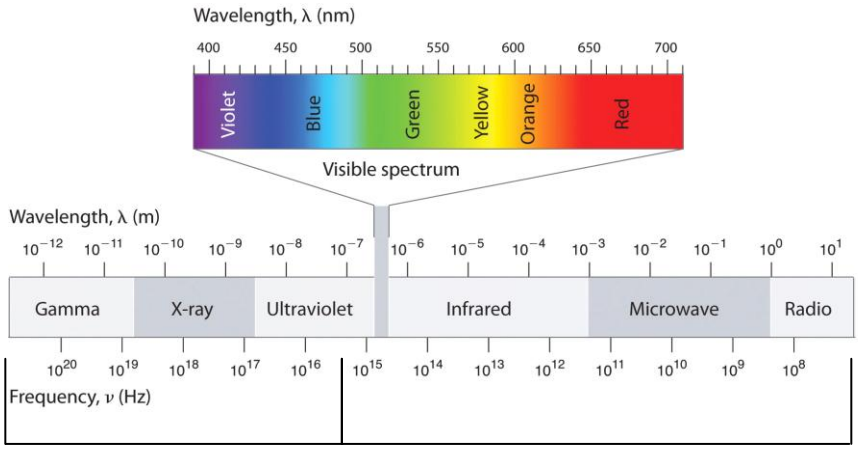
Al igual que las preferencias de los consumidores han ido evolucionando con el tiempo, el modo de aplicar el calentamiento convencional a los procesos de conservación de alimentos también lo ha hecho, como ya se ha mencionado. Dadas las nuevas tendencias de consumo y la creciente demanda de productos mínimamente procesados, los equipos empleados por la industria alimentaria, así como también los materiales de envasado y los propios tratamientos, han evolucionado con el fin de minimizar el impacto negativo del calentamiento en la calidad de los alimentos (Gonçalves et al., 2010). Si bien es cierto que dichos cambios pueden desencadenar en un incremento de los precios de los productos procesados, actualmente los consumidores parecen estar dispuestos a pagar más por aquellos productos que cumplan con sus expectativas (Balsa-Canto et al., 2002). En este sentido, el rotundo éxito de los tratamientos HTST y UHT frente a los procesos de esterilización en autoclave, la cada vez más amplia gama de alimentos pasteurizados disponibles en el mercado pese a su relativamente corta vida útil y la

preferencia de los envases de plástico flexibles como alternativa al vidrio o la hojalata, pese a que pueden requerir una mayor atención durante el procesado debido al mayor riesgo de daño a causa de los drásticos cambios de presión y temperatura a los que se puede ver sometido durante el mismo, pueden considerarse como algunos de los cambios más destacables de las últimas décadas (Awuah et al., 2007; Balsa-Canto et al., 2002).

## I.4.2. Calentamiento por microondas

### *I.4.2.1.Fundamentos*

Las microondas son ondas de radiación electromagnética no ionizante cuya frecuencia (300MHz y 300GHz) se sitúa entre la de los rayos infrarrojos y la de las ondas de radio y televisión (Figura I.9). La energía microondas presenta gran variedad de aplicaciones en el campo de los procesos térmicos empleados en la industria química y, más recientemente, también en el ámbito alimentario (Astigarraga-Urquiza y Astigarraga-Aguirre, 1995).

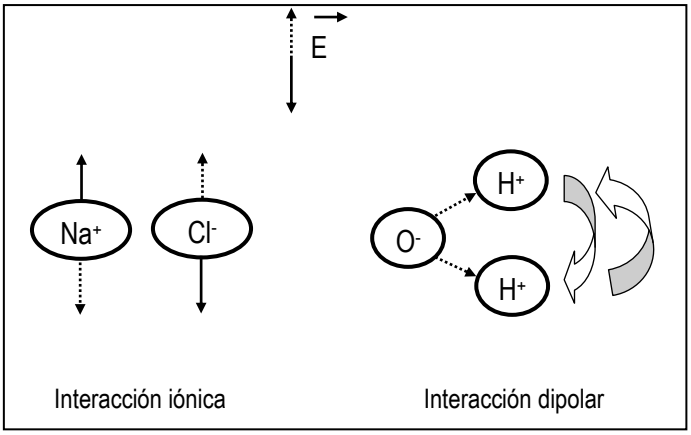



Fuente: Adaptado de Schubert & Regier (2005)

**Figura I.9.** Espectro electromagnético.

En los procesos basados en la aplicación de microondas tiene lugar una generación interna de calor o calentamiento volumétrico. Aunque de forma paralela

comienzan a producirse también fenómenos de convección y conducción, éstos ocurren a escalas de tiempo muy diferentes (Wang & Sun, 2012). Los alimentos expuestos a un campo electromagnético se calientan al absorber parte de la radiación emitida y transformarla en energía térmica a través de dos mecanismos: la rotación dipolar y la migración iónica (Figura I.10).




Fuente: Adaptado de Schubert & Regier (2005)

**Figura I.10.** Mecanismos de generación de calor en un alimento durante su exposición a energía microondas.

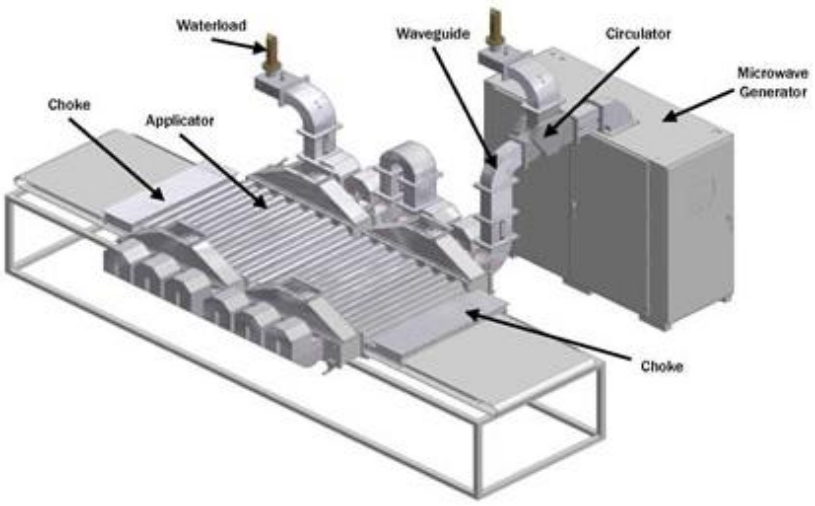
La rotación dipolar es el mecanismo de calentamiento más relevante y consiste en la reorientación de los dipolos o moléculas polares y/o cargadas negativa y positivamente, fundamentalmente agua, en el campo electromagnético, generando una fricción intermolecular (Chandrasekaran et al. 2013; Salazar-González et al., 2012; Vadivambal & Jayas, 2010; Wang & Sun, 2012). La migración iónica también contribuye en cierta medida al calentamiento del producto mediante la generación de energía cinética y su posterior conversión en energía térmica. Los iones presentes en los alimentos se ven forzados a migrar, primero en una dirección y posteriormente en la contraria, a causa del campo electromagnético alternante al que se ven expuestos y acaban colisionando entre sí y con otras moléculas (Salazar-González et al., 2012).

### *I.4.2.2. Sistemas de tratamiento por microondas*

Todo equipo microondas consta de tres componentes básicos: (i) fuente de energía microondas o magnetrón, (ii) dispositivo de guía de ondas y (iii) aplicador. Las características más relevantes de cada uno de ellos se describen a continuación (Schubert & Regier, 2005):

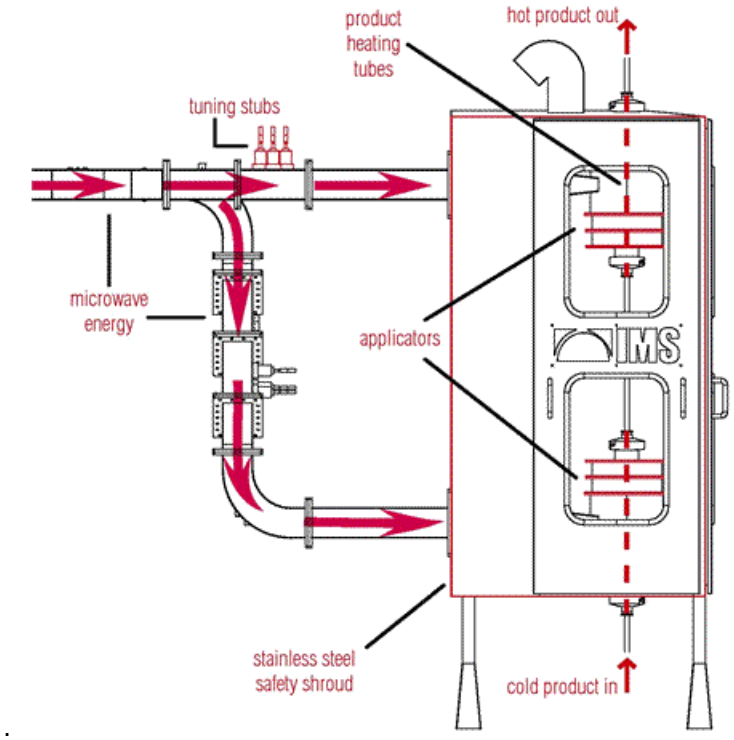
- El magnetrón, elemento generador de las microondas, es un tubo a vacío que contiene un cátodo emisor de electrones, ubicado en la parte central, que, a su vez, se encuentra rodeado por un ánodo cuya estructura contiene una serie de cavidades y que posee la frecuencia de resonancia prevista.
- Los dispositivos de guía de ondas son tubos que permiten transportar las microondas desde el magnetrón hasta el aplicador.
- El aplicador es un elemento que contiene y distribuye la energía microondas previamente a su interacción con el alimento, asegurando la máxima uniformidad del campo electromagnético generado. Se distinguen diversos tipos de aplicadores tales como el de campo cercano, monomodo y multimodo. El aplicador multimodo es el que contienen la gran mayoría de microondas, tanto de uso doméstico como industrial, ya que, en comparación con los otros tipos de aplicadores, favorece una mayor absorción de radiación por parte del producto, permitiendo incrementar la eficiencia del proceso.

Actualmente, la aplicación de energía microondas con fines domésticos es la forma de uso de esta tecnología más extendida, ya que, diversos factores dificultan su explotación a nivel industrial. Sin embargo, recientemente, se han desarrollado sistemas que permiten procesar los alimentos mediante la aplicación de energía microondas en continuo y cuyas características hacen factible su utilización por parte de la industria alimentaria de forma rentable y competitiva. A modo de ejemplo, en las Figuras I.11 y I.12, se muestran dos sistemas industriales de aplicación de microondas (sistema lineal y sistema circular) que actualmente se utilizan para procesar alimentos en países como Estados Unidos, Bélgica, Italia y Holanda (www.microwaveheating.wsu.edu, 2014).




Fuente: www.industrialmicrowave.wsu.edu (2014)

**Figura I.11.** Sistema lineal de calentamiento por microondas.




Fuente: www.industrialmicrowave.wsu.edu (2014)

**Figura I.12.** Sistema circular de calentamiento por microondas.

El sistema lineal de calentamiento por microondas (Figura I.11) presenta potenciales aplicaciones en el ámbito de la deshidratación de alimentos, pudiendo emplearse para llevar a cabo un proceso completo o bien, en combinación con

secadores convencionales, para mejorar alguna de las etapas de pre o post-tratamiento. Por otro lado, el sistema circular de calentamiento por microondas (Figura I.12) ha sido específicamente diseñado para llevar a cabo operaciones de atemperado, cocción, pasteurización y esterilización de alimentos (www.industrialmicrowave.wsu.edu, 2014).

Estos equipos, al igual que los de uso doméstico, se componen básicamente de un magnetrón, pudiendo contener uno o varios en función de la potencia de los mismos, guías de ondas y aplicadores. Además, contienen un sistema de control, que permite monitorear y controlar el proceso, así como programar o incluso automatizar las funciones del equipo en todo momento, y sensores de seguridad, mediante los que se controla de forma continua los posibles escapes o fugas de energía microondas del equipo. Particularmente, el sistema lineal de calentamiento por microondas dispone de una cinta a través de la cual circula el producto durante el proceso, mientras que, en el caso del sistema circular, el producto se transporta a través de un tubo (tubo de calentamiento).

De entre todos estos componentes, el aplicador se considera un elemento clave, ya que, al regular y distribuir el campo electromagnético previamente a su entrada en contacto con el producto, permite exponer al alimento a las microondas de forma regulada, uniforme y facilitando que éstas se absorban de manera eficiente y homogénea. Por ello, se considera la esencia del equipo y de su diseño depende en gran medida el éxito y efectividad del proceso.

### *I.4.2.3. Parámetros de influencia en el proceso*

Diversos factores relacionados con las características del alimento, el equipo microondas utilizado y las condiciones en las que se lleve a cabo el proceso pueden afectar marcadamente a la distribución de temperaturas en el producto durante el tratamiento, y por tanto, comprometer la efectividad del mismo (Chandrasekaran et al. 2013; Salazar-González et al., 2012; Vadivambal & Jayas, 2010; Wang & Sun, 2012).

Los aspectos referentes al diseño y las características propias de cada equipo microondas pueden influir sobre la uniformidad del campo electromagnético generado y, en consecuencia, sobre la mayor o menor homogeneidad de calentamiento, como es el caso de (Salazar-González et al., 2012): (i) las dimensiones del equipo, (ii) la potencia y ubicación del magnetrón, (iii) y el diseño y buen funcionamiento de los aplicadores. Por otro lado, las características del alimento tales como: (i) composición, especialmente en términos de humedad y contenido en sal, (ii) propiedades térmicas (difusividad y conductividad), (iii) propiedades dieléctricas (factor de almacenamiento y factor de pérdidas), (iv) propiedades geométricas (densidad, porosidad, forma y dimensiones) y (v) propiedades reológicas (consistencia y viscosidad), influyen sobre su interacción con el campo electromagnético, repercutiendo sobre la cantidad de radiación absorbida y la disipación de calor, consecuentemente, la distribución de temperaturas en el alimento también se ve afectada (Chandrasekaran et al. 2013; Salazar-González et al., 2012). Por último, también deben considerarse aquellos factores relativos al propio proceso como: (i) la temperatura inicial del producto, (ii) si se trata de un proceso en continuo o discontinuo, (iii) la frecuencia del campo electromagnético (2450MHz y/o 915MHz, 896MHz en Estados Unidos y Europa, respectivamente), (iv) el tiempo de tratamiento y (v) la potencia de microondas, tanto la emitida como la absorbida por el producto (Salazar-González et al., 2012; Wang & Sun, 2012).

### *I.4.2.4.Mecanismos de acción*

La inactivación tanto microbiológica como enzimática que tiene lugar durante los procesos basados en la aplicación de energía microondas se puede explicar en base a efectos térmicos (ver sección I.4.1.4.) y efectos no térmicos.

- *Efectos no térmicos*

La capacidad de las microondas para inactivar microorganismos y enzimas ha sido objeto de numerosos estudios. Algunos de ellos ponen de manifiesto la mayor efectividad de la energía microondas respecto a métodos de calentamiento

convencional. Desde 1960 se baraja la hipótesis de que la energía térmica no es la única causa de la inactivación observada en los procesos basados en la aplicación de microondas. Probablemente, Olsen et al. (1966) fueron los primeros autores en apoyar la idea de la existencia de efectos no térmicos asociados a la aplicación de energía microondas, que contribuían de forma significativa a la inactivación microbiológica y enzimática. Desde entonces, se ha especulado considerablemente al respecto y se han llevado a cabo numerosos estudios con el fin de aclarar la existencia de tales efectos (Banik et al., 2003; Shazman et al., 2007). Sin embargo, la disparidad de opiniones sobre el tema hace que, aún a día de hoy, esta cuestión siga causando gran controversia (Banik et al., 2003; Fujikawa et al., 1992). Según la revisión recientemente publicada en referencia a este tema por Chandrasekaran et al. (2013), existen diversas teorías que explican y apoyan la existencia de los efectos no térmicos asociados a las microondas. Estas teorías se mencionan y describen a continuación:

- *Calentamiento selectivo.* El alimento puede calentarse de forma selectiva, es decir, las células de los microorganismos y las enzimas pueden alcanzar temperaturas superiores a las del fluido que los rodea durante la exposición a las microondas, causando una destrucción más rápida de los mismos.
- *Electroporación.* A causa del potencial eléctrico al que se ven expuestas las células durante los tratamientos por microondas se forman poros en sus membranas, provocando cambios en su permeabilidad, pérdida del contenido celular, pérdida de funcionalidad y consecuentemente su muerte.
- *Ruptura de la membrana celular.* La rápida oscilación de las moléculas que tiene lugar durante la exposición del alimento al campo electromagnético, puede exceder el límite elástico de la membrana celular provocando su ruptura. En consecuencia, se produce la pérdida de contenido celular, pérdida de la funcionalidad celular y finalmente muerte de la célula, como se ha mencionado anteriormente.
- *Acoplamiento del campo magnético.* Esta teoría plantea que la exposición al campo electromagnético puede afectar a moléculas esenciales para la vida de la célula, como son las proteínas o el ADN.

Pese a la existencia de tales teorías, se ha demostrado la incapacidad de las microondas para inactivar enzimas y/o microorganismos en ausencia del estrés causado por bajo pH o por la energía térmica, por lo que hasta el momento, no ha sido posible corroborar la existencia de efectos no térmicos. Sin embargo, existen evidencias de que la aplicación de energía microondas potencia o magnifica de forma significativa los efectos térmicos a la hora de inactivar enzimas y microorganismos (Chandrasekaran et al. 2013).

### *I.4.2.5.Ventajas e inconvenientes*

Las principales ventajas e inconvenientes de la tecnología microondas en comparación con los métodos de calentamiento convencional se exponen y comentan brevemente a continuación.

**I. Ventajas**

- *Mayor velocidad de calentamiento.* A diferencia de los procesos basados en el calentamiento convencional, donde el calor se transmite al alimento principalmente por conducción, cuando los alimentos se exponen a la energía microondas tiene lugar una generación interna de calor o calentamiento volumétrico. Como consecuencia, la temperatura del producto aumenta mucho más rápidamente y no existe un gradiente de temperaturas tan marcado entre el interior y la superficie del mismo (De Ancos de et al., 1999).
- *Menores tiempos de proceso.* Al tener lugar un calentamiento más rápido se requiere un menor tiempo de tratamiento para conservar y/o transformar los alimentos (Chandrasekaran et al., 2013).
- *Calentamiento selectivo.* Se considera que el calentamiento por microondas es selectivo, ya que el campo electromagnético interacciona principalmente con las moléculas de agua del alimento, produciendo un aumento de su temperatura, mientras que el resto de componentes reciben calor de forma indirecta, es decir, por conducción (Fito et al., 2001).

- *Mejor preservación de la calidad del producto.* Gracias a que el alimento se calienta de forma más rápida y se requiere un menor tiempo de tratamiento, la calidad de los alimentos procesados por microondas se ve menos afectada (Salazar-González et al., 2012).
- *El alimento comienza a tratarse de forma instantánea* (Salazar-González et al., 2012).
- *Permite un ahorro energético.* Se considera que la tecnología microondas es más eficiente desde el punto de vista del consumo energético que los métodos de calentamiento convencional, ya que no requiere de agua o vapor como medio transmisor del calor (Salazar-González et al., 2012).
- *Facilita una reducción de los costes del proceso.* La mayor eficiencia energética y los menores tiempos de tratamiento asociados a la tecnología microondas, pueden dar lugar a una reducción de los costes económicos asociados a cada proceso (Vadivambal & Jayas, 2010).
- *Los equipos microondas son de fácil manejo, respetuosos con el medio ambiente y requieren poco mantenimiento* (Salazar-González et al., 2012).

**II. Inconvenientes**

- *Calentamiento heterogéneo.* Una de las principales limitaciones de la tecnología microondas es la falta de uniformidad en el calentamiento. Esto puede originar puntos fríos y puntos calientes en el producto. Como consecuencia, la inocuidad del alimento puede verse comprometida en aquellas zonas consideradas como puntos fríos, mientras que, puede haber un sobrecalentamiento de los puntos calientes (Wang & Sun, 2012). La uniformidad de calentamiento puede verse afectada por diversos factores, siendo el caso más desfavorable, es decir, aquel en el que se observa una mayor heterogeneidad de calentamiento, el procesado en discontinuo de alimentos sólidos. Por el contrario, se obtienen distribuciones de temperaturas considerablemente más homogéneas en los alimentos fluidos procesados en continuo (Vadivambal & Jayas, 2010).
- *Compleja modelización del proceso.* Modelizar los tratamientos por microondas puede resultar una tarea bastante complicada, ya que implica

considerar la distribución de las ondas electromagnéticas en el alimento al mismo tiempo que la distribución de calor. Para ello se requiere del uso simultáneo de la ecuación de Maxwell con un modelo que describa la transferencia de calor (Wang & Sun, 2012).

- *Requiere de una mayor inversión inicial* (Salazar-González et al., 2012).
- *En los procesos de tostado y horneado no se favorece el desarrollo del color y aromas típicos de estos procesos* (Wang & Sun, 2012).

### *I.4.2.6.Aplicaciones y tendencias actuales*

El calentamiento por microondas es una tecnología con gran potencial para el procesado de alimentos y uno de sus puntos fuertes es que parece preservar en mayor medida el valor nutricional y las características organolépticas de los mismos (Chandrasekaran et al. 2013; Salazar-González et al., 2012; Vadivambal y Jayas, 2007; Vadivambal & Jayas, 2010). La utilización de las microondas en los procesos alimentarios comenzó en 1940 y, a lo largo de los años, esta tecnología ha ido ganando popularidad en el ámbito doméstico, hasta convertirse en un elemento casi indispensable, y también en el industrial.

El uso de la energía microondas con fines industriales se ha ido extendiendo poco a poco. Su primera aplicación industrial a gran escala fue el secado final de chips de patata, producto que se comercializó tanto en Estados Unidos como en Europa y recibió una buena aceptación por parte de los consumidores (Chandrasekaran et al. 2013; Salazar-González et al., 2012; Vadivambal & Jayas, 2010). Con el paso del tiempo, las aplicaciones del calentamiento por microondas se han ido incrementando y, tal y como se muestra en la Tabla I.2, actualmente, se emplea con éxito en numerosos procesos de la industria alimentaria (Salazar-González et al., 2012). Algunas de sus aplicaciones más destacables son el secado de pasta, el precocinado de beicon y especialmente el descongelado de carne atemperada. De hecho, en Estados Unidos, más de 400 equipos microondas se utilizan para descongelar carne que será posteriormente sometida a algún tipo de tratamiento.

Sin embargo, la utilización de las microondas en los procesos de pasteurización y esterilización no ha mostrado tanto éxito como en el resto de sus aplicaciones potenciales (Richardson, 2001). Esto se debe en parte a que la información disponible acerca del impacto de las microondas sobre la seguridad y calidad de los alimentos sigue siendo escasa, en comparación con otras tecnologías de conservación, y a que la falta de homogeneidad en el calentamiento hace mucho más difícil el control y la validación de los procesos a la hora de asegurar la inocuidad del producto (Vadivambal & Jayas, 2010).

**Tabla I.2.** Aplicaciones de la energía microondas en procesos de la industria alimentaria.

| Aplicación | Producto |
|---|---|
| *Cocción o pre-cocción* | Platos preparados, arroz, beicon, pollo, hamburguesas, salchichas |
| *Descongelado* | Puré de patata, carne y pescado |
| *Horneado* | Pan, tartas, galletas, donuts |
| *Secado* | Miel, pistachos, pasta, brotes de soja, rodajas de fruta (plátano, manzana, etc.), rodajas de zanahoria, zumos de fruta para la obtención de concentrados, chips de patata |
| *Escaldado* | Especias, cacahuetes, algunas frutas y verduras |
| *Pasteurización* | Puré de manzana, zumo de naranja, zumo de manzana, pasta fresca, platos preparados, leche |
| *Esterilización* | Puré de patata, lonchas de carne, filetes de salmón |
| *Tostado* | Granos de café, nueces, avellanas |

Fuente: Chandrasekaran et al. 2013, Salazar González et al. (2012), Vadivambal & Jayas (2010)

No obstante, el entendimiento de las microondas y su interacción con los alimentos ha mejorado considerablemente en los últimos tiempos, así como también lo han hecho los equipos y sistemas industriales, que actualmente permiten un mejor control del proceso y aseguran una mayor uniformidad de calentamiento. Todo esto ha facilitado que las microondas comiencen también a aplicarse con éxito en los procesos de pasteurización. De hecho, en ciertos países de Europa y en Estados Unidos ya se comercializan alimentos pasteurizados por microondas tales como platos preparados a base de pasta, arroz y también salsas. Un claro ejemplo de la viabilidad económica de la pasteurización mediante la aplicación de energía microondas es la creciente producción de la empresa *TOP's foods,* ubicada en Bélgica, que comercializa una amplia gama de alimentos pasteurizados mediante esta tecnología (www.microwaveheating.wsu.edu, 2014). Por otro lado, la aplicación de la energía microondas en los procesos de esterilización sigue resultando un tema complejo, pero en el que se están produciendo grandes avances. Recientemente, *the Food and Drugs Admisnistration* (FDA) ha autorizado la esterilización de puré de patatas mediante microondas y su posterior envasado aséptico (www.microwaveheating.wsu.edu, 2014).

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# Objetivos

# II. OBJETIVOS

El objetivo general de esta Tesis es evaluar la tecnología microondas, en comparación con el calentamiento convencional, aplicada a la conservación de un puré de kiwi desde el punto de vista de la seguridad alimentaria y de la calidad nutricional, funcional y sensorial del producto.

Para ello se han planteado los objetivos específicos que se exponen a continuación:

1. Estudiar el efecto de los parámetros de proceso, potencia de microondas y tiempo, sobre las características fisicoquímicas, sensoriales, nutricionales y funcionales (compuestos bioactivos y capacidad antioxidante) de un puré de kiwi, así como sobre la inactivación de enzimas (peroxidasa, polifenoloxidasa y pectinmetilesterasa) y microorganismos (flora natural y *Listeria monocytogenes,* patógeno con capacidad de crecimiento en el producto).
2. Establecer un proceso de pasteurización por microondas que permita obtener un puré de kiwi estable, seguro desde el punto de vista microbiológico y de máxima calidad.
3. Comparar el impacto de la tecnología microondas y el calentamiento convencional en la calidad sensorial, nutricional y funcional así como la estabilidad microbiológica y enzimática de un puré de kiwi pasteurizado tras el procesado y a lo largo de la vida útil del producto.

# Plan de trabajo

# III. PLAN DE TRABAJO

A continuación se detalla el plan de trabajo seguido con el fin de alcanzar los objetivos planteados para la Tesis.

1. **Revisión bibliográfica.**
2. **Estandarización, puesta a punto de métodos y caracterización del puré de kiwi fresco.**
   - 2.1. Selección de las condiciones de triturado del puré de kiwi.
   - 2.2. Puesta a punto de métodos para determinar las siguientes propiedades y/o parámetros en puré de kiwi:
     - 2.2.1. Propiedades fisicoquímicas: humedad, actividad del agua, pH, contenido en sólidos solubles, color, viscosidad y consistencia.
     - 2.2.2. Actividad enzimática: peroxidasa, polifenoloxidasa y pectinmetilesterasa.
     - 2.2.3. Parámetros nutricionales y funcionales: capacidad antioxidante y contenido en vitaminas A, E y C, fenoles totales, flavonoides totales y taninos totales.
     - 2.2.4. Contenido en pigmentos: clorofila a, clorofila b, feofitina a, feofitina b, neoxantina, violoxantina, β-caroteno, luteína y neoluteína.
     - 2.2.5. Bioaccesibilidad de carotenoides (neoxantina, violoxantina, β-caroteno, luteína y neoluteína).
     - 2.2.6. Propiedades organolépticas: aroma, color, sabor, consistencia, granulosidad, dulzor, acidez y astringencia.
     - 2.2.7. Flora alterante y patógena.
   - 2.3. Caracterización del puré de kiwi en cuanto a las propiedades y/o parámetros previamente mencionados.
3. **Efecto del procesado por microondas sobre un puré de kiwi. Selección del tratamiento óptimo.**
   - 3.1. Determinación de la distribución de temperaturas del producto durante el calentamiento por microondas bajo distintas condiciones de proceso.

3.2. Evaluación del impacto del procesado por microondas sobre la flora patógena del puré de kiwi siguiendo un enfoque determinista y estocástico: cinética de inactivación de *L. monocytogenes*.

3.3. Evaluación del impacto del procesado por microondas sobre características fisicoquímicas, físicas, funcionales, sensoriales y sobre la actividad enzimática del puré de kiwi.

3.4. Selección de las condiciones óptimas de tratamiento para pasteurizar el producto.

**4. Efecto del procesado por calentamiento convencional sobre un puré de kiwi. Selección del tratamiento equivalente al óptimo establecido para el tratamiento por microondas**

4.1. Evaluación del impacto del procesado por calentamiento convencional sobre la flora patógena del puré de kiwi: cinética de inactivación de *L. monocytogenes*.

4.2. Evaluación del impacto del procesado por calentamiento convencional sobre la actividad enzimática previamente identificada como limitante.

4.3. Selección de un proceso de pasteurización por calentamiento convencional equivalente al tratamiento por microondas en términos de inactivación enzimática y microbiológica (*L. monocytogenes*).

**5. Comparación de la efectividad de la tecnología de microondas y del calentamiento convencional en la conservación de un puré de kiwi.**

5.1. Comparación establecida a nivel de los parámetros cinéticos obtenidos por microondas (actividad 3.2.) y por calentamiento convencional (actividad 4.1.).

5.2. Comparación establecida a nivel del cálculo de letalidad asociada a distintos procesos por microondas y por calentamiento convencional equivalentes.

5.3. Comparación establecida a nivel del impacto causado sobre la calidad y seguridad del puré de kiwi por un tratamiento de pasteurización por microondas (actividad 3.4.) y un tratamiento de pasteurización por calentamiento convencional (actividad 4.3.) equivalentes.

5.3.1. Evaluación de la aceptabilidad sensorial del puré de kiwi fresco, pasteurizado por microondas y pasteurizado por calentamiento convencional.

5.3.2. Evaluación del impacto causado por el tratamiento óptimo de microondas y el tratamiento convencional equivalente sobre: las propiedades fisicoquímicas, actividad enzimática, contenido en compuestos bioactivos y capacidad antioxidante, contenido en pigmentos y bioaccesibilidad de carotenoides, flora alterante y patógena, estabilidad durante el almacenamiento y vida útil

# Resultados

# CAPÍTULO IV.1. EFECTO DEL PROCESADO POR MICROONDAS EN LAS CARACTERÍSTICAS SENSORIALES DE UN PURÉ DE KIWI

# EFFECTS OF MICROWAVE HEATING ON SENSORY CHARACTERISTICS OF KIWIFRUIT PUREE

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## ABSTRACT

The effect of microwave processing on the characteristics of kiwifruit puree was evaluated by applying various gentle treatments. Different combinations of microwave power/processing time were applied, with power among 200–1,000 W and time among 60–340 s, and various sensory and instrumental measurements were performed with the aim of establishing correlations and determining which instrumental parameters were the most appropriate to control the quality of kiwi puree. The water and soluble solids of the product, 83 and 14/100 g sample, respectively, did not change due to treatments. For sensory assessment, an expert panel was previously trained to describe the product. Fourteen descriptors were defined, but only the descriptors 'typical kiwifruit colour', 'tone', 'lightness', 'visual consistency' and 'typical taste' were significant to distinguish between kiwifruit puree samples. The instrumental analysis of samples consisted in measuring consistency, viscosity, colour and physicochemical characteristics of the treated and fresh puree. Applying intense treatments (600 W–340 s, 900 W–300 s and 1,000 W–200 s) through high power or long treatment periods or a combination of these factors, mainly affects the consistency (flow distance decreased from 5.9 to 3.4 mm/g sample), viscosity (increased from 1.6 to 2.5 Pa/s), colour (maximum ΔE was 6 U) and taste of the product. As a result, samples were thicker and with an atypical flavour and kiwifruit colour due to increased clarity (L* increased from 38 to 43) and slight changes in the yellow–green hue (h* decreased from 95 to 94). For the instrumental determinations of colour and visual perception of consistency, the most suitable parameters for quality control are the colour coordinates L*, a*, h*, whiteness index and flow distance measured with a Bostwick consistometer.

**KEYWORDS** Kiwifruit, microwaves, descriptive sensory assessment, colour, consistency, taste

## 1. INTRODUCTION

Sensory evaluation is an essential tool in the development of new products. Physical measurements cannot normally determine consumer response or preference because psychological or sensory responses are difficult to mimic (Dubost et al. 2003). However, this type of evaluation can be characterised by imprecision, inaccuracy and uncertain repeatability (Sinija and Mishra 2011). Therefore, it is important to find a good objective method that can predict the sensory perception of the product (Segnini et al. 1999).

Instrumental measurement of fruit properties such as °Brix, acidity, texture or colour have become the cornerstones of fruit quality assessment (Oraguzie et al. 2009; Segnini et al. 1999). The industry often sets quality standards that are based on instrumental measurements. Nevertheless, the relevance of these data will depend on how well they are able to predict sensory attributes (Oraguzie et al. 2009). In sensory analysis, one of the most important tools is the quantitative characterisation of the perceivable product attributes. In the bibliography, this tool is referred to as 'descriptive analysis' or 'profiling' and uses highly trained or expert panels with an acute ability to accurately characterise products (Worch et al. 2010).

Kiwifruit is native to China (Fisk et al. 2006; Fúster et al. 1994; Jaeger et al. 2003) and has become a popular fruit because of its good organoleptic and nutritional properties. This fruit has a relatively high content of nutraceuticals (Fisk et al. 2006), as well as a higher content of vitamin C, zinc and potassium than other fruits such as oranges, apples or peaches (Beirão-da-Costa et al. 2006; Fang et al. 2008; Guldas 2003). Kiwifruit also has important quantities of organic acids (citric, quinic, malic, galacturonic, succinic, oxalic, etc.), carotenoids, phenolic compounds, aromatic components (mainly esters, alcohols, aldehydes and ketones) and minerals (phosphorus, potassium, magnesium and calcium) (Soufleros et al. 2001; Zolfaghari et al. 2010). It is cultivated principally in New Zealand, but in recent years, it has also become a commercial crop in several other countries: Australia, California, Japan, Chile and the Mediterranean countries, especially Italy and Spain (Fisk et al. 2006; Fúster et al. 1994). According to statistical data from MARM (the Spanish Ministry of the Environment, Rural Affairs and Coasts) 18,032 t of kiwifruit

were produced in Spain in 2007, mainly in Pontevedra (8,032 t), Corunna (5,620 t) and Asturias (2,100 t). Spain has become the largest European kiwifruit importer. Consumption per capita is around 2 kg/person/year and Hayward is the most consumed variety (Jaeger et al. 2003; MAPA 2010). Generally, there is a surplus production of kiwifruit, and it is a seasonal, sensitive and perishable fruit (Fang et al. 2008). Moreover, approximately 25% of kiwifruit production may not reach fresh fruit marketing outlets because of inadequate quality in terms of small size or irregular shape (Park and Luh 1985), and so these fruits must be processed into various types of products (Fúster et al. 1994; Park and Luh 1985).

To compete successfully in world markets, horticultural industries must continue to offer innovative products (Jaeger et al. 2003). Kiwifruit has great potential for industrial exploitation due to its composition, sensory characteristics and stability during preservation (Barboni et al. 2010). Traditionally, kiwifruit has been processed to obtain fruit juice, jam or dehydrated products. The use of non-conventional technologies, such as microwave energy (MW), has some advantages when compared to conventional heating. Microwave energy, which is transported as an electromagnetic wave in certain frequency bands (300 MHz to 300 GHz), heats up dielectrical materials when impinges on them. Heating generated is due to the molecular friction of permanent dipoles within the material as they try to reorient themselves with the electrical field of the incident wave (Schubert and Regier 2010). It is important to take into account that MW energy is sufficient to move the atoms of a molecule but is insufficient to cause chemical changes by direct interaction with molecules and chemical bonds. This occurs because MW are non-ionizing and their quantum energy is several orders of magnitude lower, compared to other types of electromagnetic radiation (Schubert and Regier 2010; Vadivambal and Jayas 2007).

The most important characteristic of microwaves is volumetric heating, which means that materials can absorb microwave energy directly and internally. This fact leads to higher penetrative power, faster heating rates, higher thermal efficiency and shorter processing times compared to conventional technologies (Vadivambal and Jayas 2007). All these facts seem to result in better organoleptic, nutritional and functional properties preservation, with a particular effect on colour and textural

characteristics (de Ancos et al. 1999; Igual et al. 2010a, b). Nevertheless, available information regarding the impact of microwaves on the sensory, nutritional and functional quality of products is scarce and inconsistent. Because of technical and cost factors, microwave heat treatment is not widely used for commercial purposes. The application of this technology, which seems to have a considerable potential for the processing of agricultural products in the near future, would be justified only from the standpoint of obtaining a high-quality product (Vadivambal and Jayas 2007). For this reason, the study of the impact of microwave technology on food quality is interesting.

The aim of this work was to study the effect of applying a heat treatment based on the use of microwave energy with the objective of colour and texture preservation of kiwifruit puree. Instrumental and sensory evaluation of untreated product and product heated at different power-time conditions was performed with a prior selection of the attributes of interest. A correlation between the sensory measurements and the instrumentally obtained parameters was established in order to select the most suitable instrumental parameters to describe the quality of the product. To preserve most the characteristics of the fresh fruit, gentle microwave treatments were applied, taking into account that this technology could be combined with other technologies, for instance the use of biopreservatives, to obtain high-quality and stable products. The most intense treatment was selected on the basis on a percentage of peroxidase (POD) and polyphenoloxidase (PPO) inactivation of 90% and 85%, respectively, activity reduction comparable to pasteurization treatments (Igual et al. 2010b).

## 2. MATERIALS AND METHODS

### 2.1. Sample Preparation

Kiwifruit (*Actinida deliciosa var.* Hayward) was purchased in a local supermarket. Fruit pieces (°Brix between 13.4 and 14.7) were peeled, washed with distilled water, cut into slices and finally triturated in a Thermomix (TM 21, Vorwerk, Spain), using the fourth power level for 1 min. The crushing of the fruit was done in series of 1.5 kg batches. All of them were mixed and homogenised before receiving the corresponding treatments.

### 2.2. Experimental Design

To study the simultaneous effect of the two processing variables (microwave power and process time), a central composite rotatable design was applied in order to select the treatment conditions with a reduced number of experimental trials (Cano et al. 1997; Beirão-da-Costa et al. 2006), using the Statgraphics 5.1 plus software programme (StatPoint Technologies, Inc., Warrenton, VA, USA). The range entered in the programme (300–900 W and 100–300 s) was selected taking into consideration previous experiences and ensuring that the most intense treatment would achieve a percentage of POD and PPO inactivation of 90% and 85%, respectively. Treatment conditions defined by the experimental design appear in Table 1.

**Table 1.** Microwave power levels and time used in the different treatments.

| Code | Power levels (W) | Time (s) |
|---|---|---|
| 200-200 | 200 | 200 |
| 300-100 | 300 | 100 |
| 300-300 | 300 | 300 |
| 600-60 | 600 | 60 |
| 600-200 | 600 | 200 |
| 600-340 | 600 | 340 |
| 900-100 | 900 | 100 |
| 900-300 | 900 | 300 |
| 1,000-200 | 1,000 | 200 |

### 2.3. Treatments

A household microwave oven (3038GC, Norm, China) was used to obtain the processed puree. The nine different treatments (W–s) (Table 1) were carried out immediately after the kiwifruit was triturated. For each treatment, a sample of 500 g was heated in the microwave oven in a standard size glass beaker (BKL3-1K0-006O, Labbox, Spain). Treated samples (around 85–90 °C) were immediately cooled in ice water to stop the heat treatment until the puree reached 30–35 °C. Cooked purees were then cold stored (4 °C) for 24 h before analytical determinations.

## 2.4. Analytical Determinations

All the treated samples and a non-treated sample used as control were instrumentally analysed as described below.

### *2.4.1. Physicochemical Properties*

Water and soluble solids content, water activity and pH were determined for fresh and processed kiwifruit puree. Water content ($x_w$) was measured by drying the sample to constant weight at 60 °C in a vacuum oven using AOAC 934.06 method (2000). Soluble solids were determined by measuring the °Brix in a previously homogenised sample with a portable digital refractometer Refracto 3PX at 20 °C (Metler Toledo, Switzerland). Water activity ($a_w$) was measured by using a dew point hygrometer (GBX FA-st lab, France) and pH in a digital pH-meter Basic 2 (Crison, Spain). Each analysis was carried out in triplicate.

### *2.4.2. Consistency and Viscosity*

To determine the consistency, the flow distance (millimeters per gram) of a controlled sample weight (about 40 g) for a constant time (30 s) was measured with a Bostwick consistometer (Aname, Spain), employing the procedure described by Igual et al. (2010a). Viscosity was measured using a rotational dial reading viscosimeter (LVT, Brookfield, Germany) with a R4 spindle. The viscosimeter measures the torque necessary to overcome the viscous resistance to the induced movement (Nielsen 2010). The measurement was obtained inserting the spindle in a known kiwifruit puree weight (200 g) and reading the dial at different rotational speed levels (6, 12, 30 and 60 rpm). The dial reading was corrected taking into account the corresponding factor, which depends on the spindle and the rotational speed level used in each case, to obtain a viscosity expressed in cp (Eq. 1). All measurements were done in triplicate.

$$\eta = L \cdot k \tag{1}$$

where:

$\eta$: viscosity (cp); 1 cp = $10^{-3}$ Pa·s

L: dial reading;

k: factor (Chiralt et al., 1998).

*2.4.3. Colour Measurement*

The colour of treated and fresh kiwifruit purees was measured in triplicate for each sample using a Minolta CM 3600D spectrocolorimeter (Konica Minolta Sensing, Inc., Japan). Samples were placed in size standardized sample cups (37×50×22 mm). The colour coordinates were obtained and results were obtained according to CIEL*a*b* uniform colour space (10° observer and D65 illuminant), where: the L* value is a measure of lightness (from 0 to 100); a* is a measure of chromaticity on a green (−) to red (+) axis and b* of chromaticity on a blue (−) to yellow (+) axis. Hue angle (h*), chrome (C*), total color difference with respect to non-treated kiwifruit puree sample (ΔE*), browning index (BI) (Maskan 2001; Mohammadi et al. 2008) and whiteness index (WI) (Alegria et al. 2010; Moretti et al. 2007; Zanoni et al. 2007) were calculated from previously obtained color coordinates by applying Eqs. 2 to 6. In Eq. 5, x corresponds to the triestimulus coefficient, which can also be obtained from L*, a* and b* coordinates as described by Chiralt et al. (2007).

$$h^* = a\tan\frac{b^*}{a^*} \tag{2}$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \tag{3}$$

$$\Delta E^* = \sqrt{\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2}} \tag{4}$$

$$BI = \frac{100(x - 0.31)}{0.172} \tag{5}$$

$$\mathrm{WI} = 100 - \sqrt{(100 - \mathrm{L}^*)^2 + \mathrm{a}^{*2} + \mathrm{b}^{*2}} \tag{6}$$

### 2.5. Sensory Assessment

A sensory panel with eleven assessors (four men and seven women), recruited from students and employees of the Food Technology Department (Universidad Politécnica de Valencia) aged between 25 and 50 years old, was trained over a period of 2 months (12 training sessions). Samples were cold-stored for 24 h, tempered at 25 °C before the assessment, was carried out and served in plastic disposable standard size containers identified with three-digit codes. In all cases, training, and formal assessment, was performed in a normalised tasting room (UNE 8589 2010).

#### *2.5.1. Selection of Terms and Panel Training*

The selection of descriptors was made over two 1-h sessions using the checklist method (Lawless and Heymann 1998). Assessors were provided with two samples (the non-treated kiwifruit puree and the most intensively treated one), and descriptors were listed based on sensory analysis vocabulary (UNE 5492 2010). Panellists were asked to choose the most representative attributes to describe the samples. Once the terms were selected, consensus concerning their meaning was attained. This entailed reaching a precise definition of the descriptors and how to evaluate them to quantify attribute intensity, as well as agreeing upon the tasting procedure (Table 2) (Albert et al. 2009; Escribano et al. 2010; Pagliarini et al. 2010). During the training period, all the treated (Table 1) and the non-treated samples were tasted. Tests of three different samples in each session were used by the panellists for each descriptor until the panel was homogeneous in the ranking of the samples. Panel members were then trained in the use of scales with reference samples (10-cm unstructured scales for all the attributes). Panel performance was checked by analysis of variance (ANOVA) for discrimination ability and reproducibility of the panellists.

*2.5.2. Formal Assessment*

A balanced complete block experimental design was carried out in duplicate (two different sessions) using the Compusense® programme release five 4.6 software (Compusense Inc., Guelph, Ont., Canada) to evaluate the samples. The intensity of the sensory attributes were scored on a 10-cm unstructured line scale. Samples were randomly selected and served with a random three-digit code. All the treated samples (Table 1) were subjected to formal analysis, as well as the untreated sample.

**Table 2.** Attributes, scale extremes and evaluation technique used in descriptive sensory assessment of kiwifruit puree treated with microwave.

| Attribute and scale extremes | Technique |
|---|---|
| Kiwi odour intensity (low/high) | Observe |
| Atypical odour (low/high) | Observe |
| Typical kiwi colour (low/high) | Observe |
| Tone (green/brown) | Observe |
| Lightness (light/dark) | Observe |
| Granularity (low/high) | Evenness of the sample's surface. Take a spoonful of the sample and observe its surface. |
| Visual consistency (low/high) | Take enough quantity of kiwi puree with a spoon and drop it to evaluate its visual consistency |
| Sweetness (low/high) | Taste the necessary quantity of kiwi puree to notice the intensity of sweetness |
| Acidity (low/high) | Taste the necessary quantity of kiwi puree to notice the intensity of acidity |
| Astringency (low/high) | Taste the necessary quantity of kiwi puree to notice the intensity of astringency |
| Intensity kiwi taste (low/high) | Taste the necessary quantity of kiwi puree to notice the intensity of typical kiwi taste |
| Atypical taste (low/high) | Taste the necessary quantity of kiwi puree to notice the intensity of typical kiwi taste |
| Aftertaste (low/high) | Assess the persistence of taste after ingesting kiwi puree |
| Mouth consistency (low/high) | Taste the sample and evaluate its consistency during the ingest |

### 2.6. Statistical Analyses

ANOVA with two factors (assessor and sample and their interaction) was run, with a confidence level of 95% ($p<0.05$), to check panel performance with the use of the Senpaq 4.2 package (QIstatistics, UK).

ANOVA with one factor, with a confidence level of 95% ($p<0.05$), using the Statgraphics 5.1 plus software programme (StatPoint Technologies, Inc., Warrenton, VA, USA), was applied to evaluate the differences among treatments on physicochemical and sensory data. In addition, principal component analysis (using a correlation matrix) and a Pearson correlation were made using the XLSTAT 2009 programme, with the aim of studying the correlation between physicochemical parameters and sensory attributes.

## 3. RESULTS AND DISCUSSION

### 3.1. Physicochemical Properties

No significant difference in measured physicochemical properties was found among samples (treated or nontreated). The average values (and standard deviation) obtained were 83.4 (0.7) g water/100 g product, 14.1 (0.3) g soluble solids/100 g liquid phase in the product, water activity = 0.983 (0.002) and pH=3.39 (0.07). These values are similar to those obtained by other authors for kiwifruit (de Ancos et al. 1999; Fúster et al. 1994; Zolfaghari et al. 2010).

### 3.2. Consistency and Viscosity

The effect of microwave processing on kiwifruit puree consistency and viscosity is shown in Fig. 1. According to Bourne (1982), consistency is defined as all the sensations resulting from stimulation of the mechanical and tactile receptors, especially in the region of the mouth and varying with the texture of the product. When the intensity of microwave treatment was higher, the flow distance of kiwifruit puree decreased, which means that consistency increased. Specifically, consistency of 300–100, 600–60 and fresh samples was significantly lower than the rest of the samples ($p<0.05$). However, the consistency of samples 600–200, 600–340, 900–

300 and 1,000–200 was significantly higher. The rest of the samples had an intermediate behaviour.

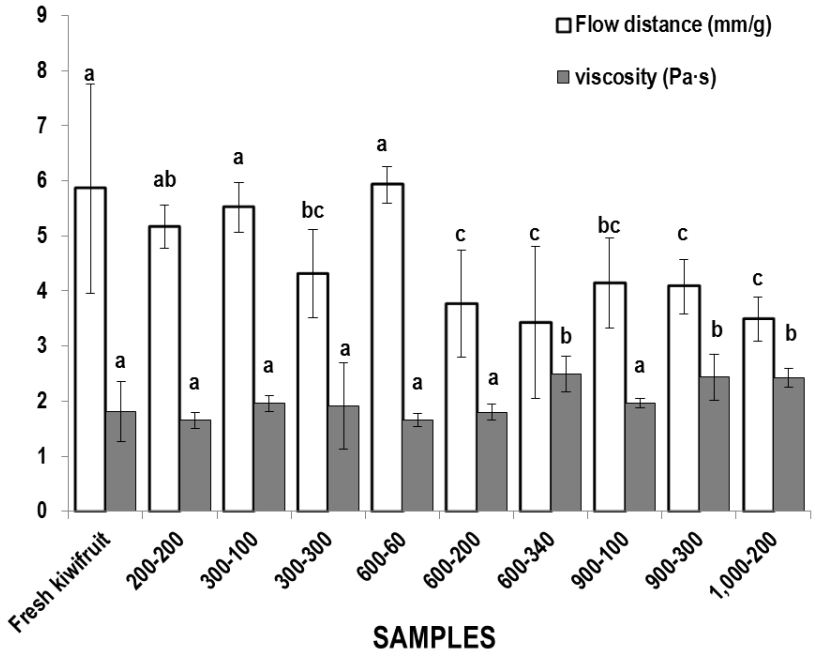


**Figure 1.** Average values (and standard deviation) of viscosity and flow distance (millimetres per gram) of fresh and treated samples. The same letter (a, b or c) indicates homogeneous groups established by the ANOVA ($p<0.05$)

The consistency increase when a higher microwave power was applied can be related to the pectin solubilisation caused by the higher temperatures reached in the product. As it has been described, no significant change in total pectin content of fruits occurs due to heating treatments. Nevertheless, when microwave is applied, the water soluble pectin fraction increases while non-extractable and oxalate soluble fractions decrease, this affecting the mechanical response of the sample (Contreras et al. 2005, 2007). An increase in the soluble pectin in the sample aqueous phase can be related with the increase in its consistency.

Viscosity is defined as the internal friction of a fluid or its tendency to resist flow (Bourne 1982). In the same way as occurs with consistency, product viscosity increased when a higher intensity microwave treatment was applied. In this case,

samples 600–340, 900–300 and 1,000–200 showed significantly higher viscosity values than the rest of the samples with a confidence level of 95%.

### 3.3. Colour

The colour of food is important for its acceptability and in consumer studies, the first attribute that determines product quality is related to colour. Colour deterioration has been studied by several researchers for a number of products. In general, microwave heating seems to cause less browning in treated products than conventionally processed products (Vadivambal and Jayas 2007).

Table 3 shows the values of quantified colour parameters. Values obtained for colorimetric coordinates (L*, a* and b*), hue angle (h*) and chrome (C*) in fresh kiwifruit were similar to those published by Fisk et al. (2006). As regards lightness, a significant increase ($p<0.05$) was observed in all cases when the most severe treatments were applied (Table 3). Samples 600–200, 600–340, 900–300 and 1,000–200 were more luminous than the rest of the samples. The a* values increased as a consequence of different treatments—although this fact can only be taken as significant in samples 300–300, 600–340, 900–300 and 1,000–200. Additionally, there were some changes in colorimetric coordinate b* values as a consequence of the processing, which generally increased when treatment intensity increased. In this manner, processed samples slightly changed to more red–yellow tones. The chrome (C*) value indicates the degree of colour saturation and is proportional to the strength of the colour. This parameter was nearly unchanged after microwave processing. Nevertheless, the hue angle (h*) values slightly decreased when microwave power increased during heating processes. This results in a displacement to a more red–yellow hue for microwave heated kiwifruit puree (Maskan 2001). Total colour difference parameter combines L*, a* and b* parameters by integrating these three characteristics to compare the colour of non-treated samples with microwaved samples. In general, the ΔE* value increases when microwave power increases, as has been also observed by de Ancos et al. (1999). According to Bodart et al. (2008), $\Delta E^*>3$ denotes differences noticeable to the human eye; thus, noticeable colour difference was only found when the most aggressive treatments were applied. Heating treatments commonly cause enzymatic

and non-enzymatic browning reactions. This fact leads a lightness reduction and, consequently, a browning index increment, as shown in results published by Maskan (2001). Nevertheless, according to Vadivambal and Jayas (2007), the opposite phenomenon sometimes occurs. The values of BI obtained in this work were very similar for all the samples (Table 3). Despite significant differences among samples ($p<0.05$) being detected, the trend seems not to be attributable to the intensity of the treatment. The whiteness index (Table 3) followed a very similar behaviour to that observed for the L* coordinate, with significantly higher values for the more intensely treated products. From this point of view, colour changes observed during treatments may be more related to degradative loss of total pigments (chlorophyll and xanthophyll) during heating (de Ancos et al. 1999; Maskan 2001), than to browning reactions.

**Table 3.** Average values (and standard deviation) of colour coordinates (L*, a* and b*), chrome (C*), hue angle (h*), total colour difference (ΔE*), browning index (BI) and whiteness index (WI) of fresh and treated samples. The same letter (a, b, c, d, e,) indicates homogeneous groups established by the ANOVA ($p<0.05$).

| Code | *L** | *a** | *b** | *C** | *h** | Δ*E** | BI | WI |
|---|---|---|---|---|---|---|---|---|
| Fresh kiwi fruit | 37.7 (1.1)a | −2.2 (0.6)a | 23 (2)bcd | 24.3 (0.5)bcd | 95 (2)bcd | – | 47 (2)c | 33.5 (1.0)a |
| 200–200 | 38.4 (1.0)a | −2.1 (0.3)ab | 22 (2)a | 22 (2)a | 95.4 (0.7)d | 2.3 (1.5)a | 44 (4)a | 34.3 (1.4)ab |
| 300–100 | 39 (2)ab | −2.03 (0.13)abc | 23 (2)abc | 23 (2)abc | 95.2 (0.5)cd | 3.0 (0.9)abc | 44 (2)ab | 35.0 (1.3)bc |
| 300–300 | 39.0 (1.8)a | −1.7 (0.5)cde | 24.4 (1.1)bcd | 24.1 (0.9)bcd | 93.9 (1.3)a | 1.6 (1.0)a | 47.4 (1.1)c | 34.0 (1.1)ab |
| 600–60 | 39 (2)a | −2.0 (0.2)abcd | 21 (2)a | 22 (2)a | 95.3 (0.6)cd | 2.3 (1.8)a | 43 (4)a | 34.3 (1.1)ab |
| 600–200 | 42.3 (1.1)cd | −1.7 (0.4)abcde | 24.5 (1.0)cd | 24.4 (1.1)cd | 94.0 (0.9)ab | 4.4 (1.1)cd | 45.2 (1.1)abc | 37.3 (0.8)d |
| 600–340 | 42.0 (1.6)cd | −1.7 (0.2)bcde | 24.0 (0.9)bcd | 24.2 (0.9)bcd | 93.9 (0.2)a | 4.3 (1.4)bcd | 45.0 (0.9)abc | 37.3 (1.2)d |
| 900–100 | 40.5 (1.0)bc | −1.8 (0.3)abcde | 24.3 (0.9)d | 24.5 (0.9)d | 94.2 (0.8)abc | 2.8 (1.1)ab | 46.3 (1.1)bc | 35.8 (0.8)c |
| 900–300 | 42.4 (1.1)d | −1.3 (0.2)e | 22.7 (1.3)ab | 22.7 (1.1)ab | 93.4 (0.5)a | 5.0 (0.8)d | 42.7 (1.7)a | 38.3 (0.8)d |
| 1,000–200 | 43.3 (1.8)d | −1.5 (0.3)de | 24.0 (1.3)bcd | 24.0 (1.4)bcd | 93.8 (0.8)a | 6 (2)d | 44.0 (1.0)ab | 38.4 (1.3)d |

### 3.4. Sensory Assessment

Significant differences ($p<0.05$) among samples were only found in the sensory descriptors 'typical kiwifruit colour', 'tone', visual consistency', 'lightness' and 'atypical taste'. As a general rule, noticeable differences increased for five considered descriptors (see Fig. 2) in treated samples compared with untreated samples when heating intensity increased. Figure 2a shows that differences were not found between fresh kiwifruit puree and processed samples (200–200, 300–100 and 600–60) as the lines in the spider plot were nearly overlapping. It has to be mentioned that the visual consistency of treated samples was slightly lower than fresh samples, although no significant differences ($p>0.05$) were found among the four samples considered. Figure 2b shows higher differences in every significant attribute between treated samples and fresh kiwifruit puree, except in 'visual consistency'. Panellists considered samples 600–200 and 900–100 less light and with lower 'typical kiwifruit colour intensity' than fresh puree. Nevertheless, observed differences between sample 300–300 and the fresh sample were not statistically significant ($p> 0.05$) with regard to these descriptors. Moreover, these three samples seemed to be significantly ($p<0.05$) browner than fresh kiwifruit puree. Although treated samples were not truly browner than the untreated sample, this fact can be related to how the attribute 'tone' was defined (Table 2).

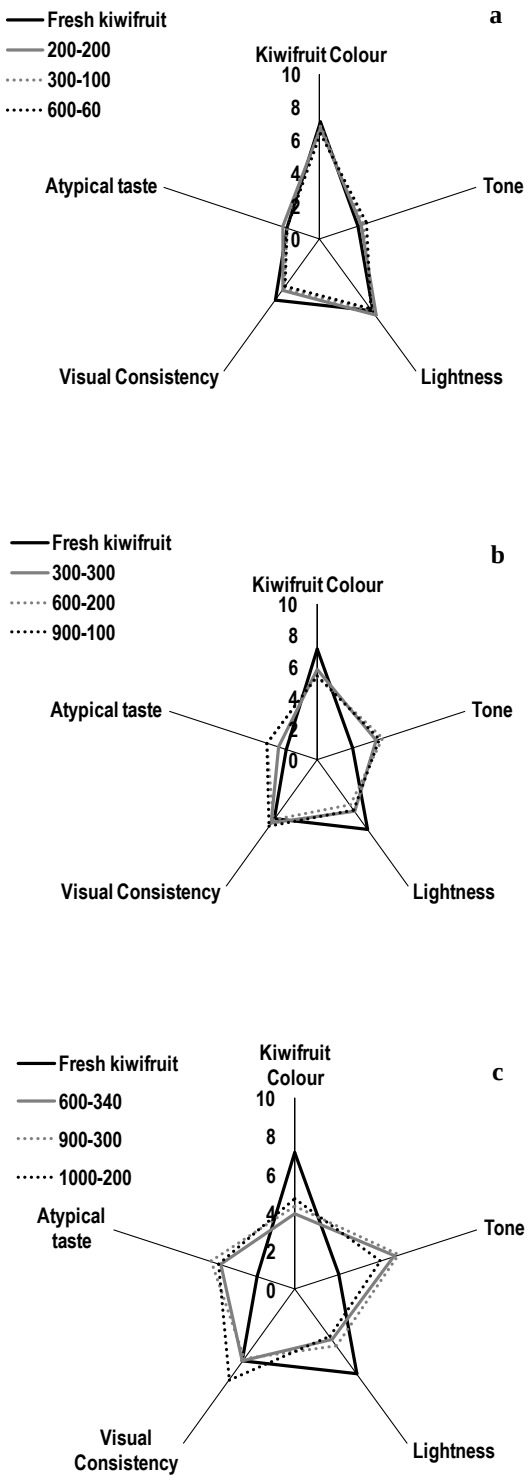


**Figure 2.** Average values (on a 1–10 scale) of panel member assessments about kiwifruit colour, tone, lightness, visual consistency and atypical taste of treated

samples: 200–200, 300– 100 and 600–60 (a), 300–300, 600–200 and 900–100 (b) and 600–340, 900–300 and 1,000–200 (c) compared to fresh sample.

Panellists probably considered that the treated samples had a less green tone; for this reason, they situated the samples on the opposite side of the scale (green/brown), and this action resulted in considering the treated sample as browner than the fresh sample. Figure 2c evidences bigger differences in assessments given to samples 600–340, 900–300 and 1,000–200 compared to fresh kiwifruit puree. In a general way, panellists considered that the three processed samples were significantly ($p<0.05$) less lightness and green, with a lower typical kiwifruit colour intensity and higher atypical taste intensity; however, they had the same visual consistency as fresh kiwifruit puree.

Figure 3 shows the two first component maps of the principal component analysis constructed using the sensory data. Two components were extracted that explain 80.59% of the data variability. The first component explained most of this variance (63.83%); for this reason, it has been used to describe all the kiwifruit puree characteristics.

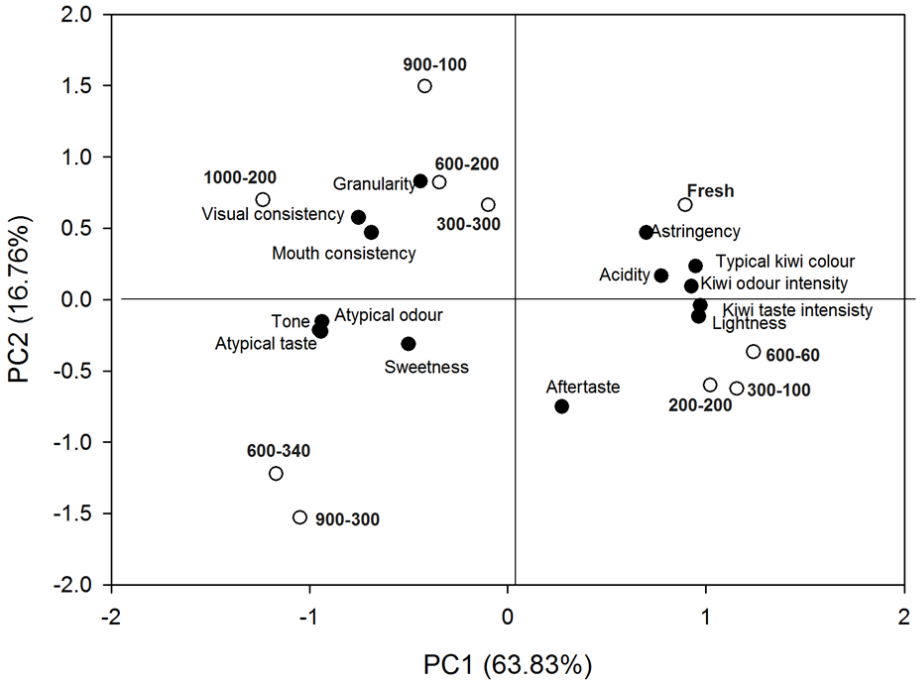


**Figure 3.** Two first components of principal component analysis plot of fresh and treated samples and sensory attributes.

This component evidenced a positive correlation with the sensory attributes 'typical kiwifruit colour intensity', 'kiwifruit odour intensity', 'lightness', 'acidity', 'astringency', 'intensity kiwifruit taste' and a negative correlation with the sensory attributes 'atypical odour', 'tone', 'atypical taste', 'visual consistency' and 'mouth consistency'. Samples 200–200, 300–100, 600–60 were characterised by a similar acidity, astringency, colour, odour and taste to the fresh kiwifruit, due to the less intensive treatments being applied to these samples. On the other hand, when the most severe treatments were applied (600–340, 900–300 and 1,000–200), the samples were characterised by a higher atypical odour and taste, higher visual and mouth consistency and more browning. Finally, the granularity and consistency of samples 300–300, 600–200 and 900–100 were higher than the rest of the samples.

### 3.5. Correlation between Instrumental and Sensory Data

A Pearson correlation matrix was constructed using the instrumental and sensory data. Correlation coefficient values obtained are summarised in Table 4. Significant ($p<0.05$) and meaningful correlations were found between some instrumental colour parameters (L*, a*, h* and WI) and sensory descriptors 'typical kiwifruit colour', 'tone' and 'lightness'. 'Typical kiwifruit colour' and 'lightness' were negatively correlated with L*, a* and WI and positively correlated with h*. The opposite situation was observed for descriptor 'tone' which was negatively correlated with h* and positively correlated with L*, a* and WI. In addition, a negative correlation was found between flow distance and 'visual consistency' determined by sensory assessment, which means that instrumental and sensory consistency were positively correlated.

**Table 4.** Correlation coefficient values between different sensory and instrumental parameters. Values in bold are statistically significant ($p<0.05$).

| Variables | Instrumentals | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sensorials** | *Moisture* | $a_w$ | *ºBrix* | *pH* | *Flow distance* | *Viscosity* | *L** | *a** | *b** | *C** | *h** | *BI* | *WI* |
| *Kiwi odour intensity* | **0.704** | 0.040 | 0.294 | -0.091 | **0.916** | **-0.720** | **-0.764** | **-0.823** | -0.444 | -0.427 | **0.846** | 0.140 | -0.471 |
| *Atypical odour* | **-0.754** | -0.131 | -0.190 | 0.091 | **-0.854** | **0.834** | **0.841** | **0.841** | 0.307 | 0.288 | **-0.830** | -0.238 | **0.735** |
| *Typical kiwi colour* | **0.815** | -0.007 | 0.199 | -0.062 | **0.896** | **-0.850** | **-0.886** | **-0.894** | -0.316 | -0.296 | **0.878** | 0.310 | **-0.700** |
| *Tone* | **-0.801** | 0.021 | -0.251 | 0.138 | **-0.877** | **0.828** | **0.868** | **0.936** | 0.331 | 0.310 | **-0.923** | -0.289 | **0.649** |
| *Lightness* | 0.612 | -0.259 | 0.521 | -0.152 | **0.946** | **-0.752** | **-0.885** | **-0.881** | -0.579 | -0.561 | **0.932** | 0.135 | -0.532 |
| *Granularity* | 0.247 | 0.467 | **-0.910** | 0.384 | -0.443 | 0.104 | 0.417 | 0.261 | **0.763** | **0.762** | -0.436 | 0.214 | -0.079 |
| *Visual consistency* | -0.167 | 0.317 | **-0.757** | 0.475 | **-0.715** | 0.520 | 0.589 | 0.561 | **0.787** | **0.779** | **-0.704** | 0.257 | 0.189 |
| *Acidity* | **0.704** | -0.171 | 0.230 | -0.410 | 0.767 | -0.619 | -0.584 | **-0.701** | -0.311 | -0.297 | **0.705** | 0.157 | -0.279 |
| *Sweetness* | -0.601 | 0.369 | 0.064 | 0.146 | -0.541 | **0.642** | **0.709** | 0.480 | 0.060 | 0.048 | -0.441 | **-0.645** | 0.405 |
| *Astringency* | **0.683** | 0.179 | 0.026 | -0.403 | 0.535 | **-0.891** | -0.606 | **-0.668** | -0.256 | -0.243 | **0.673** | 0.096 | -0.525 |
| *Intensity kiwi taste* | **0.690** | -0.099 | 0.380 | -0.185 | **0.923** | **-0.816** | **-0.888** | **-0.900** | -0.454 | -0.434 | **0.919** | 0.203 | **-0.636** |
| *Atypical taste* | **-0.791** | -0.018 | -0.215 | 0.213 | **-0.868** | **0.866** | **0.932** | **0.877** | 0.296 | 0.276 | **-0.864** | -0.410 | **0.701** |
| *Aftertaste* | -0.210 | -0.429 | **0.639** | 0.314 | 0.446 | 0.124 | -0.216 | -0.197 | -0.589 | -0.587 | 0.309 | -0.269 | 0.130 |
| *Mouth consistency* | -0.077 | 0.134 | **-0.766** | **0.649** | -0.584 | 0.548 | 0.513 | 0.509 | **0.856** | **0.851** | **-0.688** | 0.360 | 0.057 |

## 4. CONCLUSIONS

Applying intense treatments of high microwave power mainly affected the colour and taste of kiwifruit puree. This fact is shown through different instrumental and sensory parameters obtained for the treated and fresh purees. The most appropriate parameters for quality control of this product, among those considered in this study, were the colour parameters L*, a*, h*, WI as well as flow distance measured with a Bostwick consistometer. These parameters were the only ones that showed significant and meaningful correlations with sensory descriptors. As regards to instrumental analysis, the most intensive processed samples (600–340, 900–300 and 1,000–200) were significantly thicker, more viscose and had a greater lightness increment than the rest of the samples. Concerning sensory assessment, perceivable significant differences were only found between kiwifruit puree samples in some descriptors ('typical kiwifruit colour', 'tone', 'lightness', 'visual consistency' and 'typical taste'), which increased when microwave power increased. The most severely treated samples showed the highest variation in these parameters. In this sense, microwave could be considered an interesting technique to treat the samples although much care has to be taken with the intense treatments.

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# CAPÍTULO IV.2. COMPARACIÓN DEL IMPACTO DEL CALENTAMIENTO POR MICROONDAS Y CONVENCIONAL EN LA ACTIVIDAD ENZIMÁTICA Y LA CAPACIDAD ANTIOXIDANTE DE UN PURÉ DE KIWI

# COMPARISON OF MICROWAVES AND CONVENTIONAL THERMAL TREATMENT ON ENZYMES ACTIVITY AND ANTIOXIDANT CAPACITY OF KIWIFRUIT PUREE

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## ABSTRACT

Enzymes are naturally present in food and can cause product deterioration. For this reason, most food-processing steps try to reduce the enzymatic activity. The aim of this work was to compare, in terms of both the inactivation of kiwifruit puree peroxidase, polyphenoloxidase and pectinmethylesterase and also the maintenance of the antioxidant capacity of the product, the effect of a microwave treatment with a conventional thermal treatment designed to cause the same level of peroxidase inactivation (90%). The microwave power and process time that best permitted the maximisation of both the enzyme inactivation and the antioxidant capacity of the product, were selected by means of the Response Surface Methodology. The results obtained point to microwave heating as an appropriate technology with which to produce a stable kiwifruit puree, since these treatments were more effective at enzyme inactivation and antioxidant capacity retention than the conventional thermal treatment.

*Industrial relevance:* Food industry is currently focused on the development of novel and minimally processed products with improved quality. Traditional thermal processing has been assumed to require the use of high temperatures and long times to stabilise food products, which lead to dramatic losses of products' quality. Thus, a variety of different processing technologies are being explored as alternative to traditional thermal processing. The results of this study point out that more than conventional heating, microwave technology can be an appropriate means of achieving the required level of enzyme inactivation at which to obtain a stable kiwifruit puree with an improved antioxidant capacity.

**KEYWORDS** Kiwifruit, microwaves, peroxidase, polyphenoloxidase, pectinmethylesterase, antioxidant capacity.

## 1. INTRODUCTION

Different food scientists and nutrition specialists consider the consumption of fruit and vegetables as having many health benefits (Antunes, Dandlen, Cavaco, & Miguel, 2010; Du, Li, Ma, & Liang, 2009). Kiwifruit has been attributed with exceptional nutritional and sensory properties, as well as high antioxidant activity comparable to that of mangosteen, avocado, papaya, mango and cempedak (Park et al., 2006).

In recent years, consumers' food habits have changed towards a greater consumption of ready-to-eat and minimally processed fruit based products, leading to the marketing of products such as fruit juices, beverages of fruit juices mixed with milk, fruit purees or smoothies (Antunes et al., 2010; Elez-Martínez, Aguiló-Aguayo, & Martín-Belloso, 2006; Osorio, Martínez-Navarrete, Moraga, & Carbonell, 2008). This type of products has been traditionally preserved by means of conventional thermal technologies (Osorio et al., 2008; Whitaker, Voragen, & Wong, 2003). However, it usually requires the use of high temperatures combined with long process times which has been widely associated with a marked deterioration in food quality, especially with the development of cooked off-flavours, colour alteration and the loss of thermosensitive compounds (Elez-Martínez et al., 2006; Gonçalves, Pinheiro, Abreu, Brandao, & Silva, 2010; Llano, Haedo, Gerschenson, & Rojas, 2003; Maskan, 2001; Queiroz, Mendes, Fialho, & Valente-Mesquita, 2008). For this reason, alternatives to conventional processing technologies are being explored. Microwave heating has been proposed as a good alternative to conventional heating when the purpose is either drying, pre-cooking, tempering or preserving (Huang, Sheng, Yang, & Hu, 2007; O'Donnell, Tiwari, Bourke, & Cullen, 2010; Vadivambal & Jayas, 2007). Microwave energy (MW) is transported as an electromagnetic wave (0.3 GHz–300 GHz). When intercepted by dielectrical materials, MW produces an increase in the product temperature associated with dipole rotation and ionic polarisation (Schubert & Regier, 2010). This type of technology implies volumetric heating, which means that the materials can absorb microwave energy directly and internally. For this reason, compared to conventional heating methods, microwaves lead to a faster heating rate, thus reducing the process time (Huang et al., 2007;

Igual, García-Martínez, Camacho, & Martínez-Navarrete, 2010; Queiroz et al., 2008). In this way, the processing cost can be cut and the product may present better preserved sensory, nutritional and functional properties (De Ancos, Cano, Hernández, & Monreal, 1999; Igual et al., 2010).

Enzymatic and microbiological control is essential to ensure the quality and safety of fruit-based food products. However, much care has to be taken with antioxidant compounds because they are mainly responsible for the benefits to human health commonly attributed to the regular intake of vegetable origin products (Antunes et al., 2010; Du et al., 2009). Enzymes are naturally present in fruit and can cause product deterioration in many ways (Whitaker et al., 2003). Peroxidase (POD) and polyphenoloxidase (PPO) are the enzymes that are principally responsible for the deterioration of the colour and nutritive value of most fruits and vegetables (Gonçalves et al., 2010; Queiroz et al., 2008). POD can be used to evaluate the efficiency of vegetable thermal treatments because of its relatively high thermal stability (Anthon, Sekine, Watanabe, & Barrett, 2002; Lemmens et al., 2009). Pectinmethylesterase (PME) can cause changes in the rheological properties of foods by means of pectin desterification (Jolie et al., 2010; Osorio et al., 2008).

The following aspects have been considered in order to establish a process optimisation procedure with which to obtain a high quality microwaved kiwifruit puree: (i) the effect of microwave processing factors (power and time) on POD, PPO and PME inactivation as well as on the antioxidant capacity maintenance in a kiwifruit puree; (ii) the necessary treatment conditions with which to obtain the greatest enzyme inactivation and the lowest antioxidant capacity degradation in the product and (iii) a comparison of the effectiveness with which microwave and conventional thermal processing inactivate enzymes without altering the antioxidant capacity of the kiwifruit puree.

## 2. MATERIALS AND METHODS

### 2.1. Sample Preparation

Kiwifruit (*Actinida deliciosa* var. Hayward) was purchased from a local supermarket. Fruit pieces were peeled, washed with distilled water, cut into slices

and triturated with a Thermomix (TM21, Vorwerk, Spain), using the fourth power level for 1 min.

### 2.2. Treatment of kiwifruit puree

#### *2.2.1. Microwave treatment*

A household microwave oven (NORM 3038GC, China), provided with a glass turntable plate, was used to treat the kiwifruit puree. To study the effect of microwave power and process time on the inactivation of POD, PPO and PME, as well as on the antioxidant capacity of the product using the minimum number of experimental trials (Beirão-da-Costa, Steiner, Correia, Empis, & Moldão-Martins, 2006; Cano, Hernández, & De Ancos, 1997), an experimental design based on a central composite design was applied (Cochran & Cox, 1957). The power and the time were designed to vary between 300 and 900 W and between 100 and 300 s, respectively, taking into consideration the results of previous experiments that ensure that these microwave process conditions allow kiwifruit puree to be obtained with and without sensorily perceivable significant differences when compared to fresh puree (Benlloch-Tinoco, Varela, Salvador, & Martínez-Navarrete, 2011). A total of 16 running factorial points were defined by the experimental design. Samples were tempered to an initial temperature of 35 °C (no enzymatic degradation has been observed at this temperature (Rodrigo, Jolie, Van Loey, & Hendrickx, 2007; Sampedro, Rodrigo, & Hendrickx, 2008). For each treatment, a sample of 500 g was heated in the microwave oven in a standard size glass beaker (9 cm inner diameter and 12 cmlength) (BKL3-1K0-006O, Labbox, Spain). The temperature of the sample in the hottest spot, previously identified (data not shown), was continuously recorded by means of a fibre-optic probe (CR/JP/11/11671, Enelec, Spain) which was connected to a temperature datalogger (FOTEMP1-OEM, Enelec, Spain). Treated samples were immediately cooled in ice-water until the puree reached 35 °C.

#### *2.2.2. Conventional thermal treatment*

A conventional thermal treatment, which ensured a POD inactivation of 90%, was set up (Elez-Martínez et al., 2006; Llano et al., 2003; Williams, Lim, Chen, Pangborn, & Whitaker, 1986). This level of inactivation was fixed by taking into

account the industrial requirements which have to be met in order for the product to be considered as stable (Gonçalves et al., 2010; Zheng & Lu, 2011). The treatment needed to obtain this level of POD inactivation (data not shown) was 97 °C for 30 s in a thermostatic water bath (Precisterm, Selecta, Spain). After kiwifruit was triturated, 20 g of puree were introduced into TDT stainless steel tubes (13 mm inner diameter and 15 cm length) and closed with a screw stopper. A thermocouple connected to a data logger was introduced through the sealed screwed top to control the process. Prior to the treatment, the samples were preheated at 35 °C to shorten and standardise the come-up time. Under these conditions, a come-up time of 150 s was needed to reach the treatment temperature. Treated samples were immediately cooled in ice-water.

### 2.3. Analytical Determinations

Enzyme activity (POD, PPO and PME) and antioxidant capacity were measured in all the treated samples (microwaved and conventionally heated ones) and also in the non-treated sample, which was used as the control. Additionally, some physicochemical properties were determined in the fresh sample.

#### *2.3.1. Physicochemical Properties*

Water content ($x_w$) was measured by drying the sample to constant weight at 60 °C in a vacuum oven following the AOAC (2000) 934.06 method. Soluble solids were determined by measuring the °Brix in a previously homogenised sample using a portable digital refractometer, Refracto 3PX, at 20 °C (Metler Toledo, Switzerland).Water activity ($a_w$) was measured by means of a dew point hygrometer (GBX FA-st Lab, France) and pH with a digital pH-metre, Basic 2 (Crison, Spain). Each analysis was carried out in triplicate.

#### *2.3.2. Peroxidase and polyphenoloxidase activities*

##### 2.3.2.1. Extraction procedure

The enzymes were extracted using the method of De Ancos et al. (1999), with some modifications. Kiwifruit puree (10 g) was homogenised with 20 mL of 0.2 M sodium phosphate buffer (pH 6.5) containing 10 $g \cdot L^{-1}$ insoluble

polyvinylpolypyrolidone and 10 mL·L$^{-1}$ Triton X-100 with external cooling for 3 min with stop intervals every 30 s. The homogenate was centrifuged at 16,000 ×g and 4 °C for 20 min and the supernatant was collected. The volume of the obtained extract was measured. Extracts were taken in duplicate.

#### 2.3.2.2. Polyphenoloxidase activity measurement

PPO activity was assayed spectrophotometrically by placing 3 mL substrate, which consisted of a solution of 0.015 M catechol in 0.05 M sodium phosphate buffer (pH 6.5) and 0.050 mL of enzyme extract, in a cuvette (De Ancos et al.,1999). The increase in absorbance at 400 nm and 25 °C was recorded automatically for 30 min (Thermo Electron Corporation, USA). A solution containing all the components except the enzyme extract, which was replaced by 0.050 mL of sodium phosphate buffer, was used as a blank. One unit of PPO activity was defined as the amount of enzyme that caused an increase of one in the absorbance per min (Abs·min$^{-1}$·g$^{-1}$), calculated from the linear part of the obtained curve. The percentage of enzyme inactivation (I) was calculated by using Eq. (1).

$$I = \frac{A_F - A_T}{A_F} \times 100 \quad (1)$$

Where:

$A_F$: enzyme activity of fresh kiwifruit puree

$A_T$: enzyme activity of treated kiwifruit puree.

#### 2.3.2.3. Peroxidase activity measurement

POD activity was measured by spectrophotometry following the method described by De Ancos et al. (1999). Aliquots of enzyme extract (0.050 mL) were added to a reaction mixture made up of 2.7 mL of 0.05 M sodium phosphate buffer (pH 6.5) with 0.2 mL p-phenylenediamine (10 g·kg$^{-1}$) as H-donor and 0.1 mL hydrogen peroxide (15 g·L$^{-1}$) as oxidant. The oxidation of p-phenylenediamine was measured at 485 nm and 25 °C using a Thermo Electron Corporation spectrophotometer (USA). A solution containing all the components except the enzyme extract, which was replaced by 0.050 mL of sodium phosphate buffer, was

used as a blank. One unit of POD activity was defined as the amount of enzyme that caused an increase of one in absorbance per min ($Abs \cdot min^{-1} \cdot g^{-1}$), calculated from the linear part of the obtained curve. The percentage of enzyme inactivation was calculated by using Eq. (1).

*2.3.3. Pectinmethylesterase activity*

PME activity was determined following the pH decrease produced by the carboxyl groups released by the hydrolysis of methyl esters of pectin, by means of an automatic pH-stat titrator (Metrohm, Switzerland) using the method described by Rodrigo et al. (2006), with some modifications. The sample was placed in a thermostated water bath at 22 °C to control the temperature during the determination. The reaction mixture consisted of 2 mL of kiwifruit puree and 30 mL of 0.35% citrus pectin (70–75% esterification, Fluka) containing 0.117 M NaCl, which was previously adjusted to pH 6.5 with NaOH. During hydrolysis, the pH was maintained at 6.5 by the addition of 0.02 N NaOH using the automatic pH-stat titrator. The consumption of NaOH was recorded for 5 min. One unit of PME activity can be expressed as the amount of enzyme that produces 1 μmol of acid per minute at pH 6.5 and 22 °C. The enzyme activity (U/mL) and the percentage of enzyme inactivation were calculated by using Eqs. (2) and (1), respectively.

$$PME\left(\frac{U}{mL}\right) = \frac{V \cdot N \cdot 1000}{V_s \cdot t_r} \quad (2)$$

Where:

V: added volume of NaOH (mL),

N: normality of the NaOH used,

$V_s$: volume of sample (mL),

$t_r$: reaction time (min)

2.3.4. Antioxidant capacity measurement

To determine the antioxidant capacity of kiwifruit puree, the DPPH• radical scavenging capacity of kiwifruit extracts was measured as described by Igual et al. (2010) with some modifications. Kiwifruit puree was appropriately diluted with

methanol by mixing with external cooling for 30 s. The homogenate was centrifuged at 11,872 ×g for 10 min at 4 °C and the supernatant was collected. Aliquots of kiwifruit extract (0.03 mL) were combined with 3 mL of DPPH• $6.25 \cdot 10^{-5}$ M in methanol. A Thermo Electron Corporation spectrophotometer (USA) was used to measure the change in absorbance at 517 nm and 25 °C until the reaction reached a plateau (time at the steady state). A control sample, where the kiwifruit puree extract was replaced by DPPH• $6.25 \cdot 10^{-5}$ M in methanol, was used to measure the maximum DPPH• absorbance. The percentage of antioxidant capacity variation (A) was calculated using Eq. (4).

$$A = \frac{AC_T - AC_F}{AC_F} \times 100 \quad (4)$$

Where:

$AC_T$: Antioxidant capacity of the treated kiwifruit (mM Trolox/mL of kiwifruit);

$AC_F$: Antioxidant capacity of the untreated kiwifruit (mM Trolox/mL of kiwifruit)

In order to express the antioxidant capacity in terms of millimolar Trolox, a calibration curve was prepared by measuring the absorbance at 517 nm of different Trolox solutions in the range of 0.3–3 mM.

### 2.4. Statistical analysis

The statistical analysis carried out to optimise the process that consisted of a Multiple Response Optimisation procedure. To relate the experimental data with the independent variables a Response Surface Methodology was applied. Only the terms found to be statistically significant ($p<0.05$) after the analysis of variance of the corresponding regression analysis were included in the final reduced model (Mirhosseini, Tan, Hamid, Yusof, & Chern, 2009). The non-significant lack of fit in all the selected final models ($p>0.05$) confirmed the suitability of the fitted model and the non-significance of the Durbin–Watson statistic proved that there was no significant autocorrelation or serial correlation. The Statgraphics 5.1 plus software programme (StatPoint Technologies, Inc., Warrenton, VA, USA) was used.

## 3. RESULTS AND DISCUSSION

### 3.1. Kiwifruit puree characterization

Enzyme activity (POD, PPO and PME), antioxidant capacity and some physicochemical properties ($x_w$, $a_w$, °Brix and pH) of fresh kiwifruit were determined in order to control the fruit which was used as the raw material for microwave and conventional heating treatments. The obtained values (Table 1) coincide closely with those reported by other authors working on this fruit (Antunes et al., 2010; Beirão-da-Costa, Cardoso, Martins, Empis, & Moldão-Martins, 2008; De Ancos et al., 1999; Du et al., 2009; Fúster, Préstamo, & Cano, 1994; Llano et al., 2003; Zolfaghari, Sahari, Barzegar, & Samadloiy, 2010).

**Table 1.** Characteristics of fresh kiwifruit puree. Mean values and standard deviation (in brackets).

| | |
|---|---|
| Water content (g water/100 g product) | 84.1 (1.0) |
| Water activity | 0.984 (0.003) |
| ºBrix (g soluble solids/100 g product liquid phase) | 14.3 (0.3) |
| pH | 3.40 (0.07) |
| POD activity ($Abs \cdot min^{-1} \cdot g^{-1}$) | 9 (2) |
| PPO activity ($Abs \cdot min^{-1} \cdot g^{-1}$) | 6.0 (1.4) |
| PME activity ($U \cdot g^{-1}$) | 1.1 (0.2) |
| Antioxidant capacity (mM Trolox·$mL^{-1}$) | 13 (2) |

POD: peroxidase; PPO: polyphenoloxidase; PME: pectinmethylesterase

### 3.2. Effect of microwave treatment

The inactivation of POD, PPO and PME (mean value with standard deviation in brackets) in kiwifruit puree produced by processing in the desired interval of microwave power (300–900 W) and time (100–300 s) ranged from 43% (6) to 88.0% (0.7), 11.4% (0.5) to 81% (2) and −19.0% (1.3) to 57% (6), respectively. These results point out that in kiwifruit, PME and POD were the enzymes that were most resistant and labile to MW, respectively. Other authors, such as Beirão-da-Costa et al. (2008) and McFeeters, Fleming, and Thompson (1985) found PME to be a highly heat stable enzyme, as intense heat treatments were necessary to inactivate it. On the other hand, De Ancos et al. (1999) reported that the POD enzyme was more

efficiently inactivated than other enzymes in a microwaved kiwifruit puree and Terefe, Yang, Knoerzer, Buckow, and Versteeg (2010) found the same to be true in a thermally treated strawberry puree. This fact could be connected to the characteristic low pH of these fruits. Williams et al. (1986) reported that POD was less stable at pHs below 4. Despite the fact that POD was the most labile enzyme in this case, it could be considered as an adequate indicator of treatment efficiency since it has been reported to be greatly relevant in kiwifruit because of its high activity and extensive contribution to the quality of this fruit (Fang, Jiang, & Zhang, 2008). Additionally, PME residual activity can be rapidly lost during storage in acid conditions (pH<4) (McFeeters et al., 1985) and PPO activity does not seem to have a very important repercussion on kiwifruit quality in view of the lack of browning in kiwifruit tissues. This could be explained by the low polyphenolic levels and the high ascorbic acid content in this fruit, which could itself prevent the oxidation of many polyphenols (Fúster et al., 1994). On the other hand, the microwave treatment 300 W–100 s led to a PME activity promotion. This could be related to the low temperature reached in this case by the sample, around 43 °C, and the short time of exposition. This phenomenon was observed by Beirão-da-Costa et al. (2008), who found a significant increase in PME activity in kiwifruit slices subjected to a mild heat treatment prior to inactivation. The other sample submitted to 300 W reached 45 °C but the corresponding treatment was of 300 s. Under these conditions inactivation of PME was of just 4.3% (0.7). The temperature reached by the other samples was in the range 60–100 °C.

The results obtained from the enzyme inactivation study were analysed by means of the Response Surface Methodology. The models obtained from this analysis, together with the corresponding adjusted $R^2$ values and the standard error of the estimate, are summarised in Table 2. Adjusted $R^2$ values indicate the % of the variation in enzyme inactivation produced by microwave power and process time that is explained by the models. According to other authors, it can be considered that models satisfactorily represent the data in the experimental domain when $R^2$ values range between 70 and 90%, while they can be considered excellent if $R^2$ > 90% (Granato, Castro, Ellendersen, & Masson, 2010; Granato, Ribeiro, Castro, & Masson, 2010; Montgomery, 2009). In this way, the three obtained models can be

assumed to be valid predictors of POD, PPO and PME inactivation as a function of microwave power and process time, in the range considered in this study. From the equations presented in Table 2, it can be observed that increases in both factors (microwave power and time) produced significant effects, both linear and quadratic ($p<0.05$).

**Table 2.** Models explaining peroxidase (POD), polyphenoloxidase (PPO) and pectinmethylesterase (PME) inactivation and antioxidant activity variation. Adjusted $R^2$ (Adj. $R^2$) and standard error of estimate (SEE) values. P: Microwave power (W); t: process time (s).

| *Dependent variable* | *Equation* | *Adj. $R^2$* | *SEE* |
|---|---|---|---|
| 1 | $PME = -73.8202 + 0.0916 \cdot P + 0.1675 \cdot t$ | 75.15 | 10.36 |
| 2 | $POD = -75.7720 + 0.3476 \cdot P + 0.1220 \cdot t - 0.0002 \cdot P^2$ | 82.40 | 1.49 |
| 3 | $PPO = -48.6351 + 0.2353 \cdot P + 0.0802 \cdot t - 0.0001 \cdot P^2 + 0.0001 \cdot P \cdot t + 0.0003 \cdot t^2$ | 98.64 | 2.09 |
| 4 | $A = 86.2719 - 0.1539 \cdot P - 0.1822 \cdot t + 0.0001 \cdot P^2 + 0.0003 \cdot t^2$ | 90.19 | 3.30 |

1: PME inactivation; 2: POD inactivation; 3: PPO inactivation; 4: Antioxidant capacity variation

A three-dimensional plot showing POD inactivation is presented in Fig. 1. As can be observed, a significant increase in POD inactivation occurs up to 800 W of power, decreasing slightly when a higher microwave power was applied. This behaviour can be explained by the fact that the model provided positive linear and negative quadratic effects (Table 2). De Ancos et al. (1999) observed the inactivation of papaya POD to behave similarly under microwave heating. They reported an increase in peroxidase inactivation when microwave power increased from 285 to 570 W at 30 s of processing time. Thereafter, a higher power level (800 W) did not increase the POD inactivation. In accordance with other authors, a linear increase in POD inactivation was detected the longer the process lasted (Matsui, Gut, de Oliveira, & Tadini, 2008; Zheng & Lu, 2011).

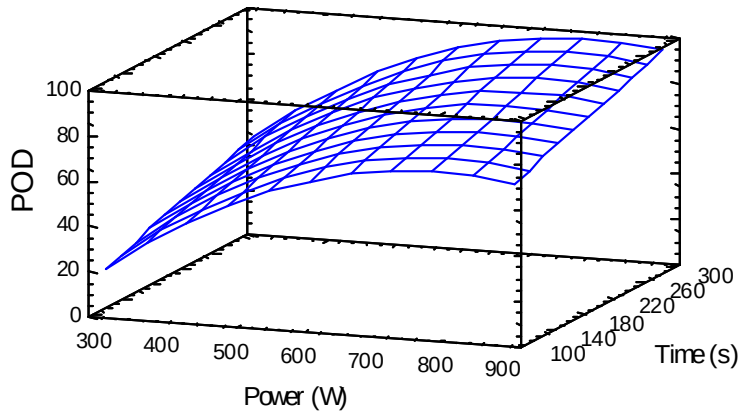


**Figure 1.** Response surface plot for the percentage change of peroxidase (POD) inactivation in kiwifruit puree as a function of microwave power and process time.

Fig. 2 shows the PPO inactivation behaviour as related to microwave power and process time based on the obtained model. As can be observed, the level of PPO inactivation rose as the microwave power increased, which had significant ($p<0.05$) linear and quadratic effects (Table 2).

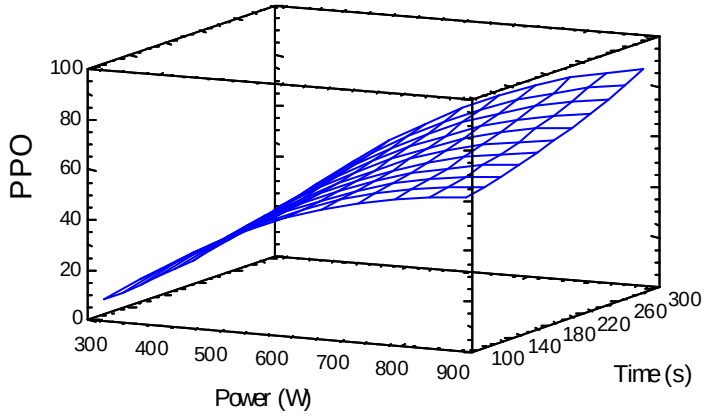


**Figure 2.** Response surface plot for the percentage change of polyphenoloxidase inactivation (PPO) in kiwifruit puree as a function of microwave power and process time.

The negative quadratic effect of this factor leads to the smaller increase in the PPO inactivation observed at greater powers. Process time also had a significant effect, in this case both positive linear and quadratic (Table 2), leading to a greater inactivation of this enzyme. Latorre, Bonelli, Rojas, and Gerschenson (2012) and

Matsui et al. (2008) found that there was a greater level of PPO inactivation in red beet and green coconut water, respectively, after longer microwave exposure.

De Ancos et al. (1999) observed that PPO inactivation in kiwifruit and strawberry was better controlled by pre-fixing the power rather than the exposure time. In addition, an interactive effect was observed between both independent variables (microwave power and process time) on PPO inactivation. As expected, in samples subjected to longer treatment times, the level of PPO inactivation rose faster as greater microwave power was applied than in kiwifruit puree subjected to shorter treatment times.

PME inactivation increased significantly ($p<0.05$) as the microwave power level rose and the processing time got longer (Fig. 3). In both cases, only a linear effect was found in PME inactivation (Table 2).

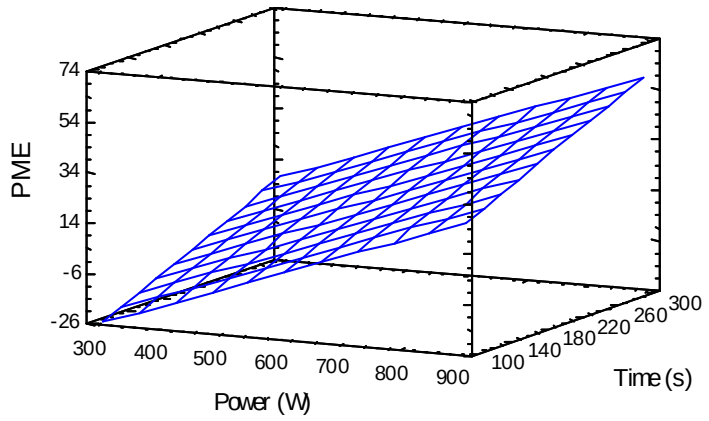


**Figure 3.** Response surface plot for the percentage change of pectinmethylesterase (PME) inactivation in kiwifruit puree as a function of microwave power and process time.

In the same way, Tajchakavit and Ramaswamy (1997) reported a linear relationship between time and PME inactivation during microwave heating of orange juice and, when microwaving orange peels, Kratchanova, Pavlova, and Panchev (2004) found that the higher the microwave power, the greater the PME inactivation.

The mean value with standard deviation of antioxidant capacity variation caused in kiwifruit puree under microwave processing ranged between 3% (2) and 36%

(0.6). Table 2 shows the model found to explain the effect of microwave power and process time on this variation and Fig. 4 shows the corresponding response surface plot based on the obtained model.

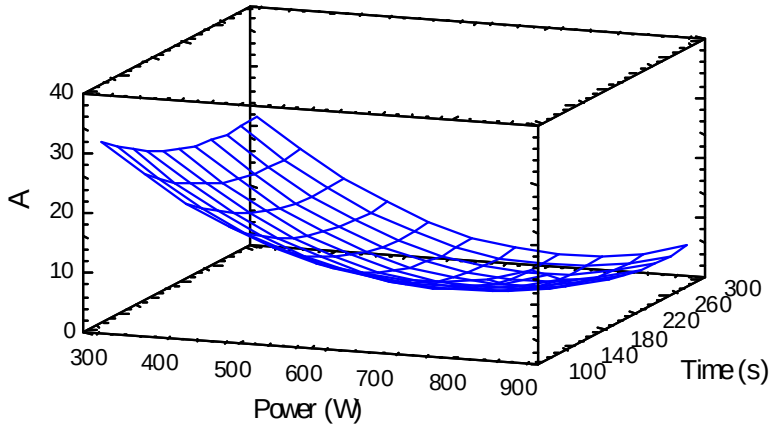


**Figure 4.** Response surface plot for the percentage change of the antioxidant capacity variation (A) in kiwifruit pure as a function of microwave power and process time.

Although applying intense thermal treatments (T>60 °C) is usually regarded as undesirable because it can induce oxidative condensation or degradation of thermolabile compounds, the opposite behaviour was found during the present study. When microwave treatments were applied, a positive variation of the antioxidant capacity of treated purees was observed, which is related with an increase in the antioxidant capacity of the product: the more intense the treatments, the lower the antioxidant capacity increase and so the smaller the antioxidant capacity variation (Fig. 4).

According to literature, the application of thermal treatment can be associated with the dissociation of conjugated forms into free phenolic acid compounds, like gallic, caffeic, ferulic and p-coumaric acids (Dewanto, Wu, Adom, & Liu, 2002; Gallegos-Infante, Rocha-Guzman, Gonzalez-Laredo, & Pulido-Alonso, 2010; Jing, Jing-Feng, Yu-Ying, & Lin-Chun, 2010). Despite the fact that the oxidative and hydrolytic enzymes that can destroy the antioxidant compounds can also be released during processing, they are deactivated by the thermal treatment thus avoiding the loss of phenolic acids (Dewanto et al., 2002). Several authors, such as

Randhir, Kwon, and Shetty (2008), Sahin, Topuz, Pischetsrieder, and Özdemir (2009) and Jing et al. (2010) reported an increase of antioxidant capacity in cereals, carob powder and sweet potatoes after being thermally treated, dried or steamed, respectively. As far as the effect of microwave heating on the antioxidant capacity preservation of treated purees is concerned, a significant repercussion of microwave power and process time was observed ($p<0.05$). The polynomial model obtained from the experimental design explained 90.19% of the data variation caused by these effects (Table 2). Both factors had negative linear and positive quadratic effects on the antioxidant capacity variation of the kiwifruit puree (Table 2). In our experimental conditions, this implied that a greater antioxidant capacity promotion was obtained when lower microwave power and shorter processing times were employed, and when greater microwave power was applied, the repercussion of the length of the processing time seemed to be less relevant.

### 3.3. Process optimisation procedure to obtain an optimum microwaved stable kiwifruit puree

In the present research work, the effect of microwave heating on the global quality of kiwifruit puree has been evaluated in a pre-established range of power and time, taking both the enzyme inactivation and the antioxidant capacity preservation as quality indicators. Through the superposition of all the obtained models (Table 2), it is possible to predict which treatment conditions (power and time combination) are better at achieving, simultaneously, the largest enzyme activity reduction (POD, PPO and PME) and the maximum increase in the antioxidant capacity within the studied range. From this multiple response optimisation, the overall optimum condition was achieved by applying 1000 W of power for 340 s. Under this optimum condition, the corresponding predicted response variables for POD, PPO and PME inactivation and antioxidant capacity variation were 90.7%, 97.5%, 77.2% and 13.0%, respectively. This treatment could be considered the adequate method by which to produce stable kiwifruit puree that is not seriously affected, since 90% of the POD activity was reduced (Gonçalves et al., 2010; Zheng & Lu, 2011) and no loss of antioxidant capacity was caused.

### 3.4. Microwave versus conventional heating

The conventional thermal treatment caused 89.2% (0.9), 64.9% (0.7) and 65% (4) POD, PPO and PME inactivation, respectively and −11% (8) antioxidant capacity variation. Although both microwave mode and conventional treatments reached the same level of POD inactivation (≈90%), a considerably greater reduction of PPO and PME enzyme activity, as well as an increase instead of a loss in antioxidant capacity, were observed in the sample subjected to the optimum microwave treatment. These results seem to indicate that microwave heating was more effective at enzyme inactivation and led to a better antioxidant capacity retention in kiwifruit puree than conventional heating. Several authors have reported similar results when working on enzyme inactivation in different fruit products processed by means of microwave technology. Tajchakavit and Ramaswamy (1997) found significantly faster PME inactivation in orange juice in the microwave heating mode than in the conventional heating mode. Matsui et al. (2008) published that the inactivation of PPO and POD during microwave processing of green coconut water was significantly faster in comparison with the conventional processes reported in the literature. Zheng and Lu (2011) found microwave heating to be more effective at inactivating POD and preserving nutritional properties in carrot than the conventional thermal treatment. All these differences could indicate the possibility of there being some contributory non-thermal effects of microwaves, making them more effective at enzyme inactivation than the conventional thermal treatment (Awuah, Ramaswamy, & Economides, 2007; Banik, Bandyopadhyay, & Ganguly, 2003; Tajchakavit & Ramaswamy, 1997; Tajchakavit, Ramaswamy, & Fustier, 1998). Although the mechanism is still unclear, Kermasha, Bisakwski, Ramaswamy, and Van de Voort (1993) proposed that enzyme inactivation under microwave heating may be the result of both the temperature and the interaction of the microwave energy with the enzyme, because the microwave field can affect the polar and/or charged moieties of proteins. This phenomenon has long been under investigation and it was Olsen, Drake, and Bunch (1966) who were probably the first ones to postulate the non-thermal effects of microwaves. Nowadays, however, it remains necessary to study this matter more thoroughly given that there are still controversial opinions (Awuah et al., 2007). In any case, it has been demonstrated that MW technology is suitable

for facing enzyme inactivation (De Ancos et al., 1999; Latorre et al., 2012; Matsui et al., 2008) in a shorter process time in comparison with other conventional technologies, which indicates that stability can be properly ensured and product quality can be effectively preserved (Igual et al., 2010; Zheng & Lu, 2011).

## 4. CONCLUSION

More than conventional heating, microwave technology can be an appropriate means of achieving the required level of enzyme inactivation at which to obtain a stable kiwifruit puree with an improved antioxidant capacity. Nevertheless, microwave power and processing time must be adequately selected as they had a significant influence on all the variables considered in the present research work. The Response Surface Methodology may be used as a suitable tool with which to optimise the process conditions that allow both the enzyme inactivation and the antioxidant capacity of kiwifruit puree to be maximised.

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# CAPÍTULO IV.3. CINÉTICA DE INACTIVACIÓN DE *LISTERIA MONOCYTOGENES* EN UN PURÉ DE KIWI PROCESADO POR MICROONDAS Y POR CALENTAMIENTO CONVENCIONAL

# *LISTERIA MONOCYTOGENES* INACTIVATION KINETICS UNDER MICROWAVE AND CONVENTIONAL THERMAL PROCESSING IN A KIWIFRUIT PUREE

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## ABSTRACT

The inactivation of *Listeria monocytogenes* in a kiwifruit puree by conventional and microwave heating was studied. Survival curves at three microwave power levels (600–1000 W) and three temperatures (50–60 °C) were obtained. Data were properly fitted by a first-order kinetic model. Processing times under both technologies were corrected to isothermal treatment for the kinetic study. Microwave heating was shown to effectively inactivate *L. monocytogenes*. In the range of microwave and conventional processing conditions assayed, the 5-$\log_{10}$ reductions of *L. monocytogenes* recommended by the FDA for pasteurized products were achieved. The level of microwave power applied had a considerable influence on the *L. monocytogenes* inactivation rate. The higher the power level, the faster the inactivation. The inactivation of *L. monocytogenes* under microwave heating at 900 W ($D_{60}$ °C= 17.35 s) and 1000 W ($D_{60}$ °C= 17.04 s) happened faster than in a conventional thermal process ($D_{60}$ °C= 37.45 s). Consequently, microwave heating showed greater effectiveness for *L. monocytogenes* inactivation than conventional heating.

*Industrial relevance:* Consumers' desires are oriented towards new foods that are convenient, easy to preserve and ready-to-eat products, being consumption of fresh fruit replaced with processed fruit products. Food industry is currently focused on the development of novel and minimally processed products with improved quality. Thus, a variety of new processing technologies are being explored as alternative to traditional thermal processing. In this work, the thermal and microwave inactivation kinetics of *Listeria monocytogenes* in a ready-to-eat kiwifruit puree were investigated so as to assess the suitability of microwave processing as an alternative to thermal processing. The results of this study point out that more than conventional heating, microwave technology can be an appropriate means of fruit product pasteurization with the possibility of offering the required safety by using a lower process time, when microwave power of a certain level is applied.


**KEYWORDS** Kiwifruit, *Listeria monocytogenes*, Microwave heating, Conventional heating, Inactivation kinetics.

## 1. INTRODUCTION

Microwave energy (MW) has been extensively used in the area of food processing for various commercial purposes (Vadivambal & Jayas, 2007). MW heating involves the use of electromagnetic waves (0.3–300 GHz) in order to generate heat in materials. This technology implies volumetric heating, as materials can directly absorb microwave energy, causing dipolar molecule oscillation and ionic polarization. Some commercially proven applications of microwave food processing include dehydration of low-moisture solids, pre-cooking of meat products and tempering of frozen foods (Vadivambal & Jayas, 2007). In recent years, the suitability of microwave heating to enhance food microbial safety (pasteurization and sterilization processes) has been successfully tested in various animal and vegetable food products (Cañumir, Celis, de Brujin, & Vidal, 2002; Huang, Sheng, Yang, & Hu, 2007; O'Donnell, Tiwari, Bourk, & Cullen, 2010). This technology has been recognized to present some advantages over conventional heating: (i) MW leads to faster heating rates, so it can approach the benefits of high temperature-short time processing whereby bacterial destruction is achieved, but thermal degradation of the desired components is reduced (Huang et al., 2007); (ii) the magnetron, the element that produces microwave radiation, can be turned on or off instantaneously; (iii) the product can be pasteurized after being packaged; and (iv) MW processing systems can be more energy efficient (De Ancos, Cano, Hernández, & Monreal, 1999). However, in spite of these advantages, there are some potential problems which are inherent in microwave processing that are contributing to delay MW exploitation to its fullest potential in food industry applications (Picouet, Landl, Abadias, Castellari, & Viñas, 2009), being the existence of a non–uniform temperature distribution, which could result in hot and cold spots in the heated product, its major limitation (Vadivambal & Jayas, 2007). Additionally, up to date, little is known kinetically about the basic general relationship between microbial inactivation in foods and MW exposure, having Fujikawa, Ushioda, and Kudo (1992), Tajchakavit, Ramaswamy, and Fustier (1998), Cañumir et al. (2002), Yaghmaee and Durance (2005) and Pina-Pérez, Benlloch-Tinoco, Rodrigo, and Martínez (2013) conducted some of the few studies regarding the kinetics of destruction of foodborne pathogens and spoilage microorganisms by microwave irradiation, respectively.

On the other hand, nowadays consumers' desires are oriented towards new foods that are convenient, easy to preserve and ready-to-eat products. Actually, consumption of fresh fruit is being replaced with consumption of processed fruit products such as fruit juices, fruit juice and milk mixture beverages, fruit purees and smoothies. In this respect, development and applicability studies on novel processing technologies are required to provide these fruit-based products with better quality and guaranteed safety in order to address consumers' expectations (Picouet et al., 2009). Although fruit products with acidic nature have not potentially been recognized as the main vehicles for foodborne illnesses, there has been increasing concern because some outbreaks have been caused by consumption of unpasteurized juices contaminated with *Escherichia coli* or *Salmonella* spp. (Buffler, 1993; Picouet et al., 2009) as well as salad vegetables or mixed salads with *L. monocytogenes* (EFSA, 2013). In this respect, the National Advisory Committee on Microbiological Criteria for Foods recommended that E. coli O157:H7 and *L. monocytogenes* be used as appropriate target organisms for fruit juices. *L. monocytogenes* is a pathogen of great concern in minimally processed because of its ubiquitous presence and psychrotrophic nature, with a particular ability to multiply at low temperatures, low water activity levels and acidic pH (Carpentier & Cerf, 2011), allowing it to reach levels high enough to cause human disease (Chan & Wiedmann, 2009). Actually, the presence of *L. monocytogenes* has been demonstrated under refrigerated conditions in a number of fruits and vegetables, such as tomatoes, oranges, strawberries and fresh-cut fruit salad (Cobo-Molinos et al., 2008). Despite the fact that the minimum pH allowing growth of this pathogen in food products has been reported to be pH = 4.6 (Carpentier & Cerf, 2011), ready-to-eat fruit-based acidic products may still represent a potential hazard to health, given the well-known ability of *L. monocytogenes* to proliferate in products stored under long cooling storage. In this respect, some authors have found that *L. monocytogenes* should be used as the target organism in evaluating lethality of UV and heat processes for apple juice (pH = 3.68), since this pathogen showed higher resistance than *E. coli*, *Salmonella enteritidis* or *Salmonella typhimurium* at this low pH (Gabriel & Nakano, 2009; Guerrero-Beltrán & Barboza-Cánovas, 2005).

In order to contribute to the acquisition of knowledge about MW processing to preserve fruit-based products safely, the objective of the present research was to compare the effectiveness of MW and conventional thermal technologies for the inactivation of *L. monocytogenes* in a kiwifruit puree.

## 2. MATERIALS AND METHODS

### 2.1. Culture preparation

The lyophilized strain of *L. monocytogenes* CECT 4032 was supplied by the Spanish Type Culture Collection. For rehydration, it was transferred to 10 mL of Tryptic Soy Broth (TSB) (Scharlab Chemie S.A., Barcelona, Spain). After 30 min, 5 mL of culture was inoculated in 500 mL of TSB and incubated at 37 °C with constant agitation (200 rpm) for 21 h to obtain cells in the stationary growth stage. The cells were centrifuged twice at 4000 ×g for 15 min at 4 °C and resuspended in 20 mL of TSB. The cells were placed in 2 mL sterile plastic cryogenic vials containing TSB supplemented with 20% glycerol in a relation of 1:1. The 2 mL samples, with an approximate concentration of $5 \cdot 10^9$ CFU/mL, were immediately stored at −80 °C until use for the microbiological studies.

### 2.2. Sample preparation

Kiwifruit (*Actinidia deliciosa* var. Hayward) was purchased in a local supermarket. Fruit pieces were peeled, washed with distilled water, cut into slices and finally triturated in a Thermomix (TM 21, Vorwerk, Spain), using the fourth power level for 1 min. The physicochemical characteristics of kiwifruit puree were determined in order to control the fruit which was used as raw material for microwave and conventional heating treatments. Each analysis was made in triplicate. Results are expressed as mean values with standard deviation in brackets. Moisture was 83.4 (0.7) g water/100 g product (AOAC 934.06 method (2000)), °Brix 14.1 (0.3) g soluble solids/100 g product (Refracto 3PX refractometer, Metler Toledo, Switzerland), water activity 0.987 (0.002) (dew point hygrometer, GBX FA-st lab, France) and pH 3.39 (0.07) (Basic 2 pH-meter, Crison, Spain).

*2.2.1. Sample inoculation*

Taking into consideration the acidic character of the product, *L. monocytogenes* growth in kiwifruit puree prepared as described above was corroborated previously to perform the inactivation experiences (data not shown). Growth of the microorganism at several temperatures was tested (4, 10 and 20 °C). Temperatures higher than 4 °C were used to simulate *L. monocytogenes* growing in cases of cold chain breaking during the shelf-life of the product. The product was inoculated by adding 1mL of the concentrated suspension so as to give an initial *L. monocytogenes* concentration of $10^7$ CFU/g. Kiwifruit puree was blended at 25 °C for 30 s with the aim of ensuring a homogeneous initial content of the bacterium.

**2.3. Treatments**

*2.3.1. Microwave pasteurization*

A microwave oven (model: 3038GC, Norm, China) provided with a turntable plate was used to treat the kiwifruit puree (MWP). The microwave oven was provided with a probe (CR/JP/11/11671, OPTOCOM, Germany) which was connected to a fiber-optic thermometer (model FOTEMP1-OEM, OPTOCOM, Germany) to continuously record the time–temperature history of the sample during microwave treatments. With the aim of identifying the coldest spot of the product, the temperature profile at six different points in the puree were obtained, in the center and in the edge, both of them at the top, the center and the bottom of the sample, with two replicates per point. Then, the probe was located at the coldest spot and the temperature profile of the inoculated puree was again registered. The safety of the process was determined at the coldest spot location, since contaminating pathogen microorganisms may survive in cold spots (Nicolaï, 1998). For each treatment, a 500 g sample was tempered to an initial temperature of 25 °C and then heated in the microwave oven in a standard size glass beaker (BKL3-1K0-006O, Labbox, Spain). Treated samples taken from the coldest spot were immediately cooled in ice-water until the puree reached 35 °C (for 10–15 s), a temperature at which no enzymatic degradation has been observed (Rodrigo, Jolie, Van Loey, & Hendrickx, 2007). Survival curves were obtained at three power levels (600, 900 and 1000 W) with processing times varying between 50 and 340 s. Three replicates of each treatment

were carried out. The power absorbed by the sample at this three nominal power levels was estimated by heating 1 kg of distilled water from 10 °C up to 20 °C at 600, 900 and 1000W, according to the norm CEI IEC 60705 (1999). Experiments were performed in triplicate and results showed an average (and standard deviation) of 427 (12) W for the 600 W, 525 (10) W for the 900 W and 725 (6) W for the 1000 W.

*2.3.2. Conventional pasteurization*

Conventional thermal pasteurization (CTP) was applied in a circulating thermostatic water bath (Precisterm, Selecta, Spain). After the kiwifruit had been triturated, 20 g of puree was placed in TDT stainless steel tubes (13 mm inner diameter and 15 cm length) and closed with a screw stopper (Rodrigo, van Loey, & Hendrickx, 2007; Sampedro, Geveke, Fan, & Zhang, 2009). A thermocouple which was connected to a data logger was inserted through the sealed screw top in order to record the time–temperature history of the sample during the treatment. Three replicates were carried out to define the average temperatura profile of the process. Previously, the samples were preheated to 25 °C to shorten and standardize the come-up time. Treated samples were immediately cooled in ice-water until the puree reached 35 °C (for 15–20 s). Inactivation was studied at 50, 55 and 60 °C for 90–1200 s.

*2.3.3. Enumeration of microorganisms*

Serial decimal dilutions of the untreated and treated samples, immediately after having been inoculated or subjected at different process times (see Tables 1 and 2), respectively, were performed in 0.1% (w/v) sterile peptone water (Scharlab Chemie S.A., Barcelona, Spain). The enumeration medium used for viable cells was Tryptic Soy Agar (TSA) (Scharlab Chemie S.A., Barcelona, Spain). The selected dilutions were incubated at 37 °C for 48 h, subsequently the counting step was carried out. The reduction of viable cells was expressed as the decimal logarithm of the quotient of the treated and untreated cells.

### 2.4. Mathematical modelling of data

Mean data of *L. monocytogenes* inactivation due to each treatment were fitted using first-order kinetics and D-values were determined in each case. The D-value represents the heating time required to reduce 90% of the existing microbial population under isothermal conditions (Eq. (1)) (Awuah, Ramaswamy, & Economides, 2007; Tajchakavit & Ramaswamy, 1997).

$$\log \frac{N}{N_0} = -\frac{t}{D} \quad (1)$$

where

N: survivor counts after treatment (CFU/g);

$N_0$: initial population of microorganism (CFU/g);

t: processing time (s);

D: D-value at the temperature studied (s).

D-values were calculated by non-linear regression according to the methodology described by Matsui, Gut, de Oliveira, and Tadini (2008). Since both treatments (MWP and CTP) applied in the present study involved non-isothermal heating conditions, correction of processing time values for come-up periods was necessary prior to kinetic data analyses. Therefore time–temperature profiles were used to calculate the effective time ($t_e$) or accumulated lethality (Eq. (2)), which represents the isothermal holding time at the selected reference temperature that causes the same level of microbial destruction as the heating actually applied (Awuah et al., 2007; Tajchakavit & Ramaswamy, 1997). During CTP a come-up time (CUT) was observed before the programmed temperature was achieved and maintained. This constant programmed temperature was considered as the reference temperature ($T_{ref}$). In the case of MWP, non-isothermal heating was observed during the treatments. Hence, for each microwave process, the maximum temperature reached during the treatment was considered as Tref (Latorre, Bonelli, Rojas, & Gerschenson, 2012; Matsui et al., 2008).

$$t_e = \int_0^\infty 10^{\left(\frac{T(t)-T_{ref}}{z}\right)} dt \quad (2)$$

### 2.5. Statistical data analysis and model evaluation

The goodness of fit between experimental and predicted data was assessed by using the adjusted regression coefficient (adjusted-$R^2$) (Eq. (3)) and the root mean square error (RMSE) (Eq. (4)). The higher the $R^2$ value and the lower the RMSE value were, the better the fit was considered. Analyses of variance (multifactor ANOVA) were run to study the effect of process variables (microwave power, temperature and treatment time) on *L. monocytogenes* inactivation. Values of log ($N/N_0$) at the selected times (Tables 1 and 2) were considered for this purpose. Mean values were compared by the least significant difference (LSD) test, with a confidence level of 95% ($p<0.05$), using the Statgraphics Centurion XV software program.

$$Adjusted - R^2 = \left[ \frac{(m-1)(1-\frac{SSQ_{REGRESSION}}{SSQ_{TOTAL}})}{(m-j)} \right] \quad (3)$$

$$RMSE = \sqrt{\frac{\sum (fitted - observed)^2}{m}} \quad (4)$$

where

m: Number of observations;

j: Number of model parameters;

SSQ: Sum of squares.

## 3. RESULTS AND DISCUSSION

### 3.1. *L. monocytogenes* inactivation by MWP and CTP

Temperature profiles of the sample subjected to different conventional and microwave treatments assayed are shown in Fig. 1. As previously reported by other authors, the temperature profiles obtained evidence that unlike conventional heating, non-isothermal heating takes exclusively place under microwave heating.

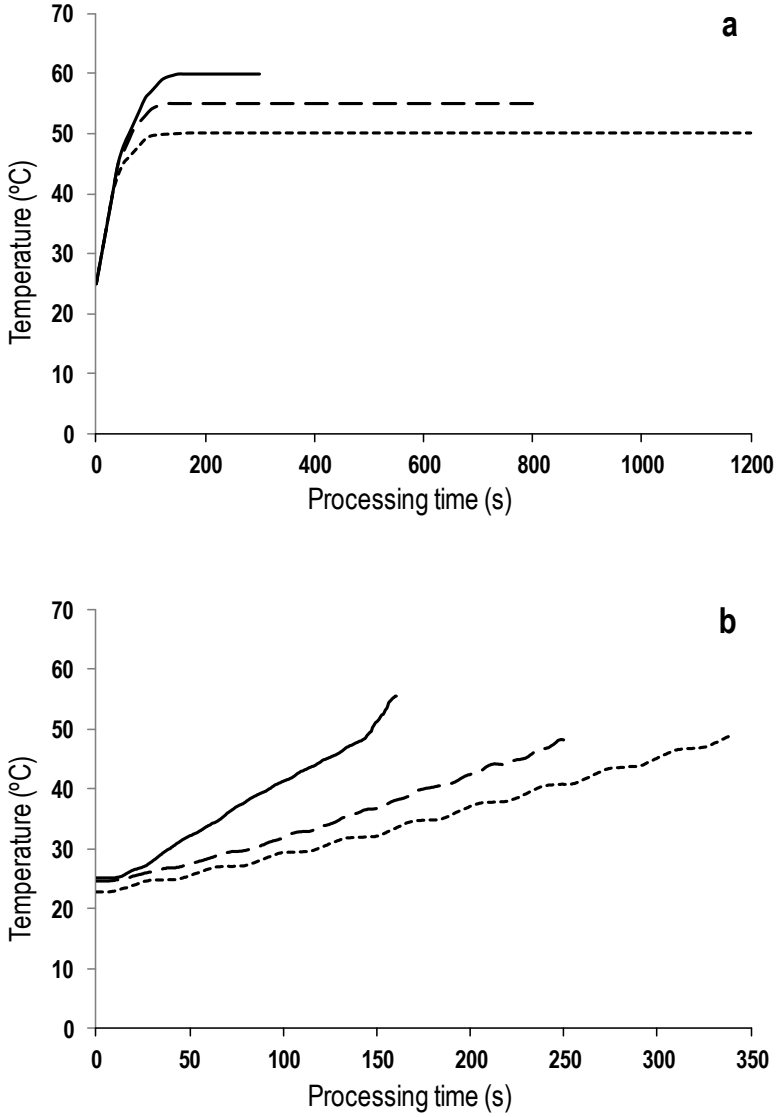


**Figure 1.** Mean kiwifruit puree temperature profile for conventional thermal processing (a) at 60°C (_____), 55°C (__ __ .) and 50°C (------) and microwave processing (b) at 1000 W (_____), 900W (__ __ .) and 600 W (------).

The inactivation of *L. monocytogenes* under microwave and conventional processing was studied in a kiwifruit puree (Figs. 2 and 3). In order to make possible the comparison between D-values obtained under both processing technologies, kinetic data transformation was performed. Treatment times were corrected by using Eq. (2) and effective times ($t_e$) values were obtained. Calculated $t_e$ represented the equivalent holding time at each processing temperature as if both treatments (MWP and CTP) had been performed under isothermal conditions (Awuah et al., 2007;

Latorre et al., 2012; Matsui et al., 2008). According to requirements and recommendations given by the FDA (2004), at least a 5-$\log_{10}$ cycle reduction of the most resistant pathogen microorganism is considered necessary to pasteurize foods by means of new technologies. This safety criterion was accomplished in the kiwifruit puree subjected to MWP when effective times were higher than 75 and 82 s for 900 and 1000 W, respectively. However, effective times higher than 355 s were required under CTP to reach the target level of *L. monocytogenes* inactivation at 55 °C, respectively. To our knowledge, the only study on microwave *Listeria* spp. Inactivation in fruit-based products has been published by Picouet et al. (2009). They found a 7-$\log_{10}$ cycles reduction of *L. innocua* in an apple puree subjected to 900 W for 35 s. On the other hand, thermal inactivation of *L. monocytogenes* in different fruit substrates has been evaluated by several authors. For example, Hassani, Álvarez, Raso, Condón, and Pagán (2005) reported that 5-$\log_{10}$ cycles of *L. monocytogenes* were inactivate in a reference medium (pH = 4) when it was subjected to 58 °C for 84 s, and Fernández, López, Bernardo, Condó, and Raso (2007) found a 4-$\log_{10}$ cycle reduction when a sucrose solution (pH = 7, $a_w$ = 0.99) was maintained at 60 °C for 60 s.

### 3.2. *L. monocytogenes* MWP inactivation kinetics

First-order kinetics has been successfully employed by several authors in order to describe microbial (*Saccharomyces cerevisiae, Lactobacillus plantarum)* and enzymatic inactivation (peroxidase, polyphenoloxidase and pectinmethylesterase) under microwave processing (Fujikawa et al., 1992; Matsui et al., 2008; Soysal & Söylemez, 2005; Tajchakavit & Ramaswamy, 1997; Tajchakavit et al., 1998). In the present study, *L. monocytogenes* survival behavior under MWP was close to linearity and the data obtainedwere fitted to first-order kinetics (Eq. (1)). The D-values and the accuracy of the fit are summarized in Table 1. The goodness of the fit was indicated by the adjusted-$R^2$ (0.992–0.996), which was significant in all cases, with a confidence level of 99%, and RMSE (0.009–0.029) values.

**Table 1.** Effective times ($t_e$) and reference temperatures ($T_{ref}$) considered to study *Listeria monocytogenes* inactivation kinetics in microwaved kiwifruit puree. $D_{60ºC}$ value (with standard error); adjusted regression coefficient ($R^2$), root mean square error (RMSE).

| ***Microwave power*** | ***$T_{ref}$ (ºC)*** | ***$t_e$ (s)*** | ***$D_{60ºC}$ (s)*** | ***$R^2$*** | ***RMSE*** |
|---|---|---|---|---|---|
| *1000 W* | 55.60 | 3<br>8<br>16<br>54<br>101 | 17.04 (0.34) | 0.996 | 0.028 |
| *900 W* | 48.45 | 10<br>14<br>22<br>33<br>58<br>89 | 17.35 (0.34) | 0.993 | 0.029 |
| *600 W* | 49.10 | 3<br>13<br>16<br>41<br>51<br>66 | 42.85 (0.13) | 0.992 | 0.009 |

Although the inactivation kinetics of *L. monocytogenes* by thermal treatment has been extensively studied in various foodstuffs (beef, milk, chicken, carrot, cantaloupe and watermelon juice, etc.) (Bolton et al., 2000; Chhabra, Carter, Linton, & Cousin, 1999; Sharma, Adler, Harrison, & Beuchat, 2005), in reference medium (Hassani, Mañas, Pagán, & Condón, 2007; Hassani et al., 2005) and in sucrose solutions (Fernández et al., 2007), there is no information available about the survival behavior of this pathogen in fruit-based products under microwave heating. Cañumir et al. (2002) reported higher D-values for microwave apple juice pasteurization when the inactivation kinetics of E. coli was evaluated, ranging between $D_{70.3\ ºC}$=25.2 s and $D_{38.3\ ºC}$= 238.8 s for 900 W and 270 W, respectively. Yaghmaee and Durance (2005) found similar D-values for microwave inactivation of E. coli in peptone water at 510 W, being $D_{55.6\ ºC}$=30 s and $D_{60.5\ ºC}$=18 s. The effect of the processing parameters, power (W) and time (s), on inactivation of *L.*

*monocytogenes* was determined by means of a multifactor ANOVA. Both factors, power and treatment time, were shown to significantly affect ($p<0.05$) the *L. monocytogenes* reduction level achieved, although no significant differences were found between 1000 W and 900 W. Both higher power level and higher effective time led to significantly higher *L. monocytogenes* inactivation ($p<0.05$).

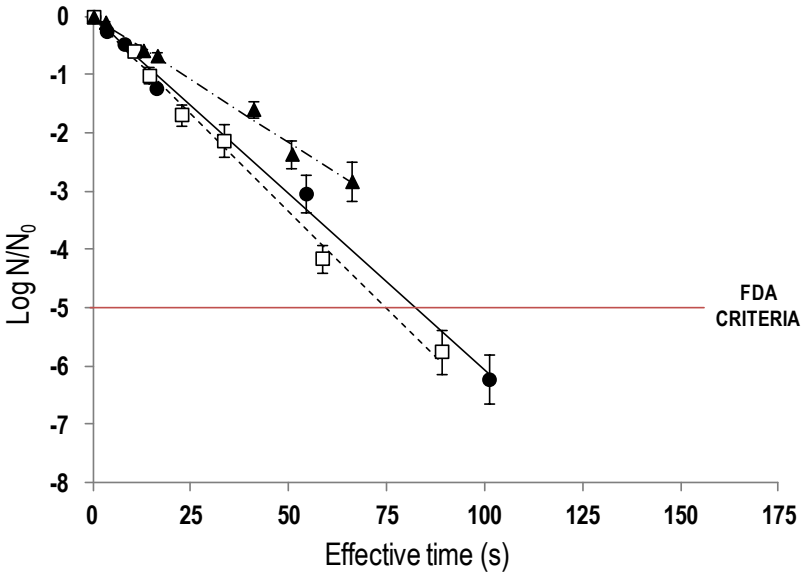


**Figure 2.** Survival curves of *Listeria monocytogenes* under microwave processing at 1000 W (experimental (●), model (_____)), 900 W (experimental (□), model (_ _ _ _ )) and 600 W (experimental (▲ ), model (__ . __ )). The plotted values and error bars represent the average of three replicates and the corresponding standard deviation.

In this respect, the higher the microwave power, the lower the effective time necessary to reach the same level of inactivation. For example, in order to achieve the FDA recommendations for pasteurized products (5-$\log_{10}$ cycles inactivation) a considerably longer effective time was required at 600 W ($t_e$= 116 s) than at 1000W ($t_e$=82 s). The power level effect can also be evaluated by means of the $D_{60\,°C}$-value comparison (Table 1).Microwave processing performed at 900 W and 1000 W led to extensively faster bacterium reduction than processing at 600 W.

### 3.3. *L. monocytogenes* CTP inactivation kinetics

Thermal processing has been widely employed for microorganism inactivation purposes. Numerous reports on the topic of thermal kinetic inactivation of *L.*

*monocytogenes* have been published. Some authors have found non-linear survival curves (Fernández et al., 2007; Peleg, Penchina, & Col, 2001; Valdramidis et al., 2006), and Weibull distribution (Fernández et al., 2007; Hassani et al., 2005; Peleg et al., 2001) and the Logistic model (Vaikousi, Koutsoumanis, & Biliaderis, 2008) between others, have been shown to fit *L. monocytogenes* survival curves appropriately. Nevertheless, when applicable, a first-order kinetic is still the way most frequently used to describe kinetic behavior under thermal treatment (Awuah et al., 2007; Hassani et al., 2005; Soysal & Söylemez, 2005; Tajchakavit & Ramaswamy, 1997; Tajchakavit et al., 1998; Zheng & Hongfei, 2011). In the present case, survival curves close to linearity were found and the data obtained were fitted to first-order kinetics (Eq. (1)). The D-values calculated and the accuracy of the fit are shown in Table 2.

**Table 2.** Effective times ($t_e$) considered to study *Listeria monocytogenes* inactivation kinetics in conventional heated kiwifruit puree. D value (with standard error); adjusted regression coefficient ($R^2$), root mean square error (RMSE)

| *Heating temperature* | *CUT (s)* | *$t_e$(s)* | *D (s)* | *$R^2$* | *RMSE* |
|---|---|---|---|---|---|
| *50 ºC* | 111 | 181<br>363<br>544<br>725 | 203.74 (3.19) | 0.990 | 0.020 |
| *55 ºC* | 115 | 55<br>110<br>164<br>329<br>438 | 70.94 (2.89) | 0.976 | 0.135 |
| *60 ºC* | 120 | 28<br>94<br>120<br>157 | 37.45 (2.68) | 0.974 | 0.082 |

CUT: come-up time.

The goodness of fit was indicated by the adjusted-$R^2$ (0.974–0.990), which was significant in all cases, with a confidence level of 99%, and RMSE (0.020–0.135). The come-up times ranged from 90 to 120 s. As expected, the inactivation rate of *L. monocytogenes* increased with temperature, as can be appreciated by visual

inspection of the survival curves (Fig. 3) and the D-values obtained (Table 2). Both temperature and treatment time significantly affected the degree of *L. monocytogenes* reduction achieved ($p<0.05$). The higher the temperature and the effective time, the higher the *L. monocytogenes* inactivation ($p<0.05$). The D-values obtained in this study were generally in the range commonly observed for this bacterium in various food products and reference medium (Hassani et al., 2005; Tajchakavit et al., 1998; Van Asselt & Zwietering, 2006). Gabriel and Nakano (2009) reported similar D-values when they studied thermal *L. monocytogenes* inactivation in clear apple juice ($D_{55\,°C}$=1.32 min).

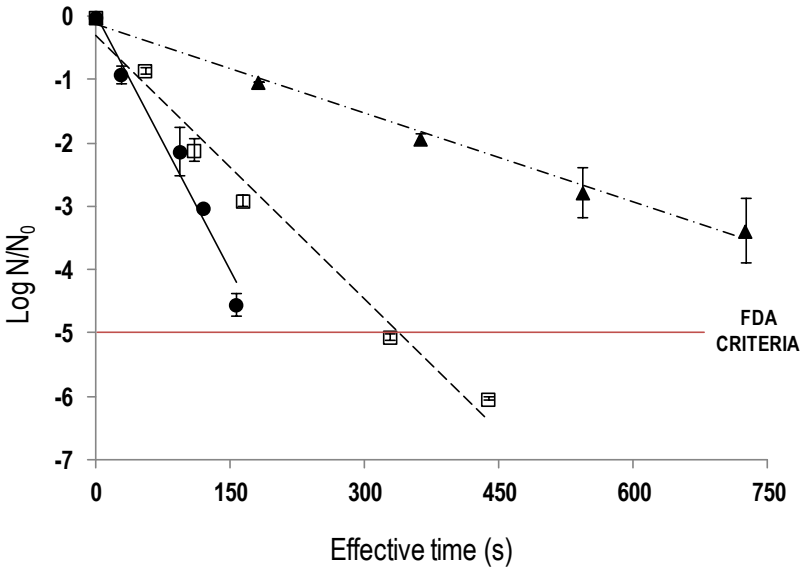


**Figure 3.** Survival curves of *Listeria monocytogenes* under conventional thermal processing at 60 °C (experimental (●), model (——)), 55 °C (experimental (□), model (– – – –)) and 50 °C (experimental (▲), model (— . —)).The plotted values and error bars represent the average of three replicates and the corresponding standard deviation.

### 3.4. Kinetic comparison: microwave versus conventional inactivation

Microwave heating has been reviewed by several authors as a good alternative to conventional thermal treatments in food preservation (Vadivambal & Jayas, 2007). In this work, the effectiveness of both technologies on *L. monocytogenes* inactivation was compared. The D-values obtained for microwave treatment were

considerably lower than those obtained for conventional heating, especially when a power level higher than 600 W was applied. For instance, the $D_{60\ °C}$-values were 17.04 s and 37.45 s for MWP (1000 W) and CTP, respectively. These data show that at 1000 W *L. monocytogenes* inactivation happened about 2 times faster than under conventional heating. These results suggest that microwave processing was much more effective in destroying the pathogen studied in kiwifruit puree than the conventional thermal treatment. These observations coincide closely with the results published by other authors. Tajchakavit and Ramaswamy (1997) reported higher effectiveness of microwave processing (700 W) on pectinmethylesterase inactivation in comparison with a conventional heating mode. Tajchakavit et al. (1998) found microwave heating (700 W) to be much more efficient for inactivating *S. cerevisiae* and *L. plantarum* in apple juice than thermal treatment. Matsui et al. (2008) reported that microwave processing led to lower residual enzyme activity than conventional thermal heating under similar treatment conditions. Soysal and Söylemez (2005), after studying kinetic inactivation of carrot peroxidase by thermal and microwave treatment (700 W), concluded that microwave technology could be more effective for inactivation of this enzyme than conventional treatment. Latorre et al. (2012) reported higher effectiveness of microwave technology (250–900 W) on peroxidase and polyphenoloxidase inactivation than conventional heating, because considerably lower D-values were obtained. However, this higher effectiveness cannot always be assumed to be true, since kiwifruit puree processed at a microwave power of 600 W led to a higher D-value ($D_{60\ °C}$ = 42.85 s) than that obtained with CTP ($D_{60\ °C}$ = 37.45 s). Similarly, Latorre et al. (2012) found a higher D-value for microwave treatment than that obtained for conventional heating when red beet samples were subjected to a relatively low microwave power level (250 W). MW technology has been proved to be effective against various foodborne pathogens of concern (Fujikawa et al., 1992) and lead to a reduction of process time in comparison with conventional technologies, which indicates that safety can be properly ensured and product quality can be effectively preserved (Soysal & Söylemez, 2005; Zheng & Hongfei, 2011).

## 4. CONCLUSION

The use of microwave energy represents a good alternative to conventional pasteurization, with the possibility of offering the required safety by using a lower process time, when microwave power of a certain level is applied. This would contribute to products of better nutritional, functional and sensory quality.

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# CAPÍTULO IV.4. EVALUACIÓN DE LA PROBABILIDAD DEL CUMPLIMIENTO DE LOS OBJETIVOS MICROBIOLÓGICOS DE PASTEURIZACIÓN DURANTE EL PROCESADO POR MICROONDAS DE UN PURÉ DE KIWI

# *ASSESSMENT OF UNCERTAINTY IN ACCOMPLISHMENT OF MICROBIAL SAFETY OBJECTIVE IN MICROWAVE PASTEURISATION OF KIWIFRUIT PUREE*

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## ABSTRACT

Temperature distribution and non-uniformity in *Listeria monocytogenes* inactivation in kiwifruit puree subjected to batch microwave processing (600–1000 W, 30–300 s) were investigated. Inactivation data were integrated into a deterministic model and a stochastic model and the two approaches were compared. Monte Carlo simulation was used to perform probabilistic predictions of the final microbial load to establish safe pasteurisation treatments and to evaluate certainty in inactivation under microwave processing. The power level of 1000 W led to the fastest heating rates and the fastest and most effective *L. monocytogenes* inactivation, but it increased the problem of non-uniform heating. The stochastic model was a useful tool for accurately predicting safe pasteurisation conditions (1000 W-6 min, 900 W-13 min and 600 W-19 min) by providing a dynamic probabilistic estimate of the pathogen inactivation. Stochastic modelling might help to establish more reliable, efficient and applicable microwave processes, contributing to optimising the risk-benefit balance of non-conventionally heated products.

**KEYWORDS** Temperature distribution, cylinder, predictive microbiology, *Listeria monocytogenes*, stochastic modelling, Monte Carlo simulation

## 1. INTRODUCTION

Microwave heating is a novel technology in food processing with bright future prospects for providing safe fruit-based products of superior quality with extended shelf-life (Elez-Martínez, Aguiló-Aguayo, & Martín-Belloso, 2006; O'Donnell, Tiwari, Bourke, & Cullen, 2010; Picouet, Landl, Abadias, Castellari, & Viñas, 2009). This technology, however, is far from being used in most of its potential commercial applications. Since an ideal microwave process should result in all food being heated to the same minimal extent for microbial safety, lack of heating uniformity is one of the key factors limiting the exploitation of this technology to its fullest potential in the food industry (Birla, Wang, Tang, & Tiwari, 2008). Some of the main drawbacks associated with uneven heating are: (i) the existence of hot and cold spots (Fujikawa, Ushioda, & Kudo, 1992), (ii) poor end quality, stability and repeatability (Vadivambal & Jayas, 2007), (iii) microbial safety concerns (Vadivambal & Jayas, 2010) and (iv) overheating of microwaved foods, with consequent sensory and nutritional detriment (Birla et al., 2008)

In recent years, quantitative microbiological risk and nutritional benefit assessment techniques (risk/benefit assessment) have increasingly been used to study and validate the applicability of novel processing technologies in academia and industry. As far as microwaves are concerned, it could be claimed that the development of quantitative microbiological risk assessment studies might be required for the scale-up of microwave processing at an industrial level, since they could act as efficient tools, contributing to making decision processes and ensuring the commercialisation of safe products treated under optimised microwave conditions. Accordingly, instead of the deterministic modelling traditionally used to establish and optimise thermal processes (Awuah, Ramaswamy, & Economides, 2007; Hassani, Álvarez, Raso, Condón, & Pagán, 2005; Peleg, Penchina, & Cole, 2000), which does not account for random variations in the input model variables and which provides fixed results for outputs, stochastic or probabilistic models that offer more realistic estimations of compliance with safety objectives by actively considering the variability inherent in biological processes (Laguerre, Hoang, & Flick, 2013; Pujol, Albert, Johnson, & Membré, 2013) should be employed. Stochastic

analysis is the starting point in an exposure assessment process and may help industry to deal with the food safety of microwave-treated products, allowing the safety/risk associated with them at each stage of the food chain to be closely predicted (Baucour, Cronin, & Stynes, 2003; Laguerre et al., 2013; Pujol et al., 2013). To date, however, stochastic assessment of microwave heating or any other non-conventional thermal process seems to have scarcely been studied (Pina-Pérez, Silva-Angulo, Rodrigo, & Martínez López, 2012). Although Monte Carlo simulation, an example of stochastic analysis, has been reported by numerous authors to profitably address various concerns in conventional thermal processes (e.g. dispersion in product quality induced by variability in packaged food thermal behaviour or temperature variability during conventional heating) (Baucour et al., 2003; Demir, Baucour, Cronin, & Abodeyeh, 2003; Membré & Zuijlen, 2011; Nicolaï, Verboven, Scheerlinck, & Baerdemaeker, 1998; Smout, Van Loey, & Hendrickx, 2000), the work published by Tanaka, Morita, Iwasaki, Verboven, Scheerlinck, and Nicolaï (2006) is, to the best of our knowledge, the only study in which thermal uniformity in infrared heating, an alternative heating technique, was investigated by means of stochastic analysis.

The objective of the present study was to investigate the impact of non-uniform microwave heating of a kiwifruit puree on the *L. monocytogenes* inactivation achieved. The effect of microwave power level on uniformity of temperature distribution and the final concentration of this pathogen was also studied. Monte Carlo approaches were used to characterise the variability in *L. monocytogenes* survival. The stochastic inactivation model built was used to optimise microwave processing of kiwifruit puree from a safety viewpoint.

## 2. MATERIALS AND METHODS

### 2.1. Culture preparation

The lyophilised strain of *L. monocytogenes* CECT 4032 was supplied by the Spanish Type Culture Collection. For rehydration, it was transferred to 10 mL of Tryptic Soy Broth (TSB) (Scharlab Chemie S.A., Barcelona, Spain). After 30 min, 5 mL of culture was inoculated in 500 mL of TSB and incubated at 37 °C with constant agitation (200 rpm) for 21 h to obtain cells in the stationary growth stage. The cells

were centrifuged twice at 4000 × g for 15 min at 4 °C and resuspended in 20 mL of TSB. The cells were placed in 2-mL sterile plastic cryogenic vials containing TSB supplemented with 20% glycerol in a relation of 1:1. The 2-mL samples, with an approximate concentration of $5 \cdot 10^9$ CFU/mL, were immediately stored at −80 °C until use for the microbiological studies.

### 2.2. Sample preparation

Kiwifruit (*Actinidia deliciosa* var. Hayward) was purchased in a local supermarket. Fruit pieces were peeled, washed with distilled water, cut into slices and finally triturated in a Thermomix (TM 21, Vorwerk, Spain), using the fourth power level for 1 min. The physico-chemical characteristics of kiwifruit puree were determined in order to control the fruit which was used as raw material for the study. Each analysis was made in triplicate. Results are expressed as mean values with standard deviation in brackets. Moisture was 83.4 (0.7) g water/100 g product (AOAC 934.06 method (2000)), °Brix 14.1 (0.3) g soluble solids/100 g product (Refracto 3PX refractometer, Mettler Toledo, Switzerland), water activity 0.987 (0.002) (dew point hygrometer, GBX FA-st lab, France) and pH 3.39 (0.07) (Basic 2 pH-meter, Crison, Spain).

#### *2.2.1. Sample inoculation*

Taking into consideration the acidic nature of the product, *L. monocytogenes* growth in kiwifruit puree prepared as described above was corroborated in a previous study (Benlloch-Tinoco, Pina-Pérez, Martínez-Navarrete, & Rodrigo, 2014). The product was inoculated by adding 1 mL of the concentrated suspension so as to give an initial *L. monocytogenes* concentration of $10^7$ CFU/g. Kiwifruit puree was blended at 25 °C for 30 s with the aim of ensuring a homogeneous initial content of the bacterium.

### 2.3. Microwave processing

A microwave oven (model: 3038GC, Norm, China) provided with a turntable plate was used to treat the kiwifruit puree. For each treatment, a 500-g sample was tempered to an initial temperature of 25 °C and then heated in the microwave oven

in a standard size glass beaker (9 cm inner diameter and 12 cm length) (BKL3-1K0-006O, Labbox, Spain) at 600, 900 and 1000 W with processing times varying between 30 and 300 s. Treated samples were immediately cooled in ice-water until the puree reached 35 °C and then used to obtain the corresponding survival curves. Ten replicates of each treatment were carried out. The power absorbed by the sample at these three nominal power levels was estimated by heating 1 kg of distilled water from 10 °C up to 20 °C at 600, 900 and 1000 W, in accordance with standard IEC 60705 (1999). Experiments were performed in triplicate and results showed an average (and standard deviation) of 427 (12) W for 600 W, 525 (10) W for 900 W and 725 (6) W for 1000 W.

The time–temperature history of the sample was continuously recorded during microwave processing by means of a fibre-optic probe (CR/JP/11/11671, OPTOCOM, Germany) which was connected to a temperature datalogger (FOTEMP1-OEM, OPTOCOM, Germany). Both the temperature profile of the sample and the microbial load were determined at six different locations in the kiwifruit puree (Figure 1) with ten replicates per point: two radial points in a cross-section of the sample and three different longitudinal locations (separated by 2 cm distance).

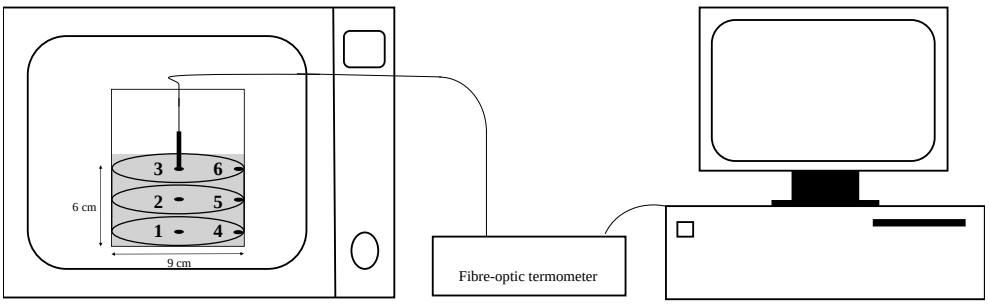


**Figure 1.** Experimental setup.

### 2.4. Modelling of survivor data

Mean data of *L. monocytogenes* inactivation due to microwave treatments at each point studied in the sample were fitted using first-order kinetics, and D-values were determined in each case. The D-value represents the heating time required to

reduce 90% of the existing microbial population under isothermal conditions (Equation 1) (Awuah et al., 2007).

$$\log \frac{N}{N_0} = -\frac{t}{D} \quad (1)$$

where

N: survivor counts after treatment (CFU/g);

$N_0$: initial population of microorganism (CFU/g);

t: processing time (s);

D: D-value at the temperature studied (s).

D-values were calculated by non-linear regression according to the methodology described by Matsui, Gut, Oliveira, and Tadini (2008). Since the microwave treatments involved non-isothermal heating conditions, correction of processing time values for come-up periods was necessary prior to kinetic data analysis. Therefore time–temperature profiles were used to calculate the effective time ($t_e$) or accumulated lethality (Equation 2), which represents the isothermal holding time at the selected reference temperature that causes the same level of microbial destruction as the heating actually applied (Awuah et al., 2007). For each microwave process, the maximum temperature reached during the treatment at each point in the sample was considered as the reference temperature ($T_{ref}$) (Matsui et al., 2008).

$$t_e = \int_0^\infty 10^{\left(\frac{T(t)-T_{ref}}{z}\right)} dt \quad (2)$$

### 2.5. Statistical data analysis and model evaluation

The goodness of fit between experimental and predicted data was assessed by using the adjusted regression coefficient ($R^2$) (Equation 3) and the accuracy factor ($A_f$) (Equation 4) (Ross, 1996). The higher the $R^2$ value and the closer the $A_f$ value to a value of 1, the better the fit. The discrepancy factor ($D_f$) (Equation 5) was used to compare deterministic and stochastic predictions (Ross, 1996). Analyses of variance (multifactor ANOVA) were run to study the effect of process variables (microwave

power, treatment time and point in the sample) on temperature and *L. monocytogenes* inactivation. Mean values were compared by the Tukey test, with a confidence level of 95% ($p < 0.05$), using the Statgraphics Centurion XV software program.

$$R^2 = \left[ \frac{(m-1)(1-\frac{SSQ_{REGRESSION}}{SSQ_{TOTAL}})}{(m-j)} \right] \quad (3)$$

where

m: Number of observations;

j: Number of model parameters;

SSQ: Sum of squares.

$$A_f = 10^{\frac{\left|\sum log\left(predicted/observed\right)\right|}{n}} \quad (4)$$

$$D_f = \left( 10^{\frac{\left|\sum log\left(predicted/observed\right)\right|}{n}} - 1 \right) \quad (5)$$

where

n: Number of observations.

## 2.6. Stochastic modelling

A stochastic model was built as a spreadsheet model in Microsoft Excel® with add-in @Risk® 5.5 (Palisade Corporation, NY, USA), which describes the inactivation as a probability distribution of *L. monocytogenes* $\log_{10}$ cycle reduction after different microwave processing conditions: microwave power (W) and time (s). The global stochastic model was built on the basis of a linear model by introducing the D-values obtained, defined by probability distribution functions, at 6 different locations in the puree (6 symmetrical reference points). The BestFit® tool (Palisade

Corporation, NY, USA) was used to predict the most accurate function defining D-values. D-values, treatment time and initial bacterial load (Log $N_0$ = N (2800000; 250000)) are defined as the inputs of the model, and the final bacterial load at each location considered in the mass is defined as the output of the system.

## 3. RESULTS AND DISCUSSION

### 3.1. Temperature distribution during microwave processing

Since the lethality of thermal treatments is closely dependent on the time–temperature history at all points in the heated food mass, the lack of heating uniformity prevalent in microwave technology is considered to have a strong influence on the applicability of microwaves for industrial processing.

In this study, the temperature distribution of kiwifruit puree subjected to batch microwave processing was investigated. The time–temperature history of a cylindrical-shaped kiwifruit puree sample was recorded at several locations (Figure 1) to assess temperature evolution along its radial and axial directions during microwave exposure at power levels in the range of 600–1000 W (Figure 2). As expected, marked temperature variations were observed in the heated sample. Temperature differences were of similar magnitude along the radial and axial directions. Both power level and treatment time had a noticeable impact on temperature distribution. Overall, the longer the time and the higher the power level, the lower the heating uniformity. The multifactor ANOVA indicated that there were significant differences ($p < 0.05$) in temperature values owing to power level, treatment time and the point in the sample at which the temperature was recorded. Significant interactions were also found between all these factors. From Figure 2 it can be seen that temperature increased monotonically with time along both the axial and the radial direction, irrespective of the power level. After 45 s of treatment, the effect of power level on heating rates started to become evident, and it was observed that the higher the power level the significantly ($p < 0.05$) faster the temperature increase at any of the locations in the sample. Temperature differences between points along the radial and axial directions became significant ($p < 0.05$) after the kiwifruit puree sample had been microwaved for at least 60 s. The sample was heated to a significantly ($p < 0.05$) greater extent at its edges. Similar behaviour

has been reported by other authors who investigated temperature distribution of cylindrical-shaped foods under microwave heating; for example, Mao, Watanabe, and Sakai (2005) found that in kamaboko samples (10 cm in diameter) heated by microwaves the high temperature domain lay near the edge. In fact, in food cylinders with dimensions similar to those of the sample used in the present study (Figure 1) the maximum temperature is expected to be at their edges (Hossan, Byun, & Dutta, 2010; Oliveira & Franca, 2002; Romano, Marra, & Tammaro, 2005), given that: (i) the low ratio between wavelength and sample size leads to a field decay near the surface (Oliveira & Franca, 2002) and (ii) a concentration of microwave energy may take place at these locations owing to some geometric effects, leading to stronger fields and therefore greater heating (Hossan et al., 2010; Zhang & Datta, 2005).

From the temperature data, point 3 was identified as the coldest spot in the sample, irrespective of the power level, with a mean final temperature (and standard deviation) of 52 °C (4) for 1000 W, 39.5 °C (1.2) for 900 W and 35.1 °C (0.4) for 600 W, although no significant differences were detected between points 3 and 2. Similarly, point 6 was shown to be the hottest spot in the puree at any of the power levels studied, with a mean final temperature of 87 °C (6) for 1000 W, 75 °C (4) for 900 W and 55.2 °C (0.8) for 600 W. However, no significant differences were observed between points 6 and 4 for 900 W and between points 6 and 1 for 600 W. Point 5 showed an intermediate temperature profile under the conditions studied (power level and treatment time).

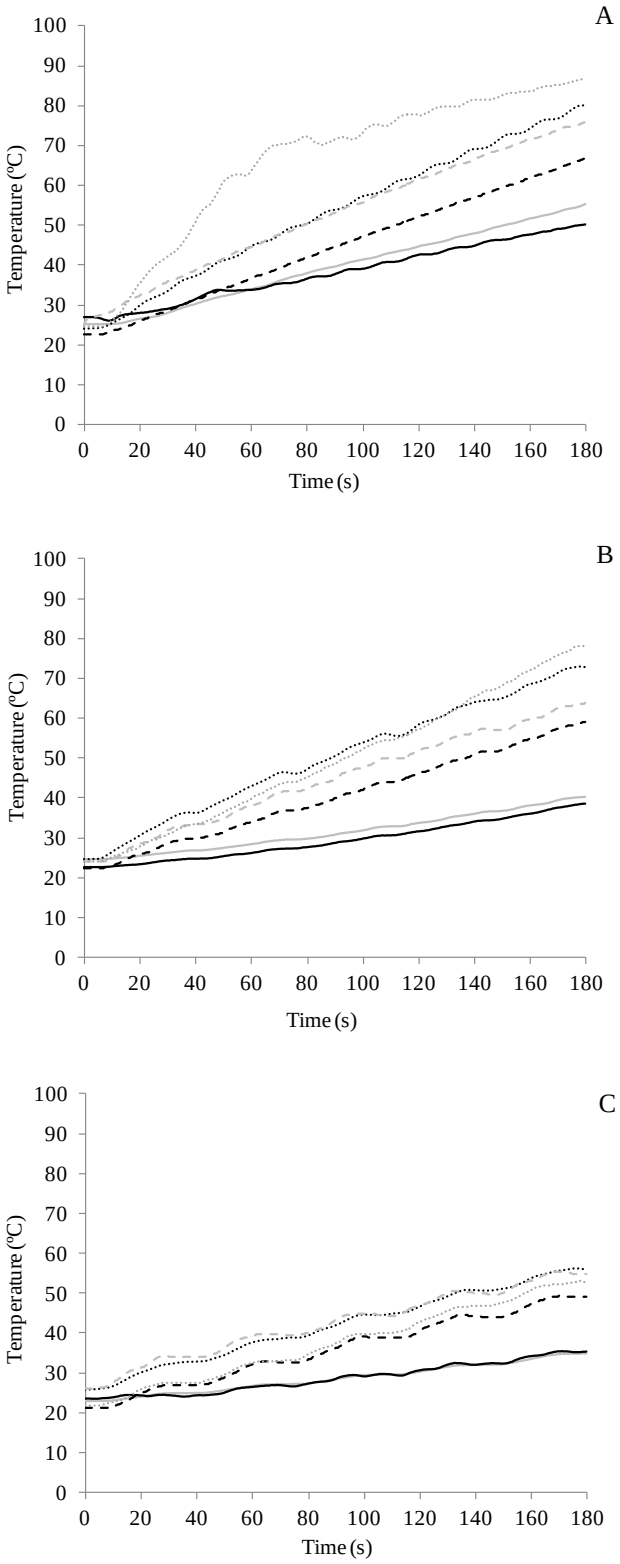


**Figure 2.** Temperature profiles corresponding to different points in the kiwifruit puree microwave-heated for 180 s at 1000 W (A), 900 W (B) and 600 W (C): 1 (– – ), 2 ( ——),3 (——),4 (.....),5 (– – ) and 6 (.....).

On the basis of the temperature differences between the hottest and the coldest spots in kiwifruit puree processed at the three different power levels, which were 20 °C for 600 W and 35 °C for 900 W and 1000 W, all calculated at a treatment time of 180 s, it could be claimed that a reduction in power level apparently provided greater heating uniformity. On the one hand, according to Vadivambal and Jayas (2010), who reviewed the temperature distribution of various food materials during microwave heating (300–900 W), differences of a similar order were found for water (22 °C), but considerably higher variations can be expected in other food products such as mashed potatoes, ready-to-eat spaghetti with bolognaise meat sauce, spaghetti with meat sauce or rice with salmon (65–70 °C). On the other hand, Goksoy, James, and James (1998) also found that heating at a reduced power level led to greater heating uniformity, which could be explained by the fact that, unlike conventional heating, in microwave processes the time scale for heat generation is shorter than that of thermal transport, owing to the low thermal conductivity of food items (Hossan et al., 2010), so reducing the heat generation rate could contribute to a decrease in the undesired lack of heating uniformity.

### 3.2. *L. monocytogenes* inactivation during microwave processing

The uneven heating typically observed in microwave processing may lead to some safety concerns. As the temperature inside the product is not uniform, the microbial load is a function of location, which means that product safety might be compromised at some under-heated points. Therefore an accurate understanding of microbial distribution in the treated sample would help to establish more reliable microwave processes.

In the present study, the uniformity of *L. monocytogenes* inactivation in kiwifruit puree heated by microwaves was investigated by assessing the survival of this pathogen along the axial and radial directions in the sample processed at three different power levels (Figure 3). The distribution of *L. monocytogenes* inactivation seemed to follow closely the temperature distribution previously described in the product (section 3.1.). Therefore it was assumed that the microbial inactivation taking place in the food system at the processing conditions assayed was defined by the heating pattern of the sample. Again, both power level and treatment time had a

strong impact on the uniformity of the pathogen reduction, and it was observed that the longer the treatment time and the higher the power level, the lower the uniformity. Greater inactivation was found at the edges of the sample, and, in general terms, the coldest and hottest spots identified in section 3.1. were the points showing the lowest and highest *L. monocytogenes* reduction levels, respectively. The multifactor ANOVA indicated that significant differences ($p < 0.05$) were observed in $\log_{10}$ cycle reduction owing to power level, treatment time and the location in the sample. However, no significant differences between 1000 W and 900 W were detected. Significant interactions were also found between all these factors. After 90 s of treatment, inactivation of *L. monocytogenes* was significantly ($p < 0.05$) faster at 1000 W and 900 W than at 600 W, irrespective of the point, which can easily be seen by comparing the inactivation data shown in Figure 3.

On the whole, the noticeable differences in the *L. monocytogenes* $\log_{10}$ cycle reduction in the treated sample (>4 $\log_{10}$ cycles) clearly showed a problem of non-uniform inactivation (Figure 3). However, this lack of uniformity seems to be in the range observed by other authors, who reported that the efficiency of microwaves in inactivating *E. coli* varied between 1 and 5 $\log_{10}$ cycles at different locations within a calcium alginate gel sample (Hamoud-Agha, Curet, Simonin, & Boillereaux, 2014).

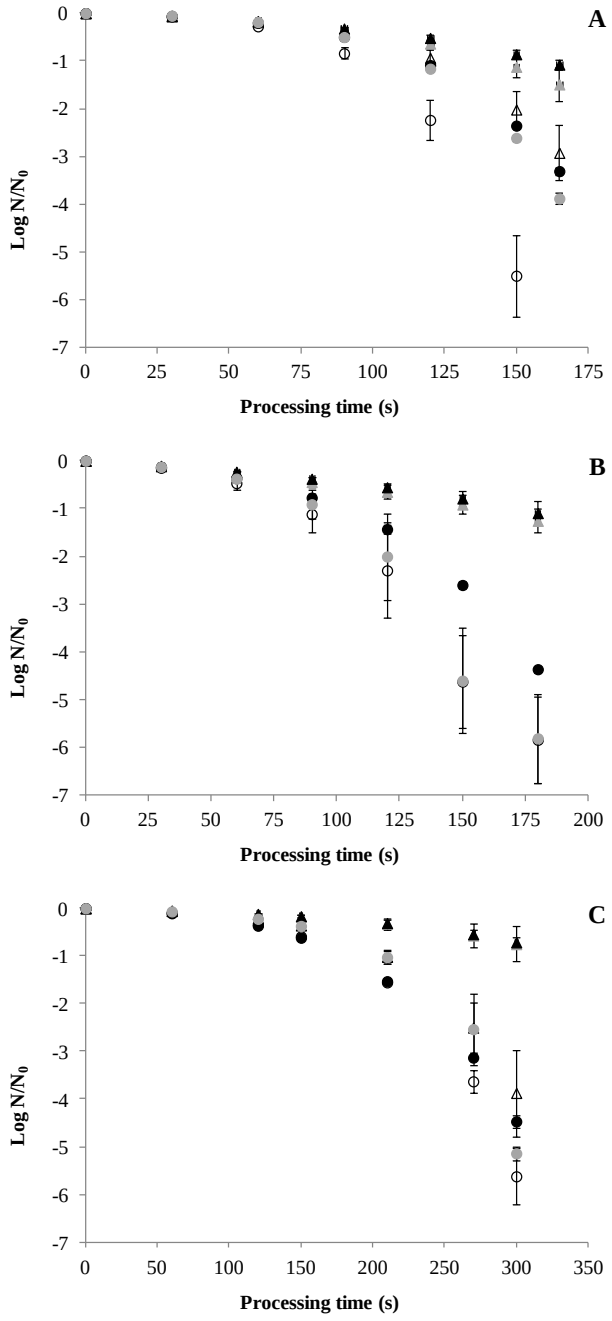


**Figure 3.** Survival curves of *Listeria monocytogenes* under microwave processing at 1000 W (A), 900 W (B) and 600 W (C) at different points in the kiwifruit puree: 1 (●), 2 (▲), 3 (▲), 4 (○), 5 (Δ) and 6 (●).The plotted values and error bars represent the average of three replicates and the corresponding standard deviation.

### 3.3. Modelling of *L. monocytogenes* inactivation. Deterministic vs. stochastic approach

In order to further investigate the distribution of *L. monocytogenes* in the microwave-treated kiwifruit puree, the kinetics of inactivation of this pathogen in the sample were calculated. In the present study, both deterministic and stochastic modelling approaches were applied for comparative purposes, taking into account the advantages of stochastic predictions from the standpoint of food safety assurance. Stochastic predictive microbiology is based on dynamic microbial population models that incorporate variability and uncertainty in empirical data and model parameters, providing a microbial load for processed products (microbiological risk assessment at industrial level) defined by probability mass functions.

The BestFit® tool was used to fit input variables (D-values, see Table 1) to the most accurate probability distribution. A stochastic model was built by introducing input variables into the global linear model, the inactivation levels being the output of the system. Monte Carlo simulation was used as an iterative mathematical tool to obtain output values defined by means of associated probability distributions. Deterministically predicted final *L. monocytogenes* levels in the puree were compared with stochastically predicted ones by using the discrepancy factor ($D_f$). The appropriate agreement found between the two approaches ($D_f$ < 25%) indicated the accuracy of the stochastic model that had been built, which was considered to give a good description of the experimental observations and the associated probability (Baranyi, Pin, & Ross, 1999). For both the deterministic and the stochastic approach, the coldest and the hottest spots showed the lowest and the highest *L. monocytogenes* inactivation rates, respectively, at 1000, 900 and 600 W.

**Table 1.** Accuracy factor and discrepancy factor defining the goodness of the correspondence between deterministic (D-values: $D_d$, average value with standard error in brackets)) and Monte Carlo stochastic (95% most probable D-values: $D_s$) model fit at different points in kiwifruit puree (1–6) for 1000, 900 and 600 W.

| Power level (W) | Point | $T_{ref}$ (°C) | $D_d$ (s) | $R^2$ | $A_f$ | $D_s$ (s) | Distribution function | $A_f$ | $D_f$ (%) |
|---|---|---|---|---|---|---|---|---|---|
| 1000 | 1 | 66.60 | 20.13 (0.14) | 0.999 | 1.06 | 20.12 | RiskExtvalue(19.93;0.23) | 1.06 | 0.50 |
| | 2 | 60.60 | 41.6 (0.9) | 0.993 | 1.17 | 35.55 | RiskBetaGeneral(0.30;0.32;31.42;40.04) | 1.26 | 15.60 |
| | 3 | 50.28 | 78 (2) | 0.986 | 1.08 | 69.08 | RiskExtvalue(67.63;2.52) | 1.17 | 11.86 |
| | 4 | 71.12 | 16.13 (0.08) | 0.998 | 1.03 | 16.05 | RiskExtvalue(15.83;0.39) | 1.03 | 0.44 |
| | 5 | 69.80 | 21.59 (0.007) | 0.999 | 1.01 | 17.59 | RiskExtvalue(17.31;0.47) | 1.20 | 20.85 |
| | 6 | 84.43 | 10.7 (0.4) | 0.978 | 1.15 | 8.59 | RiskExtvalue(7.51;1.89) | 1.26 | 20.67 |
| 900 | 1 | 63.70 | 21.5 (0.2) | 0.997 | 1.07 | 22.06 | RiskExtvalue(21.80;0.45) | 1.08 | 2.39 |
| | 2 | 42.44 | 97 (0.4) | 0.999 | 1.02 | 90.43 | RiskExtvalue(88.56;3.24) | 1.05 | 6.69 |
| | 3 | 38.69 | 159 (5) | 0.996 | 1.37 | 153.80 | RiskExtvalue(149.33;7.73) | 1.26 | 2.70 |
| | 4 | 77.94 | 13 (1) | 0.951 | 1.41 | 11.05 | RiskExtvalue(10.92;0.23) | 1.26 | 16.19 |
| | 5 | 61.83 | 31.7 (0.6) | 0.994 | 1.26 | 26.60 | RiskExtvalue(25.78;1.44) | 1.26 | 17.56 |
| | 6 | 81.84 | 9.5 (0.6) | 0.956 | 1.30 | 7.87 | RiskExtvalue(6.94;1.61) | 1.29 | 18.96 |
| 600 | 1 | 70.10 | 32.1 (0.5) | 0.992 | 1.09 | 33.20 | RiskExtvalue(31.96;2.19) | 1.09 | 3.33 |
| | 2 | 54.10 | 168 (7) | 0.983 | 1.39 | 154.24 | RiskBetaGeneral(0.20;0.30;133.09;184.52) | 1.27 | 8.50 |
| | 3 | 47.51 | 256 (2) | 0.998 | 1.04 | 227.88 | RiskBetaGeneral(0.21;0.21;202.02;254.17) | 1.11 | 11.75 |
| | 4 | 78.83 | 23.4 (0.5) | 0.989 | 1.24 | 19.80 | RiskExtvalue(19.59;0.36) | 1.27 | 15.73 |
| | 5 | 68.50 | 38.4 (0.5) | 0.992 | 1.32 | 43.78 | RiskExtvalue(43.56;0.38) | 1.27 | 12.16 |
| | 6 | 75.74 | 30.9 (0.9) | 0.971 | 1.33 | 26.14 | RiskExtvalue(24.99;1.98) | 1.24 | 15.77 |

$T_{ref}$: Reference temperatures; $R^2$: adjusted regression coefficient; $A_f$: accuracy factor; $D_f$: discrepancy factor.

Monte Carlo simulation was used to assess the impact of power level on the variability of the inactivation data at different locations in the kiwifruit puree. For explanatory purposes, distributions of *L. monocytogenes* $\log_{10}$ cycle reduction at three points in the sample (1, 3 and 5) are shown in Figure 4. The dispersion in inactivation data distribution increased at points 3 and 5 when the kiwifruit was treated at a higher power level, while the opposite behaviour was found at point 1. In other words, higher power levels led to greater heterogeneity in microbial inactivation at the points heated to a lesser extent, which may indicate that microwave processing at high power levels might increase the uncertainty of deterministic inactivation prediction results at the coldest spot in the product, thus increasing the microbiological risk.

The power level effect on the certainty of *L. monocytogenes* inactivation was assessed by comparing the cumulative frequency curves fitting the simulated $\log_{10}$ cycle reductions at various points in the sample (1, 3 and 5) subjected to 1000, 900 and 600 W for 100 s (Figure 5). As expected, meaningful differences in the probability of inactivation of the microorganism were found, depending on the microwave power employed: processing the kiwifruit puree at power levels higher than 600 W led to a remarkably higher certainty of *L. monocytogenes* inactivation, irrespective of the point evaluated. For instance, at a treatment time of 100 s, 3.5 $\log_{10}$ cycles were reduced with a probability level of 99.9% at 1000 and 900 W, while at 600 W the probability of reaching this level of inactivation was only 10% (point 1). Although significant differences were not found ($p > 0.05$) between 1000 and 900 W in terms of *L. monocytogenes* inactivation data (see section 3.2), noteworthy differences were detected in the certainty of this pathogen reduction provided by these two power levels, especially at the coldest spot in the product (Figure 5). For example, whereas 0.7 $\log_{10}$ cycles were inactivated at point 3 with a probability of 99.9% when the puree was processed at 1000 W for 100 s, at 900 W the certainty of achieving this level was only 40%. These results seem to indicate that increasing the power level from 900 W to 1000 W led to a meaningful increase in the probability of inactivation of the pathogen in the product, so 1000 W might be considered the best power level choice to process the kiwifruit puree in order to ensure the highest degree of certainty of *L. monocytogenes* inactivation.

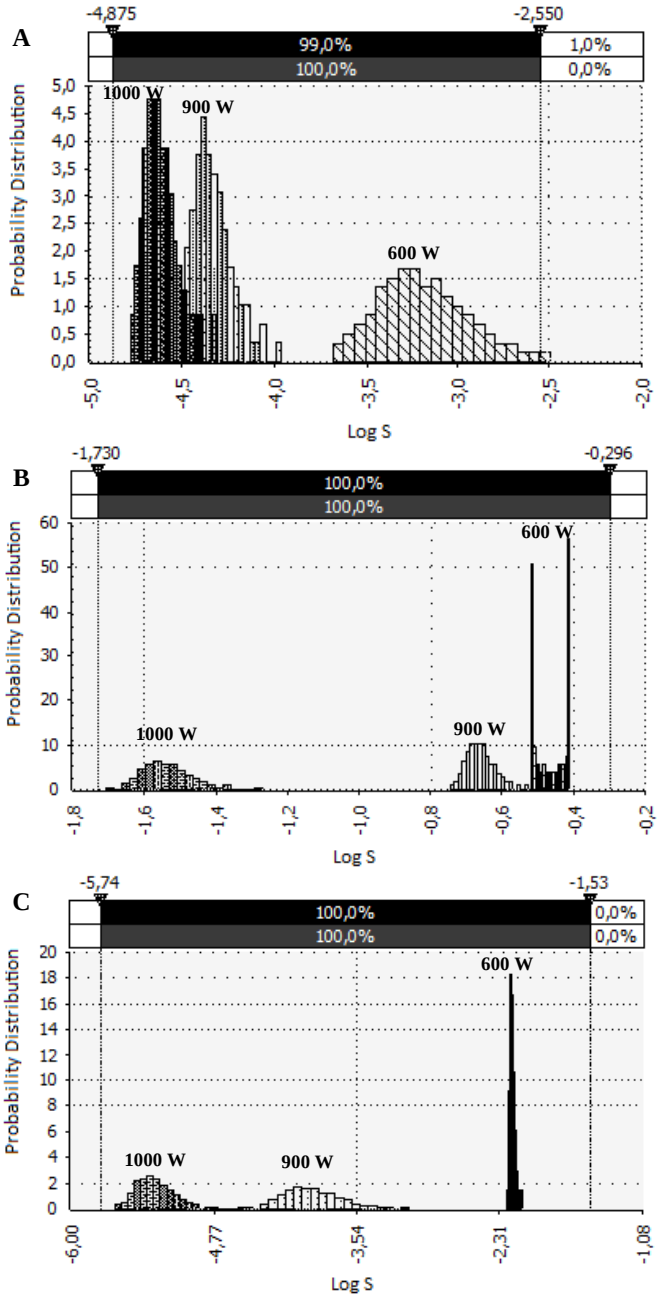


**Figure 4.** Distributions of *L. monocytogenes* $\log_{10}$ cycle reductions (Log S) in the kiwifruit puree microwave-heated for 100 s (effective time) at 1000, 900 and 600 W at various locations: point 1 (A), point 3 (B) and point 5 (C).

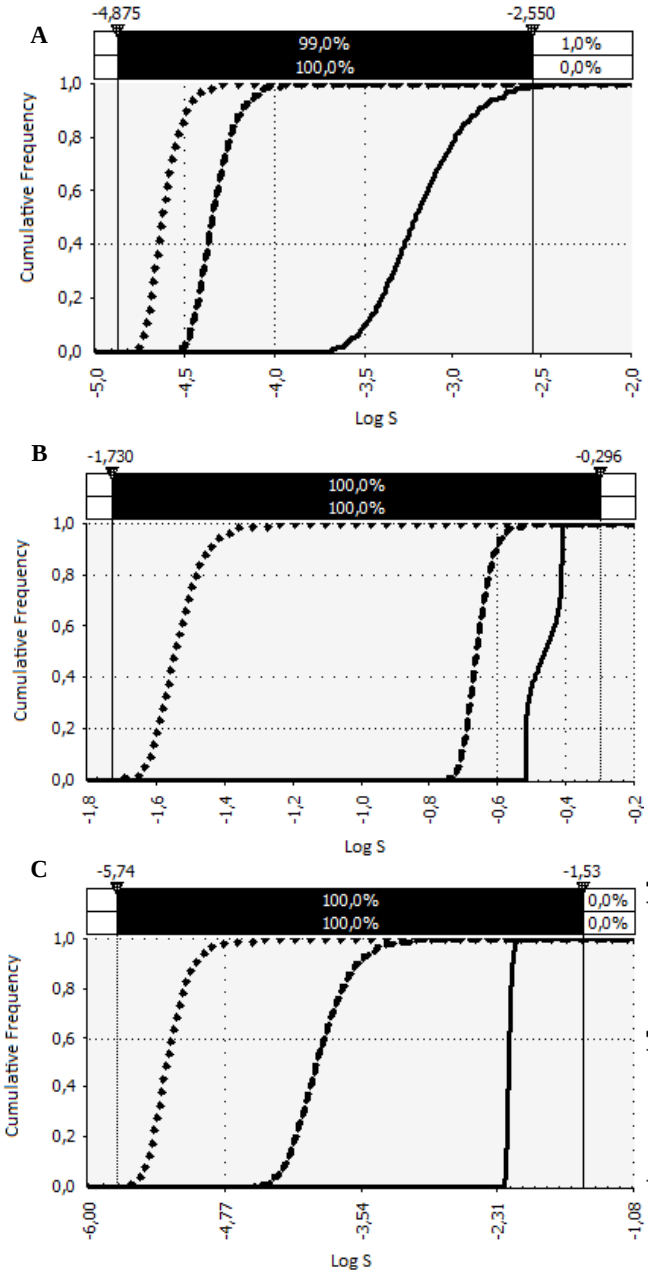


**Figure 5.** Cumulative frequency curves fitting the simulated *L. monocytogenes* $\log_{10}$ cycle reductions (Log S) in the kiwifruit puree microwave-heated for 100 s at 1000 ( .....), 900 (– – ) and 600 W (——) at various locations: point 1 (A), point 3 (B) and point 5 (C).

On the basis of the foregoing observations, 1000 W, taken as the most suitable power level to ensure the microbiological safety of the microwave-processed kiwifruit puree, was chosen to investigate the impact of processing time on pasteurisation certainty at the coldest spot in the product (Figure 6). Time was shown to have a strong influence on the degree of certainty in pasteurisation, with even slight time variations leading to marked differences in terms of probability of microbial inactivation. For example, the certainty of a *L. monocytogenes* load reduction ≥5 $log_{10}$ cycles in kiwifruit puree processed for 325 s was less than 10%, whereas when the treatment time was 350 s the probability of reaching this level of inactivation was greater than 90%. This highlights the importance of properly calculating microwave preservation treatments, given that even minor miscalculations of processing time (e.g. <7%) might cause a considerable reduction in the certainty of pathogen inactivation (80%), leading to serious safety concerns.

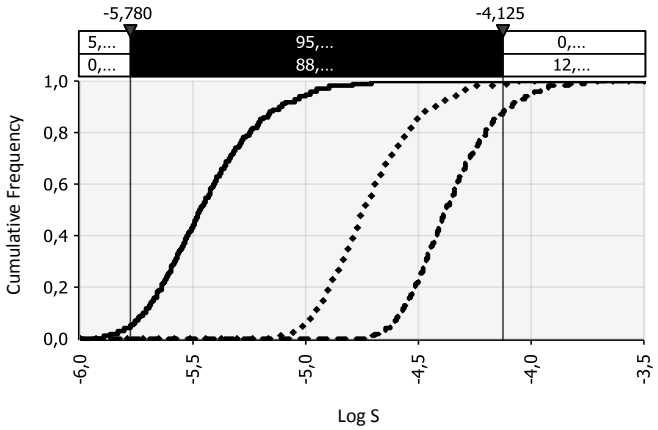


**Figure 6.** Cumulative frequency curves fitting the simulated *L. monocytogenes* $log_{10}$ cycle reductions (Log S) at the coldest spot (point 3) in the kiwifruit puree microwave-heated for 300 (– –), 325 (.....) and 350 s (——) at 1000 W.

Finally, the stochastic model was used to design a safe microwave pasteurisation treatment for the kiwifruit puree. In order to pasteurise fruit juices and other fruit-based products with similar characteristics, the Food and Drugs Administration established that at least 5 $log_{10}$ cycles of *L. monocytogenes*, taken as the pathogen of greatest concern in the product (Benlloch-Tinoco et al., 2014), must be reduced. The processing conditions that led to the accomplishment of the pasteurisation

objective were estimated at the various locations in the sample (Table 2). It was observed that in the kiwifruit puree subjected to 6 min (50.28 °C) at 1000 W, 13 min (38.69 °C) at 900 W or 19 min (47.51 °C) at 600 W at least 5 $\log_{10}$ cycles of *L. monocytogenes* are inactivated in the whole sample with a probability of 95%.

**Table 2.** Reference temperatures ($T_{ref}$) and effective treatment times ($t_e$) required to reduce 5-$\log_{10}$ cycles of *Listeria monocytogenes* at different points in kiwifruit puree (1–6) at 1000, 900 and 600 W with a probability of 95%. Temperature and time corresponding to the coldest spot are highlighted in bold.

| Power level (W) | Point | $T_{ref}$ (ºC) | $t_e$ (s) |
|---|---|---|---|
| 1000 | 1 | 66.60 | 100.56 |
| | 2 | 60.60 | 176.14 |
| | **3** | **50.28** | **348.08** |
| | 4 | 71.12 | 92.05 |
| | 5 | 69.80 | 92.60 |
| | 6 | 84.43 | 50.07 |
| 900 | 1 | 63.70 | 110.25 |
| | 2 | 42.44 | 451.05 |
| | **3** | **38.69** | **766.30** |
| | 4 | 77.94 | 61.43 |
| | 5 | 61.83 | 132.40 |
| | 6 | 81.84 | 40.06 |
| 600 | 1 | 70.10 | 162.97 |
| | 2 | 54.10 | 757.80 |
| | **3** | **47.51** | **1128.80** |
| | 4 | 78.83 | 103.08 |
| | 5 | 68.50 | 218.87 |
| | 6 | 75.74 | 132.44 |

The stochastic approach employed in the present study allows the associated probability of accomplishing the 5D pasteurisation objective with a combination of the various control factors (power level, treatment time and location in the sample) to be predicted for more efficient establishment of safe microwave pasteurisation processes. This dynamic probabilistic estimation of the inactivation of a potential pathogenic microorganism under variable processing conditions might aid the development of industrial microwave treatments that are more reliable in terms of food safety and could provide some guidance for the selection of the most

appropriate risk-reduction processing conditions, perhaps contributing to an expansion of the use of this technology at an industrial level.

## 4. CONCLUSION

The approach evolved in the present study gives a general idea of uneven heating and non-uniformity in the inactivation of *Listeria monocytogenes* during batch microwave processing of a kiwifruit puree. Additionally, this study contributes to the state of the art of stochastic modelling and interpretation of potential microbiological risks in microwave heating by estimating the final bacterial load in the treated kiwifruit puree as a function of probability.

In order to obtain safe, high-quality microwave-treated fruit-based products, processing conditions must be carefully chosen. The right balance between faster heating rates, greater effectiveness and certainty in microbial inactivation, on the one hand, and greater heating uniformity, on the other, provided by high and low power levels, respectively, must be found for each particular application.

**ACKNOWLEDGEMENTS**

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# CAPÍTULO IV.5. IMPACTO DE LA TEMPERATURA SOBRE LOS VALORES DE LETALIDAD DE UN PROCESO DE PASTEURIZACIÓN DE UN PURÉ DE KIWI POR MICROONDAS O POR CALENTAMIENTO CONVENCIONAL

# IMPACT OF TEMPERATURE ON LETHALITY OF KIWIFRUIT PUREE PASTEURIZATION BY THERMAL AND MICROWAVE PROCESSING

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## ABSTRACT

The use of pasteurization units (PU) as a measure of the lethal effect of processes was proposed with the aim of comparing conventional and novel thermal technologies. Kiwifruit puree was subjected to microwave (1000 and 900 W) and conventional (97 °C) heating. Processing conditions of the treatments were chosen to simulate a pasteurization treatment. The temperature profiles of the samples during processing were recorded at different positions. The coldest and hottest spots of the product were identified and the associated PU numbers were calculated. A significantly ($p<0.05$) higher thermal load was necessary in order to stabilize the kiwifruit puree under conventional (19.27 min) than microwave heating mode (0.03–1.8 min) at any of the conditions studied. The higher effectiveness of microwave heating could be attributed to non-thermal effects associated with this technology.

**KEYWORDS** Microwaves, thermal treatment, kiwifruit, temperature, accumulated lethality.

## 1. INTRODUCTION

Microwave heating (MW) appears to be a promising novel technology for food preservation (Vadivambal & Jayas, 2010). During recent decades, many studies have been carried out on the evaluation of MW benefits with respect to conventional heat treatments. Its suitability for pasteurization, sterilization, and dehydration processes as well as its capacity of producing safe and better quality products has been widely demonstrated (Igual, García-Martínez, Camacho, & Martínez-Navarrete, 2010). Although MW could potentially replace conventional heat processes for some specific applications (Awuah, Ramaswamy, & Economides, 2007), there are still problems that are inherent in this technology, such as non-uniform product temperature distribution (Salazar-González, San Martín-González, López-Malo, & Sosa-Morales, 2012), and that contribute to delaying the exploitation of MW to its fullest potential in the food industry.

On the other hand, improper comparison between treatments because of inadequate control of processing parameters such as sample temperature exposure, roughly selected exposure periods or poor kinetic data accommodation may be generating doubts and causing conflicting opinions regarding the superiority of this technology against conventional heat treatments. Some authors have proposed different ways of comparing microwave and conventional treatments: (i) to select processing conditions to get equal heating rates (°C/min) (Fujikawa, Ushioda, & Kudo, 1992), (ii) to reach a similar temperature profile in samples under both technologies (Welt, Tong, Rossen, & Lund, 1994), and (iii) to carry out kinetic studies (Matsui, Gut, De Oliveira, & Tadini, 2008). This lack of homogeneity in comparison procedures may result in mistaken interpretations and hinders the contrast of different research works.

In the present study, the concept of accumulated lethality, a parameter traditionally employed to evaluate conventional heat treatments, is proposed as a tool for comparison of conventional and novel thermal technologies. The lethal effect of the process is determined on the basis of the time-temperature history of the product and it is expressed as a numerical value in arbitrary units. The pasteurization unit (PU) was proposed by Shapton, Lovelock, and Laurita-Longo

(1971) as a measure of accumulated lethality but more specifically adapted for pasteurization processes.

The objective of the present research work was to assess the suitability of the PU parameter to compare the thermal load of microwave and conventional kiwifruit puree pasteurization treatments.

## 2. MATERIALS AND METHODS

### 2.1. Sample Preparation

Kiwifruit (*Actinidia deliciosa var.* Hayward) was purchased in a local supermarket. Fruit pieces were peeled and triturated in a Thermomix (TM 21, Vorwerk, Spain), using the fourth power level for one minute. The physicochemical characteristics of kiwifruit puree (water content, soluble solids, water activity, and pH) were determined in order to control the fruit which was used as raw material (data not shown).

### 2.2. Treatments

Processing conditions were chosen based on preliminary experiments to simulate a pasteurization treatment (Benlloch-Tinoco, Pina-Pérez, Martínez-Aguirre, Rodrigo, & Martínez-Navarrete, 2012). The treatments selected inactivated 90% of peroxidase enzyme and reduced more than 5-$\log_{10}$ cycles of the most important pathogenic microorganism (*Listeria monocytogenes*) (data not shown). These data correspond to the global inactivation achieved in the samples. Three replicates of each treatment were run.

#### *2.1.1. Microwave treatment*

A household microwave oven (model: 3038GC, Norm, China) was used to treat the kiwifruit puree. For each treatment, a sample weighing 500 g was tempered to an initial temperature of 25 °C and then heated in the microwave oven in a standard size glass beaker (BKL3-1K0-006O, Labbox, Spain). Two microwave treatments, based on different power-time combinations, were carried out: 1000W-200s and 900W-225s. The microwave oven was provided with a probe (CR/JP/11/11671, Enelec, Spain) which was connected to a fiber optic thermometer (FOTEMP1-OEM,

Enelec, Spain) to continuously record the time-temperature history of the sample during the microwave treatments. Because MW has traditionally been associated with non-uniform heating, the coldest and the hottest spots were identified and the temperature at these points was recorded.

*2.1.2. Conventional thermal treatment*

The conventional thermal treatment selected consisted in heating the sample at 97 °C for 30 s in a thermostatic water bath (Precisterm, Selecta, Spain). After the kiwifruit was triturated, 20 g of puree was placed in TDT stainless steel tubes (1.3 cm inner diameter and 15 cm length) and closed with a screw stopper. A thermocouple which was connected to a data logger was inserted through the sealed screw top in order to record the time-temperature history of the sample during the treatment. Three replicates were carried out to define an average temperature profile of the process. Previously, the samples were preheated to 25 °C to shorten and standardize the come-up time (150 s).

**2.3. Peroxidase enzyme determination**

Peroxidase activity (POD) was measured in all the treated samples (microwaved and conventionally heated ones) and also in the non-treated sample, which was used as a control, according to the method proposed by De Ancos, Cano, Hernández, and Monreal (1999) with the following modifications. For enzyme extraction, centrifugation was done for 20 min and the filtration step was omitted. Extracts were made in duplicate. Enzyme extract (0.050 mL) was used for the enzyme activity measurement and pH 6.5 was fitted. A solution containing all the components except the enzyme extract, which was replaced by 0.050 mL of sodium phosphate buffer, was used as a blank. One unit of POD activity was defined as the amount of enzyme that caused an increase of one in the absorbance per min ($Abs \cdot min^{-1} \cdot g^{-1}$), calculated from the linear part of the curve obtained. The percentage of enzyme inactivation (I) was calculated by using Eq. (1).

$$I = \frac{A_F - A_T}{A_F} \times 100 \quad (1)$$

Where:

$A_F$: enzyme activity of fresh kiwifruit puree;

$A_T$: enzyme activity of treated kiwifruit puree.

### 2.4. *L. monocytogenes* inactivation study

*L. monocytogenes* is recommended by the National Advisory Committee on Microbiological Criteria for Foods to be used as a target microorganism for products of similar characteristics. Kiwifruit puree, prepared as described above, was inoculated by adding 1 mL of a *L. monocytogenes* (CECT 4032, Spanish Type Culture Collection) inoculum to give a final concentration of $10^7$ CFU/g. Kiwifruit puree was blended for 30 s with the aim of ensuring a homogeneous initial content of the bacterium. After processing, serial decimal dilutions of both treatments and the untreated one were performed in 0.1% (w/v) sterile peptone water (Scharlab Chemie S. A., Barcelona, Spain). The enumeration medium used for viable cells was Tryptic Soy Agar (TSA) (Scharlab Chemie S. A., Barcelona, Spain). The selected dilutions were incubated at 37 °C for 48 h. The reduction of viable cells was expressed as the decimal logarithm of the quotient of the treated and untreated cells.

### 2.5. Pasteurization units calculation

The pasteurization units corresponding to the microwave and conventionally treated samples were calculated using Eq. (2) with a reference temperature of 80 °C (Heinz, Toepfl, & Knorr, 2003; Lau & Tang, 2002) and a z-value of 13.62 °C, previously determined for this pathogen from inactivation data under thermal processing.

$$PU = \int_0^t 10^{\left(\frac{T(t)-T_{ref}}{z}\right)} dt \quad (2)$$

Where,

t: Treatment time (s);

T(t): Product temperature at each treatment time;

$T_{ref}$: 80 °C;

z: Temperature sensitivity (°C) for *L. monocytogenes*.

### 2.6. Statistical analyses

Significant differences were evaluated by means of the corresponding analysis of variance (ANOVA) using Statgraphics Plus 5.1. Differences of $p<0.05$ were considered to be significant.

## 3. RESULTS AND DISCUSSION

Microwave and conventional heating comparison has been the base of many studies dealing with MW process applications, such as those performed by Gentry and Roberts (2005) or Igual et al. (2010). The difficulty of comparing the two technologies lies in the particular way of heating which takes place during MW treatments (Banik, Bandyopadhyay, & Ganguly, 2003). While in conventional heating a holding period is expected, in the case of MW non-isothermal heating takes place exclusively (Matsui et al., 2008). Additionally, fixing the parameters that affect the heating process, such as (i) the heating rate, (ii) the range of temperatures at which the samples are exposed, or (iii) providing appropriate sample homogenization is not usually possible. Consequently, products conventionally and microwave treated are not normally subjected to equivalent temperature-time combinations and comparing the effect of the two technologies on the product quality may prove complicated.

Given the different nature of the heating processes that take place under conventional and microwave modes, the temperature control should not be limited to the initial and the final point of the process, but the whole temperature history of the product should be taken into account. In this context, the PU parameter offers the

possibility of evaluating the complete thermal load of the heating process at any reference temperature, as if it had taken place under isothermal conditions. This implies that the product is considered to reach the reference temperature instantaneously (Matsui et al., 2008), so the effect of processing factors that could be causing differences in the nature of the heat transfer, such as (i) product characteristics including consistency, solid/liquid ratio and thermophysical properties, (ii) sample quantity, and (iii) container type, size, and shape (Awuah et al., 2007), is avoided.

The concept of accumulated lethality has been used in relation to microwaves in order to validate the lethal effect of a previously established preserving treatment (Wang, Wig, Tang, & Hallberg, 2003). It has also been employed as a tool for assessing the effect of a conventional and a combined microwave-conventional pasteurization process on the nutritional and sensory quality of asparagus by calculating the C-value (Lau & Tang, 2002). However, to date, PU has still not been used with the aim of evaluating the thermal load of various conventional and novel heating processes to perform comparisons.

In the present study the temperature profiles of kiwifruit puree samples subjected to various microwave and conventional thermal treatments were recorded in order to compare the different lethal effects of the processes (Figure 1).

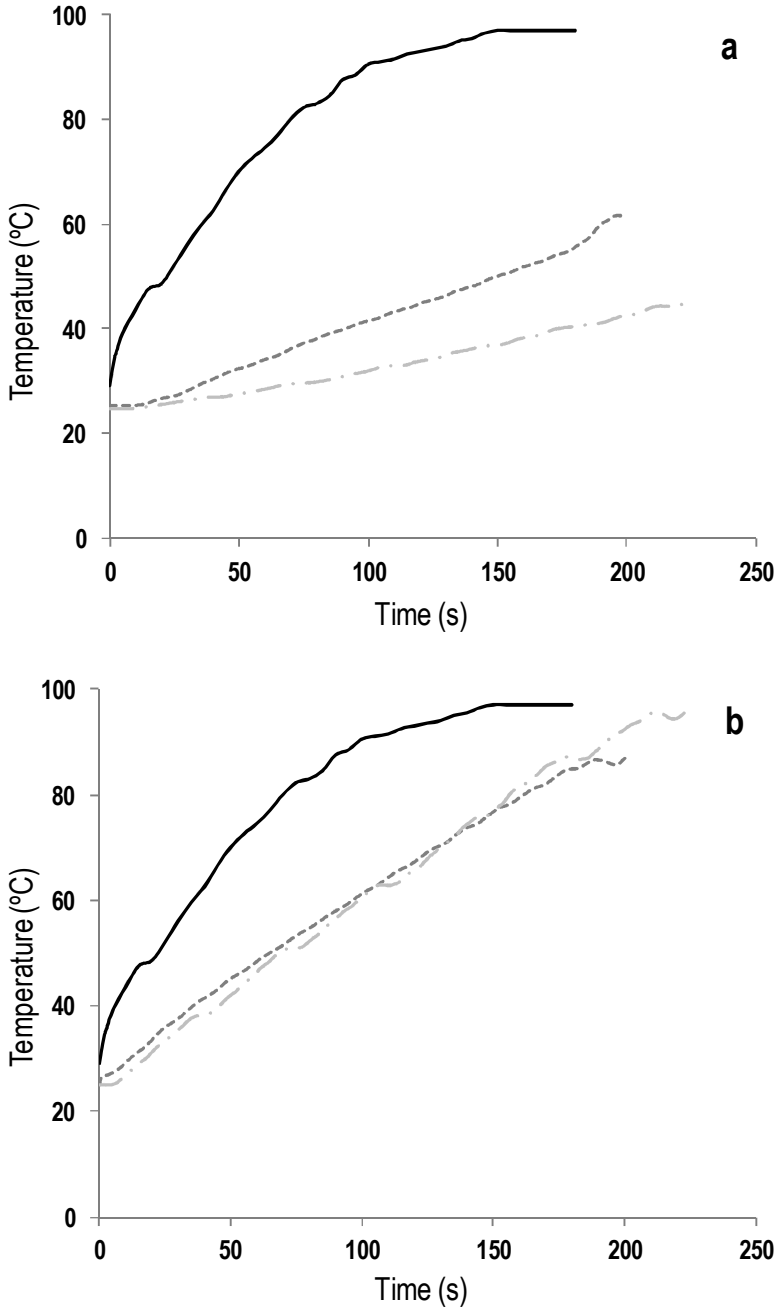


**Figure 1.** Temperature profile of kiwifruit puree subjected to conventional thermal (——) and microwave (1000 W, ..... and 900 W, — . —) processing at the coldest spot (a) and the hottest spot (b) of the product.

Mean value (and standard deviation in brackets) of the PU numbers obtained for the treatments assayed are presented in Table 1. As expected, substantial differences were found in the thermal load received by the product at the two locations studied during the microwave treatments. The PU obtained at the hottest

spot was considerably higher than the PU obtained at the coldest spot. On the other hand, the conventional heating mode required a significantly ($p<0.05$) higher thermal load to achieve the pre-set level of POD inactivation in the kiwifruit puree than any of the microwave treatments studied, irrespective of whether the comparison was carried out at the coldest or hottest spot of the sample.

**Table 1.** Mean values and standard deviation (in brackets) of pasteurization units (PU) calculated at the coldest and the hottest spot of the kiwifruit puree under conventional and microwave (1000 and 900 W) heating. The same superscript letters in columns (x,y for the coldest spot and a, b, c for the hottest spot) indicate homogeneous groups established by the ANOVA ($p<0.05$) when the different treatments are compared.

| *Treatment* | *PU (min)* | |
|---|---|---|
| | *Coldest spot* | *Hottest spot* |
| Conventional heating | 19.27 (0.13)$^{yc}$ | |
| Microwave heating | | |
| 1000W-200s | 0.046 (0.007)$^{x}$ | 1.79 (0.02)$^{a}$ |
| 900W-225s | 0.0027 (0.0014)$^{x}$ | 7.9 (1.4)$^{b}$ |

The greater effectiveness of MW with respect to conventional heating treatments for food stabilization has been widely reported by various authors, such as Matsui et al. (2008) and Soysal and Söylemez (2005). Although differences observed in MW and conventional heating processes have traditionally been attributed to the faster heating rates of MW (El-Abassy, Donfack, & Materny, 2010), in our case this premise cannot be accepted to explain the differences observed, because the PU data were calculated as if the treatments had taken place under isothermal conditions. Consequently, they might indicate the possibility of some contributory non-thermal effects associated with MW. Although other authors have reported similar findings (Banik et al., 2003), in-depth research work on this area is considered necessary.

## 4. CONCLUSIONS

The pasteurization unit seems to be a suitable parameter to evaluate the thermal load associated with conventional and microwave heating processes. This parameter can be taken as a common basis to compare the effect of different heating technologies on product quality and stability. Microwave heating required a lower thermal load than conventional heating to pasteurize the product at the power level studied, which might be attributed to some contributory non-thermal effects associated with this technology.

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# CAPÍTULO IV.6. CALIDAD Y ACEPTABILIDAD DE UN PURÉ DE KIWI PASTEURIZADO POR MICROONDAS Y CALENTAMIENTO CONVENCIONAL

# QUALITY AND ACCEPTABILITY OF MICROWAVE AND CONVENTIONALLY PASTEURIZED KIWIFRUIT PUREE

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## ABSTRACT

The development and optimisation of food preservation processes seem to be necessary in order to address consumer expectations related to secure, fresh-like foods. To this end, the sensory, nutritional and functional properties must be maximally retained. In order to contribute to the acquisition of knowledge about the adequacy of microwave processing as a means of preserving fruit-based products, the present study compares the impact of microwave heating with conventional thermal processing. The consumer acceptance of fresh and pasteurised kiwifruit puree was studied as was the content of water, soluble solids and bioactive compounds and the pH, consistency, viscosity, colour coordinates and antioxidant capacity, as well as the effect of the thermal treatment on enzyme and microbial inactivation. As bioactive compounds, the content of vitamins C, A and E and the total flavonoid, phenol and tannin content have been considered. As the obtained results show, not only was microwaved puree preferred by consumers, but it also exhibited a superior maintenance of the nutritive and functional properties of the fruit, smaller colour changes and a content of inactivated enzymes and microorganisms equal to or greater than the conventionally heated sample.

**KEYWORDS** Consumer perception, bioactive compounds, enzymes, microorganisms, microwave heating, conventional heating.

## 1. INTRODUCTION

One of the most relevant trends in food manufacturing has stemmed from the recent increased demand for convenient, easy-to-preserve and health-promoting foods (Elez-Martínez et al. 2006). Superplus production of fruits presenting appreciated sensory and nutritional value, e.g. kiwifruit (Barboni et al. 2010), may be processed and marketed as pasteurised pulp or puree products which, besides from being consumed directly as a dessert or as a complement of cooked meals, could be used as ingredients in juices, nectars, jams, ice creams, baby foods or pastry (Silva and Silva 1997). Given the current consumer expectations, the industrial sector is showing a greater interest in the development and optimisation of novel food preservation processes, intending to market high-quality minimally processed fruit-based products (Señorans et al. 2003). In this respect, sensory assessment must be considered as an essential tool to help guide any modification of the food processing step, taking great care of what consumer expectations are and what information positively affects their decision to purchase (Di Monaco et al. 2005). However, to date, there still seems to be need for sensory analyses that focus on the impact emerging technologies have on the consumer acceptance of processed products (Da Costa et al. 2000).

Microwave heating presents commercially proven applications with which to preserve fruit and vegetable products (Salazar-González et al. 2012). This technology could potentially replace conventional heat processes for some specific purposes, overcoming the slow heating rates found in conventional canning operations of thick materials (Awuah et al. 2007) and offering the possibility of obtaining safe, stable and superior quality products (Salazar-González et al. 2012). However, as the currently published information on the consumer acceptance of microwaved products is both scarce and inconsistent, it has to be said that there is still a gap in knowledge concerning the fundamental understanding of the effects of microwaves when applied to food. In this regard, in depth sensory research work is considered that could relevantly contribute to increase the knowledge of how microwaves affect food quality and, perhaps, to expand its use on an industrial level. Some of the few studies dealing with the sensory assessment of microwave

processing applied to different food products have been conducted by Igual et al. (2013), Benlloch-Tinoco et al. (2012b), Huang et al. (2007), Gerard and Roberts (2004), Guan et al. (2002), Valero et al. (2000) and Fathima et al. (2001), using different approaches to achieve their purpose. Triangle differentiation tests were employed by Gerard and Roberts (2004) and Igual et al. (2013) to compare the sensory properties of some fresh and microwaved apple juice or microwaved, conventionally heated and fresh grapefruit juice, respectively. Benlloch-Tinoco et al. (2012a, b) and Huang et al. (2007) carried out a descriptive analysis of the sensory properties of a kiwifruit puree subjected to several microwave treatments and a microwaved green tea compared with a conventionally heat processed one. Guan et al. (2002), Valero et al. (2000) and Fathima et al. (2001) evaluated the impact of microwave processing on the consumer acceptance of shelf-stable macaroni and cheese, milk and selected greens. However, no available data have been found on the acceptance of kiwifruit-based products.

In order to contribute to the acquisition of knowledge about microwave processing as a means of preserving fruit-based products, the aim of the present study was to evaluate the impact of microwave and conventional thermal processing both on the consumer acceptance and on some chemical, physical and biochemical properties of a ready-to-eat kiwifruit puree. The properties analysed were the water, soluble solid and bioactive compound content and the pH, consistency, viscosity, colour coordinates and the antioxidant capacity of fresh and pasteurised purees, as well as the effect of the heating treatments on enzyme activity and microbial inactivation.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and Standards

The following chemicals and standards were used: chlorhydric acid, sodium hydroxide, Folin-Ciocalteu reagent, sodium carbonate, aluminium chloride, methanol, tartaric acid, sodium fluoride, gallic acid and rutin. Unless otherwise stated, all chemicals employed were of analytical quality or superior. All these chemicals were from Sigma-Aldrich (St. Louis, MO, USA).

### 2.2. Sample Preparation

Kiwifruit (*Actinida deliciosa* var. Hayward) imported from Italy was purchased from a local supermarket in Valencia, Spain, in May 2013. Fruit pieces, selected on the basis of a similar appearance and a soluble solid content of about 13–14 °Brix, were peeled, washed with distilled water, cut into slices and triturated with a Thermomix (TM 21, Vorwerk, Spain), using the fourth power level for 1 min.

### 2.3. Treatments

Processing conditions were chosen based on preliminary experiments to simulate pasteurisation treatments in terms of the enzyme and microbial inactivation achieved (Benlloch-Tinoco et al. 2013; Zheng and Lu 2011). After the assay of several power-time combinations for MW and temperature-time combinations for Co, those reaching 90% of POD inactivation and 5 $\log_{10}$ cycles of *Listeria monocytogenes* inactivation, described below, were selected to carry out the present work (Benlloch-Tinoco et al. 2012a).

#### *2.1.1. Microwave treatment*

A microwave oven (model: 3038GC, NORM, China), provided with a glass turntable plate, was used to treat the kiwifruit puree. A sample of 500 g was tempered to an initial temperature of 25 °C and then heated in the microwave oven in a standard size glass beaker (9-cm inner diameter and 12-cm length) (BKL3-1 K0-006O, Labbox, Spain) at 1,000W for 340 s. The temperature of the sample in the coldest spot, previously identified (data not shown), was continuously recorded by means of a fibre-optic probe (CR/JP/11/11671, Enelec, Spain) which was connected to a temperature datalogger (FOTEMP1-OEM, Enelec, Spain). The microwave-treated samples were immediately cooled in ice water until the puree reached 35 °C.

#### *2.1.2. Conventional thermal treatment*

A vertical pilot plant-scale stainless steel batch retort (Calderería Palou S.L., Barcelona, Spain) was used to carry out the conventional thermal process. A sample of 450 g was heated in a standard size tin can (7-cm inner diameter and 11.5-cm height) at 84 °C for 300 s. Prior to the treatment, the samples were preheated at 45

°C to shorten and standardise the come-up time. Under these conditions, a come-up time of 18 min was needed to reach the treatment temperature. The product temperature was registered in the coldest spot of the sample using a thermocouple (type T) connected to a datalogger (Fluke 2176A, Fluke Corporation Inc, USA). Conventional thermally treated samples were immediately cooled in ice water until the puree reached 35 °C.

### 2.4. Sensory assessment

A total of 82 frequent kiwifruit consumers, 54 female and 27 male, aged from 18 to 65 years old took part in the study. Consumers were recruited from students and workers from both administrative and technical staff of the Instituto de Agroquímica y Tecnología de Alimentos and University of Valencia, in Paterna (Valencia), Spain. The consumers evaluated three samples of kiwifruit puree (samples treated by MW and Co, also as the untreated one) which were tempered at 25 °C before the assessment and served in plastic disposable standard size containers following a balanced complete block experimental design (samples and consumers as factors). Each sample was identified with three-digit random codes. For each sample, the consumers had to score their liking for the appearance, colour, odour, taste, sweetness, acidity, consistency and overall acceptance using a 9-point hedonic scale labelled on the left with 1="dislike very much" and on the right with 9="like very much" (Cruz et al. 2012). Additionally, the adequacy of three of the attributes (sweetness, acidity and consistency) was measured using bipolar "JAR" scales (from 1=much too little to 5= much too much, with 3=just about right) (Desai et al. 2013).

These scales usually have five points to assess whether there is too little, too much or a "just-about-right" level of an attribute (Lawless and Heymann 1998). The end-points are anchored with labels that represent levels of the attribute that deviate from a respondent's theoretical ideal point in opposite directions, whilst the central point is the ideal (Rothman 2007). Testing was carried out in a sensory laboratory equipped with individual booths (ISO 8589 1988). Data acquisition was performed by using Compusense five release 5.0 software (Compusense Inc., Guelph, Ontario, Canada).

## 2.5. Analytical determinations

The treated samples and a non-treated sample used as control were analysed as described below. Each analysis was carried out in triplicate.

### *2.5.1. Physicochemical properties, enzyme activity and antioxidant activity*

Water content, soluble solids, pH, consistency, viscosity, colour coordinates and POD, PPO and PME activities and antioxidant activity (AOA) were measured as described by Benlloch- Tinoco et al. (2012b) and Benlloch-Tinoco et al. (2013).

### *2.5.2. Bioactive Compounds*

The content of vitamins C, A and E and total phenols was measured as previously described by Igual et al. (2010) and García-Martínez et al. (2012). Total tannins were evaluated spectrophotometrically, using the Folin-Denis method, which involves the reduction of the reagent by tannin compounds, as explained by Taira (1995) but with some modifications. The extraction consisted of homogenizing 5 g of the sample (T25 Janke and Kunkel turrax) with 45 mL of 0.56 N HCl and boiled (100 °C) for 30 min. Then, the homogenate was cooled, neutralised with 2 N NaOH and centrifuged (10,000 rpm, 5 min, 4 °C). The supernatant was brought to 100 mL with distilled water. An aliquot (1mL) of this sample was mixed with 6mL of distilled water, 0.5 mL of 1 N Folin-Ciocalteu reagent. The samples were well shaken and incubated for 3 min in darkness; 1 mL of 7.5 % sodium carbonate aqueous solution and 1.5 mL of distilled water were added. Samples were allowed to stand for 1 h at room temperature before absorbance was measured at 725 nm in a UV-visible spectrophotometer (Thermo Electron Corporation, USA). The TT content was expressed as milligram of gallic acid equivalents (GAE) per 100 g of kiwifruit, using a standard curve range of 0.05–0.34 mg/mL. Total flavonoids were measured spectrophotometrically, following the method described by Djeridane et al. (2006) based on the formation of a flavonoid-aluminium complex. The extraction of TF consisted of homogenising 35 g of the sample (T25 Janke and Kunkel turrax) for 5 min with 40 mL of methanol, 10 mL of chlorhydric acid and sodium fluoride to inactivate polyphenol oxidases and prevent phenolic degradation. The homogenate

was centrifuged (10,000 rpm, 10 min, 4 °C) (P-Selecta Medifrigar BL-S, Spain) to obtain the supernatant. For total flavonoid quantification, 1 mL of the extract was mixed with 1 mL of 20 g/L $AlCl_3$ methanolic solution. After incubation at room temperature for 30 min in darkness, the absorbance of the reaction mixture was measured at 430 nm using the aforementioned spectrophotometer. The TF content was expressed as milligram of rutin equivalents per 100 g of sample, using a standard curve range of 0–0.05 mg/mL.

#### *2.5.3. Microbiological analysis*

*L. monocytogenes* inactivation was evaluated as described by Benlloch-Tinoco et al. (2014). The total mesophilic bacteria and yeast and mould counts were examined by diluting the uninoculated samples in 0.1 % (w/v) sterile peptone water (Scharlab Chemie S. A., Barcelona, Spain) and enumerating the viable cells in plate count agar (PCA, Scharlab Chemie S. A., Barcelona, Spain) and potato dextrose agar (PDA, Scharlab Chemie S. A., Barcelona, Spain) acidified with tartaric acid (10 %), adding 1 mL of TA per 10 mL of PDA, respectively. The selected dilutions were incubated at 30 °C for 48 h for TMB and at 25 °C for 5 days for Y&M.

### 2.6. Statistical analyses

An analysis of variance with one factor, at a confidence level of 95 % ($p<0.05$), was applied using the Statgraphics Centurion XV software program (StatPoint Technologies, Inc., Warrenton, VA, USA) to evaluate the differences among samples. The JAR results were analysed by penalty analysis to identify potential directions for product improvement on the basis of consumer acceptance by highlighting the most penalizing attributes in terms of liking. This technique is used to relate JAR scales to liking data, particularly in order to understand which side of the JAR scale is linked to lower hedonic ratings. The usefulness of the method is that it provides guidance for a better understanding of attribute adequacy in relation to liking in terms of direction, with the assumption that the maximum hedonic score will occur at the “just-about right” point (Rothman 2007). A cluster analysis was carried out to classify consumers according to their preference patterns. Agglomerative Hierarchical clustering was performed using Euclidian distance with

Ward's method as the aggregation criterion. XLSTAT 2009.4.03 statistical software (Microsoft, Mountain View, CA) was used to analyse all sensory data and to study the correlation between physicochemical parameters and sensory attributes using a Pearson correlation matrix.

## 3. RESULTS AND DISCUSSION

### 3.1. Consumer acceptance

#### *3.1.1. Liking tests*

A sensory analysis was performed to elucidate how the technology employed to preserve a ready-to-eat kiwifruit puree affected the consumer acceptance of the product. The treated (MW, Co) and the untreated kiwifruit puree samples were tasted to this end. Consumers scored the overall acceptance, appearance, colour, odour, taste, sweetness, acidity and texture liking of the three samples. The consumers' scores are shown in Table 1. As expected, the fresh sample showed the highest scores for all the attributes evaluated. Most consumers' liking scores significantly ($p<0.05$) decreased after both treatments (MW and Co).

The microwaved puree presented intermediate scores, between the fresh and the conventionally heated sample. However, it should be noted that no significant differences between the sweetness, acidity and consistency of the fresh sample and MW sample were found. In turn, with the aim of gaining a better understanding of consumer responses, the liking results were also analysed by clusters to see if all the consumers had the same preference pattern on the samples or not. Cluster analysis was used to identify groups of individuals that are similar to each other but different from individuals in other groups. In this case, cluster analysis was applied to the acceptability scores of panellists to partition the panel into groups showing a preference for each one of the attributes scored, and the consumers were grouped based upon the similarity of their responses to their liking scores. Cluster analysis was performed individually for each attribute.

**Table 1.** Mean values of the different sensory attributes scored by consumers (n=82) corresponding to the fresh (F), microwaved (MW) and conventionally heated (C) kiwifruit puree.

| | F | MW | C |
|---|---|---|---|
| Overall acceptance | 5.76[a] (1.67) | 4.90[b] (1.90) | 4.01[c] (1.86) |
| Appearance | 6.67[a] (1.13) | 5.51[b] (1.61) | 5.11[b] (1.53) |
| Colour | 6.96[a] (1.12) | 5.52[b] (1.69) | 5.06[b] (1.59) |
| Odour | 6.00[a] (1.56) | 5.22[b] (1.45) | 4.84[b] (1.48) |
| Taste | 5.87[a] (1.74) | 4.96[b] (1.75) | 4.13[c] (1.80) |
| Sweetness | 5.51[a] (1.61) | 5.00[ab] (1.71) | 4.60[b] (1.74) |
| Acidity | 5.39[a] (1.77) | 4.95[ab] (1.71) | 4.49[b] (1.87) |
| Consistency | 6.17[a] (1.27) | 5.74[ab] (1.40) | 5.31[b] (1.51) |

In rows, different letters denote significant differences ($p<0.05$) according to the Tukey's test.

The consumers were distributed into two clusters (Fig. 1). The number of consumers in each cluster varied from one attribute to another because consumer preferences were different for each one of the attributes scored. In this respect, cluster 1 and cluster 2 were formed by 59 and 22 consumers for "overall acceptance," 51 and 25 consumers for "appearance," 22 and 57 consumers for "colour," 47 and 28 consumers for "odour," 64 and 16 consumers for "taste," 16 and 59 consumers for "sweetness," 23 and 56 consumers for "acidity" and 27 and 43 consumers for "consistency," respectively. The mean value of the different sensory attributes scored by each consumer cluster was studied by means of a one-way analysis of variance.

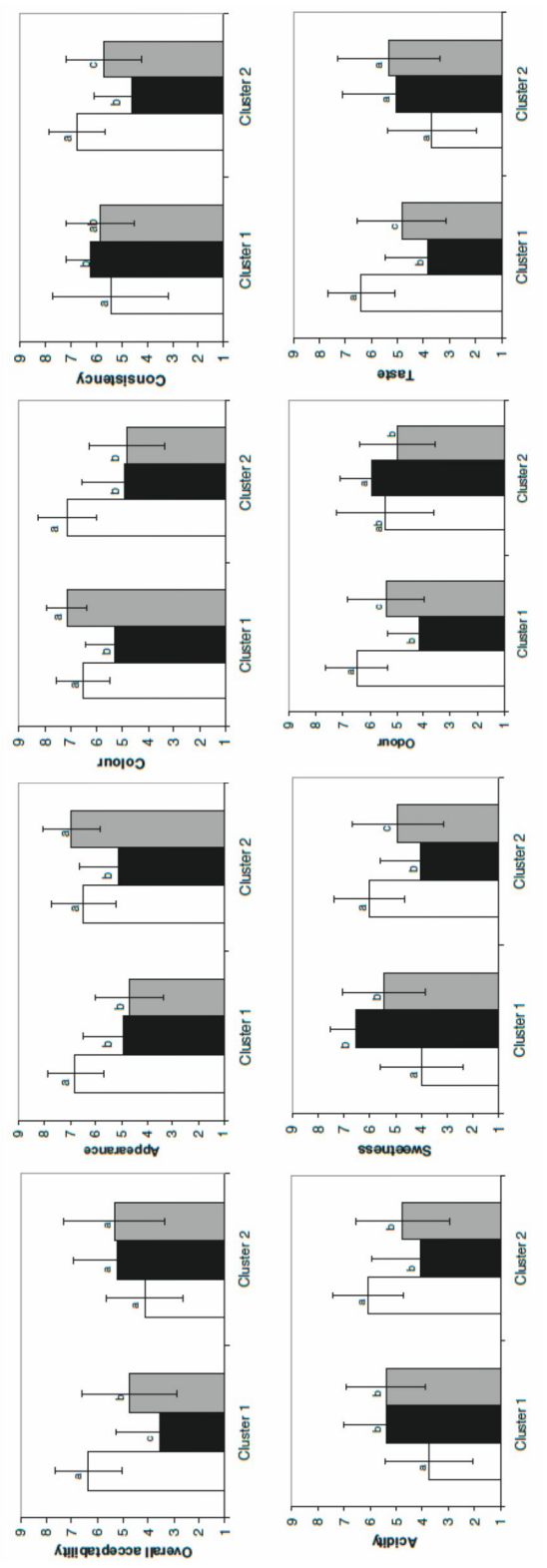


**Figure 1.** Mean values of the different sensory attributes scored by each consumer cluster corresponding to the fresh (□), conventionally heated (■) and microwaved (■) kiwifruit puree. Identical letters for each cluster indicate that there is no significant difference among the samples according to the Tukey test ($p<0.05$).

In Fig. 1, it can be seen that none of the samples studied by cluster 2 gave any significant differences ($p<0.05$) between the overall acceptance and taste liking. This cluster was formed by a small number of consumers (n=22 for overall acceptance and n=16 for taste), and in addition, between 55 and 75 % of them did not frequently eat kiwifruit. However, cluster 1 basically liked MW better than the Co sample. When odour was evaluated, small differences between samples were found by cluster 2, 50 % of which was formed by consumers that did not frequently consume kiwifruit; but again, cluster 1 preferred MW over Co puree.

For acidity and sweetness, cluster 1 seemed to like the treated samples (MW, Co) more than the fresh one, while cluster 2 had a preference for the fresh sample and liked the sweetness of the MW sample significantly more ($p<0.05$) than the Co. Although, the heating process seemed to have a noticeable impact on the perception of the samples' sweetness and acidity, most consumers (cluster 2, n=56 for acidity and n=59 for sweetness) appreciated significant differences ($p<0.05$) between the treated samples in favour of MW. Additionally, 50–57% of those consumers who gave a similar score to MW and Co samples did not frequently eat kiwifruit. As regard to appearance and colour, the majority of the consumers (cluster 1, n=51 for appearance and cluster 2, n=57 for colour) preferred the appearance and colour of the fresh sample without detecting any significant differences ($p>0.05$) between the treated ones. The rest of the subjects exhibited a significant ($p<0.05$) preference for the fresh and MW samples. On the other hand, cluster 1 found small differences in the consistency of the samples, while most of the subjects (cluster 2, n=43) liked the fresh puree better, followed by the MW one and finally the Co one. To sum up, when both treated samples were compared, cluster 1 significantly ($p<0.05$) preferred the MW puree in terms of its taste, colour, odour and overall acceptance, while cluster 2 significantly ($p<0.05$) preferred the MW puree over the Co puree in appearance, consistency and sweetness, although no significant differences ($p>0.05$) between samples were found for the other attributes scored (Fig. 1). These results are a clear indicator of the fact that consumers much prefer and more readily accept the kiwifruit puree subjected to MW than the conventionally heated one. Given the different nature of the heating processes that take place under conventional and microwave treatments, it has been recognised that MW

allows reduced processing times and so a better maintenance of the nutritive, functional and sensory properties of food. This premise has been corroborated by different studies into the sensory properties of different food products when subjected to microwave process evaluation. Several authors reported that microwave processing allowed fruit- and vegetable-based products, macaroni and cheese or milk to be obtained with acceptable or indeed enhanced sensory properties. In this respect, when the comparison between microwaved and conventionally heated samples was established, it was mostly non-perceivable differences that were found, and in some cases, MW implied a better preservation of the evaluated sensory properties (Fathima et al. 2001; Gerard and Roberts 2004; Guan et al. 2002; Huang et al. 2007; Igual et al. 2013; Valero et al. 2000).

### 3.1.2. *Attribute adequacy and its relationship with liking-penalty analysis*

In order to improve the understanding of the attributes that most affected the liking ratings of the evaluated kiwifruit samples, a penalty analysis was carried out with JAR and liking results (Laguna et al. 2013; Villegas et al. 2010). The JAR scales are used to assess the appropriateness of specific sensory attribute levels. The data obtained with these scales provide an idea of the proportion of consumers who perceive each sample in a certain way. Three groups were formed with the responses of JAR scales for their better visualisation. The first group with responses 1 plus 2 (“should be much more” and “should be some more”), the second group with response 3 (“about right”), and the third group with responses 4 plus 5 (“should be some less” and “should be much less”). Figure 2 shows the values of the percentages obtained for the three groups for the F, Co and MW samples. As can be seen, for the three samples evaluated, higher percentages than 60 % were obtained for the point “about right” in the consistency attribute (70.73, 63.41 and 69.51 % for the F, Co and MW samples, respectively). However, 48.78, 59.96 and 53.66 % of consumers considered that F, Co and MW samples, respectively, were “much less” in sweetness, and 37.80, 39.02 and 45.12 % of the consumers considered that the F, Co and MW samples, respectively, were “much more” in acidity.

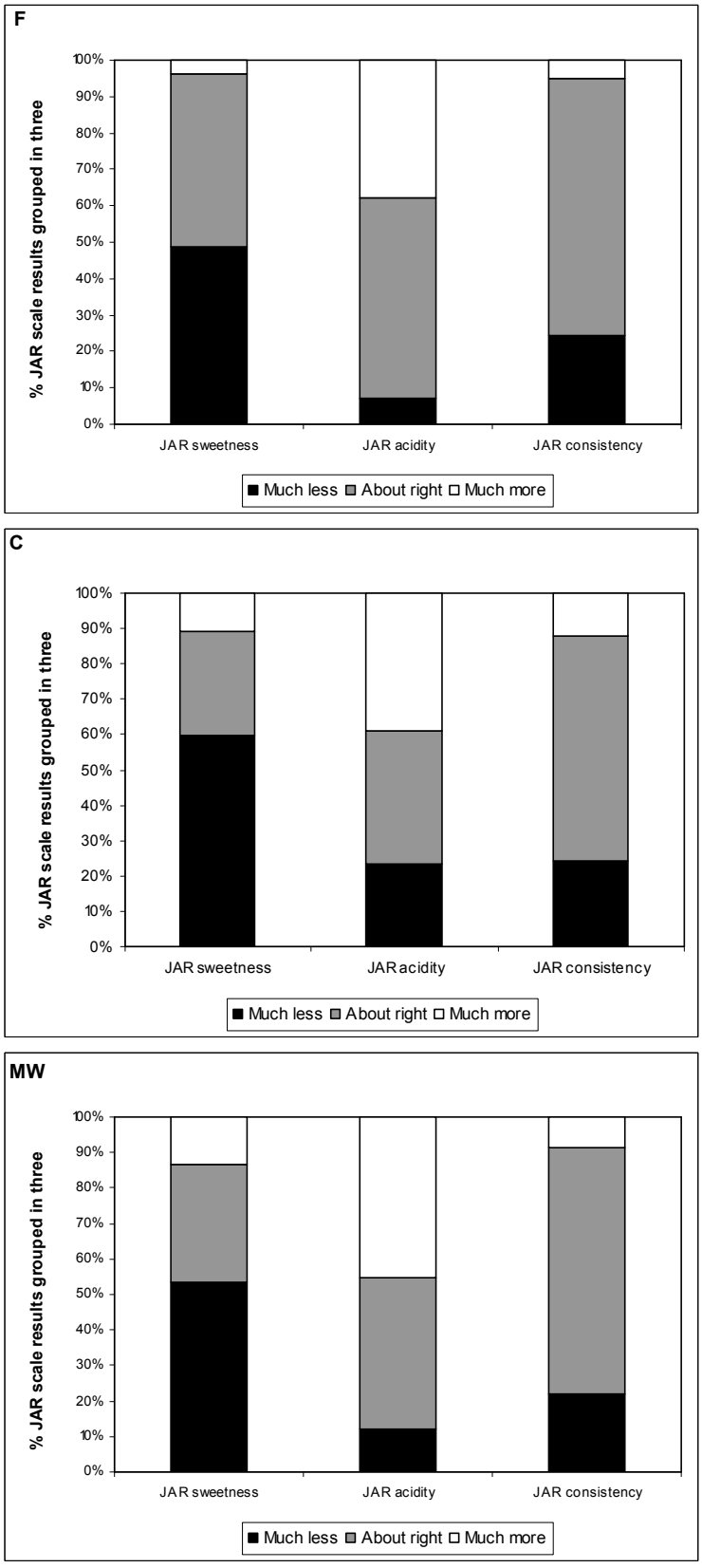


**Figure 2**. JAR scale percentages of responses grouped in three levels for the three samples evaluated.

Therefore, to determine if these attributes would be appropriate to modify, a penalty analysis was performed. The significance of penalties (drops in overall liking) was based on the proportion of consumers stating that an attribute was “not enough” (−) or “too much” (+). So, an attribute was considered significant for liking when the respondent percentage of consumers was higher than 20% (Xiong and Meullenet 2006) and the penalty score (drop in overall liking) was higher than 1. In other words, to conclude that a specific attribute is at its optimal level, a minimum of 70% of the responses is usually expected to be in the “just-about-right” group, and to conclude that an attribute is not at its optimal level, a minimum of 20 % of consumers is usually needed in the “too weak” or “too strong” categories. Significant penalties by percentage of consumers are presented in Fig. 3. Bearing these criteria in mind, the fewer the attributes located in the upper right-hand corner of the penalty plot, the better the acceptance of the kiwifruit sample. According to the obtained results, the majority of the sensory attributes evaluated in this study were found to be adequate by consumers, with only “sweetness” and “acidity” penalizing and deviating from the ideal “right point” ones. In general terms, the consumers perceived all the kiwifruit samples as “too acidic” and “not sweet enough.” Accordingly, it might be assumed that heat processing (MW, Co) did not promote this deviation from the ideal “sweetness” and “acidity” “right point” values, since this fact seemed to be mainly related to the low pH and °Brix values that are characteristic of the fresh fruit selected for the research work (see “Effect of treatments on the physicochemical properties, bioactive compounds and antioxidant activity” section).

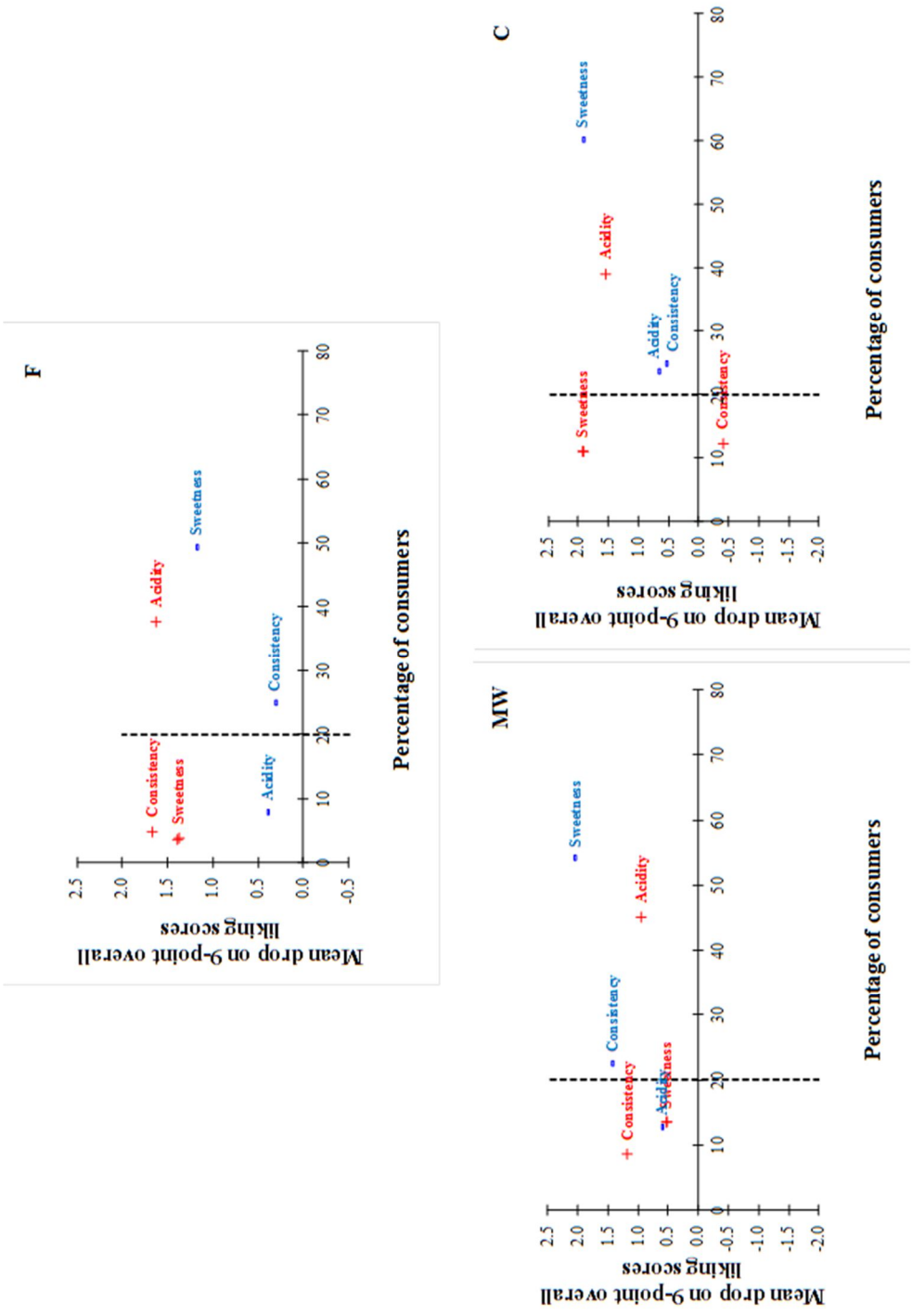


**Figure 3.** Representation of significant penalties (drops in liking) by proportion of panellists for the fresh (F), microwaved (MW) and conventionally heated (C) kiwifruit puree.

### *3.1.3. Effect of treatments on inactivation of enzymes and microorganisms*

The safety and stability of all the kiwifruit puree samples were investigated. In this respect, how effective both the microwave and conventional thermal treatments are at inactivating enzymes and microorganisms was checked. Table 2 shows POD, PPO and PME activities, TMB and Y&M counts and the $\log_{10}$ cycles reduced of *L. monocytogenes* for the treated and untreated kiwifruit purees. In general terms, the obtained values for enzyme activity (POD, PPO and PME) and the initial population of TMB and Y&M in the fresh kiwifruit puree were close to those reported by other authors working on this fruit and other similar fruit-based products (Benlloch-Tinoco et al. 2013; Picouet et al. 2009).

**Table 2.** Average values (and standard deviation) of peroxidase (POD), polyphenoloxidase (PPO) and pectinmethylesterase (PME) activity, total mesophilic bacteria (TMB) and yeast and mold (Y&M) counts and $\log_{10}$ cycles reduced of *L. monocytogenes* of fresh (F), microwaved (MW) and conventionally heated (C) kiwifruit puree.

| | F | MW | C |
|---|---|---|---|
| POD ($Abs \cdot min^{-1} \cdot g^{-1}$) | 10.2 (0.2)$^{b}$ | 1.05 (0.02)$^{a}$ | 1.11 (0.05)$^{a}$ |
| PPO ($Abs \cdot min^{-1} \cdot g^{-1}$) | 6.77 (0.07)$^{c}$ | 1.31 (0.04)$^{a}$ | 2.3 (0.2)$^{b}$ |
| PME ($U \cdot g^{-1}$) | 0.43 (0.04)$^{c}$ | 0.045 (0.011)$^{a}$ | 0.10 (0.06)$^{b}$ |
| TMB (log CFU/mL) | 3.08 (0.12)$^{b}$ | 0.27 (0.10)$^{a}$ | 0.24 (0.13)$^{a}$ |
| Y&M (log CFU/mL) | 2.88 (0.10)$^{b}$ | 0.44 (0.07)$^{a}$ | 0.46 (0.06)$^{a}$ |
| *L. monocytogenes* ($\log(N/N_0)$) | - | -7.0 (0.2)$^{a}$ | -6.96 (0.11)$^{a}$ |

In rows, different letters denote significant differences ($p<0.05$) according to the Tukey's test.

As expected, MW and Co provoked the level of enzyme inactivation and microbial decontamination required of them in order to be considered as adequate pasteurisation processes. Both treatments inactivated 90 % of POD, the enzyme selected as an indicator of treatment efficiency, (Benlloch-Tinoco et al. 2013; Zheng and Lu 2011) and reduced more than 5 $\log_{10}$ cycles of the most important pathogenic microorganism (*L. monocytogenes*), taking into consideration the characteristics of the product (FDA 2004; NACMCF 2006). Neither the POD inactivation nor the *L. monocytogenes* inactivation was found to be different

($p>0.05$), regardless of whether the samples were treated conventionally or by microwave (Table 2). In the same way, MW and Co similarly ($p>0.05$) reduced the content of TMB (2.8 $\log_{10}$ cycles) and Y&M (2.4 $\log_{10}$ cycles) in the puree. However, MW was shown to be significantly more effective at inactivating PPO and PME enzymes than the Co treatment ($p<0.05$). Other authors have reported that microwaves are more effective than conventional heating at inactivating enzymes in fruit or vegetable products, which seems to be related to the interaction of microwave energy with the polar and/or charged moieties of these compounds, affecting the non-covalent bonds and enhancing loss of protein functionality (Kermasha et al. 1993). In this regard, Tajchakavit and Ramaswamy (1997), Matsui et al. (2008) and Zheng and Lu (2011) found that MW was faster at inactivating PME in orange juice, PPO and POD in coconut water and POD in carrot, respectively, than other conventional heating methods.

#### *3.1.4. Effect of treatments on the physicochemical properties, bioactive compounds and antioxidant activity*

The physicochemical properties, the content of the major bioactive compounds and the antioxidant activity of the kiwifruit puree, before and after processing, are summarised in Table 3. The fresh sample used in this work presented the characteristic values of all the analysed properties shown in the bibliography for kiwifruit (Fiorentino et al. 2009; Park et al. 2011; Zolfaghari et al. 2010). As previously reported by other authors, kiwifruit has a high content of vitamins C and E along with a marked antioxidant activity. Actually, its content of vitamin C is even higher than that found in grapefruit, orange (Igual et al. 2010) and citric fruits which are widely recognised as a good source of this bioactive compound. Given the substantial content of such vitamins (C, E), kiwifruit is assumed to provide an antioxidant protective effect under both hydrophobic and hydrophilic conditions (Tanaka et al. 1997). All these excellent nutritional and functional characteristics were highlighted by Fiorentino et al. (2009), who defined this fruit as a unique and precious cocktail of protective phytochemicals. The parameters shown in Table 3 were used to evaluate the impact of MW and Co on the quality of the product. In Table 3, it can be observed that the TT content and pH were the sole parameters to

remain significantly ($p>0.05$) unchanged after processing. The a* and b* colour coordinates were affected in a similar way by both MW and Co. While the a* values significantly increased as a consequence of processing, the b* values significantly ($p<0.05$) decreased, these differences being higher when the Co treatment was applied. Accordingly, processed samples slightly changed to redder, less yellow tones. However, the L* coordinate was exclusively affected by MW that gave place to a significantly ($p<0.05$) more luminous kiwifruit puree (Table 3). This increase in luminosity has been previously described and could be mostly attributed to the degradative loss of pigments instead of to the typical browning reactions in heating processes (Benlloch-Tinoco et al. 2012b).

**Table 3.** Mean values (standard deviation) of content of water ($x_w$), soluble solid (ºBrix), vitamin C (Vit. C), vitamin A (Vit. A), vitamin E (Vit. E), total phenols (TP), total flavonoids (TF) and total tannins (TT), antioxidant activity (AOA), pH, consistency, viscosity, colour coordinates (L*, a* and b*) and colour diference ($\Delta$E) of fresh (F), microwaved (MW) and conventionally heated (C) kiwifruit puree.

| | F | MW | C |
|---|---|---|---|
| $x_w$ (g/100g) | 85.17 (0.13)$^b$ | 84.4 (0.2)$^a$ | 84.9 (0.2)$^b$ |
| ºBrix (g/100g LP) | 13.67 (0.06)$^a$ | 14.33 (0.06)$^c$ | 13.9 (0.2)$^b$ |
| Vit. C (mg/100g) | 75.9 (1.3)$^b$ | 75.5 (1.1)$^b$ | 55.63 (0.07)$^a$ |
| Vit. A (mg/100g) | 0.057 (0.007)$^b$ | ND$^a$ | ND$^a$ |
| Vit. E (mg/100g) | 2.45 (0.06)$^c$ | 2.22 (0.07)$^b$ | 0.41 (0.05)$^a$ |
| TP (mg GAE/100g) | 22 (2)$^c$ | 17.2 (0.5)$^b$ | 15.5 (0.2)$^a$ |
| TF (mg RE/100g) | 1.16 (0.05)$^c$ | 0.74 (0.06)$^b$ | 0.57(0.02)$^a$ |
| TT (mg GAE/100g) | 14.40 (0.10)$^a$ | 10.6 (0.8)$^a$ | 9.9 (0.3)$^a$ |
| AOA (mM Trolox/g) | 5.81 (0.05)$^c$ | 1.99 (0.06)$^b$ | 1.3 (0.3)$^a$ |
| pH | 3.33 (0.02)$^a$ | 3.33 (0.02)$^a$ | 3.34 (0.02)$^a$ |
| Flow distance (mm/g) | 5.1 (0.2)$^b$ | 3.3 (0.2)$^a$ | 3.0 (0.8)$^a$ |
| Viscosity (Pa·s) | 1.57 (0.06)$^a$ | 2.3 (0.2)$^c$ | 1.87 (0.02)$^b$ |
| L* | 40.17 (0.02)$^b$ | 41.71 (0.02)$^c$ | 39.423 (0.006)$^b$ |
| a* | -1.557 (0.006)$^a$ | 1.027 (0.006)$^b$ | 1.707 (0.006)$^c$ |
| b* | 30.700 (0.010)$^c$ | 26.74 (0.02)$^b$ | 26.60 (0.03)$^a$ |
| $\Delta$E | - | 4.98 (0.09)$^a$ | 5.29 (0.02)$^b$ |

LP: liquid phase; ND: not detected

In rows, different letters denote significant differences ($p<0.05$) according to the Tukey's test.

The total colour difference parameter was calculated with respect to the non-treated sample. Both treatments lead to colour differences which are noticeable to the human eye (ΔE*>3, Bodart et al. 2008), with the ΔE* value being significantly ($p<0.05$) higher in the Co sample. This difference in instrumental colour could be the cause of the decrease in consumer acceptability when the kiwifruit puree was heat treated (Table 1). On the other hand, as expected, both MW and Co significantly ($p<0.05$) increased the consistency and viscosity of the puree, changes that can be explained by the increase in the soluble pectin content in the aqueous phase of the product (pectin solubilisation) due to the high temperatures reached (Contreras et al. 2007). As in the colour, this increase in consistency was not well accepted by the consumers which decreased their liking in this attribute. As regard to the effect of the treatments on the bioactive compounds and the AOA of the samples, significant ($p<0.05$) losses were found in all the analysed compounds, except TT, with vitamin A being the most labile (loss of 100 %) (Table 3). The impact of the heating processes on the bioactive compound content of several fruit-based products has been reviewed by Rawson et al. (2011), who highlighted thermal pasteurisation as a treatment severe enough to reduce the levels of most bioactive compounds present in fruit, with vitamins found to be among the most heat-sensitive food components (Awuah et al. 2007). Although simple thermal decomposition would appear to be the most likely cause for these losses, their degradation may be a complex phenomenon which is also dependent on oxygen, light, pH, water solubility and the presence of chemical, metal or other compounds that could catalyse deteriorative reactions (Awuah et al. 2007). On the other hand, the changes observed in the rest of the bioactive compounds and AOA were significantly ($p<0.05$) higher when kiwifruit puree was conventionally heated. In this respect, losses of 0.5 and 26.7% of vitamin C, 9.4 and 83.3% of vitamin E, 21.8 and 29.5% of TP, 36.2 and 50.9%of TF and 65.7 and 77.6% of AOA under MW and Co were found, respectively. The results obtained point out that microwave processing allowed the nutritional and functional properties of the kiwifruit puree to be better maintained than the conventional thermal treatment. Similar results have been extensively reported in the bibliography. Igual et al. (2010) found a superior retention of ascorbic acid in grapefruit juice pasteurised by microwaves when compared to a conventional

pasteurisation treatment. Barrett and Lloyd (2012) reviewed the effect of microwave processing on the bioactive compounds of products of vegetable origin and reported that microwaves, more than conventional heating, lead to a relatively high retention of the vitamin C in most fruits and vegetables. The same authors also mention that microwaves allowed the phenolic compounds of unpeeled potatoes, tomatoes and spinach to be better retained than boiling water.

*3.1.5. Correlation between instrumental and sensory data*

A Pearson correlation matrix was constructed using the instrumental and sensory data (data not shown). Significant ($p<0.05$) and meaningful correlations were found between instrumental parameters and sensory descriptors. In this regard, sensory "appearance" ($R^2=-0.999$) and "colour" ($R^2=-0.999$) were negatively correlated with the instrumental a* parameter. Taking into consideration that negative a* values are associated with green tones, as expected, the lower the a* values corresponding to the sample, the better the consumer liking of the product's appearance and colour. Additionally, sensory "taste" was positively correlated with sensory "sweetness" ($R^2=0.999$), "acidity" ($R^2=0.999$) and "consistency" ($R^2=0.999$). In the same way, "overall acceptance" was positively correlated with sensory "taste" ($R^2=0.999$), "acidity" ($R^2=0.999$) and "consistency" ($R^2=0.999$). Accordingly, the sweeter, the more acid and the thicker the kiwifruit puree, the better the taste liking and the greater the overall acceptance of it.

## 4. CONCLUSIONS

Although pasteurisation significantly affected quality and the consumer acceptance of kiwifruit puree, differences were found depending on the technology employed to preserve the product. Based on all the sensory attributes evaluated, microwaved kiwifruit puree was clearly preferred. Microwave heating did not have an effect on sweetness and acidity and those sensory attributes of kiwifruit puree that most influence consumer acceptance. Moreover, microwave technology inactivated enzymes and microorganisms to the same, or greater, extent than conventional heating, better preserved the nutritive and functional properties of the fruit and produced smaller colour changes. Accordingly, pasteurised kiwifruit puree with

enhanced safety, stability and nutritional and functional value and consumer acceptance may be obtained by means of microwave heating.

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# CAPÍTULO IV.7. LAS MICROONDAS PERMITEN UNA PRESERVACIÓN SIMILAR O MAYOR DE LOS PIGMENTOS DE UN PURÉ DE KIWI TRAS EL PROCESADO Y DURANTE EL ALMACENAMIENTO QUE EL CALENTAMIENTO CONVENCIONAL

# CHLOROPHYLLS AND CAROTENOIDS OF KIWIFRUIT PUREE ARE AFFECTED SIMILARLY O LESS BY MICROWAVE THAN BY CONVENTIONAL HEAT PROCESSING AND STORAGE

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## ABSTRACT

The impact of microwave (1000 W-340 s) and conventional heat (97 °C-30 s) pasteurisation and storage (4, 10, 22 °C) on total and individual carotenoid and chlorophyll contents in kiwifruit puree was evaluated. Bioaccessibility of carotenoids, before and after pasteurisation and storage, was also studied. Microwaves and conventional heating led to marked changes in the chlorophyll (42–100% losses) and carotenoid (62–91% losses) contents. First- and second-order kinetics appropriately explained the degradation over time of total carotenoids and chlorophylls, respectively. Pasteurised samples showed significantly ($p < 0.05$) enhanced stability of these pigments, with microwaves ($k = 0.007–0.031$ 100 $g \cdot mg^{-1} \cdot day^{-1}$ at 4–22 °C) promoting chlorophyll stability to a greater extent than conventional heating ($k = 0.0015–0.034$ $100g \cdot mg^{-1} \cdot day^{-1}$ at 4–22 °C). Bioaccessibility of carotenoids remained significantly ($p < 0.05$) unaffected by processing and storage. These results highlight the fact that the pigment composition of microwaved kiwifruit was more similar to that of the fresh fruit and better preserved during storage.

**KEYWORDS** Microwave heating, conventional heating, pheophytin, lutein, bioaccessibility, degradation kinetics.

## 1. INTRODUCTION

Fruits have been natural components of the human diet throughout history. Although their consumption seems to have been promoted more in recent times because of their well-known nutritional value and additional associated health benefits such as chronic disease prevention (Antunes, Dandlen, Cavaco & Miguel, 2011), they have traditionally been perceived as appetising food products, given their wide variety of inviting colours and flavours, mostly conveyed by their pigment composition (Khoo, Prasad, Kong, Jiang & Ismail, 2011).

In the particular case of kiwifruit (*Actinidia deliciosa*), a comparatively low-calorie (57 kcal/100 g), nutritious fruit rich in vitamin C, potassium, folate and fibre (Drummond, 2013), chlorophylls and carotenoids are the main pigments that contribute to the characteristic bright green colour of its flesh (Nishiyama, Fukuda & Oota, 2005). The potential beneficial health properties of carotenoids, in particular, such as anti-inflammatory and anti-oxidant effects (Kaulmann & Bohn, 2014; Khoo et al., 2011), have been widely recognised and have long been considered an interesting study target. Although most investigations have traditionally focused on evaluating food carotenoid content, it should be kept in mind that the positive effect of these secondary plant compounds or any other functional compounds depends not only on their content but also on the extent to which they are bioaccessible and available for absorption after ingestion and digestion (Biehler, Hoffmann, Krause & Bohn, 2011).

On the other hand, although kiwifruit has been reported to possess great potential for industrial exploitation (Barboni, Cannac & Chiaramonti, 2010), few processed kiwifruit products are available on the international market nowadays. During processing and storage, dramatic changes are often observed in the pigment pattern of this fruit, resulting in degradation of chlorophylls into pheophytins, pyropheophytins, chlorophyllides and pheophorbides (Cano & Marín, 1992), and *cis-trans* isomerisation of carotenoids and formation of epoxides, furanoids and other degradation products of these compounds (Khoo et al., 2011). Consequently, the typical bright green colour turns to a yellowish-brown tone (Cano & Marín, 1992), and a product with an appearance very different from that of the raw kiwifruit is

obtained (Cano, 1991). Given that colour is a highly important attribute in fruit quality assessment and has a considerable influence on consumer acceptance, these undesirable changes in pigment patterns of processed kiwifruit products may represent an important limitation for their marketing.

Consequently, development and applicability studies on different processing technologies that can guarantee safety and stability while offering superior quality foods may be the key to minimising the aforementioned potential problems, and to addressing consumer expectations regarding the increased demand for ready-to-eat foods with fresh-like characteristics (Picouet, Landl, Abadias, Castellari & Viñas, 2009). In this respect, microwave heating is considered an interesting alternative to conventional heating methods to extend fruit shelf-life. Given the particular way in which heating takes place during microwave processing, when compared to conventional thermal treatments microwaves lead to a faster heating rate, approaching the benefits of high-temperature, short-time processing, reducing thermal degradation of the sensory, nutritional and functional properties of the product (De Ancos, Cano, Hernández & Monreal, 1999).

In order to investigate pigment behaviour following pasteurisation and storage of a ready-to-eat kiwifruit puree, the objectives of the present research were (i) to evaluate the effect of applying a microwave heating process on carotenoid and chlorophyll pigment of kiwifruit puree compared with a conventional heat treatment, (ii) to study the stability of these pigments during subsequent storage of the product, and (iii) to assess the impact of both heat processing and storage on the bioaccessibility of carotenoids.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and Standards

Unless otherwise stated, all chemicals employed were of analytical or superior quality. Carotenoid standards (lutein, β-carotene, 96% purity) were purchased from CaroteNature (Lupsingen, Switzerland). All other chemicals were from Sigma-Aldrich (St. Louis, MO, USA).

### 2.2. Kiwifruit preparation and processing

Eight kg of kiwifruit (*Actinidia deliciosa* var. Hayward) was purchased from a local supermarket in Spain (Mercadona S. A., Valencia, Spain) in June 2013. Fruit pieces selected on the basis of a similar soluble solids content (13–15 °Brix) were peeled with a knife, washed with distilled water (50 mL per fruit), cut into slices ca. 10 mm thick and homogenised with a Thermomix (TM 21, Vorwerk, Spain) using the fourth power level for one minute.

The kiwifruit puree obtained was aliquoted, kept below 4 °C in darkness, and then rapidly (5 min) pasteurised by means of microwave technology and conventional heating as described below. Processing conditions were chosen on the basis of preliminary experiments to simulate equivalent pasteurisation treatments in terms of the degree of enzyme and microbial inactivation they achieved (Benlloch-Tinoco, Igual, Rodrigo & Martínez-Navarrete, 2015).

#### *2.2.1. Microwave treatment*

A microwave oven (3038GC, NORM, China) provided with a glass turntable plate was used to treat the kiwifruit puree. A sample weighing 500 g was tempered to an initial temperature of 25 °C in a thermostatic water bath (Precisterm, Selecta, Spain) set at 30 °C for 3 min and then heated in the microwave oven in a standard-size glass beaker (9 cm inner diameter and 12 cm height) (BKL3-1K0-006O, Labbox, Barcelona, Spain) at 1000 W for 340 s. The temperature of the sample in the coldest and hottest spots, previously identified (data not shown), was continuously recorded by means of a fibre-optic probe (CR/JP/11/11671, Optcom, Dresden, Germany) which was connected to a temperature datalogger (FOTEMP1-OEM, Optcom). The treated samples, termed MW, showed a final temperature of 72 °C and 94 °C in the coldest and the hottest spot, respectively. They were immediately cooled in ice-water for 3 min until the puree reached 35 °C, before they were further aliquoted.

*2.2.2. Conventional thermal treatment*

The conventional thermal treatment consisted of heating the sample to 97 °C for 30 s in a circulating thermostatic water bath (Precisterm, Selecta). After the kiwifruit had been mashed, 20 g of puree was placed in TDT stainless steel tubes (1.3 cm inner diameter and 15 cm length) and closed with a screw stopper. A thermocouple, connected to a datalogger, was inserted through the sealed screw top in order to record the time–temperature history of the sample during the treatment. Prior to this heating step, the samples were preheated to 25 °C in a thermostatic water bath (Precisterm, Selecta) (30 °C for 30 s) to shorten and standardize the come-up time (150 s). The treated samples, termed C, were immediately cooled in ice-water for 45 s until the puree reached 35 °C, before further aliquoting.

**2.3. Storage study**

The heat-treated (MW, C) and the non-treated (F) kiwifruit purees were packaged into clean, sterile plastic tubes (1.7 cm inner diameter and 11.8 cm length) (ref. 525-0153, VWR, Spain) and then stored in darkness in heat-adjustable incubators at 4, 10 and 22 °C for 7, 14, 21, 35 and 63 days. The purpose of the storage at 10 and 22 °C was to observe the changes that may take place in the samples in the case of a partial, or total, rupture of the cold chain, respectively, during the shelf-life of the product. Following the storage trials, all samples were stored at –80 °C until analysis.

**2.4. Analytical procedure**

The MW and C samples as well as the F samples, which were used as a control, were analysed in triplicate as described below, at day 0 and at regular time intervals for each storage temperature tested. Bioaccessibility of carotenoids in the F, MW and C purees was evaluated in triplicate at day 0 and after 63 days of storage at 10 °C as described below. Additionally, a physico-chemical characterisation of F, MW and C purees at day 0 was carried out as described below. Analyses were run in triplicate.

*2.4.1. Physicochemical properties*

Water content ($x_w$) was measured by drying the sample to constant weight at 60 °C in a vacuum oven (Vaciotem, J.P. Selecta, Barcelona, Spain) following the AOAC 934.06 method (2000). Soluble solids were determined by measuring °Brix in a previously homogenised sample with a portable digital refractometer (Refracto 3PX, Mettler Toledo, Buchs, Switzerland) at 20 °C and pH using a digital pH-meter (Basic 2, Crison, Barcelona, Spain).

*2.4.2. Extraction of pigments*

*2.4.2.1.* Chemical extraction

Chlorophylls and carotenoids were extracted from the kiwifruit puree as described by Biehler, Mayer, Hoffmann, Krause and Bohn (2010), with some modifications. In brief, 4 g of frozen kiwifruit was weighed into a 15-mL centrifuge tube (BD Biosciences, San Jose, CA, USA) and 6 mL of methanol was added together with 0.25 g of sodium carbonate to prevent rapid conversion of chlorophylls to the corresponding pheophytins. After mixing, sonication and incubation for 5 min on ice, samples were centrifuged (Harrier 18/80 refrigerated centrifuge, MSE, London, UK) for 5 min at 2,500 × g at 4 °C. The supernatant was decanted into a 50-mL centrifuge tube, extraction was performed twice with 9 mL of a mixture of hexane and acetone (1:1, v/v) and the organic fractions were combined. Ten mL of saturated aqueous sodium chloride solution was added to the combined extracts and the mixture was shaken. The supernatant hexane phase was transferred to a 50-mL centrifuge tube, and the lower aqueous phase was re-extracted with 15 mL of hexane and combined with the first extract. The hexane extracts were weighed exactly for volume determination. A 10-mL aliquot was then pipetted from the combined extracts into a 15-mL centrifuge tube, evaporated to dryness under a stream of nitrogen in a TurboVapLVR apparatus (Caliper Life Sciences Benelux, Teralfene, Belgium) and stored at –80 °C until analysis.

2.4.2.2. Simulated *in vitro* gastrointestinal (GI) digestion

To mimic *in vivo* GI digestion conditions and to determine the amount of carotenoids potentially available for further uptake, the methodology proposed by

Bouayed, Hoffmann and Bohn (2011) was followed, with some modifications. The release of total carotenoids from the kiwifruit samples after digestion, i.e. the gastric and small intestinal phases of digestion, was evaluated by analysing aliquots from the GI digesta by UPLC as described below. The percentage of relative bioaccessibility of carotenoids was estimated by calculating the ratio between the mean levels of each carotenoid in the kiwifruit puree samples and after the *in vitro* digestion process.

2.4.2.2.1. Gastric phase and small intestinal phases

Two g of kiwifruit puree sample, 1 g of cream (10% fat) and 12 mL NaCl (0.15 M) were mixed in a 50-mL plastic centrifuge tube prior to acidification with 0.5 mL HCl (1 M), to achieve a final pH of 3, and the addition of 1 mL of porcine pepsin solution (40 mg/mL in 0.1 M HCl). The mixture was incubated for 1 h in a shaking water bath (GFL 1083 from VEL, Leuven, Belgium) at 37 °C and 100 rpm. After this, the pH was raised to 5–5.5 by adding 0.7 mL of sodium bicarbonate (0.9 M) in order to simulate the transition from the gastric phase to the intestinal phase.

Then 4.5 mL of a mixture of pancreatin and porcine bile extract (4 mg/mL pancreatin and 24 mg/mL bile extract dissolved in 0.1 M sodium bicarbonate) was added to the digesta. The pH was increased to 7–7.5 by adding 0.9 mL of sodium bicarbonate (0.1M) and the final volume was adjusted to 25 mL with NaCl (0.15 M). After this, the samples were incubated in the shaking water bath (100 rpm) at 37 °C for 2 h to complete the intestinal phase of the *in vitro* digestion process.

2.4.2.2.2. Obtaining bioaccessible fractions

Aliquots from the GI digestion (ca. 12 mL) were centrifuged (164,000 × g, 4 °C, 35 min), the supernatant (4 mL) was filtered through 0.2-µm PVDF syringe filters and extraction of pigments was performed twice with 4 mL of a mixture of hexane and acetone (1:1, v/v). The combined hexane phases were transferred to a 15-mL centrifuge tube, evaporated to dryness under a stream of nitrogen in a TurboVapLVR apparatus (Caliper Life Sciences Benelux, Teralfene, Belgium) and stored at –80 °C until analysis.

*2.4.3. Pigment identification using UPLC*

Separation, identification and quantification of carotenoids and chlorophylls was achieved on a Waters UPLC instrument (Milford, MA) including a P580 pump, a Gina 50 autosampler and a UVD340S photodiode array detector (Dionex Benelux B.V., Amsterdam, The Netherlands), simultaneously set at 409 (detection of pheophytin a), 431 (detection of chlorophyll a), 436 (detection of pheophytin b), 440 (detection of neoxanthin and violaxanthin), 450 (detection of β-carotene and lutein) and 459 (detection of chlorophyll b) nm. Separation of carotenoids was performed following the procedure described by Kaulmann, Jonville, Schneider, Hoffmann and Bohn (2014) using an RP-18 column (2.1 x 100 mm, 1.7 µm particle size) at 40 °C (Waters Inc., Zellik, Belgium). Injection volume was 4 µL. For quantification, external calibration curves based on 7 points were obtained for each compound, with concentrations ranging from 0.01 to 25 µg/mL.

**2.5. Kinetic modelling of pigment degradation**

To obtain the kinetic parameters explaining loss of pigment content in the treated and untreated kiwifruit puree during storage, the amount of total carotenoids and total chlorophylls detected in the samples was plotted vs. time at all temperatures studied. Zero-, first- and second-order kinetics were hypothesized by applying the corresponding reaction rate expression. Then the order that best fitted the experimental data (data not shown) was selected. Following this criterion, first-order (equation 1) and second-order (equation 2) kinetics were used to describe degradation of total carotenoids and total chlorophylls, respectively, over time. The time for the concentration of a compound to fall to half its initial value (half-life, $t_{1/2}$) was also determined (equations 3 and 4, corresponding to first- and second-order kinetic models, respectively).

$$\ln\frac{C}{C_0} = -k \cdot t \tag{1}$$

$$\frac{1}{C} - \frac{1}{C_0} = k \cdot t \tag{2}$$

$$t_{\frac{1}{2}} = \frac{\ln 2}{k} \quad (3)$$

$$t_{\frac{1}{2}} = \frac{1}{k \cdot C_0} \quad (4)$$

where C represents the concentration of the compound at t (mg·100 $g^{-1}$); $C_0$ the concentration of each compound at time zero (mg·100 $g^{-1}$); k the first-order ($days^{-1}$) or second-order rate constant (100 g·$mg^{-1}$·$day^{-1}$); t the storage time; $t_{1/2}$ the half-life of the compound (days).

On the other hand, the temperature dependence of the degradation of these attributes was studied by employing the Arrhenius equation (equation 5). In every case, the goodness of the fit between the experimental and predicted data was assessed by means of the adjusted regression coefficient ($R^2$-ad.) (equation 6), considering that the higher the $R^2$-ad. value, the better the fit.

$$k = k_0 \cdot e^{\frac{-E_a}{R \cdot T}} \quad (5)$$

$$Adjusted - R^2 = \left[ \frac{(m-1)(1 - \frac{SSQ_{REGRESSION}}{SSQ_{TOTAL}})}{(m-j)} \right] \quad (6)$$

where k represents the rate constant; $k_0$ the pre-exponential factor; $E_a$ the activation energy (kcal·$mol^{-1}$); R the gas constant (1.987 kcal·$mol^{-1}$·$K^{-1}$); T the absolute temperature (K); m the number of observations; j the number of model parameters; SSQ the sum of squares.

### 2.6. Statistical analyses

Assumptions of normality and equality of variance were tested by normality plots and box plots, respectively. Linear mixed models correlating carotenoid and chlorophyll content (dependent variables) with the type of sample, storage temperature and storage time (fixed factors) were developed using the SPSS Statistics 19 software program (IBM SPSS, Inc., New York, NY, USA). A p-value of

0.05 (2-sided) was assumed to reflect significant statistical differences. After significant Fisher-F tests, post-hoc tests (Bonferroni's) were conducted. Additionally, non-linear and linear regression analyses were carried out in order to estimate the kinetic parameters using the SPSS Statistics 19 software program (IBM SPSS), based on the Levenberg–Marquardt estimation method.

## 3. RESULTS AND DISCUSSION

### 3.1. Pigment composition of kiwifruit - processing effects

One of the main goals of the present research was to obtain an understanding of how the pigment composition of kiwifruit is affected by different thermal processing conditions. For this purpose, the pigment pattern of the fruit was evaluated before and after microwave and conventional heat pasteurisation (Table 1, Figure 1). Neither of the two treatments significantly affected the physico-chemical properties of the product. The mean values (± standard deviation) obtained were 84.8 ± 0.4 g water·100 g product$^{-1}$, 14.1 ± 0.3 g soluble solids·100 g liquid phase in the product$^{-1}$ and pH = 3.36 ± 0.08. In fresh kiwifruit, the mean value (± standard deviation) of total carotenoid and total chlorophyll contents was shown to be 0.53 ± 0.06 mg·100 g$^{-1}$ and 2.58 ± 0.08 mg·100 g$^{-1}$, respectively. Among the 5 different carotenoid compounds identified in this fruit, lutein, which was accompanied by two minor *cis*-isomers (neolutein A and B), was the most abundant component (68%), followed by β-carotene, neoxanthin and violaxanthin. The content of chlorophyll a and b in kiwifruit was 1.609 ± 0.003 mg·100 g$^{-1}$ and 0.49 ± 0.05 mg·100 g$^{-1}$, respectively. The most common derivatives of chlorophylls, pheophytin a and b, were also detected in the fresh fruit (Figure 1). As previously stated by Cano (1991), the presence of pheophytins in untreated kiwifruit tissues may be due to rapid conversion of chlorophylls to these derivative compounds under low pH conditions. These results are in good agreement with those published by other authors for the same fruit (Cano, 1991; Cano & Martín, 1992, De Ancos et al., 1999; McGhie & Ainge, 2002; Montefiori, McGhie, Hallet & Costa, 2009).

**Table 1.** Quantitative distribution of carotenoids and chlorophyll degradation products (mean value ± standard deviations, μg·100 $g^{-1}$) detected in fresh (F), microwaved (MW) and conventionally heated (C) kiwifruit puree during storage at 4, 10 and 22 °C in the dark.

| | T (ºC) | Day | Lutein | Neolutein A+B | β-carotene | Neoxanthin | Violaxanthin | Total Carotenoids | Pheophytin a | Pheophytin b |
|---|---|---|---|---|---|---|---|---|---|---|
| F | | 0 | 362±42$^{a}$ | 50±7$^{a}$ | 79±19$^{a}$ | 33±16$^{a}$ | 6.3±1.2$^{a}$ | 531±64$^{a}$ | 408±82$^{a}$ | 75±21$^{a}$ |
| | 22 | 4 | 241±4$^{b}$ | 42±28$^{a}$ | 28±9$^{b}$ | 13±5$^{a}$ | – | 311±16$^{b}$ | 1404±168$^{b}$ | 472± 132$^{b}$ |
| | | 7 | 152±17$^{bc}$ | 16±3$^{b}$ | 27±10$^{b}$ | – | – | 196±20$^{c}$ | 836±31$^{c}$ | 170±53$^{c}$ |
| | | 14 | 106±28$^{c}$ | 12±5$^{b}$ | 21±5$^{b}$ | – | – | 138±19$^{d}$ | 337±97$^{a}$ | 217±69$^{c}$ |
| | 10 | 7 | 132±4$^{bc}$ | 22.0±1.0$^{b}$ | 40±3$^{b}$ | 3.4±0.8$^{b}$ | 2.4±0.3$^{b}$ | 194±7$^{b}$ | 1478±66$^{b}$ | 352±28$^{b}$ |
| | | 14 | 157±23$^{bc}$ | 24.8±1.4$^{bc}$ | 36±3$^{b}$ | 2.6±0.5$^{b}$ | 1.6±0.2$^{b}$ | 218±29$^{b}$ | 881±87$^{c}$ | 501±12$^{c}$ |
| | | 21 | 161±14$^{b}$ | 27±4$^{b}$ | 32±7$^{b}$ | – | – | 220±20 | 696±29$^{d}$ | 167±65$^{d}$ |
| | | 35 | 92±26$^{c}$ | 15±5$^{cd}$ | 12±5$^{bc}$ | – | – | 119±37$^{c}$ | 280±21$^{e}$ | 79±20$^{a}$ |
| | | 63 | 11±2$^{d}$ | 9.2±1.0$^{d}$ | 7.1±1.3$^{c}$ | – | – | 27.6±0.2$^{d}$ | 290±13$^{e}$ | 49±5$^{e}$ |
| | 4 | 7 | 209±21$^{b}$ | 23±2$^{b}$ | 36±2$^{b}$ | 1.4±0.2$^{b}$ | 1.6±0.3$^{b}$ | 268±23$^{b}$ | 1418±63$^{b}$ | 389±11$^{b}$ |
| | | 14 | 157±22$^{bc}$ | 24±4$^{b}$ | 36±2$^{b}$ | 1.2±0.2$^{b}$ | 1.4±0.2$^{b}$ | 220±29$^{bc}$ | 1094±38$^{b}$ | 506±9$^{c}$ |
| | | 21 | 161±13$^{bc}$ | 27±3$^{b}$ | 33±7$^{b}$ | – | – | 222±20$^{c}$ | 715±43$^{c}$ | 294±34$^{d}$ |
| | | 35 | 132±4$^{c}$ | 15±4$^{c}$ | 17±4$^{c}$ | – | – | 164±15$^{d}$ | 392±52$^{a}$ | 149±67$^{ae}$ |
| | | 63 | 91±26$^{c}$ | 12±2$^{c}$ | – | – | – | 103±27$^{e}$ | 168±12$^{d}$ | 53±4$^{a}$ |
| MW | | 0 | 149±34$^{a}$ | 32±7$^{a}$ | 12±3$^{a}$ | 3.6±0.2$^{a}$ | 2.0±0.1$^{a}$ | 198±54$^{a}$ | 1320±304$^{ab}$ | 200±25$^{ab}$ |
| | 22 | 4 | 129±12$^{a}$ | 32±11$^{a}$ | 16±3$^{a}$ | – | 3.1±0.1$^{a}$ | 180±15$^{a}$ | 1669±150$^{a}$ | 371±18$^{c}$ |
| | | 7 | 116±15$^{a}$ | 26±8$^{a}$ | 10±2$^{a}$ | – | 2.2±0.3$^{a}$ | 154±21$^{a}$ | 1306±90$^{b}$ | 307±2$^{d}$ |
| | | 14 | 115±37$^{a}$ | 25.0±4.0$^{a}$ | 6.4±1.0$^{b}$ | – | 1.7±0.2$^{a}$ | 148±47$^{a}$ | 930±90$^{c}$ | 105±7$^{e}$ |
| | 10 | 7 | 139±9$^{a}$ | 29±2$^{a}$ | 15±4$^{a}$ | 2.2±0.4$^{b}$ | 2.3±0.5$^{a}$ | 188±14$^{a}$ | 1547±103$^{a}$ | 521±19$^{c}$ |
| | | 14 | 116±15$^{a}$ | 30±4$^{a}$ | 5.4±1.2$^{b}$ | 2.0±0.2$^{b}$ | 2.0±0.2$^{a}$ | 156±19$^{a}$ | 885±85$^{c}$ | 301±74$^{b}$ |
| | | 21 | 138±34$^{a}$ | 32±7$^{a}$ | 5.3±1.0$^{b}$ | – | 2.4±0.3$^{a}$ | 178±39$^{a}$ | 1064±197$^{ab}$ | 205±33$^{a}$ |
| | | 35 | 105±6$^{a}$ | 31±6$^{a}$ | 4.0±1.2$^{b}$ | – | 1.6±0.4$^{a}$ | 142±22$^{a}$ | 701±212$^{cb}$ | 117±11$^{d}$ |
| | | 63 | 168±20$^{a}$ | 34±4$^{a}$ | – | – | 2.1±0.2$^{a}$ | 205±26$^{a}$ | 1283±263$^{b}$ | 102±10$^{d}$ |
| | 4 | 7 | 139±8$^{a}$ | 29.0±1.1$^{a}$ | 14±4$^{a}$ | 1.3±0.5$^{b}$ | 1.7±0.7$^{a}$ | 185±15$^{a}$ | 1504±146$^{a}$ | 535±109$^{b}$ |
| | | 14 | 139±34$^{a}$ | 29.3±3$^{a}$ | 16.0±0.4$^{a}$ | 2.4±0.1$^{c}$ | 1.3±0.2$^{a}$ | 188±36$^{a}$ | 1349±269$^{a}$ | 435±25$^{bc}$ |
| | | 21 | 116±14$^{a}$ | 32±7.2$^{a}$ | 4.8±1.1$^{b}$ | – | 1.2±0.3$^{a}$ | 154±15$^{a}$ | 1208±149$^{a}$ | 331±24$^{c}$ |
| | | 35 | 105±5$^{a}$ | 31±5$^{a}$ | 3.1±0.6$^{c}$ | – | 1.4±0.5$^{a}$ | 141±10$^{a}$ | 1085±53$^{a}$ | 233±28$^{a}$ |
| | | 63 | 130±24$^{a}$ | 29±4$^{a}$ | – | – | – | 159±19$^{a}$ | 932±165$^{a}$ | 274±37$^{a}$ |
| C | | 0 | 110±48$^{a}$ | 21±6$^{a}$ | 9.1±1.0$^{a}$ | 2.4±0.8$^{a}$ | 2.1±0.2$^{a}$ | 145±71$^{a}$ | 1533±72$^{a}$ | 473±27$^{a}$ |
| | 22 | 4 | 96±6$^{a}$ | 17±2$^{ab}$ | 4.1±0.9$^{b}$ | – | 2.1±0.2$^{a}$ | 119±21$^{a}$ | 1377±24$^{b}$ | 442±52$^{a}$ |
| | | 7 | 85±6$^{a}$ | 15±3$^{ab}$ | 2.0±0.2$^{c}$ | – | 1.6±0.5$^{a}$ | 103±18$^{a}$ | 1058±49$^{c}$ | 355±16$^{b}$ |
| | | 14 | 78±14$^{a}$ | 8.9±0.6$^{b}$ | – | – | 1.1±0.2$^{a}$ | 88±17$^{a}$ | 753±48$^{d}$ | 297±23$^{c}$ |
| | 10 | 7 | 139±5$^{a}$ | 24.3±0.7$^{ab}$ | 15.0±1.1$^{b}$ | 2.3±0.2$^{b}$ | 1.9±0.4$^{a}$ | 182±14$^{a}$ | 543±4$^{b}$ | 366±8$^{b}$ |
| | | 14 | 109±9$^{a}$ | 23.8±1.4$^{ab}$ | 11±2$^{a}$ | 2.0±0.1$^{b}$ | 2.1±0.2$^{a}$ | 148±25$^{a}$ | 657±70$^{c}$ | 292±10$^{c}$ |
| | | 21 | 133.6±1.2$^{a}$ | 36±9$^{b}$ | 26±4$^{c}$ | – | 2.5±0.2$^{a}$ | 198±18$^{a}$ | 433±119$^{b}$ | 267±93$^{c}$ |
| | | 35 | 141±26$^{a}$ | 25±5$^{ab}$ | 2.8±0.7$^{d}$ | – | 2.0±0.2$^{a}$ | 170±29$^{a}$ | 333±86$^{bd}$ | 226±53$^{c}$ |
| | | 63 | 135±12$^{a}$ | 8.9±0.5$^{a}$ | – | – | 1.7±0.1$^{a}$ | 145±19$^{a}$ | 198±11$^{d}$ | 139±2$^{d}$ |
| | 4 | 7 | 139±5$^{a}$ | 24.3±0.7$^{a}$ | 15.0±1.1$^{a}$ | 2.3±0.2$^{b}$ | 1.4±0.3$^{a}$ | 182±12$^{a}$ | 1295±32$^{b}$ | 419±61$^{ab}$ |
| | | 14 | 141±26$^{a}$ | 23.8±1.3$^{a}$ | 9.6±0.5$^{a}$ | 2.0±0.1$^{b}$ | 2.1±0.2$^{a}$ | 178±31$^{a}$ | 1219±62$^{b}$ | 328±86$^{b}$ |
| | | 21 | 142±34$^{a}$ | 24±4$^{a}$ | 8.2±1.2$^{a}$ | – | 1.7±0.6$^{a}$ | 176±36$^{a}$ | 945±29$^{c}$ | 418±81$^{ab}$ |
| | | 35 | 109±9$^{a}$ | 25±2$^{a}$ | 7.4±1.3$^{a}$ | – | 1.5±0.2$^{a}$ | 143±16$^{a}$ | 851±71$^{c}$ | 417±29$^{b}$ |
| | | 63 | 102±7$^{a}$ | 17.0±1.3$^{a}$ | – | – | – | 119±13$^{a}$ | 593±34$^{d}$ | 418±5$^{b}$ |

(–): not detected. All values on wet weight basis. Three independent replicates were used to calculate each mean value and the corresponding standard deviation. Different letters in columns between the same sample at each temperature indicate significant statistical differences ($p < 0.05$) according to Bonferroni test.

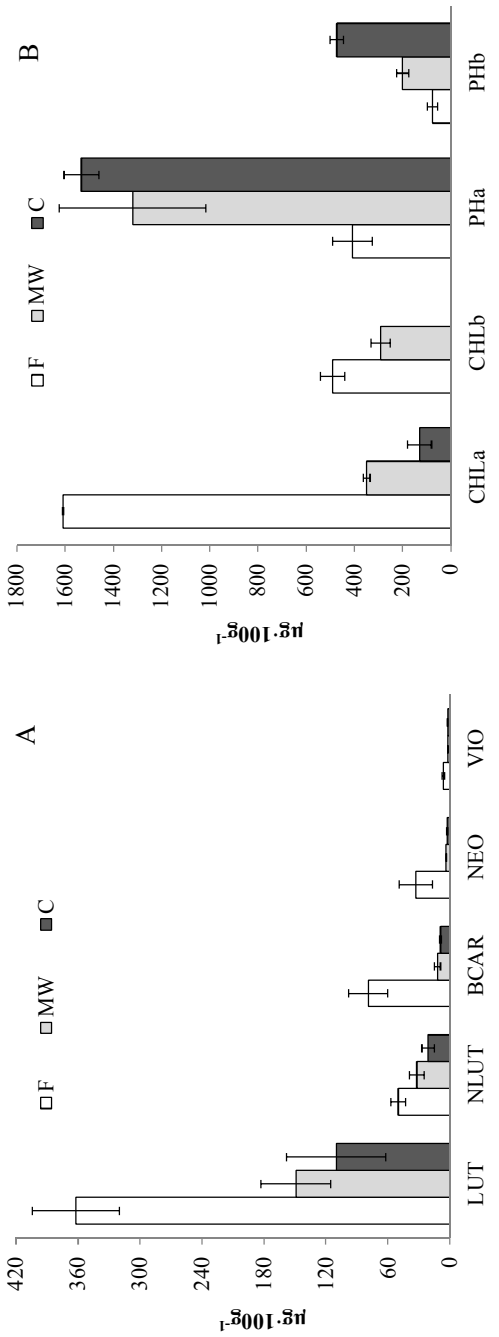


**Figure 1.** Quantitative distribution of carotenoids (a) and chlorophylls (b) in fresh (F), microwaved (MW) and conventionally heated (C) kiwifruit puree. LUT: lutein; NLUT: neolutein A+B; BCAR: β-carotene; NEO: neoxanthin; VIO: violaxanthin; CHLa: chlorophyll a; CHLb: chlorophyll b; PHa: pheophytin a; PHb: pheophytin b

The processing step (MW, C) significantly ($p < 0.05$) affected the quantitative pigment concentration of kiwifruit, both the carotenoid and chlorophyll contents being reduced in the treated puree (Figure 1). Thermal degradation, a process

promoting the formation of oxidation compounds and the decomposition of pigments into more volatile, low molecular weight, colourless components, appears to be the most likely cause for these losses (Heaton & Marangoni, 1996; Rios, Fernández-García, Mínguez-Mosquera & Pérez-Gálvez, 2008).

Carotenoids were affected more or less equally by microwave and conventional processing, with no statistically significant differences between the two processes overall. In pasteurised puree (MW, C) the total carotenoid content was reduced by 67 ± 7% and it was observed that neoxanthin (loss of 91%) and lutein (loss of 62%) were the most and least thermolabile compounds in the kiwifruit, respectively (Figure 1). However, greater resistance of carotenoids to thermal processing has been observed in other fruit products. According to Lee and Coates (2003) and Gama and de Sylos (2007), when Valencia orange juice was heat pasteurised (90 °C–105 °C for 10–30 s), losses of carotenoids ranged from 20% to 46% and 9% to 38%, respectively. Lee and Coates (1999) did not find significant changes in β-carotene and lycopene contents after thermal pasteurisation (91 °C for 10 s) of red grapefruit juice. Lessin, Catigani and Schwartz (1997) stated that carotenoid content of orange juice decreased by up to 50% during heat pasteurisation (80 °C for 2 min), and losses in carotenoid compounds of canned peaches ranged from 25% to 59%. On the other hand, although provitamin A activity has been reported to be slightly changed during pasteurisation (Gama & de Sylos, 2007; Lee & Coates, 2003), in the present study a considerable loss of β-carotene (86%) was detected in the MW and C samples. Overall, the discrepancy with data in the literature might be attributable to the great variability of carotenoid stability in different food matrices (Lee & Coates, 1999).

As expected, chlorophylls were shown to be more thermolabile than carotenoids (Cervantes-Paz et al., 2014). The chlorophyll pattern was noticeably changed after processing owing to chlorophyll degradation to pheophytins, with pheophytin a becoming the predominant compound in the treated samples (Figure 1). The MW puree showed chlorophyll a and b contents of 0.349 ± 0.014 mg·100 $g^{-1}$ and 0.29 ± 0.04 mg·100 $g^{-1}$, respectively. The chlorophyll a content in the C puree was shown to be 0.13 ± 0.05 mg·100 $g^{-1}$, while chlorophyll b was not detected in this sample,

possibly because it was more rapidly degraded in the C samples owing to chlorophyllase or some other enzymatic activity. From these data it can be claimed that microwave technology allowed significantly ($p < 0.05$) greater preservation of chlorophylls than conventional heating, which, in contrast, led to almost complete degradation of these pigments (92–100%). A similar range of chlorophyll degradation was found by Lefsrud, Kopsell, Sams, Wills and Both (2008) in kale and spinach after drying (50–75 °C). It is widely accepted that chlorophyll a is more susceptible to heat loss than chlorophyll b (Chen & Chen, 1993). However, the conventionally pasteurised kiwifruit puree presented losses of similar magnitude for both chlorophyll compounds. Similar results were published by Turkmen, Poyrazoglu, Sari and Sedat Velioglu (2006) for thermally processed peas. As pointed out by Weemaes, Ooms, Van Loey and Hendrickx (1999), the food matrix may have a strong impact on resistance of chlorophylls a and b to heat degradation, with different fruits and vegetables exhibiting dissimilar rates of degradation of these pigments.

### 3.2. Effect of storage time on pigment composition of kiwifruit puree

In order to understand the changes in pigment composition of kiwifruit puree throughout the shelf-life of the product, the stability of carotenoids and chlorophylls during storage of the pasteurised and fresh puree was investigated. Figures 1 and 2, respectively, illustrate the evolution of the total content of these pigments in the MW, C and F samples during storage at 22, 10 and 4 °C. The stability of individual carotenoid and chlorophyll compounds over time was also monitored in all the samples (Table 1).

Linear mixed models were used to evaluate the effect of storage temperature, storage time and type of sample on kiwifruit pigments. The statistical analysis indicated that storage time, processing and their interaction brought about significant ($p < 0.05$) differences in the total and individual carotenoid contents. However, no significant effect of storage temperature was detected. Carotenoids tended to be significantly ($p < 0.05$) reduced over time, their decrease being ameliorated by pasteurisation (Figure 2). Both the microwave and conventional heat treatments

promoted stability of carotenoids during storage compared with the untreated samples (F).

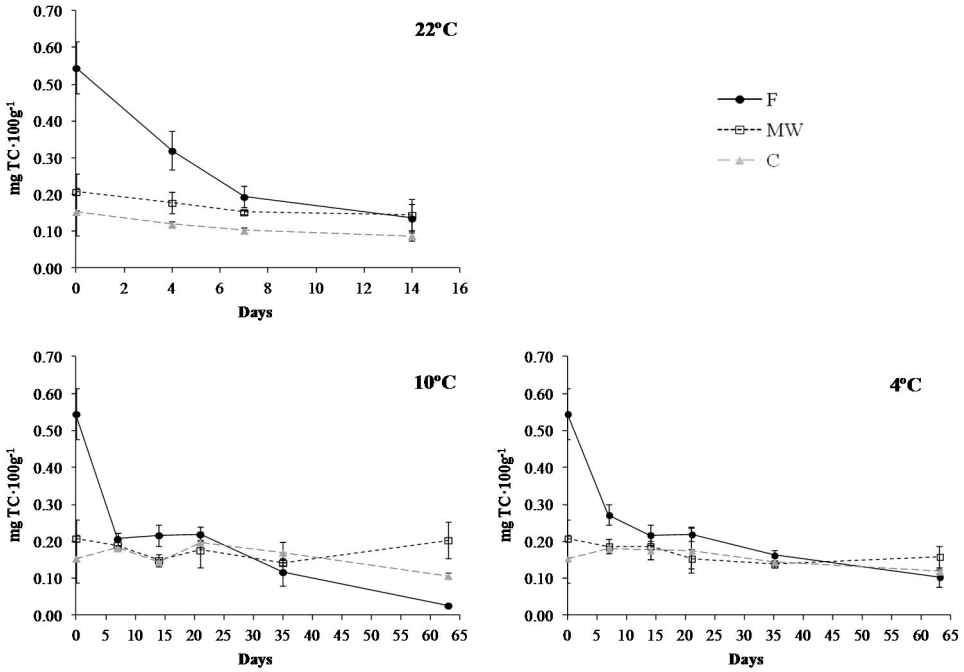


**Figure 2.** Total carotenoid content (TC) in the kiwifruit puree processed by different means (F: fresh, MW: microwaved and C: conventionally thermally treated) following storage under dark conditions at 22, 10 and 4 °C. The plotted values and error bars represent the mean of three replicates and the corresponding standard deviation. Weight is given on a wet weight basis.

However, no positive effect of processing was observed for β-carotene and neoxanthin (Table 1): β-carotene gradually degraded over time and started to disappear completely after 35 days of storage at 4 and 10 °C or 14 days at 22 °C, while neoxanthin started to disappear completely after 14 days of storage at 4 and 10 °C or 4 days at 22 °C. In this respect, despite the fact that pasteurisation had a significant ($p < 0.05$) detrimental effect on carotenoids at onset (section 3.1), no significant differences in the contents of these compounds were observed among the samples (F, MW, C) after 14 days of storage. In order to further investigate the impact of processing on the stability of carotenoids during storage, the degradation kinetics of total carotenoids were studied. As has been reported previously by various authors investigating different food matrices, total carotenoid degradation

was appropriately described by first-order kinetics (Hidalgo & Brandolini, 2008). Since no significant effect of storage temperature was observed, kinetic data were calculated only at 4 °C for each sample. The results obtained seemed to corroborate the positive effect of pasteurisation on the preservation of carotenoids over time, without revealing noticeable differences between microwave and conventional heating technology. The losses of carotenoids in the fresh kiwifruit puree (k = 0.022 ± 0.005 days–1; R2-ad. = 0.834) were almost twice as fast as in the microwaved samples (k = 0.010 ± 0.003 days–1; R2-ad. = 0.935) and the conventionally heated samples (k = 0.008 ± 0.001 days–1; R2-ad. = 0.943). According to Gama and de Sylos (2007), the principal cause of carotenoid losses is oxidative degradation, which depends on the availability of oxygen and is stimulated by heat, light, enzymes, metals, and co-oxidation with lipid hydroperoxides. Given that the treated and untreated samples in the present study were exposed to equal storage conditions in terms of temperature, light, etc., it was considered that the increased stability against enzymatic breakdown – such as via peroxidases – provided by pasteurisation (Baldermann, Naim, & Fleischmann, 2005; Lessin et al., 1997) may well explain the superior stability of carotenoids found in the MW and C samples over time.

On the other hand, all the samples exhibited rapid degradation of chlorophylls (a and b) at all temperatures investigated (22, 10 and 4 °C). These compounds were gradually converted to pheophytins, which significantly ($p < 0.05$) increased in concentration during the first few days of storage before gradually decreasing. Similarly, a transient accumulation, prior to a drastic decrease, of pheophytin and chlorophyllide was observed by other authors in stored coleslaw and spinach, respectively (Heaton & Marangoni, 1996; Yamauchi & Watada, 1991). As suggested by Weemaes et al. (1999), after complete pheophytinisation of chlorophylls pheophytins might continue to be further degraded to pheophorbides, which may eventually be converted to some colourless components by following different pathways (Heaton & Marangoni, 1996). The evolution of chlorophyll derivative compounds (ChDs), pheophytin a and b, was monitored during storage. From the statistical analysis it was seen that the total contents of chlorophylls and their derivative compounds were significantly ($p < 0.05$) affected by storage time,

processing technique, storage temperature and their interactions. On the whole, the ChD contents decreased significantly ($p < 0.05$) over time in all the samples, although here, too, pasteurisation seemed to promote a certain stability of these pigments, their degradation over time being slower in MW and C purees (Figure 3). As expected, the higher the storage temperature, the faster the degradation of these pigments over time.

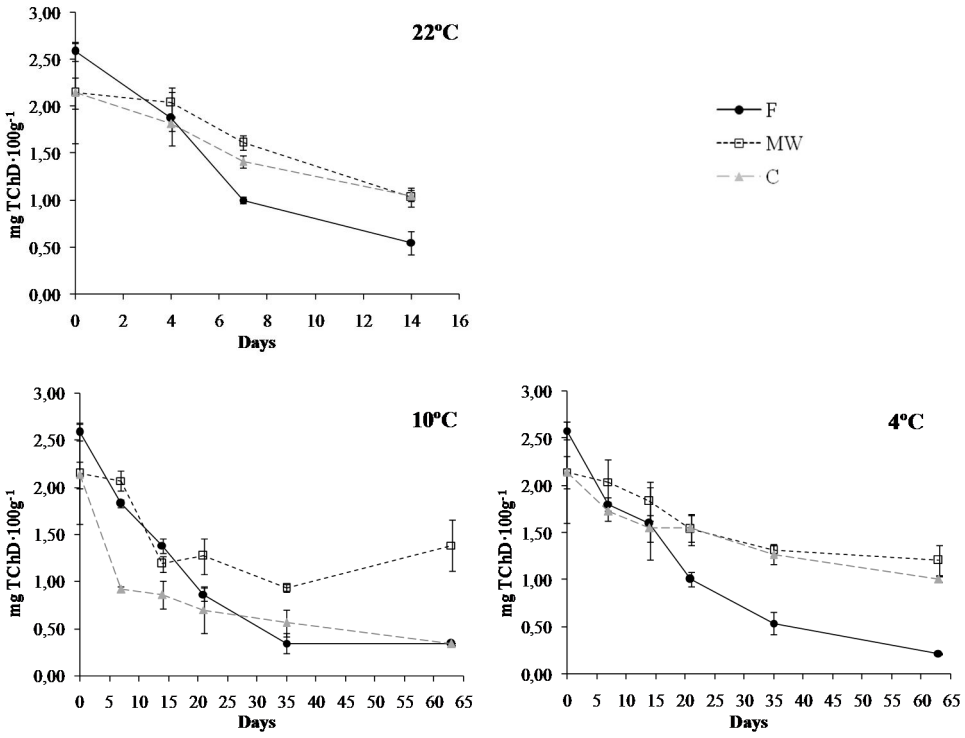


**Figure 3.** Total chlorophylls and derivative compounds (TChD) in the kiwifruit puree processed by different means (F: fresh, MW: microwaved and C: conventionally thermally treated) following storage at 22, 10 and 4 °C in the dark. The plotted values and error bars represent the mean of three replicates and the corresponding standard deviation. All values expressed on a wet weight basis. TChD: chlorophyll a, chlorophyll b, pheophytin a and pheophytin b.

In order to further study the impact of processing and storage temperature on the stability of ChDs in the kiwifruit puree, their degradation kinetics were analysed by means of a second-order model. The values of the kinetic rate constant (k) and half-life ($t_{1/2}$) for the F, MW and C samples stored at 22, 10 and 4 °C are presented in Table 2. Additionally, to determine the effect of temperature on the parameters

studied, the rate constants that were obtained were fitted to the Arrhenius equation. The activation energies ($E_a$) obtained are also shown in Table 2.

**Table 2.** Half-life times ($t_{1/2}$: days), mean values (and standard error of 3 independent replicates) of the degradation rates (k: 100 g·mg$^{-1}$·day$^{-1}$) and activation energy ($E_a$: kJ·mol$^{-1}$) of total chlorophylls in fresh (F), microwaved (MW) and conventionally heated (C) kiwifruit puree during storage at 22, 10 and 4 °C. Adjusted regression coefficient ($R^2$-ad.).

| Sample | T (ºC) | $t_{1/2}$ | k | $R^2$-ad. | $E_a$ | $R^2$-ad. |
|---|---|---|---|---|---|---|
| F | 4 | 7 | 0.056 (0.007) | 0.896 | 21.184 (7.015) | 0.901 |
| | 10 | 7 | 0.057 (0.010) | 0.829 | | |
| | 22 | 4 | 0.095 (0.009) | 0.946 | | |
| MW | 4 | 55 | 0.007 (0.002) | 0.883 | 44.718 (11.135) | 0.942 |
| | 10 | 24 | 0.016 (0.002) | 0.885 | | |
| | 22 | 12 | 0.031 (0.005) | 0.886 | | |
| C | 4 | 26 | 0.015 (0.002) | 0.893 | 38.826 (13.068) | 0.867 |
| | 10 | 14 | 0.027 (0.005) | 0.977 | | |
| | 22 | 11 | 0.034 (0.002) | 0.979 | | |

In order to describe the effect of both treatment and storage temperature on the rate of decrease of ChDs, it was considered that the lower the $t_{1/2}$ and the higher the k values, the faster the degradation of these compounds. Moreover, a higher activation energy value means a greater dependence of the kinetic rate constant on the storage temperature. From the results obtained, pasteurisation clearly contributed to stabilisation of the total ChD contents in the product over time, with the F sample showing considerably higher degradation rates and lower half-life times than the MW and C samples at any of the temperatures studied (Table 2). Microwave technology helped to prevent ChD losses during storage to a greater extent than conventional heating, with differences being particularly noticeable at 4 and 10 °C. However, as deduced from the $E_a$ values, pasteurisation treatment led to greater thermal sensitivity of these pigments, especially when microwaves were used to pasteurise the kiwifruit puree. Degradation of chlorophyll compounds is primarily attributed to enzyme activity (magnesium dechelatase, chlorophyllase, chlorophyll oxidase, peroxidase, etc.) (Heaton & Marangoni, 1996; Yamauchi & Watada, 1991). Accordingly, the higher stability of chlorophylls and derivative compounds exhibited by the treated kiwifruit puree might be associated with greater

enzymatic stability brought about by processing. In this respect, despite the fact that chlorophylls a and b were completely lost during processing and storage, pasteurising the kiwifruit puree might still help to prevent further degradation of pheophytins to colourless compounds and a consequent colour change from olive green to a lighter whitish colour, especially if the product is processed by microwave heating.

Although equal heat degradation and stability of carotenoids were observed in the MW and C samples, pasteurising the kiwifruit puree by applying microwaves may be assumed to be beneficial in order to obtain processed kiwifruit with a colour more similar to that of the fresh fruit and better maintained over time, given the greater preservation of chlorophylls brought about by this technology. The treatments compared in the present study were selected on the basis of the results of previous research, in which it was observed that the possibility of some stability-enhancing effects associated with microwaves might explain their ability to provide equal or superior enzymatic and microbial stability of kiwifruit and to preserve its nutritive and functional value (Benlloch-Tinoco et al., 2015). Taking all these aspects into account, the superiority of microwave technology over conventional heating to preserve the pigment composition of kiwifruit puree during its shelf-life may be assumed.

### 3.3. Bioaccesibility of carotenoids in kiwifruit puree

Bioactive compounds need to be released from the food matrix and solubilised in order to be available for absorption. Consequently, evaluating to what extent they become accessible in the GI tract after ingestion (bioaccessibility) represents a key feature in the assessment of the role of different food matrices as dietary sources of these compounds. In the present investigation, the bioaccessibility of carotenoids detected in kiwifruit was evaluated before and after pasteurisation and storage. Results are shown in Figure 4.

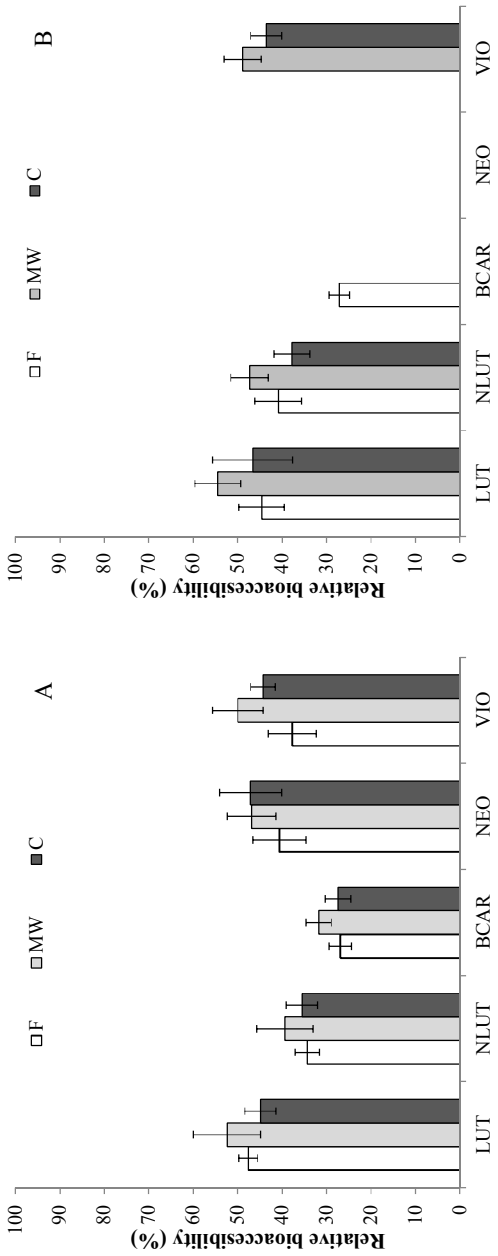


**Figure 4**. Relative bioaccessibility (%) of lutein (LUT), neolutein A+B (NLUT), β-carotene (BCAR), neoxanthin (NEO) and violaxanthin (VIO) in the fresh (F), microwaved (MW) and conventionally heated (C) kiwifruit puree (a) and the fresh (F), microwaved (MW) and conventionally heated (C) kiwifruit puree stored for 63 days at 10 °C (b), after *in vitro* simulated gastrointestinal digestion of the puree. No bar implies concentrations below detection values (1 μg·100 $g^{-1}$). Total concentration of carotenoids in the stored samples (63 days at 10 °C) was 27, 202 and 143 μg·100 $g^{-1}$ for F, MW and C, respectively.

The carotenoids identified in the kiwifruit puree showed a fractional bioaccessibility that ranged from 29 ± 3% to 47 ± 2%, with β-carotene and lutein being the least and most accessible compounds in the product, respectively. These results are in line with previous works dealing with the bioaccessibility of carotenoids in different fruit products (O'Connell, Ryan & O'Brien, 2007; Rodríguez-Roque, Rojas-Graü, Elez-Martínez & Martín-Belloso, 2014), being generally lower for the more apolar carotenes than for xanthophylls (Bohn, 2008). However, neither pasteurisation (MW, C) nor storage had a noticeable effect on the bioaccessibility of carotenoids from the kiwifruit matrix, as no significant differences among the studied samples were observed (Figure 4). A plausible explanation for the results obtained in the present study might be: on the one hand, that the severity of the pasteurisation treatments was insufficient to promote structural changes in the kiwifruit matrix, and, on the other hand, that thermal processing might not produce further destruction of previously homogenised matrices (e.g. purees), as suggested by Hornero-Méndez and Mínguez-Mosquera (2007).

In any case, as pointed out by Cilla et al. (2012), the effects of food processing on the bioaccessibility of carotenoids are more complex than the positive effects that might be expected. Although it has been extensively reported that thermal processing tends to enhance the bioaccessibility and bioavailability of carotenoids and other functional compounds in various vegetable-based food matrices, this cannot be taken for granted, since, according to Van Buggenhout et al. (2010), the data reported so far by different authors on this topic has not been found to be consistent and may largely depend on the distribution and original presence of carotenoids in various forms, such as in crystalline form or in the form of oil droplets (Schweigert et al., 2012).

## 4. CONCLUSIONS

Both processing conditions and storage time had a strong impact on the pigment composition of kiwifruit, with chlorophylls being affected to a greater extent than carotenoids. Pasteurisation enhanced the stability of pigment compounds in the kiwifruit puree. Microwaves allowed greater preservation of chlorophylls during processing and storage, a finding that might help to palliate the dramatic colour

changes typically undergone by kiwifruit-based products under these conditions. Fractional bioaccessibility, however, remained unchanged following processing and storage, suggesting only minor changes in their tissue distribution following processing. Accordingly, microwave technology may be successfully employed as an innovative tool that could aid in maintaining the natural colour of fresh kiwifruit in the pasteurised fruit and improve its market acceptance.

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# CAPÍTULO IV.8. SUPERIORIDAD DE LAS MICROONDAS SOBRE EL CALENTAMIENTO CONVENCIONAL PARA PRESERVAR LA VIDA ÚTIL Y LA CALIDAD DE UN PURÉ DE KIWI

# SUPERIORTY OF MICROWAVES OVER CONVENTIONAL HEATING TO PRESERVE SHELF-LIFE AND QUALITY OF KIWIFRUIT PUREE

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## ABSTRACT

The effect of both microwave (1000 W-340 s) and conventional heating (97 ºC-30 s) on the quality and shelf-life of kiwifruit puree was investigated. The growth of microorganisms and the evolution of enzyme activity, colour, pH, bioactive compounds and antioxidant activity in the product during storage at 4, 10 and 22 ºC were checked. The storage temperature had a significant ($p < 0.05$) impact on both the shelf–life and the nutritional and functional value of the samples: the higher the temperature, the significantly ($p < 0.05$) faster the rate of both the sample spoilage and the loss of the bioactive compounds. On the other hand, thermal processing significantly ($p < 0.05$) reduced the growth of microorganisms and the degradation rate of some bioactive compounds in a 12–59%, as well as leading to enzyme and colour stabilization. A longer shelf-life (123 days at 4 ºC) and a superior preservation of colour ($\Delta E_{SE}$=6.54) and bioactive compounds (57–67%) were obtained when microwave heating was the technology selected to process the kiwifruit puree. Microwave heating was considered a suitable means of preserving kiwifruit puree that might be successfully employed as an innovation tool with which to help safe, high-quality and minimally processed kiwifruit based-products reach the market.

**KEYWORDS** Microorganism spoliage, *Listeria monocytogenes*, enzymes, bioactive compounds, antioxidant activity, colour.

## 1. INTRODUCTION

A wide variety of minimally processed fruit-based products, such as fresh-cut fruits, fresh-squeezed fruit juices, fruit juice and milk mixture beverages, fruit purees and smoothies, are being marketed in response to the recent increase in demand for convenient, easy-to-preserve, health-promoting foods (Elez-Martínez, Soliva-Fortuny, & Martín-Belloso, 2006). Nevertheless, many fruits which are both appreciated for their sensory and nutritional value and possess a great potential for industrial exploitation, e.g. kiwifruit, still seem to be mostly limited to the fresh market outlet, ignoring their surplus production (Barboni, Cannac, & Chiaramonti, 2010).

Microwave heating has been reported to provide superior quality fruit-based products with an extended shelf-life, representing a good alternative to conventional preservation processes (Landl, Abadias, Sárraga, Viñas, & Picouet, 2010). Given the particular way of heating which takes place during microwave processing (volumetric heating), this technology leads to higher penetrative power, faster heating rates, higher thermal efficiency and shorter processing times compared to conventional heating methods. All these facts seem to result in better organoleptic, nutritional and functional properties preservation, with a particular effect on colour (Huang, Sheng, Yang, & Hu, 2007; Vadivambal & Jayas, 2007). Similarly to other novel technologies in the field of food innovation, microwaves might be a key factor either in the successful differentiation of products (Deliza, Rosenthal, Abadio, Silva, & Castillo, 2005) or in finding new uses for some fruits by helping to develop novel ways with which to process them. To this end, many comparative studies of the effect of microwave and conventional heating on various quality aspects of fruits have been conducted (Barrett & Lloyd, 2012), pointing out the advantages of microwave heating (Huang et al., 2007). However, it should be taken into consideration that despite published data on the effect of microwaves on safety and quality being available for different food systems, to date, little seems to be known of the impact of microwaves on the shelf-life and post-processing quality loss of fruit products.

The marketing of these products frequently implies a storage step, which might also relevantly contribute to their final quality. For this reason, the evolution of their

properties and the growth of microorganisms during shelf-life is an important issue to study (Rodrigo et al., 2003).

A few studies have focused on the evaluation of the shelf-life of microwaved foods of animal origin. Of these (i) Aziz, Mahrous, and Youssef (2002) studied the impact of microwave and gamma-ray processing on the shelf-life of beef when stored at 5 ºC, (ii) Göksoy, James, and Corry (2000) assessed the effect of short-time microwave energy exposures on several pathogens inoculated in chicken and the shelf-life of the product, (iii) Hebbar, Nandini, Lakshmi, and Subpramanian (2003) studied the shelf-life of microwaved and infrared-heated honey and its quality during storage and (iv) Paterson, Cranston, and Loh (1995) investigated how microwave processing helped to extend the shelf-life of beef under cold storage.

Despite the existence of one article dealing with microbial, enzymatic, physical and nutritional issues during the short storage (14 days) of an apple-based product subjected to minimal microwave processing (Picouet, Landl, Abadias, Castellari, & Viñas, 2009) and several studies evaluating the evolution of physicochemical, nutritional and functional properties during the storage of both microwaved and conventionally-heated grapefruit (Igual, García-Martínez, Camacho, & Martínez-Navarrete, 2010, 2011, 2013), no published study has been found comparing the effect of an alternative microwave process with that of conventional heat pasteurisation on the shelf-life and quality of a fruit-based product.

The aim of this study was to investigate the influence of microwave and conventional thermal pasteurization processes and storage at various temperatures (22, 10 and 4 ºC) on the pH, colour, enzyme activity, bioactive compounds and antioxidant activity of kiwifruit puree, as well as to determine the shelf-life of the product based on its microbial stability at 4 ºC.

## 2. MATERIALS AND METHODS

### 2.1. Sample preparation

Kiwifruit (*Actinidia deliciosa var.* Hayward) produced in Italy was purchased from a local supermarket. Fruit pieces selected on the basis of a similar soluble solid content (13–16º Brix) and apparent fruit quality were peeled, washed with distilled

water, cut into slices and triturated with a Thermomix (TM 21, Vorwerk, Spain), using the fourth power level for 1 min. The obtained puree was preserved in ice-water until further usage.

### 2.2. Treatments

Processing conditions were chosen based on preliminary experiments to simulate equivalent pasteurization treatments in terms of the degree of enzyme and microbial inactivation they achieved (Benlloch-Tinoco, Igual, Rodrigo, & Martínez-Navarrete, 2013; Benlloch-Tinoco, Pina-Pérez, Martínez-Navarrete, & Rodrigo, 2014). Requirements for pasteurization of fruit juices or similar fruit-based products are: (i) at least 5 $\log_{10}$ cycle inactivation of the most relevant pathogen microorganism (FDA, 2004) and (ii) no less than 90% of enzyme inactivation (Gonçalves, Pinheiro, Abreu, Brandao, & Silva, 2010). In a previous study (data not shown), several power–time combinations for microwave heating (200–1000 W and 60–340 s) and temperature-time combinations for conventional heating (90–97 ºC and 30–60 s) were assayed.

Those reaching the required level of peroxidase inactivation and *Listeria monocytogenes* reduction but causing the minimum nutritional and functional value deterioration were selected to carry out the present work (described below).

#### *2.2.1. Microwave treatment*

A microwave oven (model: 3038GC, NORM, China), provided with a glass turntable plate, was used to treat the kiwifruit puree. A sample of 500 g was tempered to an initial temperature of 25 ºC and then heated in the microwave oven in a standard size glass beaker (9 cm inner diameter and 12 cm length) (BKL3-1K0e006O, Labbox, Spain) at 1000 W for 340 s. The temperature of the sample in the coldest and hottest spots, previously identified (data not shown), was continuously recorded by means of a fibre-optic probe (CR/JP/11/11671, Optcom, Germany) which was connected to a temperature datalogger (FOTEMP1-OEM, Optcom). The treated samples were immediately cooled in ice-water until the puree reached 35 ºC.

*2.2.2. Conventional thermal treatment*

The conventional thermal treatment consisted of heating the sample to 97 ºC for 30 s in a circulating thermostatic water bath (Precisterm, Selecta, Spain). After the kiwifruit was triturated, 20 g of puree was placed in TDT stainless steel tubes (1.3 cm inner diameter and 15 cm length) and closed with a screw stopper. A thermocouple, connected to a datalogger, was inserted through the sealed screw top in order to record the time temperature history of the sample during the treatment. Prior to this heating step, the samples were preheated to 25 ºC to shorten and standardize the come-up time (150 s). The treated samples were immediately cooled in ice-water until the puree reached 35 ºC.

**2.3. Storage study**

Both the heat-treated and the non-treated kiwifruit purees were packaged in clean, sterile plastic tubes (1.7 cm inner diameter and 11.8 cm length) (ref. 525–0153, VWR, Spain) and then stored in darkness at 4, 10 and 22 ºC for a maximum of 188, 58 and 23 days, respectively. The purpose of storage at 10 and 22 ºC was to observe the changes that may take place in the samples in the case of a partial, or total, rupture of the cold chain, respectively, during the shelf-life of the product.

**2.4. Analytical determinations**

The treated samples, as well as a non-treated sample used as control, were analysed as described below. Measurements were performed in triplicate at time 0 and at regular time intervals for each storage temperature tested.

*2.4.1. Chemicals and standards*

Unless otherwise stated, all chemicals employed were from Sigma–Aldrich (Germany) and they were of analytical quality or superior.

*2.4.2. Colour, pH, enzyme activity and antioxidant activity*

Colour of kiwifruit puree samples was measured using a Minolta CM 3600D spectrocolorimeter (Konica Minolta Sensing, Inc., Japan). The colour coordinates

were obtained and results were expressed according to CIE L*a*b* uniform colour space (10º observer and D65 illuminant), where the L* value is a measure of lightness (from 0 to 100), a* is a measure of chromaticity on a green (-) to red (+) axis and b* of chromaticity on a blue (-) to yellow (+) axis. Colour differences caused by treatment and storage effects ($\Delta E_{TE}$ and $\Delta E_{SE}$, respectively) were calculated (see Table 1). To obtain $\Delta E_{TE}$, the colour of microwave and conventionally treated samples were compared with that of the non-treated sample, while for $\Delta E_{SE}$, the colour of the treated or untreated samples at the end of their shelf–life was compared with that of the newly-processed samples. To determine the pH, a digital pH-metre Basic 2 was used (Crison, Spain). Peroxidase (POD) and polyphenoloxidase (PPO) activity were determined spectrophotometrically and the $DPPH^{\cdot}$ radical scavenging capacity of kiwifruit extracts was measured to determine antioxidant activity (AOA) of the samples. All this measurements were carried out Benlloch-Tinoco, Varela, Salvador, and Martínez-Navarrete (2012) and Benlloch-Tinoco et al. (2013).

### *2.4.3. Bioactive compounds*

The vitamin C (Vit. C) and total phenol (TP) content was measured as previously described by Igual et al. (2010). Briefly, ascorbic acid and total vitamin C (ascorbic acid þ dehydroascorbic acid) were determined by HPLC (Jasco, Italy). The procedure employed to determine total vitamin C was the reduction of dehydroascorbic acid to ascorbic acid, using DL-dithiothreitol as the reductant reagent. Total phenols were quantified by using the FolineCiocalteu method.

Total flavonoids (TF) were measured spectrophotometrically, following the method described by Djeridane et al. (2006), based on the formation of a flavonoide aluminium complex. The extraction of TF consisted of homogenising 35 g of the sample (T25 Janke and Kunkel turrax) for 5 min with 40 ml of methanol, 10 ml of chlorhydric acid and sodium fluoride to inactivate polyphenoloxidases and to prevent phenolic degradation. The homogenate was centrifuged (11,872 x g, 10 min, 4 ºC) (P-Selecta Medifrigar BL-S, Spain) to obtain the supernatant. For total flavonoid quantification, 1 mL of the extract was mixed with 1 mL of 20 g/L $AlCl_3$ methanolic solution. After incubation at room temperature for 30 min in darkness, the

absorbance of the reaction mixture was measured at 430 nm using a UV–visible spectrophotometer (Thermo Electron Corporation, USA). The TF content was expressed as mg of rutin equivalents (RE) per 100 g of sample, using a standard curve range of 0–0.05 mg of rutin/mL.

#### *2.4.4. Microbiological analysis*

The survival of *L. monocytogenes* was evaluated as described by Benlloch-Tinoco, Martínez-Navarrete, & Rodrigo, 2014; Benlloch-Tinoco, Pina-Pérez, et al., 2014 with some modifications. Briefly, the kiwifruit puree subjected to both microwave and conventional heat processing and the fresh kiwifruit puree used to assess the growth of *L. monocytogenes* in the sample at various temperatures were previously inoculated with a mean value (and standard deviation) of $1 \cdot 10^7$ ($2 \cdot 10^6$) and $2.8 \cdot 10^2$ ($1.5 \cdot 10^1$) CFU/g, respectively. The total mesophilic bacteria (TMB) and yeast and mould (Y&M) counts were examined by diluting the uninoculated samples in 0.1% (w/v) sterile peptone water (Scharlab Chemie S.A., Spain) and enumerating the viable cells in Plate Count Agar (PCA, Scharlab Chemie S.A.) and Potato Dextrose Agar (PDA, Scharlab Chemie S.A.) acidified with tartaric acid (10%), by adding 1mL of tartaric acid per 10 mL of PDA, respectively. The selected dilutions were incubated at 30 ºC for 48 h in the case of TMB and at 25 ºC for 5 days in that of Y&M.

### 2.5. Kinetics modelling degradation

To obtain the kinetic parameters explaining the colour changes and the degradative loss of bioactive compounds and AOA in the treated and untreated kiwifruit puree during storage, the results of L* coordinate, Vit. C, TP and AOA obtained for the samples were plotted vs. time at all the temperatures studied. Reaction order was determined by fitting experimental data to second-order, first-order and zero-order models. Zero-order kinetic (Equation (1)) resulted to be the one that best fitted experimental data. The same was observed by (Gonçalves, Abreu, Brand~ao, & Silva, 2011; Zheng & Lu, 2011). The time for the concentration of a compound to fall to half its initial value (half-life, $t_{1/2}$) was also determined (Equation (2)).

$$C = C_0 - k \cdot t \quad (1)$$

$$t_{\frac{1}{2}} = \frac{C_0}{2k} \quad (2)$$

Where

C: concentration of the compound at t ($mg \cdot 100g^{-1}$);

$C_0$: concentration of each compound at time zero ($mg \cdot 100g^{-1}$);

k: zero-order rate constant ($mg \cdot 100g^{-1} \cdot days^{-1}$);

t: storage time (days);

$t_{1/2:}$ the half time of the compound (days).

On the other hand, the temperature dependence of the degradation of these attributes was studied by employing the Arrhenius equation (Equation (3)). In every case, the goodness of fit between the experimental and predicted data was assessed by means of the adjusted regression coefficient (adj-$R^2$) (Equation (4)), considering that the higher the adj-$R^2$ value, the better the fit.

$$k = k_0 \cdot e^{\frac{-E_a}{R \cdot T}} \quad (3)$$

Where

k: rate constant ($mg \cdot 100g^{-1} \cdot days^{-1}$);

$k_0$: the pre-exponential factor;

$E_a$: activation energy ($kJ \cdot mol^{-1}$);

R: gas constant (8.314 $J \cdot mol^{-1} \cdot K^{-1}$);

T: absolute temperature (K)

$$Adjusted - R^2 = \left[ \frac{(m-1)(1-\frac{SSQ_{REGRESSION}}{SSQ_{TOTAL}})}{(m-j)} \right] \quad (4)$$

Where

m: number of observations;

j: number of model parameters;

SSQ: sum of squares.

### 2.6. Statistical analyses

The assumptions of normality and equality of variance were tested by normality plots and box-plots, respectively. Linear mixed models correlating each one of the attributes evaluated in the present study with the type of sample (fresh, conventionally heated, microwaved), storage temperature and storage time were developed using the SPSS Statistics 19 software program (IBM SPSS, Inc., USA). A p-value of 0.05 (2-sided) was assumed to reflect statistically significant differences. Following significant Fisher-F tests, post-hoc tests (Bonferroni's) were conducted. Non-linear and linear regression analyses, based on the Levenberge Marquardt estimation method, were carried out in order to estimate the kinetic parameters using the SPSS Statistics 19 software program (IBM SPSS). Furthermore, a correlation analysis was run between all the studied components with a 5% significance level.

## 3. RESULTS AND DISCUSSION

### 3.1. Shelf-life determination based on microbial stability

In order to evaluate the impact of processing and storage on the microbial stability of kiwifruit puree, the survival of *L. monocytogenes*, taken as the pathogen of greatest concern in the product (Benlloch-Tinoco, Martínez-Navarrete, et al., 2014; Benlloch-Tinoco, Pina-Pérez, et al., 2014) was investigated (Fig. 1). At the same time, TMB and Y&M flora were also followed in the fresh (F), microwaved

(MW) and conventionally-heated (C) samples during storage at various temperatures (22, 10 and 4 ºC) (Figs. 2 and 3).

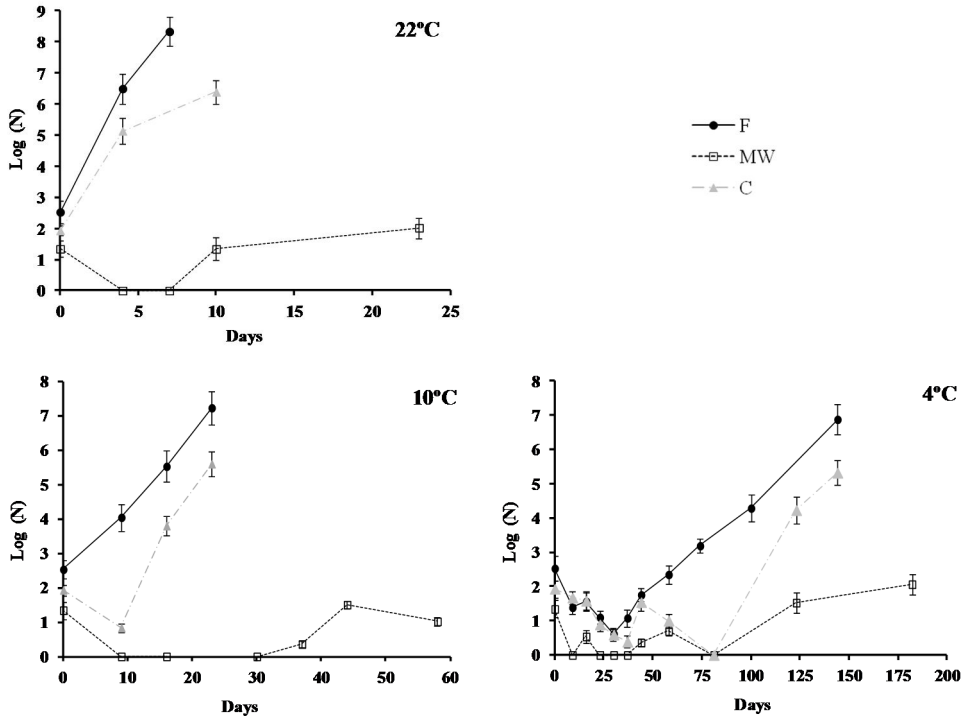


**Figure 1.** Survival of *Listeria monocytogenes* in the kiwifruit puree (F: fresh, MW: microwaved and C: conventionally thermal treated) during storage at 22, 10 and 4ºC.

Microwave and conventional thermal treatments lead to a 5.8 (0.4) and 5.1 (0.3) $log_{10}$ cycle reduction in the count of *L. monocytogenes*, a 2.1 (0.0) and 1.1 (0.2) $log_{10}$ cycle reduction in the count of TMB and a 2.10 (0.10) and 0.95 (0.13) $log_{10}$ cycle reduction in the count of Y&M, respectively. While both the microwave and conventional thermal treatments lead to equivalent *L. monocytogenes* inactivation (no significant differences), the microwave process was significantly more effective at inactivating TMB and Y&M.

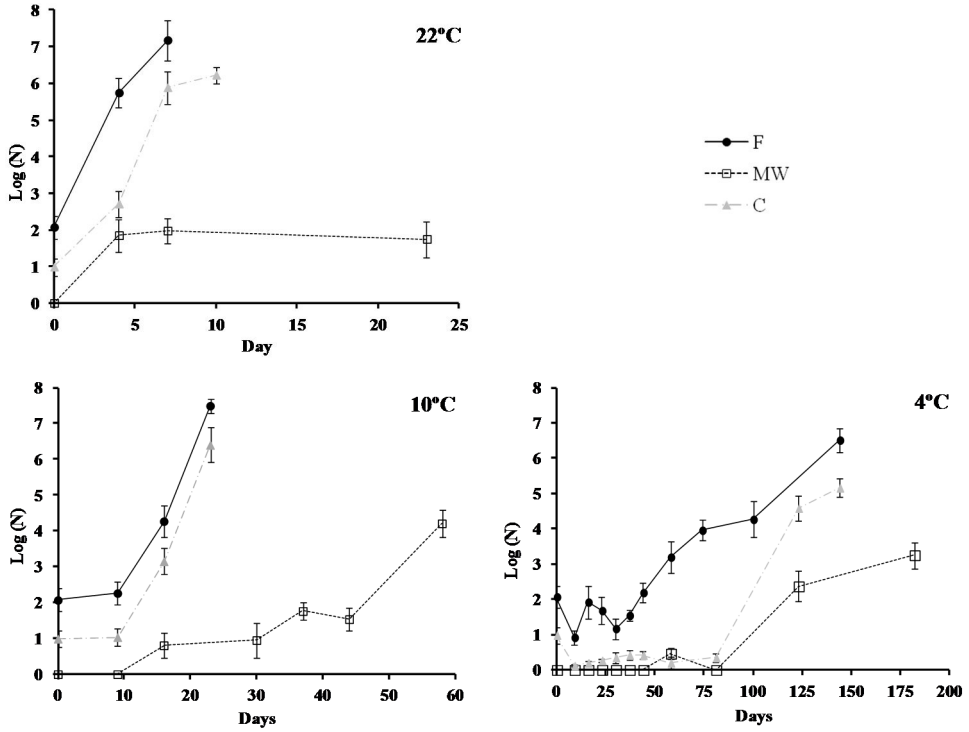


**Figure 2.** Survival of total mesophylic bacteria in the kiwifruit puree (F: fresh, MW: microwaved and C: conventionally thermal treated) during storage at 22, 10 and 4ºC.

After such a reduction in the bacterial counts brought about by processing, a growth of microorganisms was observed during storage. Linear mixed models were used to evaluate the effect of storage temperature, storage time and type of sample on their growth. Significant statistical differences were found in the counts of *L. monocytogenes*, TMB and Y&M due to all these factors and their interactions. As expected, the higher temperature led to a significantly faster growth of these microorganisms and the longer storage time to significantly higher counts (Figs. 1–3). Similarly, Rivas, Rodrigo, Martínez, Barbosa-Cánovas, and Rodrigo (2006) reported faster growth rates of microorganisms in orange and carrot juice when stored at 12 ºC than at 2 ºC. On the other hand, F and MW samples showed by far the fastest and slowest growth rate of microorganisms at any of the temperatures studied (22, 10, 4 ºC), respectively, while C sample exhibited intermediate behaviour. These differences between samples were found to be more evident at 22 ºC and 10 ºC than at 4 ºC. In this regard, while the untreated kiwifruit puree was

rapidly spoiled by microorganisms reaching 3.2, 4.0 and 4.2 $log_{10}$ CFU/g for *L. monocytogenes*, TMB and Y&M after 74 days at 4 ºC, respectively, the treated samples (MW, C) stored at 4 ºC kept microbial loads below 1 $log_{10}$ CFU/g for 74 days.

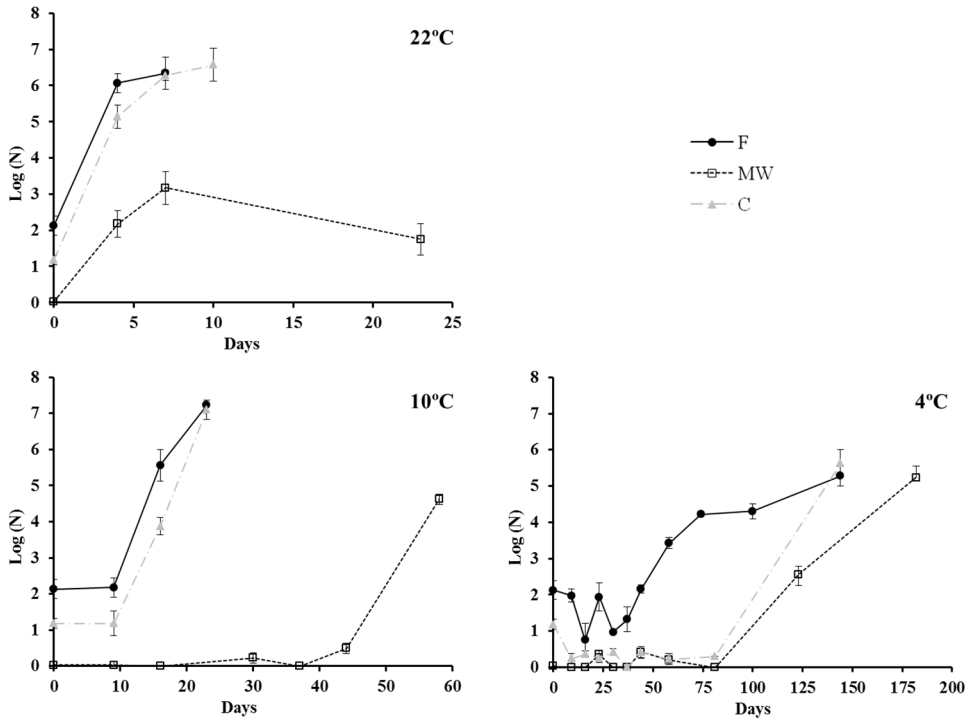


**Figure 3.** Survival of yeast and mould in the kiwifruit puree (F: fresh, MW: microwaved and C: conventionally thermal treated) during storage at 22, 10 and 4ºC.

The shelf-life of treated samples was determined at 4 ºC, taking into account the acceptable limit established by EU legislation (*L. monocytogenes* <2.0 $log_{10}$ CFU/g and TMB and Y&M < 3.0 $log_{10}$ CFU/g) (EU, 2005). On this basis, the shelf-life of C and MW treated puree was found to be 81 and 123 days, respectively (Figs. 1–3). These results are in the range of those published by other authors working on different fruits subjected to conventional thermal processes. The shelf-life of heat-pasteurized orange and carrot juice (98 ºC for 21 s) stored at 2 ºC, thermally pasteurized pomegranate (90 ºC for 5 s) stored at 5 ºC and conventionally heat-pasteurized orange juice (90 ºC for 50 s) stored at 4 ºC was found to be 70,120 and 105 days, respectively (Leizerson & Shimoni, 2005; Rivas et al., 2006; Vergara,

Martí, Mena, Saura, & Valero, 2013). On the other hand, Picouet et al., (2009) reported that an apple-puree preserved by gentle microwave heating (652 W–35 s) had a shelf-life of at least 14 days under refrigeration conditions.

Bearing in mind the results obtained in the present study, microwave heating seems to provide greater microbial stability than conventional heat processing, allowing for a better preservation of kiwifruit puree. In a similar way, other authors have found a better microbiological shelf-life for various fruit-based products when preserved by novel technologies, such as pulsed electric fields, than when heat pasteurization is used (Sampedro, Geveke, Fan, Rodrigo, & Zhang, 2009; Walkling-Ribeiro, Noci, Cronin, Lyng, & Morgan, 2010). This superiority of microwaves can be supported taking into account the results of another study in which a further comparison between microwave and conventional heating processes was performed by means of Pasteurization Units (PU), which allow treatment severity to be quantified in terms of thermal load (Benlloch-Tinoco, Martínez-Navarrete, & Rodrigo, 2014). Obtained PU (80 ºC) were 0.53 (0.05) min at the coldest spot and 19 (2) min at the hottest spot for MW, and 19.27 (0.13) min for C sample. According to these data, kiwifruit puree was not subjected to a more severe treatment when was processed under microwave heating. In other words, superiority of microwaves cannot be explained by faster heating rates or greater temperature achieved. Like it has been reported by other authors, however, this superiority might indicate the possibility of some enhanced effects associated with microwaves (Banik, Bandyopadhyay, & Ganguly, 2003; Tajchakavit, Ramaswamy, & Fustier, 1998).

### 3.2. Effect of process on enzyme activity. Stability during storage

The impact of processing and storage on kiwifruit puree enzymes was assessed by investigating the evolution of POD and PPO activity in F, MW and C samples during storage at 22, 10 and 4 ºC (Figs. 4 and 5). The enzyme activity was significantly reduced by both microwave and conventional heat treatments. The mean value (and standard deviation) of inactivation caused by these processes was 96% (2) and 95.7% (1.1) of POD and 82% (2) and 43% (4) of PPO, respectively. Despite the fact that the reduction of POD activity under microwave and

conventional heat treatments was found to be equivalent (no significant differences), the microwaved kiwifruit puree exhibited a significantly higher PPO inactivation.

On the other hand, the effect of factors, such as temperature, time and type of sample, on the evolution of enzyme activity during storage was checked by using linear mixed models. The statistically significant differences observed in POD and PPO values were caused by the storage time, type of sample and their interactions. In general terms, F sample showed a significant drop in POD and PPO activity during storage, which may be attributed to a decrease of the substrates concentration available within the kiwifruit puree over time. Additionally, PPO is believed to be irreversibly inactivated during the oxidation of substrate to product due to a free radical-catalyzed fragmentation of one or more of the six histidine residues that bind the two coppers at the active site (Whitaker, Voragen, & Wong, 2003). On the contrary, treated samples (MW, C) exhibited a slighter variation of POD and PPO activity over time. On the one hand, the residual POD activity remained mostly constant in treated purees at 4 ºC, there being no observed significant differences between MW and C samples (Fig. 4). While the main fall of POD in F puree took place after 44 days when stored at 4 ºC, varying from 8.6 (1.3) to 1.9 (0.2) $Abs \cdot min^{-1} \cdot g^{-1}$, C and MW samples stored at 4 ºC maintained the POD activity below 1.5 $Abs \cdot min^{-1} \cdot g^{-1}$for all 144 and 182 days, respectively. On the other hand, although the treated samples exhibited lower residual PPO activity than F puree over time, PPO inactivation was shown to be reversible (Fig. 5). Some reactivation of this enzyme in both the MW and C samples stored for 74 days at 4 ºC was observed, PPO activity subsequently, remaining mainly constant. Other authors have reported enzymes, e.g. peroxidase, recovering their activity after heating treatments, especially in high-temperature-short-time processed fruit and vegetables (Thongsook & Barrett, 2005).

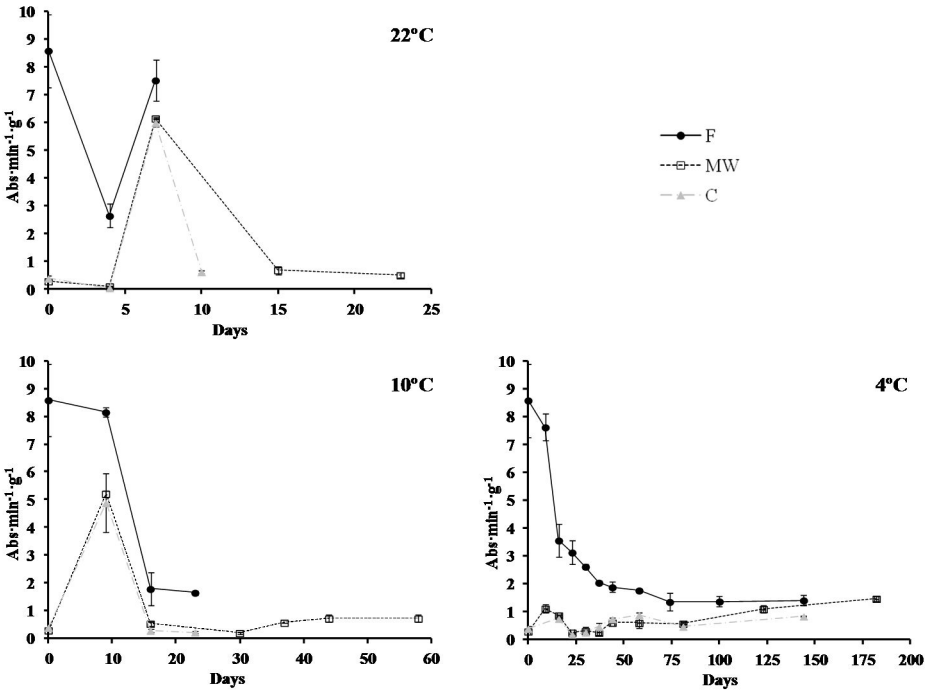


**Figure 4.** Peroxidase activity (POD) in the kiwifruit puree (F: fresh, MW: microwaved and C: conventionally thermal treated) during storage at 22, 10 and 4◦C.

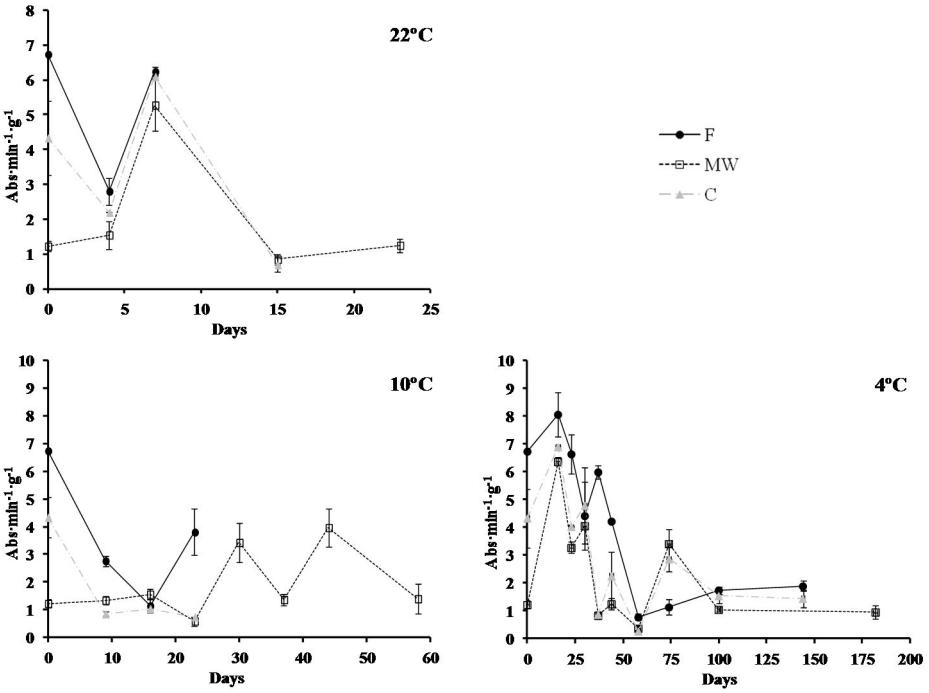


**Figure 5.** Polyphenoloxidase activity (PPO) in the kiwifruit puree (F:fresh, MW: microwaved and C: conventionally thermal treated) during storage at 22, 10 and 4 ºC.

From the results obtained, it can be seen that significantly lower residual activity and a markedly smaller variation of POD and PPO enzymes was found to take place during the storage of treated samples; this is especially true in the case of POD, one of the enzymes which most relevantly contributes to the deterioration in the colour and nutritive value of kiwifruit (Fang, Jiang, & Zhang, 2008), a fact that can be taken as an indicator of the stability provided by processing. In this respect, and taking into account the widely recognized detrimental effects of these enzymes, the microwave and conventional thermal treatments applied to pasteurized kiwifruit puree may be considered to make a meaningful contribution to the preservation of the product quality by minimizing the degradative effect of the enzyme during its shelf-life.

### 3.3. Effect of process on pH and colour. Stability during storage

The changes in the pH and L*, a*, b* colour coordinates of the kiwifruit puree brought about by processing and storage were studied. Table 1 summarises these values, together with the colour changes caused by treatment and storage effects, for treated samples, both those non-stored (storage 0 days) and those stored until the end of their shelf-life.

From the statistical analysis, it can be stated that the pH of the kiwifruit puree was not significantly affected by processing, but it significantly decreased in all the samples during storage, irrespective of the temperature, probably due to a significant growth of the microbial flora (Figs. 1–3), fact also observed by Elez-Martínez et al. (2006).

From Table 1 it can be seen that processing had a significant impact on the colour of the product, being the samples more luminous and changing to a less greenish hue after treatment. Greater colour changes were observed when the puree was conventionally heated than when microwaved. Similarly, Chandrasekaran, Ramanathan, and Basak (2013) observed that microwaving preserves colours better than other conventional thermal techniques. The statistical analysis pointed out that the storage time, type of sample and their interactions brought about significant statistical differences in L* and b*. However, a* and $\Delta E_{SE}$ values were exclusively affected by the storage time and the type of sample,

respectively. In the F sample, L* values decreased from 38.48 to 34.33 and a* values increased from -5.18 to 1.12 for the first 44 days of storage at 4 ºC; thereafter, both of them remained mostly constant. However, b* values did not show a clear trend.

**Table 1.** Mean values (and standard deviation) of vitamin C (Vit. C, mg/100g), total phenols (TP, mg GAE/100g) and total flavonoids (TF, mg RE/100g) content and antioxidant activity (AOA, mM Trolox/100g), pH, colour coordinates ($L^*$, $a^*$ and $b^*$) and colour difference due to processing ($\Delta E_{TE}$) and storage ($\Delta E_{SE}$) of microwaved (MW) and conventionally heated (C) kiwifruit puree, at the beginning and end of their shelf-life (4ºC).

| | **Beginning of shelf-life** | | **End of shelf-life** | |
|---|---|---|---|---|
| | *0 days* | | *123 days at 4ºC* | *81 days at 4ºC* |
| | **MW** | **C** | **MW** | **C** |
| Vit. C | 64.2 (0.7)a | 62.3 (0.7)a | 37.2 (0.6)b | 25.4 (1.5)c |
| TP | 25.50 (0.07)a | 22.2 (0.3)b | 13.92 (0.08)c | 9.3 (0.3)d |
| TF | 0.825 (0.004)a | 0.67 (0.02)b | 0.437 (0.013)c | 0.505 (0.010)d |
| AOA | 1211 (37)a | 1117 (27)b | 478 (35)c | 463 (41)c |
| pH | 3.85 (0.14)a | 3.75 (0.13)a | 3.35 (0.02)b | 3.15 (0.02)a |
| $L^*$ | 43.90 (0.02)a | 44.81(0.03)b | 39.76 (0.02)c | 43.67 (0.03)d |
| $a^*$ | -1.11 (0.02)a | -1.71 (0.02)b | 1.183 (0.012)c | 0.19 (0.03)d |
| $b^*$ | 26.81 (0.03)a | 22.63 (0.02)b | 24.083 (0.012)c | 27.06 (0.05)d |
| $\Delta E_{TE}$ | 7.06 (0.02)a | 7.54 (0.02)b | - | - |
| $\Delta E_{SE}$ | - | - | 6.54 (0.02)a | 7.80 (0.02)b |

Different letters in rows, indicate statistical significant differences ($p<0.05$) according to Bonfferoni test when the effect of time was evaluated.

$$\Delta E_{TE} = \sqrt{(a_F^* - a_{T_0}^*)^2 + (b_F^* - b_{T_0}^*)^2 + (L_F^* - L_{T_0}^*)^2} \quad \Delta E_{SE} = \sqrt{(a_{T_0}^* - a_{T_{Si}}^*)^2 + (b_{T_0}^* - b_{T_{Si}}^*)^2 + (L_{T_0}^* - L_{T_{Si}}^*)^2}$$

where colour coordinate subscripts refer to fresh puree (F), newly treated puree ($T_0$) and treated puree after i days of storage ($Ts_i$).

The colour of the treated samples changed in a similar way during storage although to lesser extent than the F sample, leading to a lower degree of luminosity and a redder hue angle in every case. The potential degradative impact of POD and PPO enzymes on the colour of kiwifruit puree was investigated by means of a correlation statistical analysis (Pearson's correlation) in both the treated and

untreated samples. A significant correlation between L* and POD ($R^2$=-0.3244) and PPO ($R^2$=-0.3226) was found, which indicated that a loss of luminosity over time might be attributable to the detrimental activity of POD and PPO enzymes. In this respect, colour stabilization observed in MW and C samples could be attributed to the enzymatic stability provided by processing (Figs. 4 and 5).

On the other hand, the kinetics of variation of the colour coordinates and colour differences during storage was investigated. However, the evolution of L* was the only colour coordinate that properly fitted zero-order kinetics. The values of the kinetic rate constant (k) and half-destruction time ($t_{1/2}$) calculated for the F, MW and C samples at 22, 10 and 4 ºC are given in Table 2. To determine the effect of temperature on the studied parameters, the obtained rate constants were fitted to the Arrhenius equation. The obtained activation energies ($E_a$) are also shown in Table 2. In order to describe the effect of both the treatments and temperature on the rate of decrease in L*, it was considered that the lower the $t_{1/2}$ and the higher the k values, the faster the variation of the L* coordinate. Additionally, a higher value of activation energy means a greater dependence of the kinetic rate constant on the storage temperature. In general terms, the storage temperature had a greater impact on the rate of luminosity reduction than the treatment applied, observing that the higher the storage temperature, the more quickly the L* values decreased. Although microwave heating was the only one having a positive effect decreasing the rate at which L* changed in the sample irrespective of the temperature, MW puree was the sample requiring the lowest temperature increase in order to achieve the same increase in the rate of L* reduction. Despite the fact that microwave heating leads to a greater sensitivity of the k parameter to temperature changes during storage, from the viewpoint of luminosity this technology may be preferred as a means of preserving the kiwifruit puree, since it clearly leads to the lowest rate of L* variation at any of the temperatures studied.

**Table 2.** Times of half destruction ($t_{1/2}$: days), mean values (and standard error) of the degradation rates (k: mg·100g$^{-1}$·day$^{-1}$) and the activation energy ($E_a$: kJ·mol$^{-1}$) of luminosity (L$^*$), vitamin C (Vit. C), total phenols (TP) and antioxidant activity (AOA) of fresh (F), microwaved (MW) and conventionally heated (C) kiwifruit puree during storage at 22, 10 and 4ºC. Adjusted regression coefficient ($R^2$-aj.).

| T (ºC) | L* | | | Vit. C | | | TP | | | AOA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F | MW | C | F | MW | C | F | MW | C | F | MW | C |
| 22 | 38.56 | 69.24 | 49.02 | 2.95 | 2.92 | 2.83 | 8.44 | 23.61 | 16.86 | 5.69 | 7.21 | 5.94 |
| | 0.50(0.07) | 0.317(0.003) | 0.457(0.004) | 11(2) | 11(2) | 11(2) | 1.31 (0.13) | 0.54(0.04) | 0.66 (0.03) | 107(3) | 84(8) | 94(5) |
| | 91.966 | 99.962 | 99.961 | 79.231 | 79.231 | 79.231 | 94.160 | 96.934 | 99.185 | 99.500 | 95.000 | 98.500 |
| 10 | 94.79 | 359.81 | 119.17 | 36.06 | 54.40 | 32.81 | 56.34 | 31.87 | 41.21 | 41.42 | 56.67 | 60.08 |
| | 0.20(0.03) | 0.061(0.007) | 0.188(0.002) | 0.9(0.2) | 0.59 (0.06) | 0.95(0.08) | 0.196(0.013) | 0.40(0.07) | 0.27(0.02) | 14.7(1.5) | 12(2) | 9.3(1.7) |
| | 89.189 | 86.998 | 99.886 | 82.170 | 93.113 | 91.232 | 95.831 | 83.528 | 92.006 | 95.000 | 81.000 | 86.400 |
| 4 | 223.74 | 438.97 | 203.67 | 55.01 | 80.24 | 47.22 | 96.93 | 55.43 | 58.56 | 41.42 | 56.07 | 35.36 |
| | 0.086(0.008) | 0.050(0.05) | 0.110(0.007) | 0.59 (0.05) | 0.40 (0.04) | 0.66(0.05) | 0.114(0.012) | 0.23(0.02) | 0.19(0.02) | 14.7(1.2) | 10.8(1.2) | 15.8 (1.4) |
| | 70.911 | 76.951 | 84.563 | 79.876 | 84.712 | 84.989 | 73.594 | 76.456 | 76.055 | 86.200 | 72.800 | 80.900 |
| | 64.5(1.3) | 73(2) | 53.4(0.2) | 115(3) | 131(4) | 111(3) | 94(2) | 30.3(1.3) | 47.6(0.4) | 64.5(1.3) | 73(2) | 53.5(0.2) |
| | 94.859 | 87.660 | 99.789 | 90.868 | 88.534 | 88.534 | 89.610 | 77.248 | 98.964 | 97.900 | 87.700 | 99.800 |

### 3.4. Effect of process on the bioactive compound and AOA. Stability during storage

The influence of processing and storage on the nutritional and functional value of kiwifruit puree as investigated by checking the changes in the amount of vitamin C, total phenols and total flavonoids, as well as the antioxidant activity of the puree samples during storage. Its evolution in the F, MW and C samples stored at 4 ºC is included in Fig. 6.

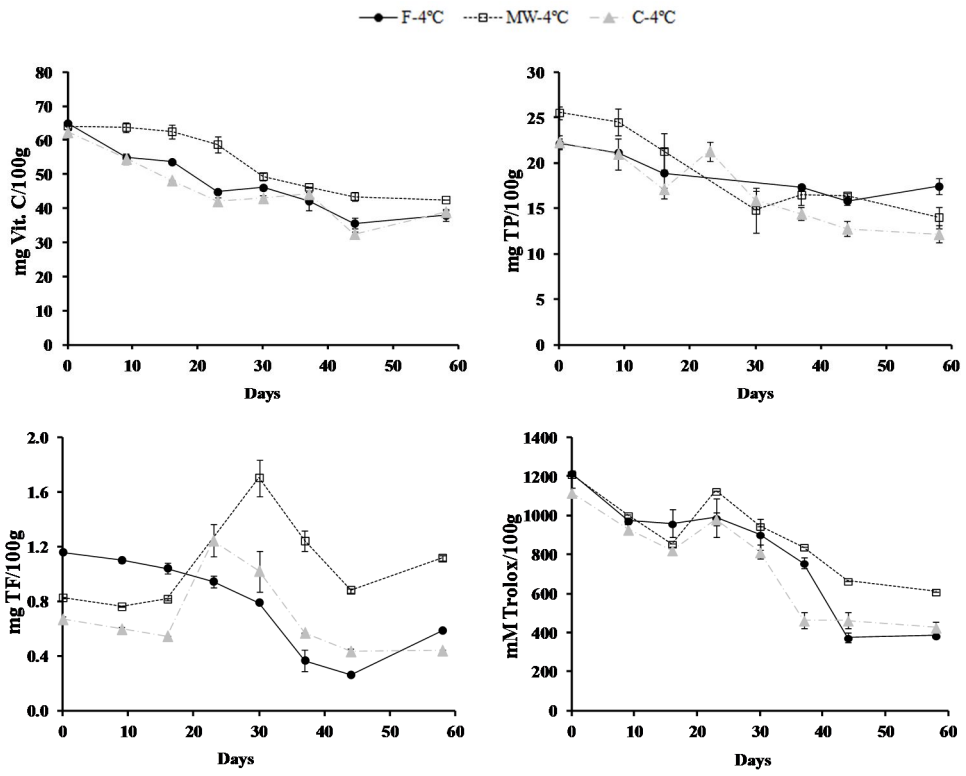


**Figure 6.** Vitamin C (Vit. C), total phenols (TP) and total flavonoids (TF) content and antioxidant activity (AOA, expressed as mM Trolox) in the kiwifruit puree (F: fresh, MW: microwaved and C: conventionally thermal treated) during storage at 4ºC.

Although processing did not provoke significant losses in the Vit. C, TP and AOA of the product, it did significantly affect the TF, reducing its content by 28.80% (0.01) and 42.38% (0.02) when the puree was microwave and conventionally treated, respectively. As the storage temperature-time and the type of sample also affected these compounds and AAO, the corresponding degradation kinetics was studied. However, the loss in TF was not appropriately described by zero-order kinetics. The

total flavonoid content decreased in kiwifruit samples during storage, the higher the storage temperature, the faster the degradation rate (data not shown). Processing clearly allowed for a better maintenance of TF during storage, especially when kiwifruit puree was microwaved (Fig. 6). Decrease of TF over time can be explained by the detrimental activity of PPO, since these compounds are widely known to be common substrate of this enzyme (Whitaker et al., 2003). Not only a smaller decrease was observed in the MW and C samples during storage, but also an increase in the total flavonoid content. In this way, despite the losses caused by processing, from day=16 onwards, the total flavonoid content was higher in the treated samples than in the untreated ones. Likewise, Kevers et al. (2007) reported that the total flavonoid content of apricot, yellow pepper, plum and green grape remained stable, or even increased, during storage.

As far as the degradation kinetics of Vit. C, TP and AOA is concerned, the values of the kinetic rate constant (k) and half-destruction time ($t_{1/2}$) for the F, MW and C samples stored at 22, 10 and 4 ºC are presented in Table 2. From the $t_{1/2}$ values obtained, whereas vitamin C may be considered to be the compound which most easily suffers degradation during storage at 22 ºC, the total phenols demonstrated they were the most stable. On the other hand, both the $t_{1/2}$ and the k values corroborated the fact that a higher storage temperature meant a faster degradation of bioactive compounds and a decrease in AOA in every sample. As expected, the processing of kiwifruit seemed to improve the stability of TP and AOA when stored at 22 ºC, leading to reduced degradation rates. However, no positive effect of pasteurization treatments (MW, C) was observed in the total phenol content of the samples stored at 10 and 4 ºC. Unlike conventional heating, microwave processing reduced the degradation rate of Vit. C at 10 and 4 ºC. On the other hand, lower rates of AOA decrease were found in the MW (10 and 4 ºC) and C purees (10 ºC). Considering that the MW sample exhibited similar or higher $t_{1/2}$ and lower k values for Vit. C and AOA than both the F and C samples, it can be pointed out that the nutritional and functional value of the kiwifruit puree was equally well or better preserved during storage when the kiwifruit puree was processed by means of microwave technology. In addition, vitamin C was the compound showing the highest activation energy values for every sample, which means that the Vit. C

degradation rates exhibited greater thermal sensitivity than TP and AOA in the treated and untreated kiwifruit puree. Moreover, the MW sample required a smaller temperature increase to achieve the same increase in the rate of Vit. C and AOA reduction than the F and C purees, while conventional heating reduced the heat sensitivity of TP and AOA with respect to the F sample.

Despite the fact that the degradation of bioactive compounds may be explained in many different ways, as a matter of fact, enzyme activity considerably contributes to the quality loss frequently observed in fruits and vegetables during storage. As has previously been mentioned (Section 3.2), POD and PPO enzymes may lead to the oxidation of polyphenolic compounds to quinines that then polymerize to dark melanin pigments, which is commonly known as enzymatic browning (Friedman, 1996). As a result, not only the colour, but also the functional value of the product, is affected. In this respect, a correlation statistical analysis (Pearson's correlation) was carried out so as to improve the understanding of the potential connection of colour changes with the loss in bioactive compounds and AOA observed in kiwifruit samples during storage. As expected, TP and TF were negatively correlated with $\Delta E_{SE}$ ($R^2$=-0.5940 and $R^2$=-0.3208, respectively) and TP were positively correlated with L* ($R^2$=0.3296). In other words, when total phenols and total flavonoids gradually decreased (Fig. 6), the luminosity of the product was reduced and greater colour differences were detected (data not shown), a fact that could be taken as an indicator of the detrimental activity of kiwifruit enzymes.

On the other hand, the nutritional and functional value of the microwave and conventionally treated kiwifruit puree at the end of their shelf-life was compared (Table 1). Despite the fact that the MW sample was stored for a longer period of time, it showed significantly higher Vit. C and TP, but lower TF, after 123 days at 4ºC. As for AOA, no differences were observed. The variation of the components brought about by both processing and storage was calculated as the difference between each compound in the treated puree at the end of its shelf-life related to the fresh puree and referred to 100 g of fresh puree. In this respect, losses of 43%, 23% and 62% in vitamin C, total phenols and total flavonoids were found for the MW sample (123 days at 4ºC) while losses of 61%, 58 and 56% were observed in

vitamin C, total phenol and total flavonoid content of the C sample (81 days at 4ºC), respectively. However, AOA was reduced by 62% in both cases. The results obtained clearly contribute to indicate the superiority of microwaves when it comes to preserving the nutritional and functional value of the product by equating or reducing the post-processing loss in bioactive compounds and AOA. In the same way, Igual et al. (2010) reported that microwave pasteurized grapefruit juices stored at -18ºC better preserved both the total phenols and antioxidant capacity when compared with fresh or conventionally pasteurized ones. Furthermore, Igual, et al. (2011) found that the use of microwaves led to a greater retention of individual grapefruit juice flavonoids during storage (4 and -18ºC) than when conventional heating was used.

## 4. CONCLUSIONS

Microwave heating may be considered a suitable means of processing kiwifruit puree and preserving the safety and quality of the product during storage. This technology led to a greater or equal degree not only of microbial and enzyme inactivation but also of the preservation of colour, bioactive compounds and antioxidant activity in comparison with conventional heating. Microwave pasteurized kiwifruit puree is not only exhibited a longer shelf–life (123 days at 4 ºC) than the conventionally heated one (81 days at 4 ºC), but also superior colour, vitamin C and total phenol maintenance over time. Accordingly, microwave technology might be successfully employed as an innovation tool with which to help safe, high-quality and minimally processed kiwifruit based–products reach the market.

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# Discusión general

# V. DISCUSIÓN GENERAL

La energía microondas aplicada a los procesos de transformación y conservación de alimentos ha sido objeto de numerosos estudios científicos. Desde que, en 1940, comenzó a utilizarse en el campo alimentario generó grandes expectativas, gracias a la particular forma de calentamiento asociada a esta tecnología, llegando incluso a considerarse que podría suponer un cambio radical en el campo de los tratamientos térmicos. Sin embargo, la realidad es que después de varias décadas, la explotación industrial de las microondas sigue siendo bastante escasa, en parte porque se trata de una tecnología que entraña cierta complejidad y que, además, presenta una serie de limitaciones que dificultan su aplicación por parte de la industria alimentaria. Así, para llevar a cabo una correcta implantación de esta tecnología, debe haber constancia de que las propiedades nutritivas y sensoriales de los alimentos se ven afectadas en menor medida por los tratamientos de microondas, pero también, que los niveles de seguridad microbiológica que se obtienen son equivalentes a los alcanzados con el procesado térmico convencional al que pretenden sustituir. Además, en la actualidad siguen siendo relativamente escasos los trabajos de investigación que abordan determinados aspectos relativos a la energía microondas aplicada al procesado de alimentos, tan relevantes como, por ejemplo, su impacto sobre determinados microorganismos patógenos y su cinética de inactivación, sobre propiedades organolépticas, sobre ciertos nutrientes y compuestos funcionales o sobre la vida útil de los productos procesados por microondas.

En este contexto, y con el fin de contribuir a ampliar el conocimiento acerca de la viabilidad de la utilización de las microondas en los procesos de conservación de alimentos, el trabajo de investigación que constituye la presente Tesis aborda el estudio de la aplicación de la energía microondas para pasteurizar un puré de kiwi como alternativa a un proceso de calentamiento convencional. Para ello, se evaluó el impacto de estas dos tecnologías en diversos aspectos de calidad y seguridad del producto, lo que posteriormente permitió diseñar tratamientos de pasteurización por microondas y por calentamiento convencional equivalentes. Seguidamente, se

comparó la efectividad de ambas tecnologías en la conservación del producto en base a diversos criterios.

## V.1 CARACTERIZACIÓN DEL PURÉ DE KIWI

Los purés de fruta son productos que están en consonancia con las actuales tendencias de consumo, ya que son saludables y que, tras ser mínimamente procesados, pueden comercializarse como derivados de fruta listos para consumir, seguros, estables y con un gran valor nutritivo y atractivas propiedades sensoriales. La caracterización del puré de kiwi, que supuso el primer paso de la investigación, reveló que esta fruta parece ser particularmente adecuada para elaborar productos de tales características, gracias a sus excelentes propiedades nutricionales y sensoriales y sus buenas aptitudes para el procesado. En términos generales, el puré de kiwi resultó ser un producto con un color, aroma y consistencia atractivos para los consumidores. No obstante, la evaluación de sus propiedades sensoriales indicó que de no prestarse la debida atención al equilibrio entre los sabores dulce y ácido de la fruta, la aceptabilidad del producto final puede verse considerablemente mermada. El color del puré es otro de los atributos que tiene una gran influencia sobre la opinión de los consumidores, por lo que debe tenerse muy en cuenta. La variedad de kiwi Hayward, con la que se ha trabajado en esta Tesis, posee un elevado contenido en clorofilas que le otorga un atractivo color verde brillante. Por otro lado, el puré de kiwi presenta una gran resistencia al crecimiento microbiano (Figura V.1.) y su excelente composición en nutrientes y compuestos fitoquímicos puede ser un reclamo para los consumidores. Desde el punto de vista tanto nutritivo como funcional, el kiwi es una fruta que destaca particularmente por su elevado contenido en vitamina C y su gran capacidad antioxidante, que además muestran cierta estabilidad durante el almacenamiento (Figura V.2.).

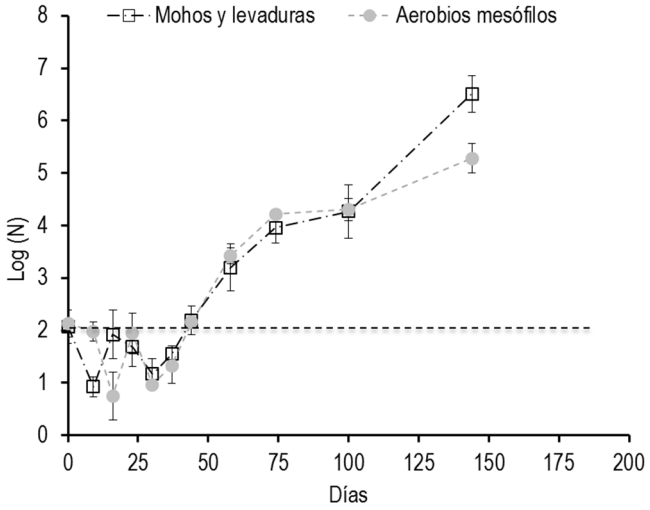


**Figura V.1.** Crecimiento de microorganismos aerobios mesófilos y mohos y levaduras en el puré de kiwi sin procesar durante el almacenamiento a 4ºC. La línea discontinua indica el contenido máximo aceptable de estos microorganismos.

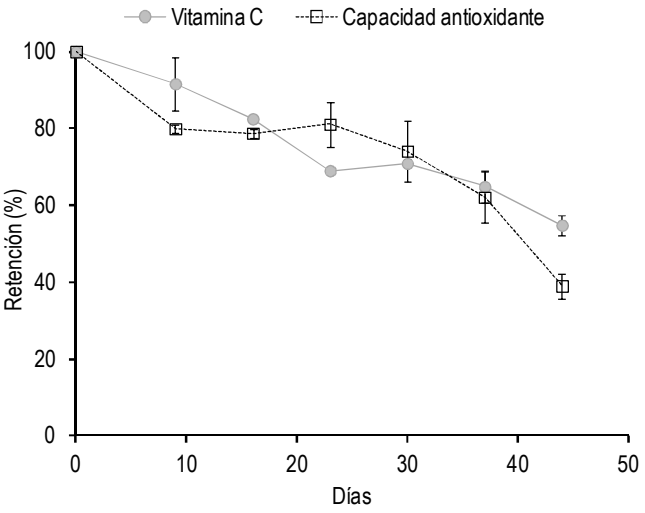


**Figura V.2.** Retención del contenido en vitamina C y capacidad antioxidante del puré de kiwi sin procesar durante su vida útil a 4ºC.

De hecho, considerando al contenido en microorganismos alterantes del producto recién preparado como un criterio en base al que definir la aceptabilidad del alimento, la vida útil de un puré de kiwi sin procesar y almacenado en refrigeración (4ºC) podría establecerse en unos 50 días (Figura V.1.). Tras este periodo de tiempo, el producto todavía retendría entorno al 55% de vitamina C y

40% de su capacidad antioxidante (Figura V.2.). Es decir, que pese a no haber recibido tratamiento alguno, un puré de kiwi almacenado durante más de mes y medio en refrigeración sería apto para el consumo, si bien es cierto, que el contenido en vitamina C y otros compuestos bioactivos no sería el propio de un producto a base de fruta de alta calidad. No obstante, la retención de tales componentes y la preservación de muchas otras características del puré podrían mejorarse fácilmente mediante el procesado, que permitiría garantizar la estabilidad de sus propiedades durante el almacenamiento.

## V.2. EFECTO DEL PROCESADO POR MICROONDAS SOBRE UN PURÉ DE KIWI. SELECCIÓN DEL TRATAMIENTO ÓPTIMO.

Uno de los objetivos de la presente Tesis fue evaluar el impacto de la energía microondas sobre diversos aspectos de calidad y seguridad del puré de kiwi, con el fin de seleccionar las condiciones de pasteurización más adecuadas. En primer lugar, se registró la temperatura del puré, en distintos puntos, durante su exposición a las microondas a varios niveles de potencia (600-1000 W), aplicadas siempre sobre la misma cantidad de muestra, y tiempos de tratamiento (30-300 s). De esta forma fue posible conocer la distribución de temperaturas del producto y ubicar el punto frío y el punto caliente bajo distintas condiciones de proceso (ver Capítulo IV.4. de Resultados, Figura 2). Tal y como era de esperar, independientemente del tratamiento recibido, las temperaturas más altas se observaron en la región externa o más próxima a la superficie del puré, concentrándose particularmente el calor generado en la zona superior de sus laterales (punto caliente), mientras que la región central del producto permaneció considerablemente más fría que el resto de puntos evaluados (punto frío). La falta de uniformidad en el calentamiento resultó más que evidente, detectándose diferencias de temperatura entre el punto más frío y el punto más caliente del orden de 20-35ºC. Todo ello se atribuyó a una capacidad de penetración limitada de las microondas hacia el interior del producto, junto a ciertos efectos geométricos que favorecen la concentración de la radiación electromagnética en determinadas zonas del alimento, como por ejemplo sus

laterales, en función de su geometría y dimensiones (Hossan et al., 2010; Oliveira & Franca, 2002; Zhang & Datta, 2005). Tanto la potencia de microondas como el tiempo de tratamiento ejercieron un impacto significativo sobre la uniformidad de calentamiento, de forma que éste fue más irregular cuanto mayor fue la potencia aplicada (mayor tasa de calentamiento) y más largo fue el tratamiento (ver Capítulo IV.4. de Resultados, Figura 2). Esto probablemente se debe a que en los procesos por microondas, los mecanismos responsables de la generación de calor y de su transferencia al resto del producto tienen lugar a escalas de tiempo totalmente distintas (Hossan et al., 2010).

Los resultados de este estudio, además de proporcionar la base a partir de la cual diseñar un tratamiento de pasteurización por microondas efectivo, indicaron claramente que la heterogeneidad del calentamiento es una limitación importante de las microondas que debe tenerse muy presente a la hora de establecer cualquier tipo de proceso basado en la aplicación de las mismas. Una de las claves del éxito del uso de esta tecnología en tratamientos de conservación de alimentos reside en la correcta selección de las condiciones de proceso que garanticen tanto la inocuidad como la calidad del producto para cada aplicación en particular, ya que, de no ser así, las ventajas asociadas a esta tecnología pueden perder sentido frente a los riesgos derivados de un calentamiento extremadamente desigual.

En base a lo anterior, se estudió la cinética de inactivación de un microorganismo patógeno con capacidad de crecimiento en el producto: *L. monocytogenes* (Figura V. 3.). Este microorganismo fue seleccionado considerando las características propias del puré de kiwi y su posible forma de consumo. Pensando que el puré sea comercializado como un alimento listo para consumir, que pueda ser ingerido sin recibir previamente ningún otro tipo de tratamiento y tras ser almacenado durante un periodo de tiempo relativamente largo en refrigeración, la presencia de *L. monocytogenes* en el mismo, patógeno que presenta una mayor resistencia frente la inactivación a bajos pH que otros microorganismos como *Escherichia coli, Salmonella enteritidis* o *Salmonella typhimurium*, podría representar un riesgo potencial para la salud de los consumidores (Gabriel & Nakano, 2009; Guerrero-Beltrán & Barboza-Cánovas, 2005).

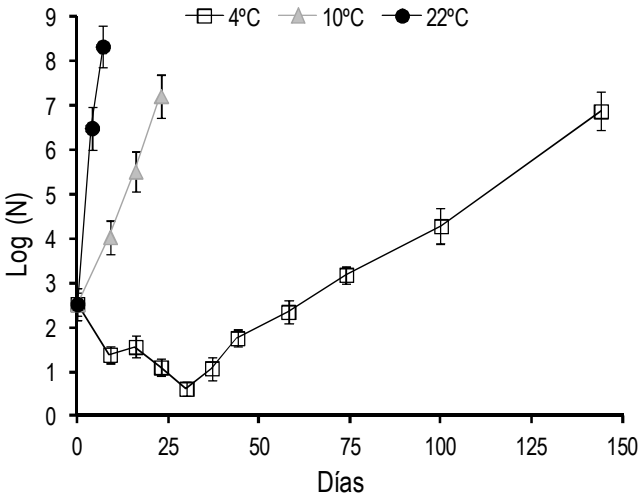


**Figura V.3.** Crecimiento de *L. monocytogenes* en el puré de kiwi sin procesar almacenado a distintas temperaturas.

Este estudio se abordó empleando dos enfoques distintos, uno determinista y otro estocástico, con fines comparativos y se llevó a cabo a varias potencias (1000, 900 y 600 W) para poder evaluar el efecto de este factor sobre la cinética inactivación de *L. monocytogenes.* Teniendo en cuenta que la Food and Drugs Admisnistration establece el objetivo de pasteurización "5D" en zumos de fruta u otros productos derivados de características semejantes (FDA, 2004), los resultados del estudio indicaron que el calentamiento por microondas es una forma efectiva de inactivar *L. monocytogenes* en puré de kiwi, llegando a reducirse al menos 5 ciclos logarítmicos de este microorganismo en las condiciones de proceso ensayadas (ver Capítulo IV.3 de Resultados, Figura 2). Por otro lado, el modelo de primer orden o lineal (modelo D-z), comúnmente utilizado en los procesos térmicos de inactivación microbiológica, describió adecuadamente la reducción de *L. monocytogenes* por microondas. Los valores de D obtenidos a partir de ambos enfoques pusieron de manifiesto el efecto significativo ($p<0,05$) del factor potencia sobre la inactivación de dicho patógeno. Además, pese a no haber detectado diferencias significativas entre las potencias de 1000 y 900 W, los resultados procedentes del estudio estocástico revelaron que 1000 W fue la potencia de microondas más apropiada para pasteurizar el puré de kiwi desde el punto de vista de la seguridad, ya que, ésta conllevó un notorio aumento de la probabilidad de

alcanzar un nivel de inactivación prefijado de *L. monocytogenes* en el punto más frío del producto (60%), respecto a la potencia de 900 W.

Seguidamente, se empleó un diseño de experimentos centrado compuesto para definir las combinaciones de potencia y tiempo idóneas para estudiar el efecto del calentamiento por microondas sobre las propiedades fisicoquímicas, funcionales y la actividad enzimática del puré de kiwi mediante el mínimo número de ensayos posible. En la Tabla V.1. se muestra la matriz del diseño experimental en la que se incluyen los resultados correspondientes a las variables respuesta estudiadas. De acuerdo con los resultados obtenidos, la aplicación de la energía microondas mostró un impacto significativo ($p<0,05$) sobre la consistencia, viscosidad, color, actividad enzimática y actividad antioxidante del producto, pero no sobre su humedad, actividad del agua, pH y contenido en sólidos solubles. La magnitud de los cambios observados fue, en todos los casos, proporcional a la intensidad de cada una de las combinaciones de potencia y tiempo empleadas. Paralelamente, un panel entrenado de catadores llevó a cabo un análisis descriptivo de las propiedades sensoriales del producto, tanto en fresco como procesado por microondas, que permitió identificar como atributos sensoriales clave del mismo el color típico de kiwi, el tono, la consistencia visual, la luminosidad y el sabor atípico. Además, dicha evaluación sensorial permitió corroborar que, al igual que sucedió con los parámetros instrumentales, los catadores percibieron cambios en las propiedades organolépticas del producto a causa del procesado por microondas, cuya magnitud fue proporcional a la intensidad de los tratamientos aplicados (ver Capítulo IV.1. de Resultados, Figura 2).

**Tabla V.1.** Matriz del diseño experimental centrado compuesto. Variables independientes ($x_i$) y promedio de los valores experimentales correspondientes a las variables respuesta ($y_i$).

| Ensayo | $x_1$ | $x_2$ | $y_1$ | $y_2$ | $y_3$ | $y_4$ | $y_5$ | $y_6$ | $y_7$ | $y_8$ | $y_9$ | $y_{10}$ | $y_{11}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1000 | 200 | 14,20 | 83,10 | 0,98 | 3,31 | 3,61 | 2,38 | 5,75 | 88,37 | 74,40 | 77,32 | 14,17 |
| 2 | 900 | 300 | 14,03 | 82,68 | 0,98 | 3,36 | 3,87 | 2,82 | 4,95 | 88,02 | 84,48 | 56,58 | 5,01 |
| 3 | 900 | 100 | 14,13 | 83,75 | 0,98 | 3,30 | 3,64 | 1,90 | 3,12 | 56,74 | 58,74 | 11,96 | 15,20 |
| 4 | 600 | 340 | 14,00 | 82,93 | 0,98 | 3,39 | 1,90 | 2,78 | 4,42 | 86,30 | 72,86 | 46,78 | 3,16 |
| 5 | 600 | 200 | 14,10 | 84,24 | 0,98 | 3,23 | 2,97 | 1,60 | 6,21 | 82,10 | 49,43 | 15,19 | 6,85 |
| 6 | 600 | 200 | 14,00 | 83,69 | 0,98 | 3,24 | 3,63 | 1,70 | 6,23 | 81,43 | 53,65 | 15,47 | 6,24 |
| 7 | 600 | 200 | 14,20 | 83,38 | 0,98 | 3,24 | 3,35 | 1,70 | 4,68 | 75,51 | 54,65 | 16,09 | 8,62 |
| 8 | 600 | 200 | 14,20 | 83,61 | 0,99 | 3,41 | 5,37 | 1,95 | 4,59 | 80,40 | 53,10 | 8,91 | 6,59 |
| 9 | 600 | 200 | 13,90 | 83,32 | 0,99 | 3,41 | 4,87 | 1,90 | 4,56 | 81,09 | 55,21 | 12,97 | 5,00 |
| 10 | 600 | 200 | 13,90 | 83,15 | 0,99 | 3,42 | 5,03 | 1,95 | 4,42 | 81,17 | 53,62 | 14,51 | 4,40 |
| 11 | 600 | 200 | 13,90 | 83,57 | 0,99 | 3,37 | 5,51 | 1,63 | 2,89 | 81,77 | 51,37 | 17,03 | 3,64 |
| 12 | 600 | 200 | 14,00 | 83,60 | 0,98 | 3,36 | 4,99 | 1,87 | 5,62 | 82,10 | 49,43 | 15,19 | 6,85 |
| 13 | 600 | 60 | 13,60 | 83,61 | 0,98 | 3,40 | 5,90 | 1,57 | 2,99 | 44,02 | 43,92 | -11,20 | 17,11 |
| 14 | 300 | 300 | 14,20 | 83,36 | 0,98 | 3,29 | 3,80 | 1,20 | 2,41 | 46,37 | 23,23 | -11,62 | 28,01 |
| 15 | 300 | 100 | 13,80 | 83,60 | 0,98 | 3,41 | 5,30 | 2,07 | 2,91 | 39,89 | 11,44 | -19,01 | 35,95 |
| 16 | 200 | 200 | 13,90 | 83,42 | 0,98 | 3,41 | 5,10 | 1,52 | 2,22 | -11,45 | -16,41 | 3,04 | 30,44 |

Donde, $x_1$: potencia de microondas (W), $x_2$: tiempo de tratamiento (s), $y_1$: sólidos solubles (g/100g), $y_2$: humedad (g/100g), $y_3$: actividad del agua, $y_4$: pH, $y_5$: distancia de avance (mm/g), $y_6$: viscosidad (Pa·s), $y_7$: diferencia de color, $y_8$: inactivación de peroxidasa (%), $y_9$: inactivación de polifenoloxidasa (%), $y_{10}$: inactivación de pectinmetilesterasa (%), $y_{11}$: variación de actividad antioxidante (%).

El estudio realizado en este sentido, permite establecer que para llevar a cabo un proceso de control de calidad de un puré de kiwi que implique una evaluación de sus propiedades ópticas y reológicas, los parámetros instrumentales más indicados son las coordenadas de color L*, a*, el ángulo de tono (h*) y el índice de blancura (WI), así como la consistencia determinada mediante un consistómetro Bostwick, al ser éstos los que mejor describen dichas propiedades del producto (ver Capítulo IV.1. de Resultados, Tabla 4).

Los resultados experimentales obtenidos para las distintas variables (Tabla V.1.) se analizaron empleando la metodología superficie respuesta, que permitió evaluar el efecto de los factores potencia de microondas ($x_1$) y tiempo de tratamiento ($x_2$) sobre las mismas. No obstante, tan sólo fue posible obtener modelos que describieran de forma significativa ($p<0,05$) el comportamiento de cuatro de las variables estudiadas, siendo éstas: inactivación de peroxidasa ($y_8$), inactivación de polifenoloxidasa ($y_9$), inactivación de pectinmetilesterasa ($y_{10}$) y variación de la actividad antioxidante ($y_{11}$). La aplicación de energía microondas implicó una reducción de la actividad enzimática del puré de kiwi, mientras que promovió la actividad antioxidante del mismo. Los modelos de regresión obtenidos indicaron que: por un lado, tanto una potencia de microondas más alta como un mayor tiempo de tratamiento implicaron un aumento significativo ($p<0,05$) de la inactivación enzimática (peróxidasa, polifenoloxidasa y pectinmetilesterasa), no obstante, el peso que tuvo cada uno de estos factores ($x_1$, $x_2$) sobre dicha inactivación varió en función de la enzima estudiada (ver Capítulo IV.2. de Resultados, Figura 1-3) y por el otro, el aumento de la actividad antioxidante fue significativamente ($p<0,05$) mayor cuanto menor fue la intensidad del tratamiento, es decir, cuando se empleó una potencia de microondas más baja y el tiempo de tratamiento fue menor (ver Capítulo IV.2. de Resultados, Figura 4). Con esta información y a partir de los modelos de regresión previamente obtenidos, se predijeron las condiciones de proceso más adecuadas para obtener un puré de kiwi estable desde el punto de vista enzimático (90% de inactivación de enzima peroxidasa, Gonçalves et al., 2010), pero sin que su valor funcional se viera comprometido, resultando ser la combinación de potencia y tiempo idónea: 1000 W-340 s. Adicionalmente, sin perder de vista el objetivo de establecer un proceso de

pasteurización por microondas, se estudió la distribución de temperaturas de la muestra sometida a este tratamiento y se comprobó la efectividad del mismo para inactivar el microorganismo patógeno de referencia. Los resultados mostraron que el tratamiento óptimo redujo 5 ciclos logarítmicos de *L. monocytogenes* en el punto más frío del puré a un 99,9% de probabilidad.

## V.3. EFECTO DEL PROCESADO POR CALENTAMIENTO CONVENCIONAL SOBRE UN PURÉ DE KIWI. SELECCIÓN DEL TRATAMIENTO EQUIVALENTE AL ÓPTIMO ESTABLECIDO PARA EL TRATAMIENTO POR MICROONDAS

El siguiente pasó consistió en establecer un tratamiento térmico convencional que fuera equivalente al tratamiento óptimo de microondas en cuanto a la inactivación de la enzima peroxidasa (90% de inactivación) y que además cumpliera el objetivo de pasteurización (5D). Esta enzima se empleó como indicador de la efectividad del tratamiento, ya que, pese a no ser la más termorresistente en el kiwi, es la que puede contribuir de forma más significativa a deteriorar la calidad mismo (Llano et al., 2003). Para ello se definieron distintas combinaciones de temperatura y tiempo (Tabla V.2.) elegidas en base a referencias bibliográficas, y se evaluó la efectividad de las mismas en la inactivación de peroxidasa, resultando ser la combinación 97ºC-30s (150 s de come-up-time) el tratamiento seleccionado.

**Tabla V.2.** Promedio (y desviación estándar) de los valores de inactivación de la enzima peroxidasa en el puré de kiwi sometido a diferentes combinaciones de temperatura y tiempo.

| Temperatura (ºC) | Tiempo (s) | Inactivación de peroxidasa (%) |
|---|---|---|
| 90 | 30 | 74 (3) |
| 90 | 60 | 80,1 (1,4) |
| 95 | 30 | 78 (5) |
| 95 | 45 | 86,2 (0.2) |
| 97 | 30 | 90 (2) |

Una vez hecho esto, se comprobó la efectividad de dicho tratamiento para inactivar el microorganismo *L. monocytogenes* (>5D).

## V.4. COMPARACIÓN DE LA EFECTIVIDAD DE LA TECNOLOGÍA MICROONDAS Y DEL CALENTAMIENTO CONVENCIONAL EN LA CONSERVACIÓN DE UN PURÉ DE KIWI.

A partir de este momento, se llevaron a cabo diferentes estudios que, de una forma u otra, permitieron comparar el impacto de ambas tecnologías en varios aspectos relacionados con la calidad y la seguridad del producto. Si bien es cierto que existen numerosos trabajos que al comparar ambas tecnologías ensalzan los beneficios de las microondas, también son frecuentes los estudios que no tienen en cuenta la posible equivalencia de los tratamientos sujetos a comparación. Además, en aquellos casos en los que se diseñan tratamientos comparables, la equivalencia de los mismos se establece en base a una serie de criterios tan diversos como, por ejemplo, fijar tasas de calentamiento similares o equiparar las temperaturas inicial y final de tratamiento, sin tener en cuenta el perfil térmico completo del producto. Todo ello, sin duda alguna, dificulta la posibilidad de contrastar los resultados obtenidos por los distintos autores y además, puede conducir a conclusiones erróneas sobre la superioridad de una u otra tecnología (Banik et al. 2003). Comparar adecuadamente dos tecnologías térmicas cuyos respectivos mecanismos de calentamiento presentan diferencias considerables, como es el caso de la energía microondas y el calentamiento convencional, puede resultar una tarea compleja. Teniendo en cuenta toda esta problemática, en la presente Tesis se plantearon las siguientes vías de comparación:

- *Comparación establecida a nivel de los parámetros cinéticos obtenidos por microondas y por calentamiento convencional*

Los estudios cinéticos son una forma factible de abordar la comparativa entre dos tecnologías térmicas, ya que, un correcto manejo de los datos durante la etapa de modelización matemática permite obtener parámetros cinéticos comparables

(Latorre et al., 2012; Matsui et al., 2008; Tajchakavit & Ramaswamy, 1997; Tajchakavit et al., 1998). Como se ha mencionado anteriormente, se utilizó un modelo de primer orden o lineal para describir la cinética de inactivación de *L. monocytogenes* por microondas (600, 900 y 1000 W) y por calentamiento convencional (50, 55 y 60 ºC) (Tabla V.3).

**Tabla V.3.** Promedio (y error estándar) de los valores de tiempo de reducción decimal de *L. monocytogenes,* a una temperatura de referencia de 60ºC ($D_{60ºC}$), obtenidos en el puré de kiwi procesado por calentamiento convencional y por microondas, a distintos niveles de potencia.

| | **Microondas** | | | **Calentamiento convencional** |
|---|---|---|---|---|
| | *1000 W* | *900 W* | *600 W* | |
| **$D_{60ºC}$ (s)** | 17,0 (0,3) | 17,4 (0,3) | 42,85 (0,13) | 37 (3) |

Al contrastar los valores de D obtenidos para el calentamiento por microondas y el calentamiento convencional a una temperatura de referencia común (60ºC) se observó claramente que el factor potencia de microondas ejerce una marcada influencia sobre la efectividad de esta tecnología en los procesos de inactivación. Cuando se trabajó a 900 y 1000 W quedo patente la superioridad de las microondas para inactivar al microorganismo estudiado. Sin embargo, se observó justo lo contrario cuando la potencia de microondas seleccionada fue 600 W. Teniendo en cuenta que la mayor efectividad de las microondas parece atribuirse a que éstas potencian o magnifican de forma significativa los efectos asociados a la energía térmica, resulta lógico pensar que la exposición del alimento a una menor tasa de radiación electromagnética (potencias de microondas bajas) podría conllevar una reducción de la efectividad de esta tecnología para inactivar microorganismos.

- *Comparación establecida a nivel del cálculo de letalidad asociada a distintos procesos por microondas y por calentamiento convencional equivalentes*

En la presente Tesis se propuso la aplicación del concepto de letalidad acumulada como una herramienta de comparación. Se estudió la viabilidad del concepto unidad de pasteurización (UP), inicialmente propuesto por Shapton et al.

(1971), para estandarizar los tratamientos térmicos de pasteurización, con el fin de cuantificar la intensidad o severidad de los procesos basados en la aplicación de microondas en comparación con los tratamientos térmicos convencionales, en términos de carga térmica. Para ello, se calculó y comparó la carga térmica asociada a diversos tratamientos por microondas (1000 W-200 s y 900 W-225 s) y un tratamiento convencional de pasteurización (97 ºC-30 s), todos ellos diseñados para causar una inactivación de la enzima peroxidasa equivalente. Los resultados obtenidos revelaron que las unidades de pasteurización no sólo resultaron ser una buena herramienta para comparar la efectividad de las microondas y el calentamiento convencional, que además podría ser de gran utilidad a la hora de evaluar cualquier otra tecnología de carácter térmico, sino que permitió demostrar la superioridad de las microondas para inactivar enzimas, ya que se observó que cuando el puré se procesó por calentamiento convencional, fue necesaria una carga térmica significativamente ($p<0.05$) mayor para inactivar un 90% de peroxidasa (ver Capítulo IV.5. de Resultados, Tabla 1). Estos datos apuntaron nuevamente a la posibilidad de que la energía microondas magnifique los efectos asociados a la energía térmica.

*Comparación a nivel del impacto causado sobre la calidad y seguridad del puré de kiwi por un tratamiento de pasteurización por microondas y un tratamiento de pasteurización por calentamiento convencional equivalentes*

Se planteó un estudio comparativo del impacto causado por la tecnología microondas y el calentamiento convencional sobre las propiedades fisicoquímicas, enzimáticas, microbiológicas y sensoriales del puré de kiwi, sobre su contenido en compuestos bioactivos y actividad antioxidante, su contenido en pigmentos y bioaccesibilidad de carotenoides, así como sobre la vida útil del mismo y la estabilidad de dichas propiedades durante la etapa de almacenamiento, en base a dos tratamientos de pasteurización equivalentes, en términos de inactivación de enzima peroxidasa (90%): el tratamiento óptimo de microondas (1000 W-340 s) y el tratamiento convencional previamente seleccionado (97 ºC-30 s).

En primer lugar, se apreció que, independientemente de la tecnología empleada para procesar el producto, y a diferencia del resto de las propiedades

evaluadas, la humedad, pH, contenido en sólidos solubles y actividad del agua del puré no sufrieron modificación alguna tras la pasteurización. Ambos tratamientos, sin embargo, conllevaron un aumento de la viscosidad y consistencia del producto y una reducción de su contenido en pigmentos, que dio lugar a cambios de color perceptibles en el mismo. Estas variaciones, a su vez, se vieron reflejadas en las propiedades organolépticas del puré. Los consumidores percibieron diferencias entre el puré de kiwi fresco y el pasteurizado en base a los atributos sensoriales relacionados con su color y consistencia, entre otros, lo que conllevó una disminución de su aceptabilidad. También se observaron pérdidas del valor nutritivo y funcional del puré a consecuencia del procesado, así como la inactivación de los microorganismos y enzimas presentes en el mismo (ver Capítulo IV.6. de Resultados, Tabla 1-3), hecho que permitió alargar su vida útil y estabilizar sus propiedades durante el almacenamiento.

Cuando se comparó la efectividad del tratamiento por microondas y el tratamiento por calentamiento convencional a la hora de garantizar la seguridad y la estabilidad del puré de kiwi, quedó constancia de la superioridad de las microondas, ya que al pasteurizar el producto mediante esta tecnología fue posible alcanzar niveles de inactivación significativamente ($p<0,05$) superiores, tanto de enzimas (polifenoloxidasa y pectinmetilesterasa) como de microorganismos alterantes (aerobios mesófilos totales y mohos y levaduras), que cuando se aplicó el tratamiento térmico convencional, mientras que ambos procesos resultaron ser equivalentes en términos de inactivación de *L. monocytogenes* (ver Capítulo IV.6. de Resultados, Tabla 2). Como era de esperar, la mayor efectividad de la tecnología microondas para inactivar tanto microorganismos como enzimas dio lugar a un producto procesado más estable. Como resultado, el puré de kiwi pasteurizado por microondas presentó un vida útil más larga (123 días a 4ºC) que el puré pasteurizado de forma convencional (83 días a 4ºC) y, en términos generales, se observó una menor variación de las propiedades del mismo durante el almacenamiento, dando lugar a un producto, sencillamente, de mayor calidad.

Con el fin de despejar cualquier duda acerca de que la superioridad de las microondas pueda venir determinada por las condiciones de proceso empleadas en

el estudio, es decir, que la mayor estabilidad enzimática y microbiológica asociada a esta tecnología pueda deberse, de algún modo, a que el tratamiento de microondas sea más intenso que el tratamiento convencional, se examinó el perfil térmico del producto durante los dos procesos (Figura V.4.) y se calculó el valor de las unidades de pasteurización correspondiente a cada uno de ellos. Los resultados obtenidos fueron: $UP_{80ºC}$=19 (2) y $UP_{80ºC}$=19,27 (0,13) min para el puré pasteurizado por microondas y por tratamiento convencional, respectivamente. De esta forma, fue posible demostrar que cuando el puré de kiwi se procesó por microondas, éste no recibió una mayor carga térmica que cuando se pasteurizó por calentamiento convencional. Obviamente, otra prueba de ello reside en el impacto causado por ambos tratamientos sobre el resto de las propiedades del producto, ya que, en caso de que el tratamiento de microondas hubiera sido más severo, cabría esperar un mayor deterioro de la calidad del puré cuando fue procesado mediante esta tecnología. Sin embargo, los resultados obtenidos en la presente Tesis indicaron que el impacto del tratamiento de microondas sobre diversas propiedades del puré de kiwi resultó ser siempre menor a aquel asociado al tratamiento convencional, tal y como se comenta en mayor profundidad a continuación.

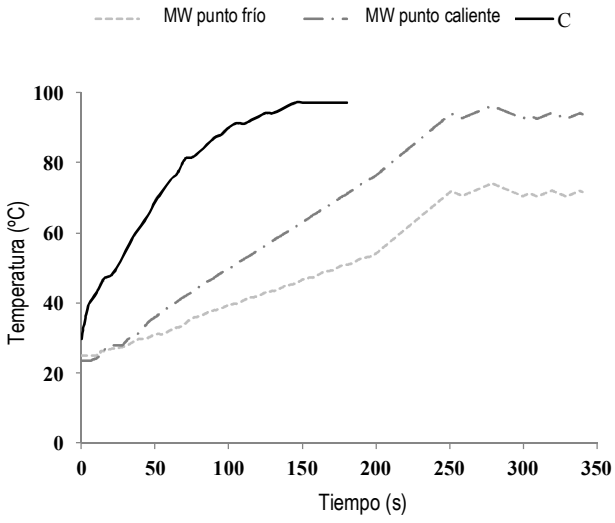


**Figure V.4.** Perfil de temperatura del puré de kiwi sometido al tratamiento óptimo de microondas (1000 W-340 s) y el tratamiento térmico convencional equivalente (97 ºC-30 s).

En lo referente a las propiedades organolépticas del producto, se llevó a cabo un estudio de consumidores que consistió en determinar la aceptabilidad sensorial del puré de kiwi, sin procesar y pasteurizado mediante las dos tecnologías objeto de estudio, además de identificar qué atributos del producto deberían ser modificados y en qué sentido deberían de producirse tales modificaciones, para incrementar su aceptación. Si bien es cierto que ninguna de las muestras presentó una aceptabilidad particularmente elevada (45-64%), el análisis de penalización permitió relacionar tales resultados con la excesiva acidez y escaso dulzor de la fruta empleada como materia prima, quedando constancia por tanto, de la particular relevancia del equilibro entre los sabores ácido y dulce a la hora de obtener productos derivados de kiwi (ver Capítulo IV.6. de Resultados, Figura 3), aspecto que, en cualquier caso, no guarda ningún tipo de relación con el tratamiento de preservación aplicado o con la tecnología empleada. Así mismo, se observó que aunque los dos tratamientos aplicados conllevaron cambios perceptibles en sus atributos sensoriales, que implicaron una reducción de su aceptabilidad, cuando el puré de kiwi se pasteurizó mediante la aplicación de microondas se logró una mayor aceptación del mismo por parte de los consumidores, ya que, en términos generales se preservaron en mayor medida sus propiedades sensoriales (ver Capítulo IV.6. de Resultados, Tabla 1).

En consonancia con estos resultados, la evaluación de las propiedades físicas, nutricionales y funcionales del producto pusieron de manifiesto que la aplicación de microondas permitió obtener un puré de kiwi pasteurizado más semejante al fresco. Tal y como se puede ver en la Tabla V.4. el tratamiento por microondas causó pérdidas significativamente menores de la actividad antioxidante, contenido en vitamina E, contenido en vitamina C y compuestos fenólicos (flavonoides totales y fenoles totales) del puré que el tratamiento convencional. Por otro lado, si bien es cierto, que el tratamiento por microondas resultó en un producto más viscoso, aseguró una mejor preservación del color (Tabla V.4.).

Más concretamente, a la hora de estudiar el impacto del procesado sobre el color del puré de kiwi, se siguieron dos enfoques: por un lado, se analizaron sus

coordenadas colorimétricas (espacio de color CIEL*a*b*) y por el otro, su contenido en pigmentos (clorofilas y carotenoides).

**Tabla V.4.** Valor medio (y desviación estándar) de las diferencias de color (ΔE), viscosidad (Pa·s) y las pérdidas (%) causadas por el tratamiento de pasteurización de microondas y el tratamiento convencional en la actividad antioxidante y contenido de algunos compuestos bioactivos y pigmentos en el puré de kiwi

| | **Microondas** | **Calentamiento convencional** |
|---|---|---|
| ΔE | 7,06 (0,02)[a] | 7,54 (0,02)[b] |
| Viscosidad | 2,31 (0,06)[b] | 1,98 (0,08)[a] |
| Vitamina E | 10 (4)[a] | 83 (2)[b] |
| Vitamina C | 1,2 (1,0)[a] | 26,7 (0,13)[b] |
| Flavonoides totales | 36 (5)[a] | 51 (2)[b] |
| Fenoles totales | 21 (2)[a] | 28,8 (1,0)[b] |
| Actividad antioxidante | 65,7 (1,0)[a] | 78 (4)[b] |
| Carotenoides totales | 67 (7)[a] | 67 (7)[a] |
| Clorofilas totales | 64 (5)[a] | 98 (3)[b] |

*Diferentes letras (a, b) indican distintos grupos homogéneos ($p<0,05$).

De acuerdo con los resultados obtenidos, la presencia de clorofilas otorga al producto un color verde brillante muy apreciado por los consumidores, sin embargo, la rápida conversión de estos pigmentos en feofitinas puede alterar en gran medida el color original del puré de kiwi, dificultando considerablemente las etapas de procesado y almacenamiento del mismo. Además, se observó que aunque ambos tratamientos causaron una reducción similar del contenido en carotenoides totales del producto, hubo una menor degradación de las clorofilas cuando éste se procesó mediante la aplicación de microondas, que cuando la pasteurización se llevó a cabo por calentamiento convencional, hecho que, a su vez, se tradujo en una menor variación de las coordenadas colorimétricas del puré (Tabla V.4.). Sin embargo, ni la etapa de pasteurización, independientemente de la tecnología empleada, ni el posterior almacenamiento, mostraron efecto significativo alguno sobre la bioaccesibilidad de los carotenoides en el producto.

En lo que a la etapa de almacenamiento respecta, tanto el color como el contenido en compuestos bioactivos del producto sufrieron ciertas modificaciones.

En términos generales, la pasteurización permitió garantizar una mayor estabilidad de la actividad antioxidante, luminosidad y compuestos bioactivos del puré de kiwi durante el almacenamiento (menores valores de las constantes cinéticas de degradación) (ver Capítulo IV.8. de Resultados, Tabla 2), a excepción de los compuestos fenólicos (Tabla V.5.). En todos los casos, el efecto positivo del procesado fue mayor cuando el producto se pasteurizó mediante la aplicación de energía microondas.

**Tabla V.5.** Valor medio (y error estándar) de la constante cinética de degradación de fenoles totales ($mg \cdot 100g^{-1} \cdot día^{-1}$) en el puré de kiwi fresco, pasteurizado por microondas y pasteurizado por calentamiento convencional durante el almacenamiento a 4 y 10ºC.

| | Fresco | Microondas | Calentamiento convencional |
|---|---|---|---|
| **4ºC** | 0,114 (0,012) | 0,23 (0,02) | 0,19 (0,02) |
| **10ºC** | 0,196 (0,013) | 0,40 (0,07) | 0,27 (0,02) |

Teniendo en cuenta que este tipo de compuestos actúan como sustrato de la enzima polifenoloxidasa, esta observación podría estar relacionada con el hecho de que la inactivación de dicha enzima a causa del tratamiento de microondas y del tratamiento convencional resultó ser reversible (ver Capítulo IV.8. de Resultados, Figura 5). Según fuentes bibliográficas, la actividad de la enzima polifenoloxidasa ejerce un escaso efecto sobre la calidad del kiwi, hecho que en parte, se atribuye a que su alto contenido en vitamina C puede prevenir la oxidación de gran parte de los fenoles presentes en el mismo (Fúster et al., 1994, Okuse et al., 1981). Sin embargo, los resultados obtenidos en la presente Tesis revelan un efecto perjudicial notorio de esta enzima sobre los compuestos fenólicos del puré. Por tanto, se considera que tanto la inactivación de la enzima polifenoloxidasa como los fenómenos de reactivación asociados a la misma son factores a tener muy en cuenta a la hora de procesar y comercializar productos a base de kiwi de alta calidad, en los que se asegure una óptima preservación de su valor funcional, ya que, de lo contrario, su contenido en fenoles totales puede verse seriamente reducido (45-58%) a lo largo de la vida útil del producto.

Por otro lado, los cambios de color comúnmente observados en los productos a base fruta durante el almacenamiento se explican fundamentalmente a través de dos vías: pardeamiento enzimático y cambios en el contenido y distribución de los pigmentos en el producto. Con el fin de evaluar cuál de estos dos mecanismos jugó un papel más determinante en la alteración del color del puré de kiwi durante el almacenamiento, se llevó a cabo un análisis de correlación (correlación de Pearson) entre el contenido en compuestos fenólicos y pigmentos y las coordenadas colorimétricas tanto del producto fresco como de aquel sometido a ambos procesos de pasteurización (Tabla V.6.). Los datos obtenidos señalaron a la degradación de pigmentos, particularmente la pérdida de clorofilas, como la principal causa de los cambios de color observados a lo largo de la vida útil del puré de kiwi. Teniendo en cuenta que, como se ha mencionado anteriormente, la energía microondas causó menores pérdidas de clorofilas durante la pasteurización que el calentamiento convencional, y que además, aseguró una mayor estabilidad de las mismas durante el almacenamiento (ver Capítulo IV.7. de Resultados, Tabla 1 y Tabla 2), la aplicación de la tecnología microondas parece ser la apuesta más segura en vistas a elaborar y comercializar un puré de kiwi listo para consumir con un color más atractivo y estable.

**Tabla V.6.** Coeficientes de correlación de Pearson entre algunos parámetros de color y el contenido en pigmentos y fenoles totales en el puré kiwi fresco y pasteurizado, por calentamiento convencional y por microondas.

| | L* | a* | ΔE |
|---|---|---|---|
| **Clorofilas totales** | 0,506 | 0,826 | 0,842 |
| **Carotenoides totales** | - | 0,890 | - |
| **Fenoles totales** | - | 0,500 | 0,582 |

*Correlación significativa para p<0,05

En términos generales, todos los resultados obtenidos a partir de la comparación establecida entre los dos tratamientos de pasteurización equivalentes apuntaron a una clara superioridad de la tecnología microondas frente al calentamiento convencional para preservar la calidad del puré de kiwi, garantizar su seguridad y alargar su vida útil. Nuevamente, la posibilidad de que la energía microondas

magnifique los efectos asociados a la energía térmica parece ser la explicación más factible para justificar este hecho.

Por tanto, se llegó a la conclusión de que, independientemente de cuál fuera la vía empleada para establecer una comparación entre la tecnología microondas y el calentamiento convencional, la aplicación de las microondas a los procesos de pasteurización de productos a base de fruta ofrece numerosas ventajas, garantizando una inactivación microbiológica y enzimática equivalente o superior y preservando en mayor medida las propiedades nutritivas, funcionales y sensoriales del producto. Todo ello podría traducirse en que la aplicación de la energía microondas de alguna manera contribuya de forma positiva a potenciar la comercialización de productos procesados a base de fruta, como por ejemplo alimentos de conveniencia y listos para consumir obtenidos a partir de kiwi, que cumplan con las recientes expectativas de los consumidores, en cuanto a una mayor calidad y semejanza al alimento fresco, sin que, en ningún caso, la innocuidad de los mismos se vea comprometida.

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# Conclusiones

# VI. CONCLUSIONES

A continuación se exponen las principales conclusiones obtenidas del estudio realizado.

- La energía microondas dio lugar a un calentamiento heterogéneo del puré de kiwi, ubicándose el punto más frío en la zona central del producto y el punto más caliente en la zona superior de sus laterales. La distribución de temperaturas dependió, en gran medida, de las condiciones de potencia y tiempo en las que se llevó a cabo el proceso, por lo que el impacto negativo de la falta de uniformidad puede verse reducido mediante un adecuado diseño del tratamiento.

- El calentamiento por microondas resulta efectivo frente a la inactivación de las enzimas peroxidasa (90-95%), polifenoloxidasa (83%) y pectinmetilesterasa (89%), de *Listeria monocytogenes* (> 5 reducciones logarítmicas) y de la flora alterante mesófila, hongos y levaduras propios del puré de kiwi (valores de recuento post tratamiento inferiores a 1 ciclo logarítmico), alcanzándose mayores niveles de inactivación, en todos los casos, al aumentar la potencia de microondas y el tiempo de tratamiento.

- Las coordenadas de color L* (luminosidad) y a* (índice de rojo-verde), el tono, el índice de blancura y la consistencia medida mediante un consistómetro Bostwick fueron los parámetros instrumentales más adecuados para llevar a cabo el control de calidad rutinario de un puré de kiwi, tanto fresco como procesado por microondas, al ser éstos los que mejor describen la percepción sensorial del producto.

- Los modelos cinéticos son herramientas útiles que permiten predecir el comportamiento de la propiedad evaluada. En este caso, la inactivación de *Listeria monocytogenes* por microondas y por calentamiento convencional y los cambios observados durante el almacenamiento en cuanto a la luminosidad, actividad antioxidante, vitamina C, compuestos fenólicos y carotenoides del puré, tanto fresco como procesado por ambas tecnologías, se describieron

adecuadamente mediante un modelo de primer orden. Sin embargo, las clorofilas mostraron una cinética de degradación de segundo orden. La dependencia de las constantes cinéticas de inactivación (D) y de degradación (k) obtenidas con respecto a la temperatura del producto durante el procesado y el almacenamiento se ajustaron de manera correcta al modelo de Bigelow (z) y la ecuación de Arrhenius ($E_a$), respectivamente.

- Un diseño experimental centrado compuesto permitió estudiar el efecto de los factores potencia de microondas y tiempo de tratamiento sobre distintas variables de calidad del puré de kiwi. Se observó una dependencia lineal de la enzima pectinmetilesterasa respecto a los factores estudiados, mientras que se requirieron de modelos cuadráticos para describir el comportamiento de las enzimas peroxidasa y polifenoloxidasa y de la actividad antioxidante. Este diseño permitió además obtener una combinación óptima de potencia y tiempo (1000 W-340 s) que maximizó la inactivación enzimática y minimizó el deterioro de sus propiedades funcionales.

- Un enfoque estocástico en la modelización de la inactivación de *Listeria monocytogenes* permitió corroborar la inocuidad (5 reducciones logarítmicas) en el punto frío del producto tras ser sometido al tratamiento de microondas previamente establecido como óptimo con un 99,9% de probabilidad.

- En base al nivel de inactivación de peroxidasa alcanzado, enzima que resultó ser un buen indicador de la efectividad del proceso, se estableció un tratamiento convencional (97ºC-30s), equivalente al óptimo seleccionado por microondas, con fines comparativos.

- Al establecer la comparación entre tecnologías en base a la cinética de inactivación de *L. monocytogenes*, el procesado por microondas resultó ser más efectivo que el calentamiento convencional, dando lugar a un menor tiempo de reducción decimal (D) cuando éste se llevó a cabo a una potencia de microondas, como mínimo, de 900 W.

- Al establecer la comparación entre tecnologías en base a la carga térmica recibida por el producto, el cálculo de las unidades de pasteurización (UP) permitió demostrar la mayor efectividad de las microondas, al requerirse de un menor valor de UP para alcanzar un nivel de inactivación de peroxidasa equivalente cuando el producto se procesó mediante la aplicación de esta tecnología que cuando se sometió al calentamiento convencional.

- Al establecer la comparación entre tecnologías en base a tratamientos de pasteurización equivalentes (1000 W-340 s y 97 ºC-30s) quedó constancia de la superioridad de las microondas para preservar la calidad y la seguridad del producto.

- La pasteurización por microondas causó una menor alteración de las propiedades organolépticas del puré, resultando en una mayor aceptabilidad sensorial del mismo y afectó de forma similar a su consistencia que el tratamiento térmico convencional. El resto de propiedades fisicoquímicas del producto evaluadas no se vieron afectadas por el proceso de pasteurización, independientemente de la tecnología empleada.

- La pasteurización por microondas dio lugar a niveles de inactivación de *L. monocytogenes* similares, una mayor inactivación de las enzimas polifenoloxidasa y pectinmetilesterasa y de la flora alterante del puré de kiwi y una mayor estabilidad tanto microbiológica como enzimática durante el almacenamiento que el procesado térmico convencional, resultando la vida útil de puré pasteurizado por microondas más larga (123 días a 4 ºC).

- La pasteurización por microondas, aunque afectó de forma similar al contenido en vitamina A del producto, causó menores pérdidas de la actividad antioxidante, contenido en vitamina C y E y compuestos fenólicos del mismo y, en general, durante el almacenamiento, dio lugar a una degradación de su valor nutricional (vitamina C) y funcional (actividad antioxidante) menor o igual que la pasteurización convencional.

- La pasteurización por microondas afectó de forma similar a los carotenoides del producto que el tratamiento térmico convencional, pero permitió preservar en mayor medida su contenido en clorofilas, tanto tras la etapa de procesado como durante el almacenamiento, dando lugar, por tanto, a un puré de kiwi procesado con un color no sólo más similar al propio del producto fresco sino también más estable. La bioaccesibilidad de los carotenoides en el puré no se vio afectada ni por el procesado ni por el posterior almacenamiento.

- A modo de conclusión general, la energía microondas mostró una clara superioridad frente al calentamiento convencional para preservar la seguridad y la calidad de un puré de kiwi, tras el procesado y a lo largo de su vida útil, hecho que parece estar relacionado con una magnificación del efecto de la energía térmica a causa de la exposición del alimento a la radiación electromagnética (microondas). Por ello, se recomienda la aplicación de la tecnología microondas como una alternativa interesante al calentamiento convencional a la hora de pasteurizar un puré de kiwi, así como de otras frutas de características similares, que puede contribuir a ampliar la gama de productos a base de fruta de alta calidad disponibles en el mercado, que cumplan con las más recientes exigencias de los consumidores.

The main conclusions reached are exposed as follows:

- Microwaves gave rise to non-uniform heating of the kiwifruit puree, with the coldest and the hottest spots being located at its central region and its edges, respectively. The temperature distribution of the product markedly varied with the microwave power level and processing time. Therefore, the negative impact of uneven heating may be reduced if the most suitable processing conditions are selected.

- Microwave heating causes effective inactivation of peroxidase (90-95%), polyphenoloxidase (83%) and pectinmethilesterase (89%) enzymes, *Listeria monocytogenes* (>5 $\log_{10}$-cycles) and total mesophilic bacteria, yeasts and moulds (with counting values below 1 $\log_{10}$-cycle after processing). In all cases, the higher the power level and the longer the treatment time, the greater the inactivation achieved.

- The colour parameters L* (luminosity), a* (green-red scale), tone and whiteness index as well as flow distance measured with a Bostwick consistometer were found to be the most appropriate parameters for quality control of fresh and processed kiwifruit puree, given that, they showed the most significant and meaningful correlations with the sensory perception of the product.

- Kinetic modelling is a useful tool to predict the behaviour of the property investigated. In this study, microwave and conventional heating inactivation of *L. monocytogenes*, as well as, changes observed during storage in luminosity, antioxidant activity and content of vitamin C, phenolic compounds and carotenoids of the fresh and treated kiwifruit puree, irrespective of the heating technology, were properly described by first-order-kinetic models. The dependence of the kinetic rate constants obtained (D-value, k) on the processing and storage temperature was appropriately explained by the Bigelow's model (z) and Arrhenius' equation ($E_a$), respectively.

- A central composite experimental design was used to study the effect of power level and treatment time on several quality attributes of the kiwifruit puree. A linear relationship between pectinmethilesterase inactivation and the aforementioned processing factors was observed, whilst quadratic models were required to describe the behaviour of peroxidase and polyphenoloxidase enzymes and antioxidant activity. This experimental design allowed to select an optimum combination of microwave power level and time (1000 W-340 s) that gave rise to a maximum level of enzyme inactivation and minimum degradation of the functional value of the product.

- The stochastic approach used to address the inactivation of L. monocytogenes allowed to confirm the safety (reduction of 5 $\log_{10}$-cycles) of the product in the coldest spot after having been subjected to the optimum microwave treatment previously selected, with a probability level of 99.9%.

- On the basis of the level of peroxidase inactivation reached, which turned out to be a suitable indicator of treatment efficiency, a conventional thermal treatment (97 ºC-30 s), equivalent to the optimum microwave treatment previously established, was set.

- The comparison established between both technologies, when based on the inactivation kinetics of *L. monocytogenes*, pointed out greater effectiveness of microwave processing over conventional heating, with microwaves leading to lower decimal reduction times (D-values), as long as the operating power level was set, at least, at 900 W.

- The comparison between both technologies, when based on the thermal load of the processed product, was established by calculating the corresponding pasteurization units (PU), which proved greater effectiveness of microwaves, given that, a lower value of PU was required to reach an equivalent level of peroxidase inactivation in the product heated by means of microwaves than in the puree subjected to conventional heating.

- The comparison between both technologies, when based on equivalent pasteurisation treatments (1000 W-340 s and 97 ºC-30 s), highlighted superiority of microwaves to preserve safety and quality of the product.

- Microwave pasteurisation better preserved the sensory properties of the kiwifruit puree, giving rise to a processed product with greater acceptability, while the impact of this process on its consistency was similar to that of the conventional thermal process. All other physicochemical properties studied in the product were found to be unaffected by the pasteurisation step, irrespective of the processing technology.

- Microwave pasteurisation caused a similar reduction of L. monocytogenes, greater inactivation of polyphenolxidase and pectinmethilesterase enzymes and the spoiling flora of the kiwifruit puree and led to superior microbial and enzymatic stability during storage than the conventional thermal treatment, with the microwave process giving rise to a product with a longer shelf-life (123 days at 4ºC).

- Although microwave pasteurisation had a similar impact on the vitamin A content of the product than the conventional heat treatment, it caused lower loss of antioxidant activity and the content of vitamin C and E and phenolic compounds. Additionally, on the whole, it gave rise to lower or similar degradation of the nutritional (vitamin C) and functional value (antioxidant activity) of the pure during storage.

- Microwave pasteurisation presented a similar effect on carotenoids of the product than the conventional process, but it permitted a better preservation of the chlorophylls content, after the processing step and during storage, with microwaves, therefore, leading to a pasteurised product with not only more similar colour to that of the fresh puree, but also with greater stability. Bioaccessibility of carotenoids in the kiwifruit puree was unaffected by processing or storage.

- As a general conclusion, microwave energy showed clear superiority over conventional heating to preserve quality and safety of a kiwifruit puree, after processing and during storage, fact that might indicate the possibility of some enhanced effects associated with microwaves. Accordingly, microwave technology is proposed as an interesting alternative to conventional heating in order to pasteurise a kiwifruit puree or other fruit puree with similar characteristics, which can contribute to increase the range of high-quality fruit-based products that reach the market and are likely to meet new consumers' expectations.

# Anexos

# VII. ANEXOS

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ORIGINAL PAPER

## Effects of Microwave Heating on Sensory Characteristics of Kiwifruit Puree

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**Abstract** The effect of microwave processing on the characteristics of kiwifruit puree was evaluated by applying various gentle treatments. Different combinations of microwave power/processing time were applied, with power among 200–1,000 W and time among 60–340 s, and various sensory and instrumental measurements were performed with the aim of establishing correlations and determining which instrumental parameters were the most appropriate to control the quality of kiwi puree. The water and soluble solids of the product, 83 and 14/100 g sample, respectively, did not change due to treatments. For sensory assessment, an expert panel was previously trained to describe the product. Fourteen descriptors were defined, but only the descriptors 'typical kiwifruit colour', 'tone', 'lightness', 'visual consistency' and 'typical taste' were significant to distinguish between kiwifruit puree samples. The instrumental analysis of samples consisted in measuring consistency, viscosity, colour and physicochemical characteristics of the treated and fresh puree. Applying intense treatments (600 W–340 s, 900 W–300 s and 1,000 W–200 s) through high power or long treatment periods or a combination of these factors, mainly affects the consistency (flow distance decreased from 5.9 to 3.4 mm/g sample), viscosity (increased from 1.6 to 2.5 Pa/s), colour (maximun $\Delta E$ was 6 U) and taste of the product. As a result, samples were thicker and with an atypical flavour and kiwifruit colour due to increased clarity ($L^*$ increased from 38 to 43) and slight changes in the yellow–green hue ($h^*$ decreased from 95 to 94). For the instrumental determinations of colour and visual perception of consistency, the most suitable parameters for quality control are the colour coordinates $L^*$, $a^*$, $h^*$, whiteness index and flow distance measured with a Bostwick consistometer.

**Keywords** Kiwifruit · Microwaves · Descriptive sensory assessment · Colour · Consistency · Taste

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### Introduction

Sensory evaluation is an essential tool in the development of new products. Physical measurements cannot normally determine consumer response or preference because psychological or sensory responses are difficult to mimic (Dubost et al. 2003). However, this type of evaluation can be characterised by imprecision, inaccuracy and uncertain repeatability (Sinija and Mishra 2011). Therefore, it is important to find a good objective method that can predict the sensory perception of the product (Segnini et al. 1999).

Instrumental measurement of fruit properties such as °Brix, acidity, texture or colour have become the cornerstones of fruit quality assessment (Oraguzie et al. 2009; Segnini et al. 1999). The industry often sets quality standards that are based on instrumental measurements. Nevertheless, the relevance of these data will depend on how well they are able to predict sensory attributes (Oraguzie et al. 2009). In sensory analysis, one of the most important tools is the quantitative characterisation of the perceivable product attributes. In the bibliography, this

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# Comparison of microwaves and conventional thermal treatment on enzymes activity and antioxidant capacity of kiwifruit puree

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ABSTRACT

Enzymes are naturally present in food and can cause product deterioration. For this reason, most food-processing steps try to reduce the enzymatic activity. The aim of this work was to compare, in terms of both the inactivation of kiwifruit puree peroxidase, polyphenoloxidase and pectinmethylesterase and also the maintenance of the antioxidant capacity of the product, the effect of a microwave treatment with a conventional thermal treatment designed to cause the same level of peroxidase inactivation (90%). The microwave power and process time that best permitted the maximisation of both the enzyme inactivation and the antioxidant capacity of the product, were selected by means of the Response Surface Methodology. The results obtained point to microwave heating as an appropriate technology with which to produce a stable kiwifruit puree, since these treatments were more effective at enzyme inactivation and antioxidant capacity retention than the conventional thermal treatment.

*Industrial relevance:* Food industry is currently focused on the development of novel and minimally processed products with improved quality. Traditional thermal processing has been assumed to require the use of high temperatures and long times to stabilise food products, which lead to dramatic losses of products' quality. Thus, a variety of different processing technologies are being explored as alternative to traditional thermal processing. The results of this study point out that more than conventional heating, microwave technology can be an appropriate means of achieving the required level of enzyme inactivation at which to obtain a stable kiwifruit puree with an improved antioxidant capacity.



## 1. Introduction

Different food scientists and nutrition specialists consider the consumption of fruit and vegetables as having many health benefits (Antunes, Dandlen, Cavaco, & Miguel, 2010; Du, Li, Ma, & Liang, 2009). Kiwifruit has been attributed with exceptional nutritional and sensory properties, as well as high antioxidant activity comparable to that of mangosteen, avocado, papaya, mango and cempedak (Park et al., 2006).

In recent years, consumers' food habits have changed towards a greater consumption of ready-to-eat and minimally processed fruit-based products, leading to the marketing of products such as fruit juices, beverages of fruit juices mixed with milk, fruit purees or smoothies (Antunes et al., 2010; Elez-Martínez, Aguiló-Aguayo, & Martín-Belloso, 2006; Osorio, Martínez-Navarrete, Moraga, & Carbonell, 2008). This type of products has been traditionally preserved by means of conventional thermal technologies (Osorio et al., 2008; Whitaker, Voragen, & Wong, 2003). However, it usually requires the use of high temperatures combined with long process times which has been widely associated with a marked deterioration in food quality, especially with the development of cooked off-flavours, colour alteration and the loss of thermosensitive compounds (Elez-Martínez et al., 2006; Gonçalves, Pinheiro, Abreu, Brandao, & Silva, 2010; Llano, Haedo, Gerschenson, & Rojas, 2003; Maskan, 2001; Queiroz, Mendes, Fialho, & Valente-Mesquita, 2008). For this reason, alternatives to conventional processing technologies are being explored. Microwave heating has been proposed as a good alternative to conventional heating when the purpose is either drying, pre-cooking, tempering or preserving (Huang, Sheng, Yang, & Hu, 2007; O'Donnell, Tiwari, Bourke, & Cullen, 2010; Vadivambal & Jayas, 2007). Microwave energy (MW) is transported as an electromagnetic wave (0.3 GHz–300 GHz). When intercepted by dielectrical materials, MW produces an increase in the product temperature associated with dipole rotation and ionic polarisation (Schubert & Regier, 2010). This type of technology implies volumetric heating, which means that the materials can absorb microwave energy directly and internally. For this reason, compared to conventional heating methods, microwaves lead to a faster heating rate, thus reducing the process time (Huang et al., 2007; Igual, García-Martínez, Camacho, & Martínez-Navarrete, 2010; Queiroz et al., 2008). In this way, the processing cost can be cut and the product may present better preserved sensory, nutritional and

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# *Listeria monocytogenes* inactivation kinetics under microwave and conventional thermal processing in a kiwifruit puree

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ABSTRACT

The inactivation of *Listeria monocytogenes* in a kiwifruit puree by conventional and microwave heating was studied. Survival curves at three microwave power levels (600–1000 W) and three temperatures (50–60 °C) were obtained. Data were properly fitted by a first-order kinetic model. Processing times under both technologies were corrected to isothermal treatment for the kinetic study. Microwave heating was shown to effectively inactivate *L. monocytogenes*. In the range of microwave and conventional processing conditions assayed, the 5-$\log_{10}$ reductions of *L. monocytogenes* recommended by the FDA for pasteurized products were achieved. The level of microwave power applied had a considerable influence on the *L. monocytogenes* inactivation rate. The higher the power level, the faster the inactivation. The inactivation of *L. monocytogenes* under microwave heating at 900 W ($D_{60\,°C}$ = 17.35 s) and 1000 W ($D_{60\,°C}$ = 17.04 s) happened faster than in a conventional thermal process ($D_{60\,°C}$ = 37.45 s). Consequently, microwave heating showed greater effectiveness for *L. monocytogenes* inactivation than conventional heating.

*Industrial relevance:* Consumers' desires are oriented towards new foods that are convenient, easy to preserve and ready-to-eat products, being consumption of fresh fruit replaced with processed fruit products. Food industry is currently focused on the development of novel and minimally processed products with improved quality. Thus, a variety of new processing technologies are being explored as alternative to traditional thermal processing. In this work, the thermal and microwave inactivation kinetics of *Listeria monocytogenes* in a ready-to-eat kiwifruit puree were investigated so as to assess the suitability of microwave processing as an alternative to thermal processing. The results of this study point out that more than conventional heating, microwave technology can be an appropriate means of fruit product pasteurization with the possibility of offering the required safety by using a lower process time, when microwave power of a certain level is applied.



## 1. Introduction

Microwave energy (MW) has been extensively used in the area of food processing for various commercial purposes (Vadivambal & Jayas, 2007). MW heating involves the use of electromagnetic waves (0.3–300 GHz) in order to generate heat in materials. This technology implies volumetric heating, as materials can directly absorb microwave energy, causing dipolar molecule oscillation and ionic polarization. Some commercially proven applications of microwave food processing include dehydration of low-moisture solids, pre-cooking of meat products and tempering of frozen foods (Vadivambal & Jayas, 2007). In recent years, the suitability of microwave heating to enhance food microbial safety (pasteurization and sterilization processes) has been successfully tested in various animal and vegetable food products (Cañumir, Celis, de Brujin, & Vidal, 2002; Huang, Sheng, Yang, & Hu, 2007; O'Donnell, Tiwari, Bourk, & Cullen, 2010). This technology has been recognized to present some advantages over conventional heating: (i) MW leads to faster heating rates, so it can approach the benefits of high temperature-short time processing whereby bacterial destruction is achieved, but thermal degradation of the desired components is reduced (Huang et al., 2007); (ii) the magnetron, the element that produces microwave radiation, can be turned on or off instantaneously; (iii) the product can be pasteurized after being packaged; and (iv) MW processing systems can be more energy efficient (De Ancos, Cano, Hernández, & Monreal, 1999). However, in spite of these advantages, there are some potential problems which are inherent in microwave processing that are contributing to delay MW exploitation to its fullest potential in food industry applications (Picouet, Landl, Abadias, Castellari, & Viñas, 2009), being the existence of a non-uniform temperature distribution, which could result in hot and cold spots in the heated product, its major limitation (Vadivambal & Jayas, 2007). Additionally, up to date, little is known kinetically about the basic general relationship between microbial inactivation in foods and MW exposure, having Fujikawa, Ushioda, and Kudo (1992), Tajchakavit, Ramaswamy, and Fustier (1998), Cañumir et al. (2002), Yaghmaee and Durance (2005) and Pina-Pérez, Benlloch-Tinoco, Rodrigo, and Martínez (2013) conducted some of the few studies

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Short communication

# Impact of temperature on lethality of kiwifruit puree pasteurization by thermal and microwave processing

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ABSTRACT

The use of pasteurization units (PU) as a measure of the lethal effect of processes was proposed with the aim of comparing conventional and novel thermal technologies. Kiwifruit puree was subjected to microwave (1000 and 900 W) and conventional (97 °C) heating. Processing conditions of the treatments were chosen to simulate a pasteurization treatment. The temperature profiles of the samples during processing were recorded at different positions. The coldest and hottest spots of the product were identified and the associated PU numbers were calculated. A significantly ($p < 0.05$) higher thermal load was necessary in order to stabilize the kiwifruit puree under conventional (19.27 min) than microwave heating mode (0.003–8 min) at any of the conditions studied. The higher effectiveness of microwave heating could be attributed to non-thermal effects associated with this technology.



## 1. Introduction

Microwave heating (MW) appears to be a promising novel technology for food preservation (Vadivambal & Jayas, 2010). During recent decades, many studies have been carried out on the evaluation of MW benefits with respect to conventional heat treatments. Its suitability for pasteurization, sterilization, and dehydration processes as well as its capacity of producing safe and better quality products has been widely demonstrated (Igual, García-Martínez, Camacho, & Martínez-Navarrete, 2010). Although MW could potentially replace conventional heat processes for some specific applications (Awuah, Ramaswamy, & Economides, 2007), there are still problems that are inherent in this technology, such as non-uniform product temperature distribution (Salazar-González, San Martín-González, López-Malo, & Sosa-Morales, 2012), and that contribute to delaying the exploitation of MW to its fullest potential in the food industry.

On the other hand, improper comparison between treatments because of inadequate control of processing parameters such as sample temperature exposure, roughly selected exposure periods or poor kinetic data accommodation may be generating doubts and causing conflicting opinions regarding the superiority of this technology against conventional heat treatments. Some authors have proposed different ways of comparing microwave and conventional treatments: (i) to select processing conditions to get equal heating rates (°C/min) (Fujikawa, Ushioda, & Kudo, 1992), (ii) to reach a similar temperature profile in samples under both technologies (Welt, Tong, Rossen, & Lund, 1994), and (iii) to carry out kinetic studies (Matsui, Gut, De Oliveira, & Tadini, 2008). This lack of homogeneity in comparison procedures may result in mistaken interpretations and hinders the contrast of different research works.

In the present study, the concept of accumulated lethality, a parameter traditionally employed to evaluate conventional heat treatments, is proposed as a tool for comparison of conventional and novel thermal technologies. The lethal effect of the process is determined on the basis of the time-temperature history of the product and it is expressed as a numerical value in arbitrary units. The pasteurization unit (PU) was proposed by Shapton, Lovelock, and Laurita-Longo (1971) as a measure of accumulated lethality but more specifically adapted for pasteurization processes.

The objective of the present research work was to assess the suitability of the PU parameter to compare the thermal load of microwave and conventional kiwifruit puree pasteurization treatments.

## 2. Material and methods

### 2.1. Sample preparation

Kiwifruit (*Actinidia deliciosa* var. Hayward) was purchased in a local supermarket. Fruit pieces were peeled and triturated in a

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ORIGINAL PAPER

# Quality and Acceptability of Microwave and Conventionally Pasteurised Kiwifruit Puree

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**Abstract** The development and optimisation of food preservation processes seem to be necessary in order to address consumer expectations related to secure, fresh-like foods. To this end, the sensory, nutritional and functional properties must be maximally retained. In order to contribute to the acquisition of knowledge about the adequacy of microwave processing as a means of preserving fruit-based products, the present study compares the impact of microwave heating with conventional thermal processing. The consumer acceptance of fresh and pasteurised kiwifruit puree was studied as was the content of water, soluble solids and bioactive compounds and the pH, consistency, viscosity, colour coordinates and antioxidant capacity, as well as the effect of the thermal treatment on enzyme and microbial inactivation. As bioactive compounds, the content of vitamins C, A and E and the total flavonoid, phenol and tannin content have been considered. As the obtained results show, not only was microwaved puree preferred by consumers, but it also exhibited a superior maintenance of the nutritive and functional properties of the fruit, smaller colour changes and a content of inactivated enzymes and microorganisms equal to or greater than the conventionally heated sample.

**Keywords** Consumer perception · Bioactive compounds · Enzymes · Microorganisms · Microwave heating · Conventional heating

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## Nomenclature

| | |
|---|---|
| MW | Microwave heating |
| POD | Peroxidase |
| Co | Conventional heating |
| JAR | Just-about-right |
| PPO | Polyphenol oxidase |
| PME | Pectin methylesterase |
| TP | Total phenols |
| TT | Total tannins |
| TF | Total flavonoids |
| TMB | Total mesophilic bacteria |
| Y&M | Yeast and mould |
| $\Delta E^*$ | Colour difference |

## Introduction

One of the most relevant trends in food manufacturing has stemmed from the recent increased demand for convenient, easy-to-preserve and health-promoting foods (Elez-Martínez et al. 2006). Superplus production of fruits presenting appreciated sensory and nutritional value, e.g. kiwifruit (Barboni et al. 2010), may be processed and marketed as pasteurised pulp or puree products which, besides from being consumed directly as a dessert or as a complement of cooked meals, could be used as ingredients in juices, nectars, jams, ice creams, baby foods or pastry (Silva and Silva 1997).

Given the current consumer expectations, the industrial sector is showing a greater interest in the development and optimisation of novel food preservation processes, intending to market high-quality minimally processed fruit-based products (Señorans et al. 2003). In this respect, sensory assessment must be considered as an essential tool to help guide any modification of the food processing step, taking great care of

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# Superiority of microwaves over conventional heating to preserve shelf-life and quality of kiwifruit puree

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ABSTRACT

The effect of both microwave (1000 W-340 s) and conventional heating (97 °C-30 s) on the quality and shelf-life of kiwifruit puree was investigated. The growth of microorganisms and the evolution of enzyme activity, colour, pH, bioactive compounds and antioxidant activity in the product during storage at 4, 10 and 22 °C were checked. The storage temperature had a significant ($p < 0.05$) impact on both the shelf-life and the nutritional and functional value of the samples: the higher the temperature, the significantly ($p < 0.05$) faster the rate of both the sample spoilage and the loss of the bioactive compounds. On the other hand, thermal processing significantly ($p < 0.05$) reduced the growth of microorganisms and the degradation rate of some bioactive compounds in a 12–58%, as well as leading to enzyme and colour stabilization. A longer shelf-life (123 days at 4 °C) and a superior preservation of colour ($\Delta E_{ab} = 6.54$) and bioactive compounds (57–67%) were obtained when microwave heating was the technology selected to process the kiwifruit puree. Microwave heating was considered a suitable means of preserving kiwifruit puree that might be successfully employed as an innovation tool with which to help safe, high-quality and minimally processed kiwifruit based-products reach the market.



## 1. Introduction

A wide variety of minimally processed fruit-based products, such as fresh-cut fruits, fresh-squeezed fruit juices, fruit juice and milk mixture beverages, fruit purees and smoothies, are being marketed in response to the recent increase in demand for convenient, easy-to-preserve, health-promoting foods (Elez-Martínez, Soliva-Fortuny, & Martín-Belloso, 2006). Nevertheless, many fruits which are both appreciated for their sensory and nutritional value and possess a great potential for industrial exploitation, e.g. kiwifruit, still seem to be mostly limited to the fresh market outlet, ignoring their surplus production (Barboni, Cannac, & Chiaramonti, 2010).

Microwave heating has been reported to provide superior quality fruit-based products with an extended shelf-life, representing a good alternative to conventional preservation processes (Landl, Abadias, Sárraga, Viñas, & Picouet, 2010). Given the particular way of heating which takes place during microwave processing (volumetric heating), this technology leads to higher penetrative power, faster heating rates, higher thermal efficiency and shorter processing times compared to conventional heating methods. All these facts seem to result in better organoleptic, nutritional and functional properties preservation, with a particular effect on colour (Huang, Sheng, Yang, & Hu, 2007; Vadivambal & Jayas, 2007). Similarly to other novel technologies in the field of food innovation, microwaves might be a key factor either in the successful differentiation of products (Deliza, Rosenthal, Abadio, Silva, & Castillo, 2005) or in finding new uses for some fruits by helping to develop novel ways with which to process them. To this end, many comparative studies of the effect of microwave and conventional heating on various quality aspects of fruits have been conducted (Barrett & Lloyd, 2012), pointing out the advantages of microwave heating (Huang et al., 2007). However, it should be taken into consideration that despite published data on the effect of microwaves on safety and quality being available for different food systems, to date, little seems to be known of the impact of microwaves on the shelf-life and post-processing quality loss of fruit products. The marketing of these products frequently implies a storage step, which might also relevantly contribute to their final quality. For this reason, the evolution of their properties and the growth of microorganisms during shelf-life is an important issue to study (Rodrigo et al., 2003).

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# Chlorophylls and carotenoids of kiwifruit puree are affected similarly or less by microwave than by conventional heat processing and storage

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ABSTRACT

The impact of microwave (1000 W – 340 s) and conventional heat (97 °C – 30 s) pasteurisation and storage (4, 10, 22 °C for up to 63 d) on total and individual carotenoids and chlorophylls in kiwifruit puree was evaluated. Bioaccessibility of carotenoids, before and after pasteurisation and storage, was also studied. Microwaves and conventional heating led to marked changes in the chlorophyll (42–100% losses) and carotenoid (62–91% losses) content. First- and second-order kinetics appropriately explained the degradation of total carotenoids and chlorophylls over time, respectively. Pasteurised samples showed significantly ($p < 0.05$) enhanced stability of these pigments, with microwaves ($k = 0.007$–$0.031$ 100 g mg$^{-1}$ day$^{-1}$ at 4–22 °C) promoting chlorophyll stability to a greater extent than conventional heating ($k = 0.0015$–$0.034$ 100 g mg$^{-1}$ day$^{-1}$ at 4–22 °C). Bioaccessibility of carotenoids remained ($p < 0.05$) unaffected by processing and storage. These results highlighted that the pigment composition of microwaved kiwifruit was more similar to that of the fresh fruit and better preserved during storage.


## 1. Introduction

Fruits have been natural components of the human diet throughout history. Although their consumption seems to have been promoted more in recent times because of their well-known nutritional value and additional associated health benefits such as chronic disease prevention (Antunes, Dandlen, Cavaco, & Miguel, 2010), they have traditionally been perceived as appetising food products, given their wide variety of inviting colours and flavours, mostly conveyed by their pigment composition (Khoo, Prasad, Kong, Jiang, & Ismail, 2011).

In the particular case of kiwifruit (*Actinidia deliciosa*), a comparatively low-calorie (57 kcal/100 g), nutritious fruit rich in vitamin C, potassium, folate and fibre (Drummond, 2013), chlorophylls and carotenoids are the main pigments that contribute to the characteristic bright green colour of its flesh (Nishiyama, Fukuda, & Oota, 2005). The potential beneficial health properties of carotenoids, in particular, such as anti-inflammatory and anti-oxidant effects (Kaulmann & Bohn, 2014; Khoo et al., 2011), have been widely recognised and have long been considered an interesting study target. Although most investigations have traditionally focused on evaluating food carotenoid content, it should be kept in mind that the positive effect of these secondary plant compounds or any other functional compounds depends not only on their content but also on the extent to which they are bioaccessible and available for absorption after ingestion and digestion (Biehler, Hoffmann, Krause, & Bohn, 2011).

On the other hand, although kiwifruit has been reported to possess great potential for industrial exploitation (Barboni, Cannac, & Chiaramonti, 2010), few processed kiwifruit products are available on the international market nowadays. During processing and storage, dramatic changes are often observed in the pigment pattern of this fruit, resulting in degradation of chlorophylls into pheophytins, pyropheophytins, chlorophyllides and pheophorbides (Cano & Marín, 1992), and *cis–trans* isomerisation of carotenoids and formation of epoxides, furanoids and other degradation products of these compounds (Khoo et al., 2011). Consequently, the typical bright green colour turns to a yellowish-brown tone (Cano & Marín, 1992), and a product with an appearance very different from that of the raw kiwifruit is obtained (Cano, 1991). Given that colour is a highly important attribute in fruit quality assessment and has a considerable influence on consumer acceptance, these undesirable changes in pigment patterns of processed kiwifruit products may represent an important limitation for their marketing.

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