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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-log₁₀ 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 *Listeria monocytogenes* inactivation rate. The higher the power level, the faster the inactivation. The inactivation of *Listeria 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 *Listeria monocytogenes* inactivation than conventional heating.

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, Brujin, & Vidal, 2002; Huang, Sheng, Yang, & Hu, 2007; O'Donnell, Tiwari, Bourk, & Cullen, 2010). This technology has been recognised to present some advantages over conventional heating: (i) MW leads to faster heating rates, so it can approach the benefits of high temperatureshort 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 et al. (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 is 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, Abriouel, Ben Omar, Lucas, Valdivia, & Gálvez, 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. moncytogenes* 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 *Escherichia 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 one minute. 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 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. 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 fibre-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 thermal 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 et al., 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 temperature 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 (equation 1) (Awuah, Ramaswamy, & Economides, 2007; Tajchakavit & Ramaswamy, 1997).

$$\log \frac{N}{N_{\circ}} = -\frac{t}{D}$$
(1)

where

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

N₀: 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 both treatments (MWP and CTP) applied in the present study involved non-isothermal heating conditions, correction of processing time values for come-up periods were necessary prior to kinetic data analyses. 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, Ramaswamy, & Economides, 2007; Tajchakavit & Ramaswamy, 2007). 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 T_{ref} (Latorre, Bonelli, Rojas, & Gereschenson, 2012; Matsui et al., 2008).

$$t_e = \int_0^\infty 10^{\left(\frac{T(t) - T_{ref}}{z}\right)} dt$$
(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) (equation 3) and the root mean square error (RMSE) (equation 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/N₀) 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.

$$Adjusted - R^{2} = \left[\frac{(m-1)(1 - \frac{SSQ_{REGRESSION}}{SSQ_{TOTAL}})}{(m-j)}\right]$$
(3)
$$RMSE = \sqrt{\frac{\sum (fitted - observed)^{2}}{m}}$$
(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 Figure 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. The inactivation of *L. monocytogenes* under microwave and conventional processing was studied in a kiwifruit puree (Figures 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 equation 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-log₁₀ cycles 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-log₁₀ 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-log₁₀ cycles of *L. monocytogenes* were inactivated 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-log₁₀ 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 behaviour under MWP was close to linearity and the data obtained were fitted to first-order kinetics (equation 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. 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, McMahon, Doherty, Sheridan, McDonell, Blair, & Harrington, 2000; Chhabra, Carter, Linton, & Cousin, 1989; Sharma, Adler, Harrison, & Beuchat, 2005), in reference medium (Hassani et al., 2005; Hassani, Álvarez, Raso, Condón, & Pagán, 2007) and in sucrose solutions (Fernández et al., 2007), there is no information available about the survival behaviour 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°C}=25.2 s to D_{38,3°C}=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 510W, being $D_{55.6^{\circ}C}=30$ s and $D_{60.5^{\circ}C}=18$ 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). 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-log₁₀ cycles inactivation) a considerably longer effective time was required at 600 W (t_e=116 s) than at 1000 W $(t_e=82 \text{ s})$. The power level effect can also be evaluated by means of the D_{60°C}-values 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, Geeraerd, Gaze, Kondjoyan, Boyd, Shaw, Van Asselt, & Zwietering, 2006), and Weibull distribution (Hassani et al., 2005; Fernández et al., 2007; 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 behaviour under thermal treatment (Awuah et al., 2007; Hassani et al., 2005; Soysal & Söylemez, 2005; Tajchavit & Ramaswamy, 1997; Tajchavit et al., 1998; Zheng & Lu, 2011). In the present case, survival curves close to linearity were found and the data obtained were fitted to first-order kinetics (equations 1). The D-values calculated and the accuracy of the fit are shown in Table 2. 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 (Figure 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 (Tajchakavit et al., 1998; Hassani et al., 2005; van Asselt & Zwietering, 2006). Gabriel and Nakano (2009)

reported similar D-values when they studied thermal *L. monocytogenes* inactivation in clear apple juice ($D_{55^{\circ}C}=1.32$ min).

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 & Javas, 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 $D_{60^{\circ}C}$ -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 ($D_{60^\circ C}$ =42.85 s) than that obtained with CTP ($D_{60^\circ C}$ =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 (Fujikava 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 & Lu, 2011).

4. CONCLUSIONS

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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5. REFERENCES

Anaya, I., Aguirrezabal, A., Ventura, M., Comellas, L., & Agut, M. (2008). Survivability of *Salmonella cells* in popcorn after microwave oven and conventional cooking. *Microbiological Research*, *163*, 73-79.

Awuah, G. B., Ramaswamy, H. S., & Economides, A. (2007). Thermal processing and quality: Principles and overview. Chemical Engineering and Processing 46, 584-602.

Banik, S., Bandyopadhyay, S., Ganguly, S., 2003. Bioeffects of microwave – a brief review. *Bioresource Technology*, 87, 155-159.

Bolton, D. J., McMahon, C. M., Doherty, A. M., Sheridan, J. J., McDonell, D. A., Blair, L. S., & Harrington, D. (2000). Thermal inactivation of *Listeria monocytogenes* and *Yersinia enterocolitica* in minced beef laboratory conditions and in sous-vide prepared minced and solid beef cooked in a commercial retort. *Journal of Applied Microbiology*, 88, 626-632.

Buffler, C. R. (1993). *Microwave cooking and processing: Engineering fundamentals for the food scientist.* New York: Van Nostrand Reinhold.

Cañumir, J. A., Celis, J. E., de Brujin, J., & Vidal, L. V. (2002). Pasteurization of Apple juice by using microwaves. *LWT- Food Science and Technology*, *35*, 389–392.

Carpentier, B., & Cerf, O. (2011). Review – Persistence of *Listeria monocytogenes* in food industry equipment and premises. *International Journal of Food Microbiology*, *145*, 1-8.

Chan, Y. C., & Wiedmann, N. (2009). Physiology and genetic of *Listeria* monocytogenes survival and growth at cold temperatures. *Critical Reviews in Food Science and Nutrition, 49,* 237-256.

Chhabra, A. T., Carter, W. H., Linton, R. H., & Cousin, M. A. (1999). A predictive model to determine the effects of pH, milkfat, and temperature on thermal inactivation of *Listeria monocytogenes*. *Journal of Food Protection*, *62*, 1143-1149.

CEI IEC 60705. 1999. Household microwave ovens—methods for measuring performance. IEC publication 60705:1999, Geneva: 3rdIntl. Electrotechnical Commission.

Cobo-Molinos, A., Abriouel, H., Ben Omar, N., Lucas, R., Valdivia, E., & Gálvez, A. (2008). Inactivation of *Listeria monocytogenes* in Raw Fruits by Enterocin AS-48. *Journal of Food Protection*, 71 (12), 2460-2467.

De Ancos, B., Cano, M. P., Hernández, A., & Monreal, M. (1999). Effects of microwave heating on pigment composition and color of fruit purees. *Journal of the Science of Food and Agriculture*, *79*, 663-670.

European Food Safety Authority. (2013). Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 1 (outbrake data analysis and risk ranking of food/pathogen combinations). *EFSA journal*, *11*(*1*), 3025-3163.

Elez-Martínez, P., Aguiló-Aguayo, I. & Martín-Belloso, O. (2006). Inactivation of orange juice peroxidase by high-intensity pulsed electric fields as influenced by process parameters. *Journal of the Science of Food and Agriculture*, 87, 71-81.

Fernández, A., López, M., Bernardo, A., Condó, S. & Raso, J. (2007). Modelling thermal inactivation of *Listeria monocytogenes* in sucrose solutions of various water activities. *Food Microbiology*, 24, 372-379.

Food and Drug Administration, Department of Health and Human Services. (2004). Juice HACCP hazards and controls guidance. http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocum ents/Juice/ucm072557.htm.Accessed 8 March 2012.

Fujikawa, H., Ushioda, H., & Kudo, Y. (1992). Kinetics of *Escherichia coli* destruction by microwave irradiation. *Applied and Environmental Microbiology*, *58* (*3*), 920-924.

Gabriel, A. A., & Nakano, H. (2009). Inactivation of *Salmonella, E. coli and Listeria monocytogenes* in phosphate buffered saline and apple juice by ultraviolet and heat treatments. *Food Control*, *20*, 443-446.

Gaze, J. E., Brown, G. D., Gaskell, D. E., & Banks, J. G. (1989). Heat Resistance of *Listeria monocytogenes* in homogenates of Chicken, Beef Steak and Carrot. *Food Microbiology*, 6, 251-259.

Guerrero-Beltrán, J. A., & Barboza-Cánovas, G. V. (2005). Reduction of *Saccharomyces cerevisiae*, *Escherichia coli* and *Listeria innocua* in apple juice by ultraviolet light. *Journal of Food Process Engineering*, 28, 437–452.

Hassani, M., Álvarez, I., Raso, J., Condón, S., & Pagán, R. (2005). Comparing predicting models for heat inactivation of *Listeria monocytogenes* and *Pseudomonas aeruginosa* at different pH. *International Journal of Food Microbiology*, *100*, 213-222.

Hassani, M., Mañas, P., Pagán, R., & Condón, S. (2007). Effect of a previous heat shock on the thermal resistance of *Listeria monocytogenes* and *Pseudomonas aeruginosa* at different pHs. *International Journal of Food Microbiology*, *116*, 228-238. Huang, Y., Sheng, J., Yang, F., & Hu, Q. (2007), Effect of enzyme inactivation by microwave and oven heating on preservation quality of green tea. *Journal of Food Engineering*, *78*, 687-692.

Latorre, M. E., Bonelli, P. R., Rojas, A. M., & Gerschenson, L. N. (2012). Microwave inactivation of red beet (*Beta vulgaris* L. var. conditiva) peroxidase and polyphenoloxidase and the effect of radiation on vegetable tissue quality. *Journal of Food Engineering*, 109, 676-684.

Matsui, K. N., Gut, J. A. W., de Oliveira, P. V., & Tadini, C. C. (2008). Inactivation kinetics of polyphenol oxidase and peroxidase in green coconut water by microwave processing. *Journal of Food Engineering*, *88*, 169-176.

Nicolaï, B. (1998). Optimal control of microwave combination ovens for food heating. 3rd Karlsruhe Nutrition Symposium. European Research towards Safer and Better Food. Review and Transfer Congress. Proceedings Part 2 Edited by V. Gaukeland W.E.L. Spieß. pp. 328–332.

Osorio, O., Martínez-Navarrete, N., Moraga, G., & Carbonell, J. V. (2008). Effect of thermal treatment on enzymatic activity and rheological and sensory properties of strawberry purees. *Food Science and Technology International*, *14*(5), 103-108.

Peleg, M., Penchina, C. M., & Col, M. B. (2001). Estimation of the survival curve of *Listeria monocytogenes* during non-isothermal treatments. *Food Research International*, *34*, 383-388.

Picouet, P. A., Landl, A., Abadias, M., Castellari, M., & Viñas, I. (2009). Minimal processing of a Granny Smith apple purée by microwave heating. *Innovative Food Science & Emerging Technologies*, *10*(*4*), 545-550.

Pina-Pérez, M. C., Benlloch-Tinoco, M., Rodrigo, D., & Martínez, A. (2013). *Cronobacter sakazakii* Inactivation by Microwave Processing. *Food and Bioprocess Tecnology*, DOI: 10.1007/s11947-013-1063-2.

Rodrigo, D., van Loey, A., Hendrickx, M. (2007). Combined termal and high pressure color degradation of tomato puree and strawberry. *Journal of Food Engineering*, *79*, 553-560.

Rodrigo, D., Jolie, R., Van Loey, A., & Hendrickx, M. (2007). Thermal and high pressure stability of tomato lipoxygenase and hydroperoxide lyase. *Journal of Food Engineering*, 79, 423-429.

Sampedro, F., Geveke, D. J., Fan, X., & Zhang, H. Q. (2009). Effect of PEF, HHP and thermal treatment on PME inactivation and volatile compounds concentration of an

orange juice-milk based beverage. *Innovative Food Science and Emerging Technologies*, 10, 463-469.

Schubert, H., & Regier, M. (2010). The microwave processing of foods. London: Woodhead.

Sharma, M., Adler, B. B., Harrison, M. D., & Beuchat, L. R. (2005). Thermal tolerance of acid-adapted and unadapted *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in cantaloupe juice and watermelon juice. *Letters in Applied Microbiology*, *41*, 448-453.

Tajchakavit, S., & Ramaswamy, H. S. (1997). Thermal vs. microwave inactivation kinetic of pectin methylesterase in orange juice under batch mode heating conditions. *LWT-Food Science and Technology*, *30*, 85-93.

Tajchakavit, S., Ramaswamy, H. S., & Fustier, P. (1998). Enhanced destruction of spoilage microorganisms in apple juice during continuous flow microwave heating. *Food Research International 31*, (10), 713-722.

Vadivambal, R., & Jayas, D. S. (2007). Changes in quality of microwave-treated agricultural products – a review. *Biosystems Engineering*, *98*, 1-16.

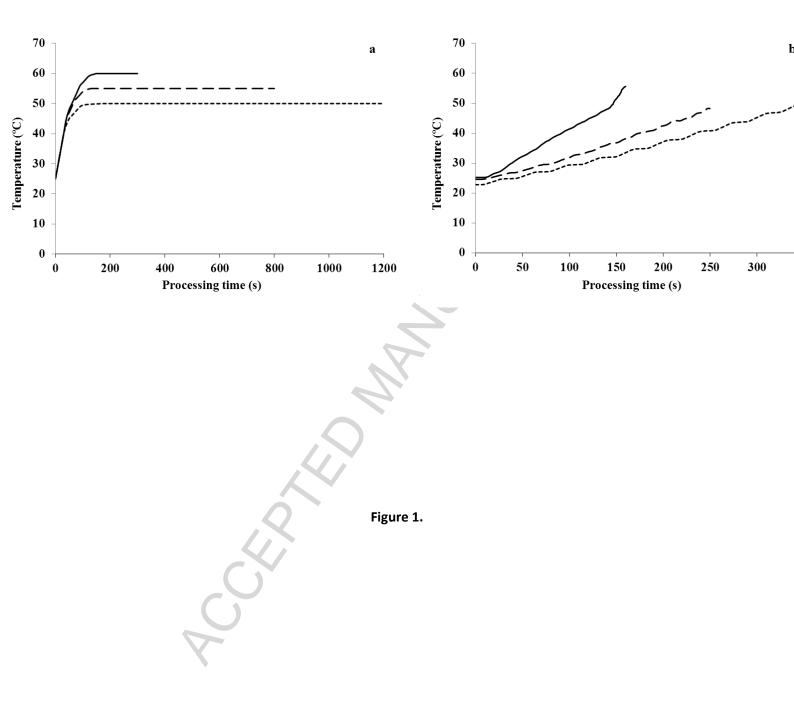
Vaikousi, H., Koutsoumanis, K., & Biliaderis, C. G. (2008). Kinetic modelling of nonenzymatic browning of apple juice concentrates differing in water activity under isothermal and dynamic heating conditions. *Food Chemistry*, *107*, 785-796.

Valdramidis, V. P., Geeraerd, A. H., Gaze, J. E., Kondjoyan, A., Boyd, A. R., Shaw Van Asselt, E. D., & Zwietering, M. H. (2006). A systematic approach to determine global thermal inactivation parameters for various food pathogens. *International Journal of Food Microbiology*, *107*, 73-82.

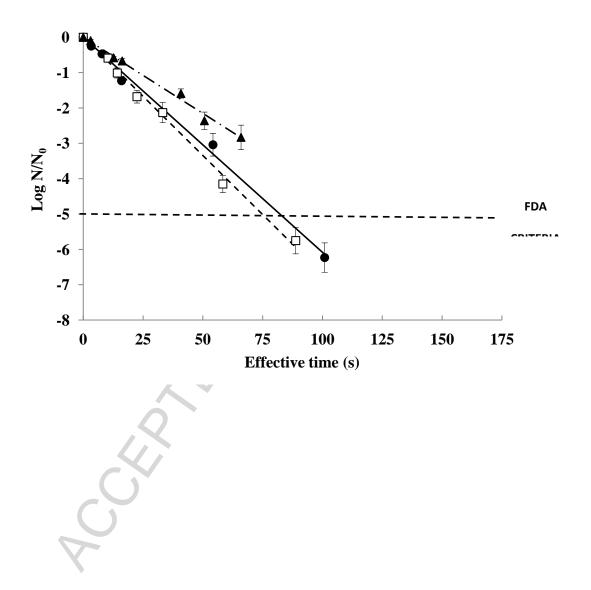
Yaghmaee, P., & Durance, T. D. (2005). Destruction and injury of Escherichia coli during microwave heating under vacuum. *Journal of Applied Microbiology*, *98*, 498-506.

Zheng, H., & Hongfei, L. (2011). Effect of microwave pretreatment on the kinetics of ascorbic acid degradation and peroxidase inactivation in different parts of green asparagus (*Asparagus officinalis L.*) during water blanching. *Food Chemistry*, *128*, 1087-1093.

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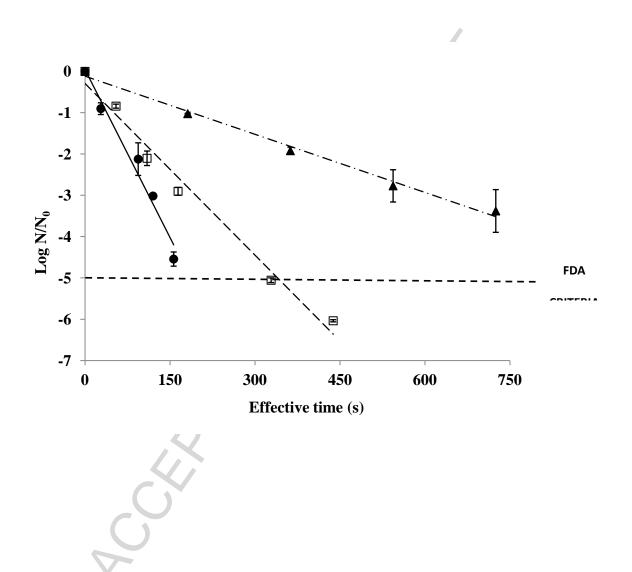


Figure Captions

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 (_____).

Figure 2. Survival curves of *Listeria monocytogenes* under microwave processing at 1000 W (experimental (\bullet), model (____)), 900 W (experimental (\Box), model (____)) and 600 W (experimental (\blacktriangle), model (____)). The plotted values and error bars represent the average of three replicates and the corresponding standard deviation.

Figure 3. Survival curves of *Listeria monocytogenes* under conventional thermal processing at 60 °C (experimental (\bullet), model (___)), 55 °C (experimental (\Box), model (___)) and 50 °C (experimental (\blacktriangle), model (___)). The plotted values and error bars represent the average of three replicates and the corresponding standard deviation.

Table 1. Effective times (t_e) and reference temperatures (T_{ref}) considered to study *Listeria monocytogenes* inactivation kinetics in microwaved kiwifruit puree. $D_{60^{\circ}C}$ value (with standard error); adjusted regression coefficient (R²), root mean square error (RMSE)

			2			
Microwave power	T _{ref} (⁰C)	t _e (s)	$D_{60^{\circ}C}(s)$	R ²	RMSE	
1000 W		3	()			
	55.60	8	5			
		16	17.04 (0.34)	0.996	0.028	
		54				
		101				
900 W	<	10		0.993	0.029	
	\bigcirc	14	17.35 (0.34)			
		22				
	48.45	33				
		58				
		89				
2		3	42.85 (0.13)	0.992	0.009	
		13				
	40.40	16				
600 W	49.10	41				
		51				
		66				

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)

K), root mean	square enc		I	Q.		
Heating temperature	CUT (s)	t _e (s)	D (s)	R ²	RMSE	
50 ºC	111	181	203.74 (3.19)	0.990	0.020	
		363				
		544				
		725				
55 ° C	115	55		0.976	0.135	
		110	70.94 (2.89)			
		164				
		329				
		438				
60 ºC	120	28				
		94	37.45 (2.68)	0.974	0.082	
		120				
		157				

CUT: come-up time.

Highlights

We studied conventional (CTP) and microwave (MWP) pasteurization for kiwifruit puree. > The kinetic parameters for *Listeria monocytogenes* inactivation were determined. > Safety criteria were satisfied in the range of MWP and CTP conditions assayed. > Power level showed an extensive influence on the *L. monocytogenes* inactivation rate. > Higher effectiveness of MWP on the inactivation of *L. monocytogenes* was found.

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