Inactivation kinetics of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in apple juice by microwave and conventional thermal processing

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Abstract

The inactivation kinetics of *Escherichia coli* O157:H7 (CECT 4972) and *Listeria monocytogenes* (CECT 4032) inoculated in apple juice were evaluated under conventional isothermal treatments at four temperatures (55, 60, 65 and 70 ℃) and microwave heating at four power levels (400, 600, 800 and 1000 W). For both thermal treatments, it was possible to achieve a 5-$\log_{10}$ reduction, as recommended by the FDA. The survival curves were non-log-linear and were well described by the Weibull model. To compare treatments, the survival ratio was calculated over the time temperature history of the microwaved samples based on the Weibull parameters for conventional heating and the it was compared with the experimental data. Results show that microwave microbial inactivation was more efficient than predicted in 18 out of 28 tests, which suggests the existence of enhanced inactivation under microwave heating.

Industrial relevance

The growing demands for natural and nutritious foods have promoted the development of preservation technologies to ensure consumer safety and to preserve the original nutritional and organoleptic characteristics of the food. Among the emerging technologies, microwave heating has shown great potential for the continuous pasteurization of fluid foods offering rapid volumetric heating, lower surface temperatures and possible enhanced effects. The results of this study shows that microwave pasteurization of apple juice is a promising technology to enhance microbial safety.

Keywords

Apple juice • *Listeria monocytogenes* • *Escherichia coli* • microwave heating • conventional heating • inactivation kinetics.

Highlights

- Conventional and microwave thermal treatments of apple juice were evaluated.
- Weibull parameters for inactivation of *E. coli* and *L. monocytogenes* were estimated.
- FDA standard of 5-log10 was reached in microwave and conventional conditions.
- Microbial inactivation showed to be more efficient under microwave hearting.

1 Introduction

Fruit juices are not traditionally recognized as vehicles for foodborne illness due to their inherent acidity, which inhibits the multiplication of most pathogens. Nevertheless, outbreaks involving *Escherichia coli* O157:H7 associated with the consumption of apple juice and apple cider led the FDA to require all fruit juice processors to implement a Hazard Analysis and Critical Control Points (HACCP) plan

(CDC, 1996; FDA, 2004). The final critical control point demands a system that achieves at least a 5-$\log_{10}$ reduction of the most resistant organism of public health concern (FDA, 2004). *E. coli* O157:H7 and *Listeria monocytogenes* were identified as pertinent bacterial pathogens for juices safety by the National Advisory Committee on Microbiological Criteria for Foods (FDA, 1997).

Currently, conventional pasteurization, combined with other preservation methods, ensures microbiological food safety and stable shelf life. However, commercial pasteurization of apple juice involves heat treatment at 77 - 88 ºC for 25 - 30 s, which results in detrimental changes in the sensory and nutritional qualities of the juice (Tajchakavit et al., 1998).

Alternative technologies are required to satisfy the growing demand for minimally processed foods. Microwave heating has entered the field of food science for various industrial purposes, such as dehydration, cooking, blanching, sterilization and pasteurization. Continuous flow microwave heating is an emerging application for pasteurizing liquid foods. The use of microwave heating instead of conventional heat exchangers offers some advantages for thermal processing, such as rapid volumetric heating, lower equipment surface temperature, and possible preservation of food quality (Heddleson and Doores, 1994; Chandrasekaran et al., 2013).

Various studies investigated the interaction of microwave radiation with biological systems, specifically microbial inactivation in fluid foods (Salazar-González et al., 2012; Benlloch-Tinoco et al., 2014; Pina-Pérez et al., 2014; Sung and Kang, 2014). However, the literature available regarding microbial inactivation in apple juice by microwave heating is scarce. The studies published in this topic include the inactivation of *Saccharomyces cerevisiae* and *Lactobacillus plantarum* during continuous-flow microwave heating (Tajchakavit et al., 1998), the effect of microwave

76 pasteurization in apple juice inoculated with *E. coli* (Cañumir et al. 2002) and the
77 continuous-flow microwave pasteurization for inactivation of *E. coli* in apple cider
78 (Gentry and Roberts, 2005). Moreover, there are no comparative studies regarding the
79 achieved lethality considering similar treatment conditions for conventional and
80 microwave heating.

81 Some authors have carried out studies to establish the target microorganism for
82 the pasteurization of apple juice using other emerging technologies, such as ultraviolet
83 light, combined heat and ultraviolet light and gaseous ozone (Guerrero-Beltrán and
84 Barbosa-Cánovas, 2005; Gabriel and Nakano, 2009; Choi et al., 2012; Gouma et al.,
85 2015). The 5-$\log_{10}$ reduction of the most resistant pathogen depends on the specific
86 processing conditions and technology.

87 The purposes of this study were: 1) to investigate the influence of microwave
88 heating on the inactivation of *E. coli* O157:H7 and *L. monocytogenes* in apple juice; 2)
89 to describe the inactivation kinetics of these microorganisms; 3) to compare the
90 effectiveness of microwave and conventional heating in the inactivation rate of
91 foodborne pathogens in apple juices.

92

93 **2 Materials and methods**

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95 2.1. Bacterial strain, culture preparation and inoculation

96 *E. coli* O157:H7 (CECT 4972, isolated from human diarrhea, Innsbruck,
97 Austria) and *L. monocytogenes* (CECT 4032, isolated from a case of meningitis after
98 eating soft cheese, Colindale, London, UK) were provided by the Spanish Type Culture
99 Collection and prepared according to the method described by Benlloch-Tinoco et al.
100 (2014). The stock culture (1 mL of cell suspension with supplemental 1 mL glycerol

20%, v/v, added after the stationary phase was reached) was stored frozen at –80 ºC. One milliliter of unfrozen *E. coli* O157:H7 and *L. monocytogenes* were cultured in 500 mL of Tryptic Soy Broth (TSB, Scharlab Chemie S.A., Barcelona, Spain) at 37 °C for 24 h under constant agitation (200 rpm) to reach the stationary growth stage. The cultures were centrifuged twice using an Avanti J-26XP centrifuge (Beckman Coulter, Palo Alto, USA) with a rotor JLA-16.250 (Beckman Coulter, Palo Alto, USA) at 4000 g, 4 °C for 15 min, and the pellets were resuspended in 50 mL of TSB. The cells were dispensed in cryogenic vials T310-2A (Simport, Saint-Mathieu-de-Beloeil, Canada) adding 2 mL of TSB with glycerol 20% (v/v) in a 1:1 ratio, and stored at – 80 °C until used for the inactivation kinetics studies. For *E. coli* and *L. monocytogenes* inoculum enumeration, samples were thawed and it was proceeded according to Section 2.4. The approximate concentration in cultures of *E. coli* and *L. monocytogenes* were $4 \cdot 10^9 \pm 7 \cdot 10^8$ CFU. mL^{-1} and $7 \cdot 10^9 \pm 9 \cdot 10^8$ CFU. mL^{-1}, respectively.

The experiments were performed using a commercial apple juice (Don Simon, J. García Carrión S.A., Spain) with a pH of 3.5 and soluble solids of 12.4 °Brix (25 ºC) purchased at a local market in Valencia (Spain). The inoculums of *L. monocytogenes* and *E. coli* (2 mL) were thawed and mixed in the apple juice (8 mL) to obtain initial viable counts of 10^6 CFU. mL^{-1}.

2.2. Conventional thermal treatment

A glass capillary tube (Blaubrand®, BRAND GMBH), previously sterilized at 240 °C for 24 h, was filled with 200 µL of the inoculated apple juice. Both ends were immediately sealed with oxygen flame. The tube was carefully sealed to avoid the heating of the inoculated apple juice. The conventional thermal treatment was carried out by immersion of the capillary tubes for different time intervals (selected between 10

and 180 s) in a circulating thermostatic Haake DC5 water bath (Thermo Fischer Scientific, Waltham, USA), which was kept at 55, 60, 65 or 70 °C. Each combination of the temperature and time (sets of ten capillaries) was conducted in two repetitions and both repetitions were considered for estimating the inactivation kinetic parameters. After the processing time, the capillary tubes were immersed in ice-water bath until the apple juice reached 25 °C. A type-T thermocouple (Control Company, Webster, USA) connected to a data logger LZ64 (Digitron instrumentation, Cambridge, UK) was inserted into one capillary tube with apple juice to obtain the average time-temperature profile of the set of ten capillaries. Prior to the thermal treatment, all samples were kept at 25 °C to standardize the come-up time (CUT).

2.3. Microwave thermal treatment

Batch microwave processing was performed using an adapted microwave oven (model 3038GC, Norm, China) at 2450 MHz with a turntable plate. A fiber-optic temperature probe CR/JP/11/11671 (Optocom, Dresden, Germany) was placed in a borosilicate glass test tube (6-mm inner diameter and 68-mm length) with 1 mL of inoculated apple juice, with the tip at the center of the liquid, and connected to a datalogger FOTEMP1-OEM (Optocom, Dresden, Germany) to continuously record the time-temperature history. The test tube was placed vertically at the center of the microwave cavity. All the samples started at an initial temperature around 25 °C and were then heated in the microwave oven. Once the processing time was achieved, the test tube was quickly removed from the cavity and inserted in an ice-water bath. Since the fiber-optic probe was fixed in the oven, the temperature data during cooling was not recorded. For the inactivation study, the inoculated apple juice was subjected in

triplicates to microwave heating at four different power levels (1000 W, 800 W, 600 W and 400 W) and different heating times between 50 and 390 s.

2.4. Enumeration of survivors

Right after microwave and conventional treatments, sample aliquots were diluted in duplicate with sterile 1%-peptone water (Scharlab Chemie S.A., Barcelona, Spain), and 0.1 mL of diluent spread-plated onto Tryptic Soy Agar (TSA, Scharlab S.A., Barcelona, Spain). The plates were then incubated at 37 °C for 24 h for *E. coli* and 48 h for *L. monocytogenes*. Afterwards, the counting step was performed. The reduction of viable cell count was expressed as the decimal logarithm of the quotient of the treated (N) and untreated samples (N_0).

2.5. Absorbed microwave power

The mean of the volumetric microwave power absorption, P'_{abs} (W/mL), was calculated from the temperature rise of the 1.0 mL sample from $T_{initial}$ to T_{final} using Eq. (1):

$$P'_{abs} = \frac{m \cdot c_p \cdot (T_{final} - T_{initial})}{t \cdot (1.0\ \text{mL})} \quad (1)$$

where m is the mass of the sample (kg), c_p is the average specific heat (J/°C·kg) of the mixture of apple juice (0.8 mL), TSB (0.1 mL) and glycerol (0.1 mL) and t is the microwave processing time (s). In order to estimate the average specific heat of the sample, the mixture of apple juice and TBS (0.8 + 0.1 mL) was assumed to have the density and specific heat of apple juice reported by Constenla et al. (1989), while the density and specific heat of glycerol (0.1 mL) came from Cheng (2008) and Righetta et al. (1998), respectively. The specific heat estimate was obtained as:

$$c_p = \frac{(0.9)\cdot\rho_{apple}\cdot c_{p,apple} + (0.1)\cdot\rho_{glycerol}\cdot c_{p,glycerol}}{(0.9)\cdot\rho_{apple} + (0.1)\cdot\rho_{glycerol}} \quad (2)$$

where densities (ρ_{apple}, $\rho_{glycerol}$) and specific heats ($c_{p,apple}$, $c_{p,glycerol}$) were calculated at the average temperature of the sample.

2.6. Modeling of survival curves

The inactivation Weibull model in decimal logarithmic form (Eq. (3)) was fitted to the data of surviving population for conventional and microwave thermal treatments *versus* processing time through non-linear regression (Statgraphics Centurion XV version 15.2.11, StatPoint Technologies, Warrenton, USA) to obtain a mathematical description of the survival curves:

$$\log(S) = -\left(\frac{t}{\alpha}\right)^{\beta} \quad (3)$$

where S is the survival ratio (N/N_0) after processing time t, α is the scale parameter (s^{-1}) that represents the time of the first decimal reduction and β is the shape parameter (-) (Mafart et al., 2002). The goodness of the fit was assessed from the adjusted coefficient of determination (R^2_{adj}), the mean squared error for the prediction of $\log(S)$ (MSE) and the parity charts of predicted *versus* experimental survival ratios.

Analogous to the Bigelow z value, van Boekel (2002) shows that the temperature dependence of the scale parameter (α) can be modeled by a logarithmic relationship as given by Eq. (4):

$$\alpha = a\cdot 10^{-bT} \quad (4)$$

where a and b are parameters and $z' = {}^{1}/_{b}$ is the temperature increase necessary to reduce the scale parameter by 90%. Since the conventional treatments in hot water bath could be assumed isothermal, Eq. (4) was fitted to the α *versus* T data to obtain the z' value for *E. coli* and *L. monocytogenes* inactivation in apple juice.

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195 2.7. Comparing conventional and microwave treatments on *E. coli* and *L.*
196 *monocytogenes* inactivation

197 Unlike the conventional heat treatments using the hot water bath, the microwave
198 treatments were not isothermal. In order to compare the inactivation of *E. coli* and *L.*
199 *monocytogenes* in apple juice using conventional and microwave heating, the adjusted
200 Weibull model for conventional heating was used to predict the survival ratio for the
201 recorded temperature history $T(t)$ of the microwave treatments, which were compared
202 to the experimental values.

203 Peleg et al. (2005) showed that the momentary inactivation rate for the Weibull
204 model derived from Eq. (3) is:

$$\left(\frac{\mathrm{d}\log(S)}{\mathrm{d}t}\right)_T = -\frac{\beta}{\alpha}.\left(-\log(S)\right)^{\frac{\beta-1}{\beta}} \tag{5}$$

205 which can be numerically integrated to provide the survival rate for a non-isothermal
206 treatment (Chen at al., 2007):

$$\log(S) = -\sum_i \frac{\beta_i}{\alpha_i}.\left(-\log(S_{i-1})\right)^{\frac{\beta_i-1}{\beta_i}}.\Delta t \tag{6}$$

207 where Weibull parameters α and β are evaluated at the momentary temperature T_i of
208 the time temperature profile and Δt is the time increment. An initial value $\log(S_0) =$
209 1×10^{-6} was used as suggested by Peleg and Penchina (2000).

210 Using the recorded time temperature profile of the microwave treatments and the
211 adjusted Weibull parameters for conventional heating, Eq. (6) provided the predicted
212 survival ratio, $\log S_{pred}$, for that given treatment. This result was compared with the
213 experimental survival ratio of the microwave treatment, $\log S_{mw}$. If non-thermal effects
214 of the microwaves in the microbial inactivation are present, then the predicted survival

ratio would be significantly lower than the experimental value, assuming that the kinetic model for conventional heating is well adjusted and provides a reliable prediction.

3. Results and discussion

3.1. Time-temperature profiles of apple juice

Some of the recorded time-temperature histories of apple juice samples inoculated with *E. coli* and *L. monocytogenes* during the processing time under conventional and microwave heating are shown in Fig. 1. Results from the microwave heating of pure apple juice showed that the inoculation of *E. coli* or *L. monocytogenes* did not result in significantly different time-temperature profiles, indicating that the thermal and dielectric properties of the mixture are close to those of the apple juice.

The capillary tube subjected to conventional heating conditions had a come-up time (CUT) of 8 s and come-down time (CDT) of 6 s, making the contribution of heating and cooling rates insignificant when compared with the holding times, which were between 50 and 390 s. Therefore, isothermal conditions were assumed for conventionally treated samples.

The mechanism of microwave heating, conversion of electromagnetic energy into heat by selective absorption and dissipation, is different from conventional heating through conduction and convection (Dorantes-Alvarez and Parada-Dorantes, 2005). Microwaves generate heat constantly and, consequently, there is no holding period (Tajchakavit et al., 1998; Brewer, 2005; Benlloch-Tinoco et al., 2014). The time-temperature profiles during the microwave treatment presented almost linear heating ramps at different rates depending on the power levels (Fig. 1). Some tests were carried out to evaluate the contribution of the CDT to the time-temperature profile, and the

CDT (less than 14 s) was verified to be insignificant when compared with the heating time.

The volumetric microwave power absorption of the samples (P'_{abs}) was calculated from Eq. (1) and it was linearly correlated with the power setting (P_{input}= 400, 600, 800 and 1000 W) through Eq. (7) (R^2 = 0.961, P'_{abs} in W/mL and P_{input} in W). The power absorbed by the sample, $P'_{abs} \cdot (1.0\ \text{mL})$, was smaller than the power setting (P_{input}) because most of the power was lost due to the reflection in the microwave cavity and the small dimensions of the sample. However, the linear trend of the time-temperature profiles (Figs. 1B and 1D) indicate that the energy absorbance was constant during the heat treatments.

$$P'_{abs} = (8.65 \cdot 10^{-7}) \cdot {P_{input}}^2 + (7.06 \cdot 10^{-4}) \cdot P_{input} \quad (7)$$

3.2. Inactivation kinetics by conventional and microwave treatments

The adjusted parameters with their standard deviations and fit criteria are presented in Tables 1 and 2. For conventional heating, the model was fitted for each processing temperature (isothermal treatment), while the model was fitted for each power level for microwave processing (non-isothermal treatment). However, the power absorbed by the sample depends on the shape and dimensions of the sample and microwave cavity. Consequently, the specific power absorption (P'_{abs} from Eq.(7)) is also included in Table 2. Results are valid assuming uniform temperature distribution.

The goodness of fit for the Weibull model was described by the R^2_{adj} and MSE values. The adequacy of the Weibull model to represent *E. coli* and *L. monocytogenes* inactivation in apple juice under conventional and microwave treatments can be seen in Figs. 2 and 3 through the parity charts of predicted microbial reduction by the adjusted model ($\log S_{mod}$) *versus* that of experimental microbial reduction ($\log S_{exp}$).

Figs. 2A, 2C, 3A and 3C show the adjusted inactivation curves of the Weibull model for the processing conditions. For both microorganisms, the number of survivors dropped over time for a given temperature or power. For *E. coli* under conventional heating, no inactivation was detected at temperatures under 55 ℃, whereas it was "completely" inactivated, *i.e.*, under detection levels, for at least 10 s at temperatures above 70 ℃. For *L. monocytogenes*, the safety criterion given by the FDA (2004) was satisfied by conventional treatment at 55 ℃ for 180 s. This criterion requires that at least a 5-$\log_{10}$ reduction of the most resistant organism of public health concern must be achieved. In the case of microwave heating, the FDA standard was reached when processing times were higher than 110 s for *E. coli* and 130 s for *L. monocytogenes* at a power setting of 1000 W ($P'_{abs} = 1.57$ W/mL).

Gentry and Roberts (2005) reported that a 5-$\log_{10}$ reduction of *E. coli* 25922 was obtained in apple cider using a continuous-flow microwave system at 2000 W for about 270 s (mean residence time). In the work by Picouet et al. (2009), after a treatment by microwave radiation at 652 W and 35 s, 7-$\log_{10}$ reduction of *L. innocua* in apple purée was achieved. However, they observed a 1-$\log_{10}$ reduction of *E. coli* O157:H7 after 35 s at the same power of 652 W. As observed by other authors, microbial inactivation was faster for increasing microwave power (Fig. 3) (Cañumir et al., 2002; Sung and Kang, 2014; Valero et al., 2014; Benlloch-Tinoco et al., 2014).

Even though the classical inactivation model with D and z values (Bigelow, 1921) is widely accepted and practiced to describe the inactivation of microorganisms (Tajchakavit et al., 1998; Gentry and Roberts, 2005; Benlloch-Tinoco et al., 2014) and enzymes (Matsui et al., 2008; Riener et al., 2008; Aguiar et al., 2012), microbial survival behavior can change according to the conditions and can be better described by the Weibull model in Eq. (3) (van Boekel, 2002; Surowsky et al., 2014; Rojas et al.,

289 2017). In our work, both microorganisms in the apple juice samples exposed to the
290 microwave energy as well as the conventional heating showed semi-logarithmic
291 survival curves with upward ($\beta < 1$) or downward ($\beta > 1$) concavities (Figs. 2 and 3).
292 The downward concavities in Fig. 3 are consistent with the temperature increase that
293 occurs during microwave treatment (Fig. 1B, 1D), which increases the inactivation rate.

294 The data in Tables 1 and 2 show that, for both microorganisms treated with
295 conventional or microwave heating, the α values presented a negative log-linear
296 dependence with temperature or power level (Eq. (4)), while the β values for *E. coli* and
297 *L. monocytogenes* inactivation do not seem to indicate a clear trend with the temperature
298 or power level.

299 Peleg and Penchina (2000) related changes in the curvature of survival curves to
300 physiological effects. A concave upward curve indicates that the most sensitive
301 members of the population are inactivated first and the more resistant ones remain. A
302 downward concavity may be explained by accumulated damage to the microbial
303 population, which leads the surviving cells to become increasingly more vulnerable.
304 Table 2 and Fig. 2A indicate that the *E. coli* inactivation curves have an upward
305 concavity ($\beta < 1$) indicating the presence of different heat resistances in the population.
306 On the other hand, the concavity of the inactivation curves of *L. monocytogenes*
307 depended on the temperature as can be seen in Table 2 and in Fig. 2C. For longer
308 processing times at the lowest temperature (55 °C), the effect of accumulated damage
309 could be observed, while for shorter times at the highest temperature (70 °C), the effect
310 of thermal resistance variation in the population was more prominent.

311 The temperature dependence on the α parameter for conventional treatment was
312 properly modeled as described in Eq. (4). The z' values and the α values at a reference
313 temperature of 70 °C of *E. coli* and *L. monocytogenes* in apple juice are shown in Table

3. The z' value of *E. coli* is similar to that found in the literature; Enache et al. (2001) compared the thermal resistance of seven serotypes with three strains in apple juice (pH 3.7) and the z value for each strain of *E. coli* O157:H7 ranged between 4.9 and 5.5 ℃. The heat resistance of *L. monocytogenes* (z' value) determined in our study was also in accordance with Mazzota (2001), who reported a z value of 6.3 for *L. monocytogenes* in the stationary phase in apple juice (pH 3.9).

3.3. Comparing conventional and microwave treatments on *E. coli* and *L. monocytogenes* inactivation

As explained in section 2.7, Eq. (6) was used to predict the final survival ratio of *E. coli* and *L. monocytogenes* based on the recorded time temperature profiles of microwave processing at four power levels (examples in Figs. 1B and 1D) using the Weibull kinetic parameters determined for conventional heating (Tables 1 and 3). The isothermal treatment was considered the reference treatment for the nature of the survival of microorganisms. Since the dependence of β with temperature was not clear, it was decided to use a piecewise linear approximation of the data in Table 1. The scale parameter was calculated for each temperature using a modified form of Eq. (4) using the z' and $\alpha_{70°C}$ values from Table 3:

$$\alpha = \alpha_{70°\mathrm{C}} \cdot 10^{\left(\frac{70°\mathrm{C}-T}{z'}\right)} \quad (8)$$

Fig. 4 shows the parity charts of the survival ratio predicted for the microwave-treated samples based on the kinetics adjusted for conventional heating ($\log S_{pred}$) *versus* the experimental survival ratio after microwave processing ($\log S_{mw}$) for *E. coli* (Fig. 4A) and *L. monocytogenes* (Fig. 4B). If the inactivation of microorganisms were similar under conventional and microwave heating, the points would be distributed evenly around the 45º dotted line as in Figs. 2B and 2D; however, results show that for

most of the microwave experiments, the microbial inactivation was more efficient than predicted (18 out of 28). The largest differences in survival ratio were observed in the experiments with shorter duration of microwave exposure. Moreover, larger differences were obtained for experiments with *E. coli* than with *L. monocytogenes*.

The distinction between the thermal and non-thermal effects during microwave heating of foods on pathogenic microorganisms is a controversial topic, since conflicting scientific viewpoints can be found in the literature (Tajchakavit et al., 1998; Anantheswaran and Ramaswamy, 2001; Shazman et al., 2007; Shaheen et al., 2012; Valero et al., 2014; Benlloch-Tinoco et al., 2014). Results from this work suggest that microwave heating can be more effective for the inactivation of *E. coli* and *L. monocytogenes* in apple juice than conventional thermal processing. There seems to exist enhancement effects associated with microwave radiation.

In a review about the specific effects of microwave radiation on bacterial cells, Shamis et al. (2012) report results from the literature that suggests that microwaves enhance enzyme and bacterial inactivation in comparison with conventional heating. Regarding enzyme inactivation, the hypothesis of instantaneous temperature effect may be proposed: some molecules can receive microwave energy at a higher rate than can be dissipated, leading to a high instantaneous temperature that cannot be directly measured. Since this effect is thermal in nature, authors suggest that the expression "specific microwave effect" is more suitable that "non-thermal effect". For the case of bacterial inactivation, besides the enzyme activity reduction, there are evidences that microwaves may increase the porosity of the cell membrane, thus changing their morphology.

Microwave radiation demonstrated to be a promising technology to enhance the microbial safety of pasteurized and sterilized foods. Therefore, further studies should focus on the development of industrial equipment for scaling up microwave

363 pasteurization processes thus allowing the development of products and applications
364 (Orsat and Raghavan, 2005).
365
366 ## 4 Conclusion
367 Microwave heating presents a promising alternative for conventional heating
368 since it is effective against *E. coli* and *L. monocytogenes* in apple juice (inactivation up
369 to 5-$\log_{10}$ cycles). For most of the microwave treated samples, the inactivation was
370 higher than that predicted from the time temperature history. However, to confirm the
371 existence of these enhancement effects, obtaining identical time-temperature profiles by
372 conventional and microwave treatment will be required, besides comparing the survival
373 ratios. Additionally, future works are necessary to evaluate the quality and safety of
374 apple juice pasteurized by microwave heating, for example, the study enzyme
375 inactivation since they are usually more resistant than pathogenic and spoilage
376 microorganisms.
377
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513 **FIGURE CAPTIONS**

514

515 **Fig. 1.** Examples of time-temperature histories of apple juice samples inoculated with *E.*
516 *coli* during conventional and microwave treatments (A and B, respectively), and for
517 samples inoculated with *L. monocytogenes* during conventional and microwave
518 treatments (C and D, respectively).

519

520 **Fig. 2.** *E. coli* (A) and *L. monocytogenes* (C) survival curves under conventional
521 thermal treatment (experimental data and adjusted Weibull model curves) and the parity
522 chart of *E. coli* (B) and *L. monocytogenes* (D) between experimental and model
523 predicted inactivation data.

524

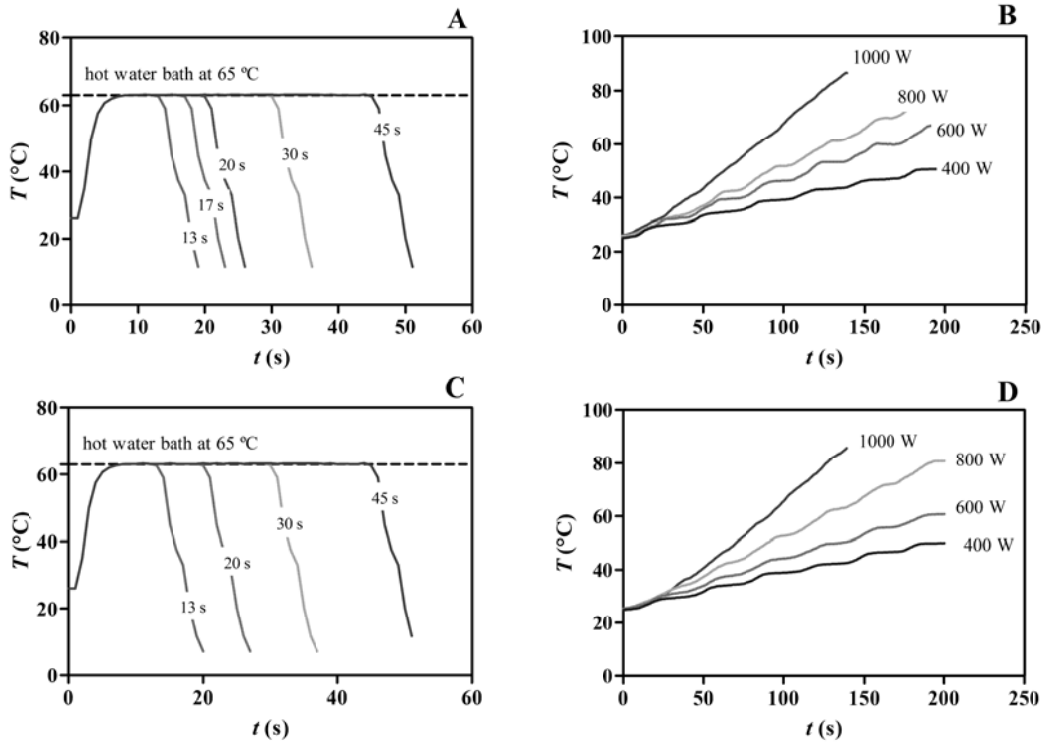
525 **Fig. 3.** Survival curves for *E. coli* (A) and *L. monocytogenes* (C) after microwave
526 thermal treatment (experimental data and adjusted Weibull model curves) and the parity
527 chart of *E. coli* (B) and *L. monocytogenes* (D) between experimental and model
528 predicted inactivation data.

529

530 **Fig. 4.** The survival ratio of *E. coli* (A) and *L. monocytogenes* (B) by microwave
531 treatment ($\log S_{mw}$) *versus* the survival ratio predicted from the time temperature
532 history and the adjusted Weibull model for conventional heating ($\log S_{pred}$). The 45º
533 dashed lines indicate equivalent survival ratio.

534

536 **FIGURE 1**



537

538

FIGURE 2

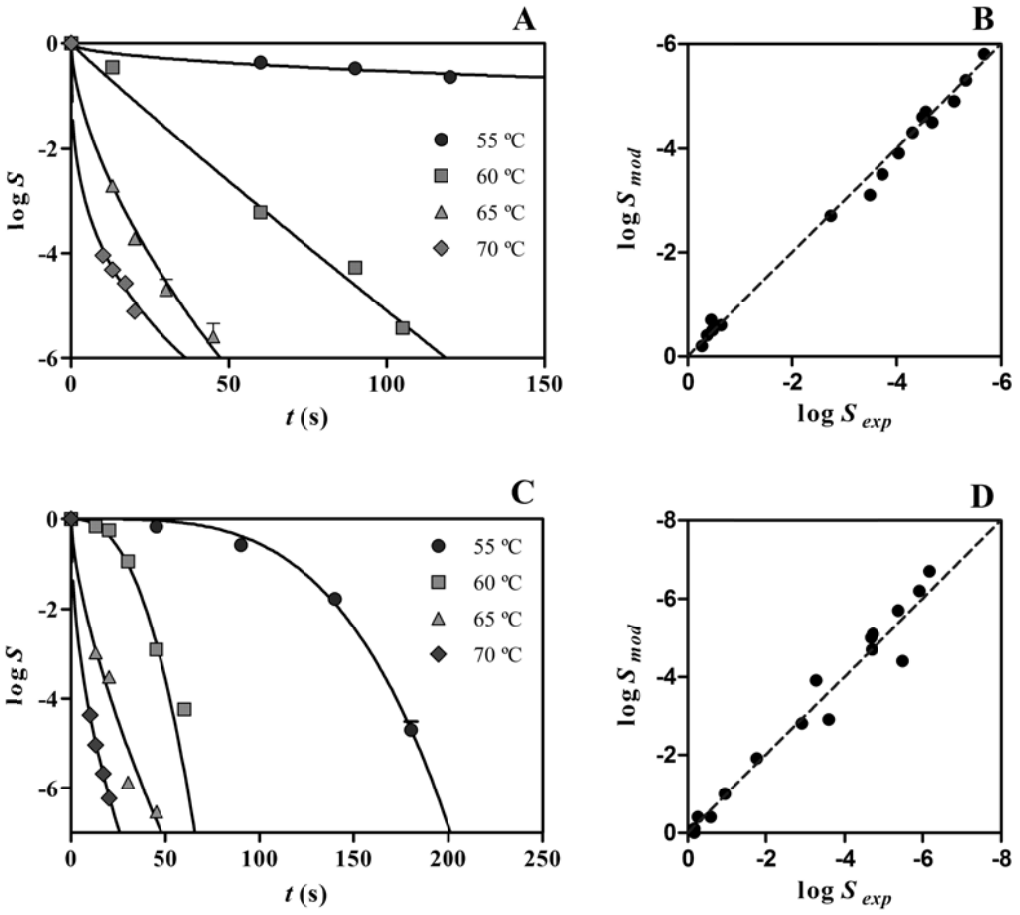
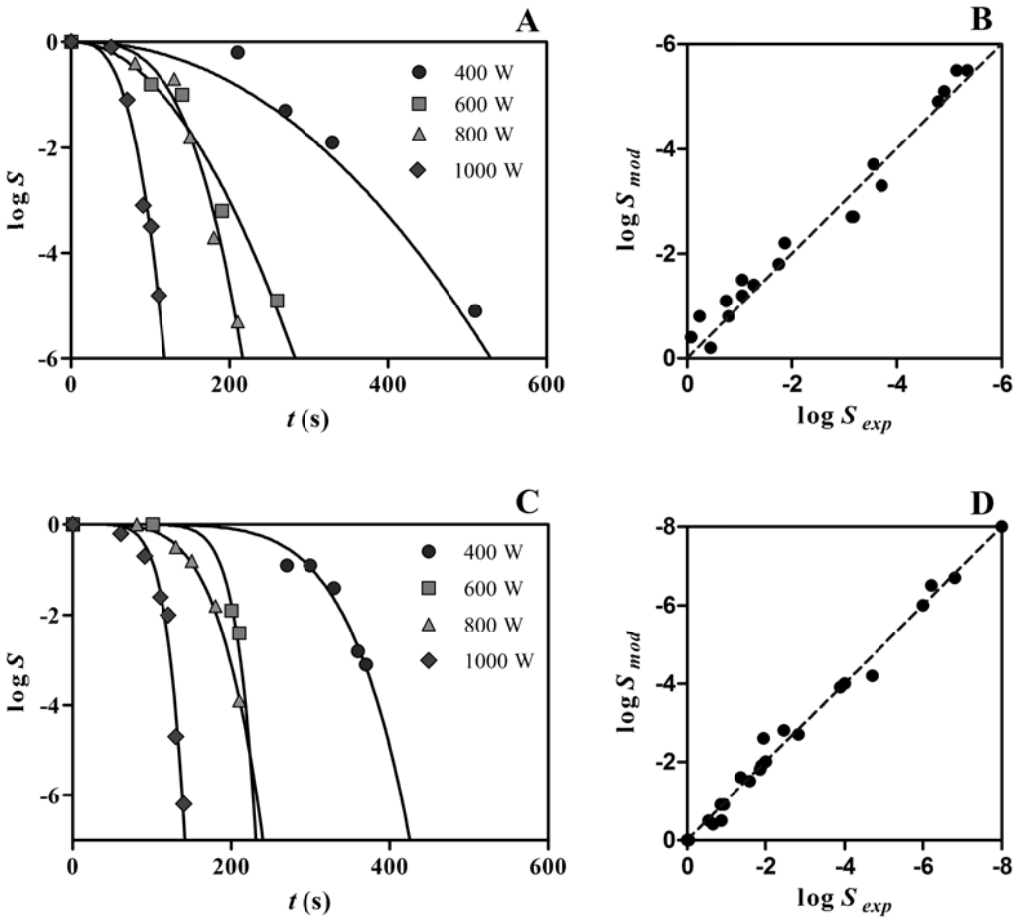
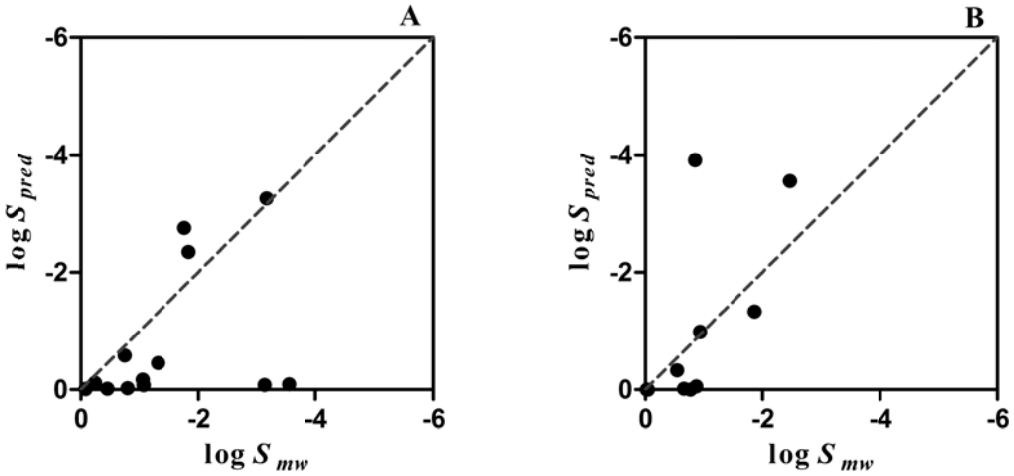


FIGURE 3



546 **FIGURE 4**



547

548

548 **Table 1.** Adjusted Weibull kinetic parameters for inactivation of *E. coli* and *L.*

549 *monocytogenes* in apple juice under conventional heating at different temperatures

T (°C)	*E. coli*				*L. monocytogenes*			
	α (s^{-1})	β (-)	MSE	R^2_{adj}	α (s^{-1})	β (-)	MSE	R^2_{adj}
55	354 ± 24	0.51 ± 0.01	0.0052	99.68	117.8 ± 3.2	3.64 ± 0.36	0.0213	99.27
60	18.23 ± 0.89	0.96 ± 0.03	0.0710	98.49	29.84 ± 0.8	2.47 ± 0.50	0.0223	98.71
65	2.56 ± 0.53	0.61 ± 0.03	0.0539	98.22	2.69 ± 0.29	0.68 ± 0.02	0.1230	97.95
70	0.158 ± 0.072	0.33 ± 0.03	0.0129	99.65	0.53 ± 0.15	0.50 ± 0.04	0.0073	99.89

550

551

552 **Table 2.** Adjusted kinetic parameters for inactivation of *E. coli* and *L. monocytogenes*

553 in apple juice subjected to microwave heating at four power levels

P (W)	P'_{abs} (W/mL)	*E. coli*				*L. monocytogenes*			
		α (s^{-1})	β (-)	MSE	R^2_{adj}	α (s^{-1})	β (-)	MSE	R^2_{adj}
400	0.42	228.9 ± 7.2	2.13 ± 0.11	0.1091	96.53	302.8 ± 5.3	5.73 ± 0.09	0.1783	95.78
600	0.74	114.7 ± 11.4	1.99 ± 0.29	0.1009	97.41	188.6 ± 1.8	9.60 ± 0.51	0.0067	99.65
800	1.12	126.5 ± 3.6	3.34 ± 0.31	0.1339	95.15	155.3 ± 0.7	4.48 ± 0.21	0.1120	97.20
1000	1.57	65.3 ± 2.9	3.06 ± 0.37	0.4265	88.59	102.8 ± 2.5	6.08 ± 0.47	0.0626	91.66

554

555

556 **Table 3.** *E. coli* and *L. monocytogenes* z' and $\alpha_{70°C}$ values obtained in apple juice
557 treated by conventional heating

	z'(°C)	$\alpha_{70°C}$ (s)	MSE	R^2_{adj}
E. coli	4.56 ± 0.25	0.161 ± 0.042	0.1093	98.88
L. monocytogenes	6.17 ± 0.23	0.511 ± 0.073	0.0983	98.48

558

559