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ORIGINAL PAPER

Effect of high hydrostatic pressure and high-pressure homogenisation on *Lactobacillus plantarum* inactivation kinetics and quality parameters of mandarin juice

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Abstract The inactivation kinetics of *Lactobacillus plantarum* in a mandarin juice treated by thermal treatment (45–90 °C), high-pressure homogenisation (HPH) (30–120 MPa at 15 and 30 °C) and high-pressure processing (HPP) (150–450 MPa at 15, 30 and 45 °C) were fitted to different Weibullian equations. A synergic effect between pressure and temperature was observed in HPH and HPP treatments achieving 2.38 log cycles after 120 MPa at 30 °C for 10 s (final *T* of 45 °C) and 6.12 log cycles after 400 MPa at 45 °C for 1 min (final *T* of 60 °C), respectively. A combined treatment of 100 MPa at 15 °C for 10 s and 300 MPa at 15–30 °C for 1 min in HPH and HPP, respectively, was needed to the first logarithm microbial population decline. Weibull model accurately predicted microorganism inactivation kinetics after HPH and HPP processing when displaying single shoulder or tail in the survivor curves, whereas when a more complex trend was observed after thermal treatment, the double-Weibull equation was found more appropriate to explain such behaviour. Equivalent treatments that achieved the same degree of microbial inactivation (77 °C–10 s in thermal processing, 120 MPa–10 s at 30 °C in HPH processing and 375 MPa–1 min at 30 °C in HPP) were selected to study the effects on quality parameters. The application of dynamic pressure led to a decrease in sedimentable pulp, transmittance and juice redness, thus stabilising the opaqueness and cloudiness of mandarin juice. Pectin methyl esterase (PME) was found to be highly baroresistant to static and dynamic pressure. Carotenoid content remained unaffected by any treatment. This study shows the potential of high-pressure homogenisation as an alternative for fruit-juice pasteurisation.

Keywords *Lactobacillus plantarum* · Modelling · Mandarin juice · High-pressure homogenisation · High-pressure processing · Quality

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Introduction

The potential of using pressure as a food preservation mechanism has been known for over two centuries [1]. In the last two decades, different processing equipment has been developed, allowing high-pressure values to be applied (100–1,000 MPa) in a confined space (pressure vessel) containing a fluid (usually water) that acts as the pressure-transmitting medium. This technology is known as high-pressure processing (HPP) [2]. Pressure is applied isostatically, that is, equally and instantaneously transmitted in all parts of food, and in combination with mild heat inactivates pathogenic and spoilage vegetative microorganisms and quality deteriorative enzymes, thus extending the shelf-life and keeping the original properties of the food [3].

HPP technology has been successfully applied in several industrial sectors such as meat, seafood and vegetable products [4]. Recently, the US Food and Drug Administration (FDA) has accepted pressure-assisted thermal-sterilisation (PATS) as a process for the commercial application in low-acid foods. This process combines heat with high pressure to produce commercially sterile low-acid food [5]. HPP technology has proved effective in the inactivation of different microorganisms in orange juice, using pressure values of up to 500 MPa for several minutes

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(*E. coli* including O157:H7 strain, *S. aureus*, *L. monocytogenes*, *Salmonella* spp. and *L. mesenteroides*) [6–9].

Homogenisation has been used in the food industry for many years to decrease the particle size of different food products, especially in milk and milk derivatives, where is essential to diminish the fat globule size using pressure values in a range of 10–69 MPa [10]. Recently, new homogenisation equipment has been developed able to reach pressure values of up to 400 MPa, the so-called dynamic high pressure (DHP) or high-pressure homogenisation (HPH) [11]. Unlike HPP technology, HPH is a continuous process with treatment time lasting several seconds, which makes it more suitable for liquid food processing. A considerable temperature increase is also produced in HPH processing, depending on the fluid viscosity (estimated as 1.5–2.5 °C per 10 MPa) [12]. Several authors have demonstrated the efficacy of HPH technology to inactivate different microorganisms in orange juice (*E. coli* O157:H7, *E. coli* 058:H21, *L. innocua*, *S. aureus*, *Penicillium* ssp., *S. cerevisiae*, *L. plantarum* and *L. mesenteroides*) by using pressure values up to 400 MPa [13–16] or combining pressure and temperature on the stabilisation of chilled fruit juices [17].

When comparing different technologies, it is very important to choose the correct parameters to achieve similar inactivation levels of selected microorganism(s) or enzyme(s). This study establishes the conditions that obtain the same degree of *Lactobacillus plantarum* inactivation by thermal, HPH and HPP treatments. Due to its ability to grow in acidic environments and its thermoresistance, this microorganism produces an undesirable buttermilk flavour by the production of diacetyl, a fermented flavour due to the presence of ethanol and organic acids (lactic acid and acetic acid), and swelling of packages due to the production of carbon dioxide and trace fermentation products [18, 19]. *L. plantarum* has been used by several authors to study the impact of thermal treatment, as well as non-thermal technologies like pulsed electric fields (PEF), HPP and HPH, in acidic products such as orange juice, orange–carrot juice and orange juice–milk beverage [15, 20–24]. The choice of an adequate predictive model that explains the microorganism behaviour against a new preservation technology is very useful when developing tools that enhance the food safety, such as the HACCP and risk assessment systems [25]. As far as the authors' knowledge, there are no studies on modelling the microbial inactivation after HPH processing.

The impact of technology on selected quality parameters is of utmost importance when applying a pasteurisation process to citrus juice. Pectinmethylesterase activity (PME) is one of the most important parameters. PME destabilises the cloud stability leading to a clarified product with low commercial value. Studies of orange-juice processing conditions have demonstrated the thermal and high-pressure stability of orange PME (600–700 MPa for 1–3 min combined with mild temperatures 50–60 °C) [26–28]. By contrast, HPH processing conditions have not been established yet. Furthermore, it is important to assess the stability of the physical–chemical parameters traditionally used as quality indexes in citrus juices, placing emphasis on colour, turbidity and carotenoid content.

The objective of the present work was to compare *L. plantarum* inactivation kinetics in mandarin juice after HPP, HPH and thermal equivalent processes and to study their effect on selected quality parameters.

Materials and methods

Samples

Clementine mandarins (*Citrus reticulata*, cv. *Nules*) were harvested from an orchard in Valencia and immediately used for juice preparation. The fruits were washed by immersion in tap water, drained and squeezed in an industrial extractor with finger cups (Exzel, Luzzysa, Valencia, Spain). Raw juice was sieved in a screw finisher (0.7 mm diameter) and immediately processed by HPP, HPH or thermal treatment.

Lactobacillus plantarum culture

The freeze-dried microorganism provided by the Spanish Type Culture Collection (CECT 220) was dissolved in 15 mL of MRS broth (Scharlau Chemie S.A., Barcelona, Spain) and kept for 30 min at 37 °C. Then, the content was placed in 500 mL of MRS broth with continuous agitation (200 rpm) at 37 °C until reaching the stationary growth stage (16–20 h). A volume of 500 mL of the culture was centrifuged twice (Beckman J-25) at 3,220*g*, 5 min and 4 °C, and dissolved in 100 and 50 mL of MRS broth, respectively. The content was transferred to 2-mL vials with 1 mL of suspension and 1 mL of glycerol 20% diluted with MRS broth (cryoprotectant) and kept at −80 °C. A negative control was carried out immediately after the microorganism was added to the juice and after the treatment to ensure inoculum homogeneity. Viable counts were based on duplicate counts from appropriate peptone water dilutions in MRS agar and incubated at 37 °C for 48 h.

Treatment of samples

HPH treatment

Triplicate HPH treatments were applied to mandarin juice samples in a high-pressure homogeniser (Panda 2 K, GEA

Process Engineering Inc., Italy) (Table 1). The flow rate of the juice in the homogeniser was constant (7.5 L/h). The residence time of the sample at maximum temperature was calculated from the moment the sample entered the treatment chamber until it was collected at the outlet tubing (10 s) and was considered as the treatment time. Experiments were performed with 2.0 L of inoculated mandarin juice (1×10^7 CFU/mL), the majority of which was passed through the homogeniser to ensure temperature equilibration. After treatment, 100 and 250 mL of mandarin juice were collected for microbial and quality analyses, respectively. The homogenised samples were cooled rapidly in an ice-water bath.

HPP treatment

Triplicate HPP experiments were performed in a laboratory-scale 2.4-L vessel high-pressure processor (EPSI, Belgium) (Table 1). The pressure medium was a water–ethylenglycol mixture (80:20). A thermostated mantel, which surrounded the vessel, was connected to a cryostat keeping the vessel wall temperature constant during the experiment. Temperature was recorded by a thermocouple placed inside the vessel. The inoculated samples (2×10^8 CFU/mL) for microbial analysis were filled in 2-mL eppendorf tubes, whereas 200-mL polyethylene plastic bags were used for the quality study. After pressure release, the samples were immediately cooled in ice-water.

Table 1 Process conditions

Description	Operating conditions (HPH)	Operating conditions (HPP)
Pressure range (MPa)	0–120	0–450
Come-up time (s)	1	90
Holding time (s)	10	10/60
Decompression time (s)	1	15
Initial temperature (°C)	15	15
	30	30
		45
Final temperature after pressurisation at highest pressure (°C)	35.1	30.6
	46.5	46.1
		60.3
Final temperature after holding time at highest pressure (°C)	–	26.6
		42.2
		57.1
Final temperature after decompression at highest pressure (°C)	–	12.5
		27.8
		40.2

Thermal treatment

Triplicate heat inactivation tests were carried out using 0.1 mL ring-marked microhaematocrit capillary tubes. *L. plantarum* cells suspended in the mandarin juice were centrally injected into the tubes, sealed by pulling off both ends in an oxygen gas flame and heated in a stirred water bath. Four capillary tubes were prepared for each temperature (45–90 °C) for 10 s. After heating, the capillary tubes were cooled in an ice/water bath. Just before thermal treatment, 150 mL of juice was inoculated with the thawed microorganism, reaching a final concentration of 8×10^6 CFU/mL. For quality purposes, the mandarin juice was pasteurised in a plate heat exchanger equipped with nominal 10-s hold-time tube (FT74X/HTST/UHT, Armfield Inc., UK).

Mathematical models

All survivor curves after HPH, thermal and HPP processing showed deviations from the log-linear behaviour. Different mathematical models have been used to describe the non-linear microorganism inactivation patterns, with Weibull distribution function-based models the most widely applied to explain microorganism inactivation kinetics after non-thermal processing [21, 22, 29–37]. Assuming that the pressure and temperature resistance of the individual cells of a microbial population is governed by a Weibull distribution, two Weibullian equations were applied to two valid replicates of *L. plantarum* inactivation kinetic data where the independent variable (t) was substituted by independent variable (P) in HPH and HPP treatments and (T) in thermal processing by using the following models:

Weibull model [38]:

$$\mathrm{Log}\,(N) = \mathrm{Log}\,(N_0) - \left(\frac{P}{a_\mathrm{P}}\right)^n \quad \text{(HPP and HPH treatment)} \tag{1}$$

$$\mathrm{Log}\,(N) = \mathrm{Log}\,(N_0) - \left(\frac{T}{a_\mathrm{T}}\right)^n \quad \text{(Thermal treatment)} \tag{2}$$

where N is the surviving cells after the treatment expressed as CFU/mL, N_0 the initial microbial population (CFU/mL), P is the independent variable in Eq. 1 as the pressure applied in the HPP and HPH treatment (MPa), T is the independent variable in Eq. 2 as the temperature applied in the thermal treatment (°C), a_P is the pressure of the first logarithm decline for the microbial population (MPa), a_T is the temperature of the first logarithm decline for the microbial population (°C) and n the shape parameter. The n value gives an idea of the form of the curve, if $n > 1$ the curve is convex (it forms shoulders), if $n < 1$ the curve is concave (it forms

tails) and if $n = 1$ the curve is a straight line and can be described by linear models.

Weibull model has been shown to be sufficiently robust and simple to describe both downward concave and upward concave behaviours [38]. However, when more complex survivor curves are observed, a double-Weibull equation was proposed assuming that the population was composed by two groups with different resistance to stress, and each group following Weibull distribution [39]. Subpopulation 1 was characterised to be more sensitive to stress that subpopulation 2 ($a_1 < a_2$). This equation can be expressed as follows:

$$\mathrm{Log}(N) = \mathrm{Log}\left(10^{\frac{N_0}{1+10^{\alpha}}} \times 10^{\left(-\frac{P}{a_{\mathrm{P1}}}\right)^{n+\alpha}} + 10^{\left(-\frac{P}{a_{\mathrm{p2}}}\right)^{n}}\right) \quad (3)$$

(HPP and HPH treatment)

$$\mathrm{Log}(N) = \mathrm{Log}\left(10^{\frac{N_0}{1+10^{\alpha}}} \times 10^{\left(-\frac{T}{a_{\mathrm{T1}}}\right)^{n+\alpha}} + 10^{\left(-\frac{T}{a_{\mathrm{T2}}}\right)^{n}}\right) \quad (4)$$

(Thermal treatment)

where α is equivalent to $\mathrm{Log}(N_{01}/N_{02})$ and can be graphically expressed as the difference between log (N_0) and the logarithm of the population size where the inflection of the survivor curve is observed; a_{P1} and a_{P2}, the pressure of the first logarithm decline for the two subpopulations (MPa); and a_{T1} and a_{T2}, the temperature of the first logarithm decline for the two subpopulations (°C).

PME activity

PME activity was determined according to a previously published method [40] based on a modification of the originally published procedure [41]. Juice samples of 5 mL adjusted to pH 7.8 with NaOH were mixed with 20 mL of a 0.2 M NaCl solution with 0.5% pectin (Grinsted Pectin MRS 351, Danisco, Denmark), also previously adjusted with NaOH to the same pH value. The pH decrease was caused by the carboxylic groups generated by PME during the de-esterification of the pectin solution at pH 7.8, and ambient temperature (22 °C) was recorded periodically with time intervals depending on enzyme activity. The initial slope of the curve fitted to the time course of pH against the incubation time was considered as PME activity expressed in nkat/mL. Values were given by the mean of three replicates.

Physical measurements

Cloudiness and suspended pulp

The method involved using a graduated centrifuge tube with a conical bottom, filled with 10 mL of juice. The sample was centrifuged at 3,000*g* for 10 min at 20 °C. The supernatant was collected, and its transmittance at 650 nm was analysed for cloudiness with a UV/visible spectrophotometer (Ultrospec 330 pro, Amaersham Bioscience, USA). The suspended pulp was measured using a drained tube, kept in the oven at 37 °C for 24 h, and the precipitate was weighed. Suspended pulp was expressed as % (w/w). Values were given by the average of two replicates.

Colour

Colour was measured with a Hunter colorimeter (Labscan II, Hunter associates Laboratory, USA) [42]. Samples were contained in optical glass cells 3.8 cm high and 6 cm in diameter. The results were obtained in a CIELAB system for illuminant D65 and a 10° angle of vision. The registered parameters were as follows: L^* (brightness), a^* (red–green component) and b^* (yellow–blue component), h_{ab} (hue, attribute related to the differences in absorbance at different wavelengths and considered the qualitative attribute of colour) and C_{ab} (chroma, quantitative attribute of colourfulness). The global colour difference (ΔE) was calculated by using the following equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (5)$$

Depending on the value of ΔE, the colour difference could be estimated such as not noticeable (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3), well visible (3–6) and great (6–12). The values provided were the average of four replicates.

Total carotenoid content

The method employed is a modification of that previously described [43]. The process was carried out in the dark to avoid carotenoid degradation. A centrifuge tube filled with 10 mL of juice and 20 mL of extraction mixture HAE (hexane: acetone: ethanol 50:25:25) was stirred for 30 s in a homogeniser (IKA-WERKE yellow line DI 25 basic, Germany) and then centrifuged at 2,880*g* for 10 min at 4 °C. The organic phase was recovered and centrifuged again until colourless. The organic extract was dried at 38 °C in a rotary evaporator (Buchi R II, BÜCHI Labortechnik, Switzerland), maintained overnight in darkness and saponified with diethyl ether and 10% KOH in methanol. The saponified extract was collected, and diethyl ether was added until it became colourless. The total content of carotenoids was measured in a spectrophotometer (Ultrospec 3300 pro UV/visible spectrophotometer, Amersham Biosciences, USA) in a wavelength ranging from 270 to 540 nm. Values were given by the average of two replicates.

Statistical analysis

Two valid replicates were used to check how well the model fits to the experimental microbial inactivation curve by calculating the adjusted R^2 defined as follows:

$$\text{Adj.}\, R^2 = \left[1 - \frac{(m-1)\left(1 - \frac{\text{SSQ}_{\text{regression}}}{\text{SSQ}_{\text{total}}}\right)}{m-j}\right] \quad (6)$$

where m is the number of observations, j is the number of model parameters and SSQ is the sum of squares.

The predictive models were validated with a third set of experimental data not used for model fitting, by calculating the accuracy factor (Af) defined as follows [44]:

$$\text{Af} = 10^{\frac{\sum |\text{Log(predicted/observed)}|}{n}} \quad (7)$$

where n is the number of observations. The predicted and observed values are referred to as the final number of microorganisms. The interpretation of this statistic is closer to 1 the Af values, the better the model predicts the data.

The statistical analysis was performed using the software Statgraphics® Centurión XV (Statistical Graphics Corp., USA) applying a nonlinear regression. The effect of different treatments on the kinetic parameters was assessed by a univariant analysis of variance (ANOVA) with a significance level of 95% ($p = 0.05$) using the Tukey's test.

Results and discussion

Effect of processing on *L. plantarum* inactivation

Figure 1 shows *L. plantarum* inactivation after combined thermal (15 and 30 °C) and HPH treatment. An increase in initial temperature produced an increase in the inactivation level achieving 2.4 log cycles after 120 MPa and 30 °C (final temperature 46.1 °C) (Fig. 1). The microbial inactivation mechanism exerted by HPH is believed to be due to the mechanical destruction of cell integrity, caused by different mechanisms such as spatial pressure and velocity gradients, turbulence, impingement and cavitation, which occur in liquids during high-pressure homogenisation where the pressure is rapidly released [45]. Several authors have observed greater *L. plantarum* baroresistance to HPH treatment in orange juice. For instance, a multiple-cycle treatment of 5 passes of 200 MPa at 25 °C reduced 2.3 log cycles of *L. plantarum* [16], whereas a single cycle of 250 MPa reduced completely initial loads of *L. plantarum* (7.1 log cycles) [15]. In addition, higher resistance of Gram-positive bacteria (among them *L. plantarum*) to HPH treatment than Gram-negative bacteria has been shown,

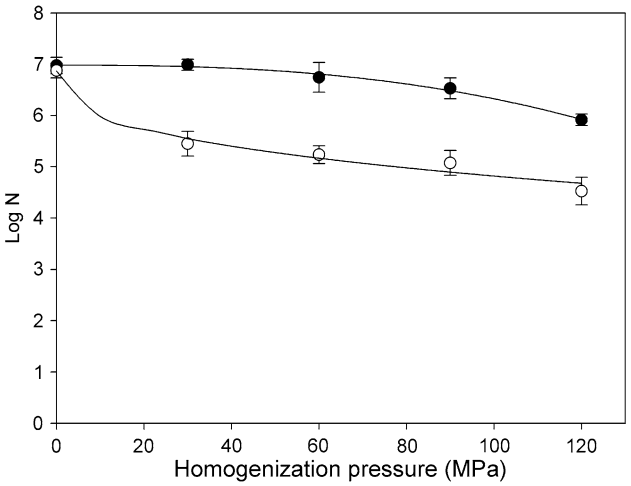

Fig. 1 *L. plantarum* inactivation kinetics after HPH treatment at 15 °C (*filled circle*) and 30 °C (*open circle*) for 10 s in mandarin juice adjusted to the Weibull model. *Error bars* represent the standard deviation

possibly due to the higher peptidoglycan content of the cell wall [46].

Table 2 shows the calculated kinetic parameters and the adjusted R^2 and Af values of the Weibullian equations (Eqs. 1, 3) used to describe *L. plantarum* inactivation kinetics after HPH treatment. Survivor curve at 15 °C showed a shouldering effect, and an increase in pressure level (up to 60 MPa) did not significantly affect the microorganism inactivation. A shouldering phenomenon in the survivor curves of *L. monocytogenes*, *E. coli* and *S. aureus* after HPH treatment has been also observed and pressure values up to 30–85 MPa (depending on the microorganism studied) did not affect the cells inactivation [47]. The shouldering phenomenon could be explained by the theory that there is a statistical distribution of individual resistance of microorganisms to pressure or to sublethal injury of the microbial cells. The sublethal injury phenomenon seems unlikely because the mechanical stresses to which microbial cells are exposed causing the physical disruption of the cells, breaking them into very small fragments rather than from physiological or metabolic damage, and this seems to cause little or no sublethal injury [45]. Several authors have demonstrated the absence of sublethal injury after HPH treatment in *L. innocua*, *E. coli*, *S. aureus*, *Y. enterocolitica*, *S. typhimurium* and *L. monocytogenes* by using selective environment such as the addition of salt, SDS or low pH, specific selective medium for the microorganism or a propidium iodide staining [13, 14, 46, 48–51].

Survivor data at 30 °C showed a higher inactivation rate at low pressure levels (30 MPa) followed by a tail, indicating a residual fraction resistant to the treatment. Kinetic parameters of Weibull (a) and double-Weibull (a_{P1}, a_{P2})

Table 2 Kinetic parameters, Adj. R^2 and Af values of Weibull and double-Weibull models fitted to *L. plantarum* survival curves on mandarin juice after HPH treatment

P (MPa)	Weibull				Double-Weibull					
	a_P (MPa)	n	Adj. R^2	Af	α	a_{P1} (MPa)	a_{P2} (MPa)	n	Adj. R^2	Af
15	116.90 ± 0.30[a]	3.06 ± 0.06	0.999	1.007	1.27 ± 0.01	111.18 ± 0.82	210.08 ± 1.05	3.40 ± 0.03	0.999	1.006
30	13.98 ± 0.02	0.36 ± 0.06	0.983	1.010	2.00 ± 0.01	13.90 ± 0.03	27.85 ± 0.01	0.36 ± 0.01	0.966	1.012

[a] Standard deviation

models decreased significantly as temperature increased, indicating lower microorganism resistance to the treatment ($p < 0.05$). An increase in α value of double-Weibull model at 30 °C indicated a decrease in the ratio of the most resistant cells of the population corresponding to a higher inactivation level of the resistant subpopulation. Kinetic parameter of the sensitive population (a_{P1}) was close to a value of Weibull model at both temperatures. Fitted values were close to experimental ones (Adj. R^2 of 0.999–0.983 and 0.999–0.966 for Weibull and double-Weibull models at 15 and 30 °C, respectively), and predictions were accurate (Af values of 1.007–1.010 and 1.006–1.012, respectively).

Temperature plays an important role in microbial inactivation by HPH and HPP treatments as an increase in pressure necessarily implies a temperature increase. In order to establish the impact of temperature on *L. plantarum* pressure inactivation, microbial cells inoculated in mandarin juice were exposed to a range of temperatures (45–90 °C) for 10 s (equal treatment time as HPH treatment) (Fig. 2). No inactivation was observed at the maximum temperature reached in HPH treatment (46 °C), whereas HPH processing at 15 °C (30 °C outlet T) only reduced one log cycle of microbial cells demonstrating a synergism between dynamic pressure and temperature. Several studies have addressed the higher inactivation achieved by HPH processing by increasing the temperature. This temperature effect was explained by changes in the physical properties of the cell membrane (hydrogen and hydrophobic bonds being weakened), making bacterial membranes less resistant to high pressure [52]. On the other hand, temperature seemed also influence some of the inactivation mechanisms proposed by HPH, such as cavitation and turbulence. The flow pattern of a fluid will be more turbulent at high temperature as a result of a reduction in fluid viscosity. Increased turbulence will in turn increase cavitation and hence microbial inactivation [53].

Different mathematical models (Eqs. 2, 4) were fitted to the survivor data after thermal treatment obtaining the corresponding kinetic parameters (Table 3). Results showed a more complex trend (sigmoidal shape) in the survivor curve. Kinetic parameter of resistant fraction (a_{T2}) tended to infinity when double-Weibull equation was fitted

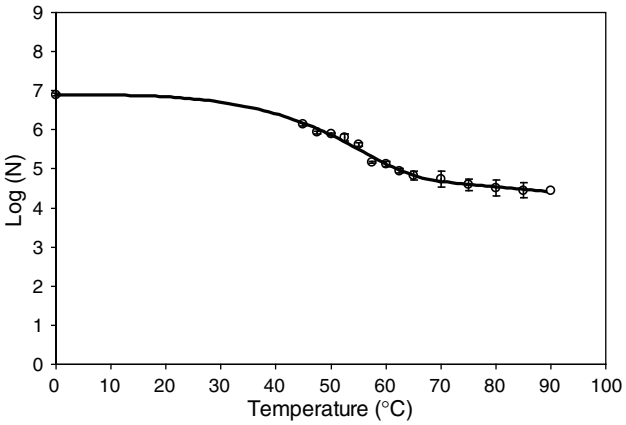

Fig. 2 *L. plantarum* inactivation kinetics after thermal treatment (10 s) in mandarin juice adjusted to the double-Weibull model. *Error bars* represent the standard deviation

to a sigmoidal shape survivor curve [39]. In this case, Weibull model was unable to accurately predict (prediction error of 36%) such behaviour, and double-Weibull equation was found to be more suitable with a prediction error of 9%.

HPP technology has been used to effectively inactivate *L. plantarum* in different matrixes, such as model beer and beer, milk buffer, phosphate buffer (pH 7.0) and citrate buffer (pH 4.2) [54–57], achieving 5 log reductions at values of 400–500 MPa from 30 s to several minutes. In our study, for the same processing conditions as HPH treatment (30–120 MPa at 15, 30, 45 °C) for 10 s, HPP did not produce a significant effect on microorganism viability (data not shown). This fact demonstrates that due to the 'static' nature of HPP, greater pressure and longer treatment time values are necessary to achieve microbial inactivation. In this sense, different inactivation patterns have been observed for several microorganisms (Gram positive and Gram negative) by HPH and HPP, which would suggest different inactivation mechanisms [46]. This is certainly true for processing by high-pressure homogenisation where other physical factors than pressure (such as the effects commented earlier) are mainly responsible for microbial inactivation.

In order to obtain higher levels of inactivation by HPP, pressure values were increased by 150–450 MPa at 30 °C (final T of 51.2 °C) for 10 s. Under these conditions, better results were obtained, with onset of inactivation after

Table 3 Kinetic parameters, Adj. R^2 and Af values of Weibull and double-Weibull models fitted to *L. plantarum* survival curves in mandarin juice after thermal treatment

Weibull				Double-Weibull					
a_T (°C)	n	Adj. R^2	Af	α	a_{T1} (°C)	a_{T2} (°C)	n	Adj. R^2	Af
32.58 ± 6.00	1.14 ± 0.17	0.921	1.036	2.76 ± 0.35	43.11 ± 1.88	–	2.38 ± 0.28	0.987	1.009

250 MPa and achieving 1.03 log cycles after 450 MPa (data not shown). However, these treatments did not obtain an equal reduction level to the maximum inactivation achieved by HPH treatment (2.4 log cycles). Consequently, a range of 200–400 MPa for 1 min at 15, 30 and 45 °C was selected (Fig. 3). No significant differences in the microbial inactivation rate were observed between treatments at 15 °C and 30 °C, reaching 4.43 log units at 400 MPa and 30 °C (final temperature of 52.1 °C) ($p > 0.05$). However, a temperature increase to 45 °C achieved a significant rise in microorganism inactivation, reaching 6.12 log units at 400 MPa (final temperature of 60.3 °C) (Fig. 3). As in the case of HPH treatment, a synergic effect was observed between pressure and temperature above 30 °C.

Diverse mathematical models (Eqs. 1, 3) were applied to the *L. plantarum* survivor data after HPP treatment obtaining the corresponding kinetic parameters (Table 4). Results revealed a shouldering effect for all temperature ranges, indicating low-treatment lethality under the initial treatment conditions, or the appearance of sublethal injury. This phenomenon was also observed in *L. plantarum* inoculated in model beer, where a shouldering effect was observed in the survivor curves at 200 MPa and room temperature [57]. No significant differences were evident in kinetic values (a, a_{P1}, a_{P2}) ($p > 0.05$) at both

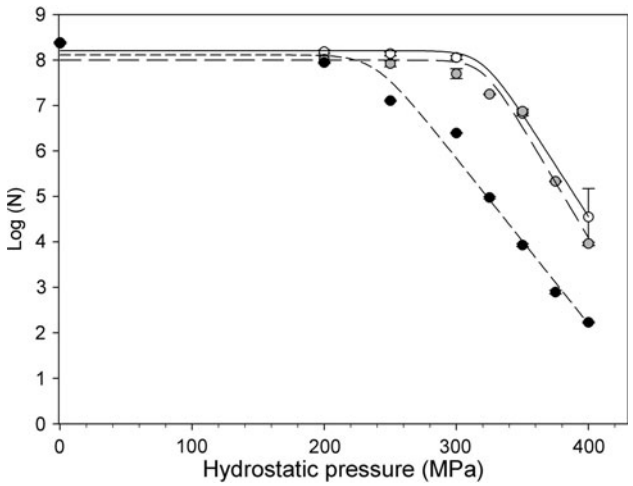

Fig. 3 *L. plantarum* inactivation kinetics after HPP treatment at 15 °C (*open circle*), 30 °C (*grey circle*) and 45 °C (*filled circle*) for 1 min in mandarin juice, adjusted to Weibull model. *Error bars* represent the standard deviation

temperatures. At 45 °C, a reduction in kinetic values were observed corresponding to a lower microorganism treatment resistance. An increase in temperature also lowered the ratio of the resistant fraction for the population (greater α value), indicating a higher degree of inactivation of resistant cells. In addition, HPP α values were close to the thermal treatment one (Table 3) but higher than the HPH treatment ones (Table 2), indicating that temperature and static pressure were able to inactivate in a higher extent the resistant fraction than homogenisation pressure. Weibull model fitted the experimental data well (Adj. R^2 of 0.952–0.990) with Af values of between 1.012–1.066.

Effect of processing on quality parameters

Equivalent treatments were chosen for each technology based on *L. plantarum* HPH inactivation (2.4 log cycles at 120 MPa and 30 °C), interpolating that value in the equation that best fitted the experimental data for each technology, that is, the double-Weibull model (Eq. 4) for the thermal treatment and the Weibull model (Eq. 1) for the HPP treatment. For thermal treatment, a combination of 77 °C–10 s and for HPP a combination of 375 MPa for 1 min at 30 °C were inferred. Table 5 shows the effect of equivalent treatments on quality parameters. Suspended pulp decreased significantly after the application of pressure and was more pronounced after HPH treatment. HPH treatment is known to reduce particle size, converting part of the sedimentable pulp into colloidal pulp. In addition, transmittance was reduced significantly after HPH treatment leading to a more visually opaque juice, which may be due to greater stabilisation of the juice cloud. PME was decreased by 26, 39 and 88% after HPH, HPP and thermal treatment, respectively, showing a high enzyme baroresistance. Eighty per cent of PME inactivation was observed after 5 passes of 250 MPa at 37 °C [58]. Carotenoid content was not affected by any treatment. Colour parameters a and b significantly decreased for all treatments ($p < 0.05$) and was more pronounced after HPH treatment, possibly due to a decrease in pulp particle size that affected juice opaqueness. The ΔE values of treated samples indicated significant colour differences compared to control.

Synergism between temperature and pressure was observed in the inactivation of *L. plantarum* in mandarin juice achieving a significant reduction by combining thermal

Table 4 Kinetic parameters, Adj. R^2 and Af values of Weibull and double-Weibull models fitted to *L. plantarum* survival curves on mandarin juice after HPP treatment

T (°C)	Weibull				Double-Weibull					
	a_P (MPa)	n	Adj. R^2	Af	α	a_{P1} (MPa)	a_{P2} (MPa)	n	Adj. R^2	Af
15	334.96 ± 7.92[a]	7.50 ± 0.85	0.990	1.012	2.81 ± 0.23	320.27 ± 36.55	668.33 ± 88.60	6.00 ± 0.96	0.965	1.037
30	330.04 ± 7.42	7.55 ± 0.86	0.983	1.021	2.56 ± 0.36	314.41 ± 18.74	651.28 ± 100.23	6.00 ± 0.65	0.962	1.040
45	128.23 ± 30.15	2.41 ± 0.47	0.952	1.066	3.08 ± 0.09	224.88 ± 27.55	445.02 ± 52.87	3.28 ± 0.74	0.974	1.026

[a] Standard deviation

Table 5 Effect of thermal, HPP and HPH treatments on quality parameters of mandarin juice

Parameter	Fresh	Thermal	HHP	HPH
Pulp (%)	2.44 ± 0.11[a]	2.95 ± 0.05	2.26 ± 0.09	2.00 ± 0.13
Transmittance	84.10 ± 0.09	66.40 ± 0.12	82.20 ± 0.07	12.26 ± 0.03
Carotenoids (μg/mL)	7.21 ± 0.91	7.70 ± 0.47	6.81 ± 0.05	7.09 ± 0.22
PME (nkat/mL)	0.98 ± 0.01	0.11 ± 0.01	0.59 ± 0.03	0.72 ± 0.01
Colour (L^*)	53.30 ± 0.33	48.55 ± 0.25	47.93 ± 0.41	48.17 ± 0.46
Colour (a^*)	9.39 ± 0.09	7.08 ± 0.18	7.26 ± 0.25	3.70 ± 0.08
Colour (b^*)	58.65 ± 0.36	53.38 ± 0.58	52.34 ± 0.69	50.60 ± 0.62
ΔE	–	7.46	8.55	11.11

[a] Standard deviation

and HPH treatment (120 MPa–30 °C–2.4 log cycles) and thermal and HPP (400 MPa–45 °C–6.12 log cycles). Weibull model was able to accurately predict *L. plantarum* survivor data after HPH and HPP treatments when displaying downward and upward concave behaviours. When a more complex survivors curve was observed after thermal treatment, Weibull model was unable to accurately describe microorganism inactivation trend and the double-Weibull equation was found to better predict such behaviour. The application of dynamic pressure stabilised the opaqueness and cloudiness of mandarin juice. High PME baroresistance was observed. Carotenoid content remained unaffected by any treatment. Colour was affected by the treatments being more pronounced after HPH processing. In order to achieve an equivalent microbial reduction to the traditional thermal processing or HPP, further studies are needed to implement the ultra-high-pressure homogenisation process for fruit-juice pasteurisation.

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