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Effect of thermosonication batch treatment on enzyme inactivation kinetics and other quality parameters of cloudy apple juice.

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Abstract

Cloudy apple juice has been treated by thermosonication in batch mode as an alternative processing to thermal treatment. Thermosonication was found to be effective to inactivate polyphenol oxidase; however, pectinmethylesterase was found to be more resistant. An increase of the working ultrasound amplitude and the amount of energy supplied to the juice led to lower enzyme residual activities.

Enzyme inactivation kinetics were determined at different temperatures (from 44 to 67 ºC). Inactivation data were described by the first order kinetic model and the Weibull model, both models yielding good fitting. Thermosonication treatment caused a homogenization effect reflected in the shifting of the particle size distribution towards smaller diameters. The effect of the nature of dissolved gases in the juice on enzyme inactivation was studied by displacing the air dissolved in the juice by bubbling nitrogen or carbon dioxide, previous to the thermosonication experiments. Higher inactivation rates were obtained by displacing the air with nitrogen.

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Industrial relevance: Consumers demand of natural and fresh-like products has driven the food industry to investigate alternative technologies to replace conventional food heat treatments that may affect food quality. Among these technologies, thermosonication treatment is an attractive technology that can inactivate microorganisms and enzymes. This work shows that some enzymes that cause deleterious effect on cloudy apple juice can be more effectively inactivated by thermosonication than by thermal treatment, in the same temperature range, reducing the damages caused by heating

Keywords: Cloudy apple juice, thermosonication, enzyme inactivation, dissolved gasses.

1. Introduction

Cloudy apple juice is a good source of bioactive compounds such as ascorbic acid, polyphenols, and pectins. However, enzymatic browning causes serious product deterioration. One of the most important enzymes responsible for enzymatic browning is polyphenol oxidase (PPO). PPO catalyzes the oxidation of phenolic compounds to quinones, which will subsequently react with other compounds to form brown pigments that reduce juice quality. Other important quality parameter in cloudy apple juice is the cloud stability. One of the most accepted theories of cloud loss in juices, is based on the action of pectinmethylesterase (PME). This enzyme causes pectin demethylation that could expedite pectin precipitation with calcium ions present in the juice, causing clarification and turbidity loss (Briongos et al., 2016)

Thermal treatments are widely used in the food industry to inactivate microorganisms and enzymes that cause deleterious effects on foods. However, consumers demand of natural and fresh-like products has driven the food industry to investigate alternative technologies to replace conventional food heat treatments, wich may affect food quality in terms of nutritional and physicochemical parameters. Among these technologies, ultrasonication is an attractive

technology that is considered to be simple, reliable, environmentally friendly and highly effective in achieving microbial decontamination (Dias et al., 2015). Inactivation of microorganisms and enzymes by sonication is attributed to physical (cavitation, mechanical effects) and chemical (formation of free radicals) effects (O'Donnell, Tiwari, Bourke, & Cullen, 2010). Inactivation of enzymes by ultrasonication has been attributed to the formation of localized hot spots upon collapse of bubbles, shear forces created by microstreaming and shock waves, as well as generation of free radicals through sonolysis of water (Kadkhodaee & Povey, 2008). Sonochemical activity (production of radicals) is influenced by operational parameters, being the effect of frequency and gas addition the most significant (Gielen et al., 2016). Furthermore, the presence of gases (air, oxygen or an inert gas) in the solution has been reported as necessary for enzyme inactivation (Grintsevich, Adzerikho, Mrochek, & Metelitza, 2001). In any case, enzyme inactivation mechanisms are specific to each enzyme and depend on their amino acid composition and their conformational structure (Anaya-Esparza et al., 2017).

When ultrasound is combined with heating, the resulting technique, thermosonication (TS), is usually more effective as preservation technique even TS temperatures being lower than those used in thermal treatments. TS treatment has been found to be more efficient than thermal treatment for the inactivation of different types of enzymes responsible for deterioration of fruits and vegetable juices, among them, pectin methylesterase, PPO, lipoxygenases and peroxidases (Baltacioglu, Bayindirli, & Severcan, 2017). Recently, Anaya Esparza et al. (Anaya-Esparza et al., 2017) reviewed the advantages and limitations offered by the application of TS on different fruit and vegetable juices. In this work, the effect of TS on PPO and PME inactivation in *Golden Delicious* cloudy apple juice has been studied. The effect of different variables, such as amount of energy transmitted to the juice, expressed as power density, PD (W/mL), ultrasoung (US) amplitude, temperature and treatment time, on enzyme activity has been determined. In addition,

the effect of the nature of the dissolved gas (air, nitrogen and carbon dioxide) on enzyme inactivation has been evaluated. No previous studies have been found in the literature regarding the effect of this variable on fruit juices processed by TS. Finally, enzyme inactivation by TS was compared with previous results obtained by high pressure carbon dioxide (HPCD) treatment, as non-thermal technology (Illera et al., 2018), and with thermal treatment alone in the same temperature range. The effect of TS on other quality parameters of cloudy apple juice, such as particle size distribution (PSD), color and total polyphenols and hydroxymethylfurfural content has been also determined.

2. Materials and methods

2.1 Juice preparation

Golden delicious apples were peeled, cut in cubes and submerged in a 0.3 % L-ascorbic acid solution to avoid enzymatic browning during processing. Apple cubes were wiped and immediately squeezed with a screw juice extractor. The liqueur was filtered with 2 layers of cheesecloth. The pH of the juice obtained was 3.89 ± 0.01 and the soluble solids content was 12.5 ± 0.1 °Brix. The juice was stored frozen at -18 °C until further use. Carbon dioxide (99.9 %) was supplied by Air Liquide S.A. (Spain). Nitrogen (98.5 %) was obtained by a Zefiro 25 LCMS nitrogen generator.

2.2 Thermosonication

A 750 W Sonics Material[TM] with a 13 mm probe was used for thermosonication. Samples were processed at a constant ultrasound frequency of 20 kHz. Cloudy apple juice was introduced in a thermostated vessel ($\Phi = 4.8$ cm, V = 199 cm^3) and the probe was submerged in the juice at a constant depth of 2 cm from the bottom of the vessel. According to the manufacturer the US amplitude is 79 μm when the amplitude control is set at 100 % and the 13 mm probe can handle volumes in the range from 50 to 150 mL.

Firstly, TS was performed in a continuous mode and fixing the amplitude at different levels (from 25 to 100 %), keeping constant the rest of operating variables: TS time (15 min), treated volume of apple juice (80 mL) and temperature of the jacketed water (40ºC). This way, the effect of amplitude on PSD and PPO inactivation was first analyzed. During each TS experiment, the temperature profile was registered. The energy input was recorded after the experiment. The ultrasonic power density, PD, was evaluated as:

$$PD = \frac{E}{t \cdot V} = \frac{P}{V} \qquad\qquad [1]$$

where E, is the energy input, J, t is the ultrasonication time, s, P, the ultrasonic power (J/s =W) and V is the sample volume, mL.

TS experiments were also performed by varying the treated sample volume from 60 to 120 mL, in continuous and in pulse (5 s on and 5 s off) modes, at maximum amplitude, during 15 min of TS and keeping constant the temperature of the jacketed water at 40ºC. By varying the sample volume at fixed amplitude, the power density was also varied (see equation 1) and its effect on PPO inactivation was analyzed.

Enzyme inactivation kinetics for PME and PPO were determined at maximum amplitude for a treated sample volume of 80 mL at different TS temperatures, by varying the temperature of the jacketed water from 20 to 50ºC.

Previous to the TS treatment, cloudy apple juice was preheated (90 s (Sulaiman, Soo, Yoon, Farid, & Silva, 2015)) to the initial temperature of TS (temperature of the jacketed water) in all the experiments, then temperature was continuously recorded during treatment. No enzyme inactivation was observed during the preheating time (90 s) at the initial TS temperatures essayed in this work, from 20 to 50ºC.

The effect of the nature of the dissolved gas in the juice was also considered. Previous to the thermosonication experiments, air dissolved in the juice was displaced by bubbling nitrogen or

carbon dioxide in the sample at atmospheric pressure until oxygen concentration was below 0.4 mg/L (YSI ProODO optical dissolved oxygen meter). After that, TS experiments were performed as previously described.

2.3   Physical-chemical analyses

2.3.1.   Determination of enzyme activity

Polyphenol oxidases. PPO activity was determined by adding 100 µL of apple juice into 2.9 mL of substrate solution consisting of a 0.05 M catechol (Sigma Aldrich) solution prepared in a 0.1 M phosphate buffer (pH 6.5). Oxidation of catechol was determined immediately by the absorbance increase at 420 nm by using a Jasco V-750 spectrophotometer equipped with a Peltier thermostated cell holder at 30 ℃. The PPO activity was determined by using the very first linear part of the reaction curve (Illera et al., 2018).

Pectinmethylesterase. Samples were analyzed by using an automatic titrator system (Metrohm ® Titrando). A pectin solution (1 %) (Alfa Aesar ® pectin citrus) was prepared in NaCl (0.3 M). This solution was used as substrate. 50 mL of pectin solution were mixed with 1 mL of cloudy apple juice and pH was adjusted to 7.5 with NaOH 0.005 N. During pectin hydrolysis at 30 ℃, pH was maintained at 7.5 by adding NaOH 0.005 N. The amount of NaOH added for 15 min was recorded. One PME activity unit (UPE) is defined as the micromoles of carboxylic groups produced per minute and mL of juice, at pH 7.5 and 30 ℃ (Briongos et al., 2016).

Relative residual activities of PPO and PME were evaluated as:

$$\text{Residual activity} = \frac{\text{Enzyme specific activity after TS treatment}}{\text{Enzyme specific activity before TS treatment}} \cdot 100\% \qquad [2]$$

As previously explained, during the preheating time (90s) no inactivation was observed, therefore the enzyme activity right before TS treatment was considered to be the enzyme activity of the untreated juice.

2.3.2. Determination of hydrogen peroxide, nitrate and nitrite formation

One of the mechanisms proposed on enzyme inactivation by US is the formation of free radicals. In the literature, hydrogen peroxide (H_2O_2) formation has been determined as an estimation of cavitation intensity (Raviyan, Zhang, & Feng, 2005). However, H_2O_2 generation during an ultrasound treatment in a food system is complicated due to the presence of ions and other colloidal components (Raviyan et al., 2005). Therefore, in this work, H_2O_2 generation during TS has been determined in a citrate buffer solution at the same pH as the one of the apple juice (pH = 3.9). Formation of nitrite and nitrate in citrate buffer solutions was also determined, since its sonochemical formation in water has been known for a long time (Supeno & Kruus, 2000).

Different citrate buffer solutions (pH = 3.9) in the presence of dissolved air, nitrogen or carbon dioxide, prepared as indicated in section 2.2, were thermosonicated (80 mL of solution at 60ºC) for 20 minutes. Samples were collected at different time intervals, to determine the H_2O_2, nitrate and nitrite formation during the sonication process.

H_2O_2 was determined spectrophotometrically by a colorimetric method based on the one described by Mead et al. (Mead, Sutherland, & Verrall, 1976). 1 mL of solution A [1g NaOH, 33g KI, and 0.1g $(NH_4)_6Mo_7O_24H_2O$ in 500mL H_2O] was mixed with 1 mL of solution B [10g $C_8H_5O_4K$ in 500 mL H_2O] and subsequently mixed with 2 mL of the thermosonicated buffer solution taken at different time intervals. Absorbance at 350 nm was registered with time and it was found that 45 min was time enough not to observe any further change in the reaction medium color. Citrate buffer solution, not subjected to thermosonication, was used as a blank. The concentration of H_2O_2 was calculated based on a standard curve at different H_2O_2 concentrations.

Nitrate and nitrite analysis were performed by using Dr. Lange Küvetten-Test analysis kits and a Hach Lange DR2800 spectrophotometer. LCK 339 kit was used for measuring nitrates. This

measurement is based on the reaction of nitrate ion with 2,6-dimethylphenol that causes the formation of 2,6-dimethyl-4-nitrophenol. For measuring nitrites, LCK 341 kit, which is based on the reaction of nitrite with aromatic primary amines, producing the formation of an azo dye, was used.

2.3.3. Particle size distribution

Particle size distribution of cloudy apple juice was determined by laser diffraction at 750 nm with a Mastersizer 2000 (Malvern® Inst., MA). Particle size distribution was calculated by the Fraunhofer model. Size distributions (volume fractions against particle size) before and after thermosonication treatment were calculated and the weight-average sizes expressed as:

- The equivalent surface area mean diameter: $D(3,2) = \sum n_c d_{lc}^3 / \sum n_c d_{lc}^2$ [3]

- The equivalent volume mean diameter: $D(4,3) = \sum n_c d_{lc}^4 / \sum n_c d_{lc}^3$ [4]

where d_{lc} is the diameter of the particle and n_c is the percentage of particles.

Other useful parameters $D_{v,0.9}$, $D_{v,0.1}$ and $D_{v0.5}$ correspond to the particle size bellow which, 90%, 10% and 50% of the particles lie respectively.

2.3.4. Color, hydroxymethyl furfural content and total polyphenol content

The cloudy apple juice color was determined by using a Beckman DU-650 spectrophotometer with diode-array of UV–vis (Beckman Instruments). Following the CIE recommendations, illuminant D65 (daylight source) and a 10° standard observer (perception of a human observer) were used. L*, a* and b* values were obtained representing brightness, red to green color and yellow to blue color. Color difference (ΔE) and chroma (C) were evaluated as:

$$\Delta E = \sqrt{\left(L^*_{before} - L^*_{after}\right)^2 + \left(a^*_{before} - a^*_{after}\right)^2 + \left(b^*_{before} - b^*_{after}\right)^2}$$ [5]

$$C = \sqrt{(a^*)^2 + (b^*)^2}$$ [6]

Non enzymatic browning in the juice has been measured as hydroxymethylfurfural (HMF) content (Queiroz, da Silva, Lopes, Fialho, & Valente-Mesquita, 2011). 0.7 mL of juice were mixed with 0.7 mL of ethanol in a 1.5 mL Eppendorf tube. The mixture was centrifuged at 12000 g for 10 min. After that, 1 mL of the supernatant was mixed with 1 mL of a trichloroacetic acid solution (734 mM) and 1 mL of a thiobarbituric acid solution (25 mM) in a closed recipient. Samples were incubated at 40 °C during 50 min in an agitated batch and absorbance was measured at 443 nm. A blank was also prepared with distilled water instead of juice. A calibration curve was prepared using different concentrations of HMF from 0.5 mg/L to 10 mg/L.

Total phenolic compounds were determined by using the Folin-Ciocalteau reagent (VWR). 100 μL of the juice were mixed with 2.8 mL of water, and subsequently with 100 μL of the Folin-Ciocalteau reagent. After that, 2 mL of sodium carbonate 7.5% (w/v) were added and the reaction started. Absorbance was measured after 60 minutes of reaction at 750 nm. A blank was also prepared using water instead of juice. A calibration curve was prepared with standard solutions of gallic acid by following the same colorimetric method.

2.4 Kinetic data analysis

The inactivation kinetic data at different TS conditions were fitted to the first-order kinetic model and the non-linear Weibull model.

The first-order kinetic model can be written as:

$$\log A/A_o = -kt \tag{7}$$

where A_o is the initial activity of the enzyme, A is the residual activity at different treatment times, k is the inactivation rate constant at the operating conditions (min^{-1}) and t is the treatment time (min).

From the inactivation rate constant, the decimal reduction time (D: time needed to achieve a 90 % reduction of enzyme activity) can be evaluated. z_T, defined as the temperature increase needed for a 90% reduction of the D value, was determined as the negative reciprocal slope of the regression line of log D as function of T:

$$\log[D_1/D_2] = (T_2 - T_1)/z_T \quad\quad\quad [8]$$

Furthermore, the dependence of the inactivation constant on temperature can be expressed through Arrhenius equation:

$$\ln(k_1/k_2) = (E_a/RT)(1/T_2 - 1/T_1) \quad\quad\quad [9]$$

where T_2 and T_1 are temperatures corresponding to the decimal reduction times D_1 and D_2 or constant k_1 and k_2, respectively. R is the universal gas constant and E_a, the activation energy (kJ/mol).

The non-linear Weilbull model can be written in the power-law form (Van Boekel, 2002):

$$\log(A/A_o) = -(1/2.303)(t/\alpha)^\beta \quad\quad\quad [10]$$

where α is the scale parameter (a characteristic time) and β is the shape parameter. The time required to achieve a number of decimal reductions, d, can be calculated by using the shape and scale parameters (Van Boekel, 2002):

$$t_d = \alpha \left(-ln(10^{-d})^{\frac{1}{\beta}} \right) \quad\quad\quad [11]$$

2.5   Statistical analysis

Statistical analyses were conducted using the software Statgraphics X64. The results are presented as a mean ± standard deviation of at least three replicates. The significance of the

differences was determined based on an analysis of the variance with the Tukey's honestly significant difference (HSD) method at p-value ≤ 0.05.

To estimate the kinetic parameters of the different models essayed in this work, non-linear regression was performed by using the Marquardt algorithm (Statgraphics X64).

3. Results and discussion

3.1 Temperature profile

Figure 1 shows the temperature profile for a 15 min TS treatment in a continuous mode at a fixed amplitude of 76 μm (100%), for a temperature of the jacketed water of 40°C, at different volumes of treated apple juice (60, 80, 100 and 120 mL), which are equivalent to power densities of 1.57, 1.36, 1.21 and 1.09 W/mL, respectively (equation 1). The temperature profile was similar for all the treated volumes; although, the power density increased by decreasing the treated volume. A sharp temperature increase was observed during the first three minutes of TS, then, it reached a plateau with a mean value of 60 ± 1 °C. Similar temperature profiles were obtained for the other temperatures essayed of the jacketed water, 20, 30, 45 and 50°C, reaching temperatures in the plateau region of 44 ± 1, 52 ± 1, 64 ± 1 and 67 ± 1°C, respectively (data not shown).

Figure 1 also shows the temperature profile for a pulse treatment. Total treatment time under pulse mode, t_p, was determined as:

$$t_p = t_c \left(1 + (1/R)\right) \tag{12}$$

where t_c is the corresponding time of exposure in a continuous experiment, 15 min, and R is the ratio on/off, in this work equal to 1. Experiments under pulse mode were carried out at the same amplitude (76 μm), temperature of the jacketed water (40°C) and amount of treated apple juice (80 mL), equivalent to a power density of 1.18 W/mL, as the continuous experiments. Power

density in the pulse mode was determined by considering only the effective sonication time (tc), although total treatment time (tp) was 30 min, including the interval length. Likewise to a continuous mode, a sharp initial increase in the temperature was observed and then, the temperature remained constant. However, the temperature increase observed in the pulse mode is less than in a continuous mode.

3.2   Effect of the amplitude

The effect of the TS amplitude was studied in the range of 25 to 100% (19 to 76 µm) in a continuous mode. These experiments were carried out with 80 mL of cloudy apple juice treated for 20 min at a temperature of the jacketed water of 40° C (Figure 2). A linear increase with amplitude was observed for both, power density and final temperature reached during TS (PD (W/mL) = 0.020·Amplitude (µm) - 0.238, R^2 = 0.986; T(ºC) = 0.258·Amplitude (µm) + 42, R^2 = 0.994).

PPO residual activity decreased linearly with amplitude, obtaining the lowest residual activity, 36 ± 3 %, at the maximum amplitude (PPO RA (%) = -1.326·Amplitude (µm) + 140, R^2=0.990). It was observed that, at the lowest amplitude essayed in this work, PPO activity after TS treatment was higher than in the untreated sample, as indicated by a residual activity value higher than 100%, as determined by equation 2. This behavior has been attributed to the homogenization of the juice, due to the acoustic energy that could cause the release of the enzymes usually bound to cell walls (Başlar & Ertugay, 2013). The increase of the ultrasound effects by increasing amplitude has been also related to the increase of the effective size of the zone of liquid undergoing cavitation and the range of sizes of bubbles that undergo cavitation at higher amplitudes (Raso, Mañas, Pagán, & Sala, 1999). Baltacioglu et al. (2017) also found that

residual enzyme activity decreased with increasing amplitude when studying the inactivation of the mushroom PPO by ultrasonication,.

The effect of the amplitude on the particle size distribution of cloudy apple juice is shown in Figure 3. Particle size distribution of untreated cloudy apple juice ranged from 0.5 µm to 550 µm with three maxima at 0.8, 30 and 240 µm (see Figure 3). After TS treatment under different amplitudes, the particle range moved towards smaller particle sizes with two main peaks at the lowest amplitude essayed (19 µm) at 0.6 and 159 µm, one main peak at 0.17 µm for 50 % of amplitude (38 µm) and at 0.14 µm at the two highest amplitude essayed (57 and 76 µm). Based on these results it can be concluded that TS reduces the size of juice particles leading to a better uniformity and stability, especially at high working amplitude. Ertugay & Baslar (2014) related cloud stability of apple juice to the size of the juice particles. These authors reported that particle size above 0.5 - 0.65 µm in apple juice are unstable and settle down, whereas those below 0.5 µm are held in suspension by Brownian motion and do not settle. Therefore, by working at higher amplitude, lower residual activity was reached and also cloud stability was improved. Cheng et al. (L. H. Cheng, Soh, Liew, & Teh, 2007) concluded in their study of sonication of guava juice that, due to the high shearing effect that occurs during cavitation, TS can fragment colloidal pectin molecules that would be more stable in the colloidal system.

Table 1 lists the values of D[3,2] and D[4,3] of fresh cloudy apple juice and after TS treatment at different amplitudes. These values indicate that TS treatment promotes dissociation, since they were significantly lower after TS than before treatment. This effect can also be observed in the values of d(0.1), d(0.5) and d(0.9) in Table 1.

Based on these results, further TS experiments were performed at 100% amplitude. In any case, it must be highlighted that probe erosion, as an unavoidable side effect of sonication, is faster when working at higher amplitudes.

3.3 Effect of power density on enzyme inactivation at different operating temperatures.

Figures 4a and 4b show PPO and PME residual activity, respectively, after 15 min of TS in the temperature range from 52 to 67 °C (temperature of the plateau, see Figure 1), for different treated juice volumes (60, 80, 100 and 120 mL), which correspond to different power densities, in continuous mode and in pulse mode.

Comparing both figures, it can be observed that PME is more resistant to TS treatment than PPO. At 60ºC, no PME inactivation was observed at any of the treated volumes (power density ranged from 1.21 to 1.57 W/mL), even a slight increase was found, probably due to the release of enzyme bound to cell walls (Ertugay & Baslar, 2014). The greater stability of PME compared to PPO has been also found in thermal treatments and other non-thermal treatments such as HPCD (Illera et al., 2018) and it has been attributed to the more complex structure of PPO, with three or four subunits in higher plants, that makes it more susceptible to inactivation. Additionally, PME in fruit juices is more difficult to be effectively inactivated, since PME is bound to the plant cell wall, which contains natural stabilizing factors (Zhou, Zhang, Leng, Liao, & Hu, 2010).

For PPO, at the highest temperature (67ºC), nearly complete inactivation (3 ± 1%) was obtained when working at power density values greater than 1.15 W/mL. Figure 4a shows that to reach a certain inactivation degree, lower values of power density are needed when working at higher temperatures.

A linear relationship between enzyme residual activity and PD (W/mL) was found at the different working temperatures (for PPO at 52ºC: RA = -77·PD + 181, R^2= 0.990; at 60ºC: RA =

-166·PD +278, R^2 = 0.987; at 67ºC: RA = -332·PD + 396 R^2 = 1 (only two points); for PME at 67ºC RA = -43·PD + 110, R^2= 0.920). The values of these slopes indicate a greater effect of the power density in enzyme inactivation when working at higher temperatures. This suggests that the synergic effect between temperature and ultrasound is more effective when working at high temperatures.

Figure 4a also shows that when working in a pulse mode, the effect of power density on PPO enzyme inactivation is lower than when working in a continuous mode (RA= -30·AED + 100, R^2= 0.867). Although pulse ultrasound can be considered as an energy saving operational mode (Al-Juboori, Yusaf, & Bowtell, 2015), in this study, it has been found to be less effective than continuous mode for PPO inactivation.

Other values have been found in the literature for PPO and PME inactivation in apple juice. Values of PPO inactivation in apple juice in *Golden delicious* by TS treatment, with a 22 mm diameter probe (24 kHz) (200 mL of apple juice in a 250 mL vessel) in a pulse mode (50% and 100% of cycle), were reported by Baslar and Ertugay (Başlar & Ertugay, 2013). These authors observed that the inactivation degree increased sharply with amplitude and temperature, observing an increase in the inactivation degree from 5% to 30% when amplitude increased from 50 to 100 µm at 50ºC, for 10 min of TS and pulse 1:1. At 60 ºC, these authors reported much higher inactivation degrees, reaching values of 30% and 80% at 50 and 100 µm of amplitude respectively for 10 min of TS and pulse 1:1. Sulaimna et al. (Sulaiman et al., 2015) reported values of PPO residual activity, around 17%, for PPO of Royal Gala apple puree when treated by TS for 15 min at 58ºC and 210 µm of amplitude in a continuous mode, with a 3 mm diameter probe, which corresponds to 1.3 W/g (25g in apple puree in a 34.4 mL vessel). These authors found that, under these conditions, nearly complete inactivation of PPO was reached at

temperatures of 72-73 °C. Abid et al. (Abid et al., 2014) studied the TS of Fuji (*Malus Domestica*) variety apple juice (80 mL of apple juice in a 100 mL vessel), with a 0.5 inch probe at 20 kHz, and a value of the power density of 0.3 W/mL in a pulse mode (5s on and 5 s off). No value of the amplitude was reported. They reported residual activities of 97, 53 and 6 % for PPO at 20, 40 and 60°C, respectively after 10 minutes of TS. For PME, these authors reported similar values of residual activities 97, 52 and 7 % at 20, 40 and 60°C, respectively after 10 minutes of TS.

In this work, lower inactivation degree, i.e. higher residual activity, was obtained for PME of *Golden deliciuous*, reaching the lowest residual activity of 50% at 67°C after 15 min of TS at 79 μm of amplitude and 1.44 W/mL. In this regard, it must be highlighted that PME from cloudy *Golden deliciuous* apple juice was found to be one of the apple cultivars presenting strongest thermostability (30% of residual activity after heating 5 min at 100 °C) (Teleszko, Nowicka, & Wojdyło, 2016), although mechanism of TS and thermal treatment is probably different. Regarding PPO activity, higher residual activities have been also obtained compared to the results found in the literature. This could be attributed to the lower working amplitude, 79 μm (maximum amplitude reported by the supplier for this type of probes). The variety of data on PPO inactivation from apple juice indicates that enzyme inactivation depends on many factors such as source, sub-type, environmental and physicochemical conditions (pH, temperature) (X. F. Cheng, Zhang, & Adhikari, 2013). Islam et al. (Islam, Zhang, & Adhikari, 2014) reviewed the inactivation mechanisms and factors affecting the enzyme inactivation by ultrasound; however, further investigation is needed to understand the specific mechanism by which the different enzymes in apple juice are inactivated.

TS treatment was compared with thermal treatment by heating the cloudy apple juice during 15 min in the same temperature range as for the TS process, 52 ± 1, 60 ± 1 and 67 ± 1ºC. Heating the juice at the lowest temperatures essayed in TS experiments 52ºC and 60ºC did not cause any loss of enzyme activity but even a significant increase ($p \le 0.05$), compared to the untreated juice (RA for PPO: 114 ± 12, 106 ± 16, RA for PME: 135 ± 10, 157 ± 8 at 52ºC and 60ºC, respectively). At the highest temperature essayed, 67ºC a significant decrease ($p \le 0.05$), in enzyme activity by 24 ± 4 % for PPO (RA = 76 ± 4 %) and 9 ± 2 % for PME (RA = 91 ± 2 %) was observed. These results prove that TS significantly improved the inactivation of PPO and PME.

3.4 Inactivation kinetics of PPO at different operating temperatures.

The TS inactivation kinetics of PPO was determined in the temperature range from to 52 to 67ºC final TS temperature. PPO of cloudy apple juice was inactivated faster by increasing operating temperature (Figure 5). First order kinetic model has been successfully used in the literature to correlate thermosonication inactivation kinetic data (Terefe, Buckow, & Versteeg, 2014). In this work, kinetic data were fitted to the first order kinetic model and the Weibull model. The corresponding kinetic parameters are listed in Table 2, together with the quality of the fitting.

For the first order kinetic model, the inactivation rate constant, k, increased with temperature; therefore, D-values for PPO inactivation decreased with temperature from 130 min at 52ºC to 18 min at 67ºC. Başlar and Ertugay (Başlar & Ertugay, 2013) reported lower D values for PPO of *Golden delicious* apple juice, 146, 66.4 and 12.9 min at 40, 50 and 60ºC respectively when working at 100 μm of amplitude by ultrasound. D values for PPO of *Malus domestica* cv Roya Gala apple juice ranged from 49 to 4 min at 33 and 72ºC, respectively, at 1.3 W/g, 210 μm of amplitude (Sulaiman et al., 2015).

The temperature sensitive parameters, z_T and E_a, were evaluated through the slopes of plotting equations 8 and 9, respectively, yielding 17.5 ± 0.8 ºC and 123 ± 4 kJ/mol, respectively. Both sensitivity parameters values show that the inactivation of PPO by TS is rather temperature sensitive. Process with high activation energy values are very temperature sensitive (Levenspiel, 1999) as well as processes with low z_T values.

In the literature, values of the same order were obtained for PPO inactivation of mushroom crude extract in the temperature range from 55 to 75ºC, with $z_T = 13.8$ ºC and $E_a = 183 \pm 32$ kJ/mol (X. F. Cheng et al., 2013). Baslar and Ertugay (2013) reported a similar value of z_T of 19 ºC for PPO inactivation by thermosonication of *Golden delicious* apple juice, same variety as in this work. These authors did not report a value for E_a; however, as previously mentioned, these authors reported D values in the temperature range from 40 to 60ºC and the activation energy could be easily evaluated through Arrhenius equation by using the inverse of the D-values ($D=1/k$), obtaining a value of 105 kJ/mol, similar to this work. On the contrary, other values reported for PPO of *Malus domestica* cv. Royal Gala apple puree inactivation by TS showed lower temperature sensitivity at 1.3 W/g, with values for activation energy and z_T of, 52 kJ/mol and 39ºC respectively, in the temperature range from 33 to 72ºC (Sulaiman et al., 2015).

The Weibull parameters are also listed in Table 2. Scale, α, and shape, β, parameters were used to calculate the time required to inactivate 1 log ($t_{d=1}$) of PPO. Similar to the D-values for the first order kinetic model, $t_{d=1}$ decreased with temperature. The $t_{d=1}$ calculated by the Weibull model were lower than the corresponding D values obtained by the first order kinetic model. Therefore, in this case, the use of the first order kinetic model could lead to an overprocessing estimation for PPO inactivation. According to van Boekel (2002), the dependence on temperature of both parameters was analyzed. It was found that the scale parameter, α, was statistically significant dependent on temperature, when tested at the 95% significance level for a

linear relationship. The shape factor, β, was higher than 1 indicating a downward concavity of the enzyme inactivation curve (Van Boekel, 2002), but it was not statistically significant dependent on temperature, when tested at the 95% significance level for a linear relationship. According to van Boekel (2002), the scale parameter, α, could be modelled in a similar way to the classical D value of the first order kinetic model, suggesting a linear dependence of the log α on temperature and considering that β did not depend on temperature:

$$log\,\alpha = a_1 - b_1 T \tag{13}$$

A z_T' value can be also defined:

$$z_T' = 1/b_1 \tag{14}$$

The value of the z_T' is given in Table 2, together with the quality of the fitting. Although the concept of z_T and z'_T is different since z_T is obtained from the linear part, whereas z'_T takes also into account the nonlinear part (Van Boekel, 2002), similar values were obtained for both parameters ($z_T = 17.5 \pm 0.8$ ºC and $z'_T = 20 \pm 1$ ºC). An Arrhenius type equation was also considered to relate the inverse of the scale parameter, $1/\alpha$, with temperature. Although $1/\alpha$ cannot be considered a kinetic constant, the value obtained from the slope was found to be close to the E_a obtained by applying the first order kinetic model, being 107 ± 6 kJ/mol

3.4. Effect of the nature of dissolved gases

The effect of the nature of dissolved gases on PPO inactivation kinetics is shown in Figure 6a at three different thermosonication temperatures, 44, 52 and 60 ºC (temperature of the plateau, see Figure 1) when treating 80 mL of apple juice at 100% of amplitude. Regarding the effect of nitrogen, faster inactivation kinetics were obtained compared to the inactivation kinetics in the presence of dissolved air in the apple juice at the same thermosonication temperature. The effect of dissolved carbon dioxide was studied only at 60ºC. In this case, initial inactivation reaction

rates (initial slope of the curve) in the presence of CO_2 was of the same order as in the presence of air (Figure 5), however, faster reaction rates were obtained at longer TS times and therefore, lower residual activity after 20 min of TS was reached in presence of CO_2, 18 ± 2 %, than in the presence of air, 36 ± 5 % at the same operating conditions. Nevertheless, the best results in terms of inactivation rate and final RA were obtained when the air was displaced by nitrogen, reaching a RA of 5 ± 2 % at 60ºC for 20 min of TS.

Figure 6b shows the PME inactivation kinetics at 60ºC (temperature of the plateau) when dissolved air was displaced by nitrogen. After 20 min of TS, a minimal RA of 55 % was reached while no inactivation of PME was observed at the same temperature when TS was performed in presence of air (Figure 4b). However, at these conditions, results obtained with CO_2 did not bring any improvement in the inactivation process of PME compared with the results obtained in the presence of air and inactivation was neither observed.

PPO and PME inactivation kinetic data obtained displacing the air by N_2 or CO_2 were also fitted to the first order kinetic model and the Weibull model (Table 3). In general, a better fitting was obtained by the Weibull model.

For the first order kinetic model, D-values were evaluated. It can be clearly observed that by displacing the dissolved air by previously bubbling nitrogen into the apple juice, D- values were lower than those reported in Table 2. At 60 ºC, in the presence of air, the D-value for PPO was 50 min, while it decreased down to 11 min in the presence of nitrogen and to 36 min in the presence of carbon dioxide. The temperature sensitive parameters, z_T and E_a, in the presence of nitrogen were evaluated through the slopes of plotting equations 8 and 9, respectively, yielding 20 ± 4 ºC and 106 ± 18 kJ/mol respectively.

Regarding the Weibull model, the time required to achieve one decimal reduction, $t_{d=1}$, was calculated by using the shape and scale parameters (Table 3). Displacing the air by nitrogen led

to lower values of $t_{d=1}$, compared to the values reported in the presence of air. It was found that the scale parameter, α, was statistically significant dependent on temperature, when tested at the 95% significance level for a linear relationship; but the shape factor, β was not. According to equations 13 and 14, z_T' for the Weibull was evaluated as 18 ± 4 ºC, being similar to z_T for the first order kinetic model. An Arrhenius type equation was also considered to relate the inverse of the scale parameter, $1/\alpha$, with temperature. From the slope, a value close to the activation energy obtained by applying the first order kinetic model was obtained, 117 ± 24 kJ/mol.

These results indicated that the nature of the dissolved gas in the apple juice played an important role in enzyme inactivation. The effect of the nature of dissolved gases on enzyme inactivation has not been frequently studied in the literature. Cheng et al. (L. H. Cheng et al., 2007) studied the sonication process on guava juice quality with previous carbonation of the sample. These authors proposed that dissolved carbon dioxide could reduce surface tension and could create more nuclei cavitation. However, they found that after sonication or a combination of carbonation + sonication, PPO showed higher activity than the untreated sample.

Gielen et al. (Gielen et al., 2016) discussed the different effect of the dissolved gases on sonochemistry considering the solubility and thermal properties of the gases as well as the different radical production. In this work, the H_2O_2 rate production was determined in a citrate buffer solutions at the same pH as apple juice (pH = 3.9) at 60 ºC, 100 % of amplitude, 80 mL of treated volume during 20 min. H_2O_2 rate formation was determined in three types of citrate solutions with dissolved air and by displacing the air by bubbling nitrogen or carbon dioxide previous to TS experiments. No H_2O_2 production was observed during the first five minutes of TS in the presence of nitrogen or air, according to our analytical method. After that, it was found that the H_2O_2 production as a function of time followed a linear relationship, with a H_2O_2 rate production of $(3.6 \pm 0.2) \cdot 10^{-8}$ mol/(Lmin) ($R^2 = 0.994$) in air, and $(3.78 \pm 0.05) \cdot 10^{-8}$ mol/(Lmin)

($R^2 = 0.999$) for nitrogen. In the presence of CO_2, no H_2O_2 formation was observed after 15 min of TS; subsequently, H_2O_2 formation rate was similar to that obtained with nitrogen and air ($3.81 \cdot 10^{-8}$ mol/(Lmin), from 15 min to 20 min). In any case, these results indicated that CO_2 partially inhibited the formation of H_2O_2 during TS. These results agreed partially with the radical yield formation obtained by Gielen et al.(2016) in the presence of different gases in water at 24 ± 2 ºC. These authors also found, that CO_2, although being a highly soluble gas (39.2 mmol/L versus 0.85 and 0.71 mmol/L for air and nitrogen respectively, at 293 K and 1 atm), inhibited radical formation due to low bubble temperature attributed to its lower polytropic index and a higher thermal conductivity compared to air and nitrogen (Gielen et al., 2016). The similar H_2O_2 rate production for air and nitrogen obtained in this work, could be then attributed to their similar thermal properties. However, Gielen et al. (Gielen et al., 2016) found that nitrogen reduced radical yield by the production of radical scavengers and a lower bubble volume. In any case, no relation can be established between H_2O_2 production rate in presence of the different dissolved gases and enzyme inactivation, since higher inactivation rates were determined when nitrogen displaced the dissolved air of the apple juice.

Nitrate and nitrite rate production was also determined during TS for the three buffer solutions at the same operating conditions. The highest nitrate rate production was obtained when nitrogen was previously dissolved in the buffer, ($2.6 \pm 0.2 \cdot 10^{-7}$) mol/(Lmin) ($R^2 = 0.982$) and the same value was obtained for CO_2 and air, $1.8 \pm 0.2 \ 10^{-7}$ mol/(Lmin), $R^2 = 0.910$ and 0.960 respectively. Nitrite rate formation was lower than nitrate for all the gases being $3.2 \pm 0.2 \cdot 10^{-8}$, $3.3 \pm 0.4 \cdot 10^{-8}$ and $1.5 \pm 0.2 \cdot 10^{-8}$ mol/(Lmin), for nitrogen, air and CO_2 ($R^2 = 0.993, 0.960, 0.967$) respectively. These results could partially explain the higher inactivation rate for PPO and PME

found by displacing the air with nitrogen. However, further studies are needed to understand the effect of the nature of the dissolved gas on enzyme inactivation by TS.

3.5. Comparison with HPCD

Among the different non-thermal technologies, the use of high pressure carbon dioxide (HPCD) treatment is having also growing attention to inactivate certain microorganisms and enzymes. Typically, operating pressure does not exceed 50 MPa and temperature ranges between 20 and 50 °C (Briongos et al., 2016), being lower than the temperature employed in this work in TS. Figure 7 compares the PPO inactivation kinetics by TS at 44 °C, temperature of the plateau, (V=80 mL and 100 % of amplitude) in the presence of air and by displacing the air with nitrogen, with results obtained in a previous study on inactivation kinetic of PPO from apple juice at 45°C by HPCD at 20 MPa (Illera et al., 2018). At 44 °C, in the presence of air, short treatment times of TS led to an increase of PPO activity. As indicated in section 3.2, this increase in enzyme activity has been attributed to the release of enzymes bond to cell walls due to the acoustic shock waves (Başlar & Ertugay, 2013) or to the activation of PPO latent forms at low intensities and short treatment times. In any case, at longer treatment times, PPO was inactivated. Figure 7 shows that, at the same operating temperature, HPCD treatment at 20 MPa led to lower PPO residual activity values. This indicates a different enzyme inactivation mechanism for these two non-thermal technologies.

3.6. Effect of thermosonication on different quality parameters of cloudy apple juice

Some quality parameters have been determined right after TS treatment at 60 °C (final TS temperature), 100 % of amplitude, 15 min of treatment time and 80 mL treated volume of apple juice.

Color parameters. Table 4 lists the L*, a*, b* parameters of cloudy apple juice before and after different TS treatments. As general trend, there were significant differences in all color attributes after TS. The lightness (L*) slightly increased, while the red and yellow components decreased compared to the untreated juice. The increase in lightness after TS has been previously reported in the literature and it has been attributed to a homogenization effect of sonication (Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2008). A decrease in a* and b* parameters has been also observed in different juices, such as orange juice (Tiwari et al., 2008) or soursop juice (Dias et al., 2015).

Color difference, ΔE, (Equation 4) is also listed in Table 4. In all TS experiments, a color difference was observed compared to the untreated juice. According to Yuk et al. (Yuk, Sampedro, Fan, & Geveke, 2014), the color difference could be estimated to be not noticeable (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3), well visible (3–6) and great (6–12). Based on this classification, at 60°C, the change in color was noticeable (1.5-3) while at 67°C was well visible (3-6). According to color parameters, the chroma value presented the lowest value at 67°C and the lowest treated volume, 60 mL (higher power density). Therefore, although higher TS temperatures, led to higher inactivation rates, change in color was more visible.

Color changes during TS have been attributed to Maillard reactions that may occur at long treatment time and high temperature, as well as to cavitation, that involves various physical, chemical and biological reactions (Anaya-Esparza et al., 2017).

Total polyphenolic compounds. Total polyphenolic compounds (TPCs) slightly increased after TS compared to the untreated apple juice. Significant differences of the total polyphenolic compounds were found at 60 and 67°C and in the apple juice treated by previously displacing the air with nitrogen (Table 4). This TPCs increase after TS has been attributed to the enhanced disruption of cell walls that might lead to the release of some chemically bound phenolic

compounds (Bhat, Kamaruddin, Min-Tze, & Karim, 2011). However, as pointed out by Bah et al. (Bhat et al., 2011), this disruption cell wall process may cause a series of other oxidation reactions that might involve changes in color as it has been observed by determining the color parameters. Abid et al. (Abid et al., 2014) reported a significant increase of chlorogenic acid, caffeic acid, epicatechin and phloridzin content in sonicated *Malus domestic cv. Fuji* apple juice in an ultrasound bath at 20 ºC (60 mL in a 100 mL thermostated vessel, 70% of amplitude and 25 kHz frequency).

Different results on TPC after TS of different juice*s* have been previously reported in the literature. Some authors observed an increase of TPC after TS of pinapple and pear juices at 54 and 78ºC, respectively (Costa et al., 2013; Zafra-Rojas et al., 2013), while a reduction between 15-30% was observed after TS of cantaloupe melon (Fonteles et al., 2012).

Hydroxymethylfurfural. Table 4 also lists the HMF content after TS treatment of cloudy apple juice. It can be observed that the HMF value remains constant around 2 mg HMF/L for all the TS conditions used in this work, indicating that non-enzymatic browning reactions were not significant during TS processing.

Conclusions

The present study reports the effect of different operating variables of the thermosonication process on enzyme inactivation and other important quality parameters of cloudy apple juice.

It has been observed that working US amplitude increased enzyme inactivation and significantly enhanced the particle size distribution of cloudy apple juice by shifting the particle size towards lower particle diameters compared to the untreated juice.

The nature of the dissolved gas in the cloudy apple juice has been found to play an important role on enzyme inactivation, observing faster inactivation kinetics when nitrogen displaced the air dissolved in the juice.

Total phenolic compounds increased after TS, and color change was noticeable. Hydroxymethylfurfural content did not change after different TS treatments.

Further investigations are needed to elucidate the enzyme inactivation mechanism and reach a better understanding and optimization of the TS process

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Figure captions:

Figure 1. Temperature profile of cloudy apple juice during thermosonication of different volumes of cloudy apple juice in a continuous mode (Δ 60 mL-1.57 W/mL, □ 80 mL-1.36 W/mL, ◇ 100 mL-1.21 W/mL, ○ 120 mL-1.09 W/mL) and pulse mode (x 80 mL-1.18 W/mL). 100 % of amplitude (76 μm), 40ºC of the jacketed water.

Figure 2. Effect of the amplitude on PPO residual activity (□), final temperature of the juice (Δ) and power density, W/mL (○) for a treated volume of 80 mL and 40ºC of the jacketed water. Lines represent the linear regression.

Figure 3. Particle size distribution of fresh cloudy apple juice (a ——) and treated by TS at different amplitudes 19 μm (25% of maximum amplitude, b − − − −), 38 μm (50% of maximum amplitude, c ·—·—·), 57 μm (75% of maximum amplitude, d ▬ ▬ ▬), 76 μm (100% of maximum amplitude, e ·······). Treated volume, 80 mL and 40ºC of the jacketed water.

Figure 4. Enzyme residual activity after 15 min of thermosonication at100 % of amplitude (79 μm) and different temperatures (o 52ºC, □ 60ºC, Δ 67ºC, T of the plateau) in a continuous mode and in pulse mode at 54ºC (T of the plateau) (×) as a function of AED, W/mL (a) PPO (b) PME. Lines represent the linear relationship.

Figure 5. PPO inactivation kinetics during TS treatment at 100 % of amplitude (79 μm) at different operating temperatures (Δ 52ºC, □ 60ºC, ◇ 64ºC, ○ 67ºC, T of the plateau). Treated volume = 80 mL. Continuous lines represent the Weibull model.

Figure 6. PPO (6a) and PME (6b) inactivation kinetics during TS treatment at 100 % of amplitude (79 μm) at different operating temperatures (◇ 44ºC; Δ 52ºC, □, ■ 60ºC, T of the plateau) in presence of nitrogen (hollow symbols) and carbon dioxide (full symbols). Treated volume = 80 mL. Lines represent the Weibull model (continuous: N_2, dashed line: CO_2).

Figure 7. Comparison of PPO inactivation kinetics at 44 - 45 ºC for different non-thermal treatments: Δ HPCD at 20 MPa, $\Diamond$ TS in the presence of N_2 (V=80 mL, 100 % of amplitude), $\Box$ TS in the presence of air (V=80 mL, 100 % of maximum amplitude).
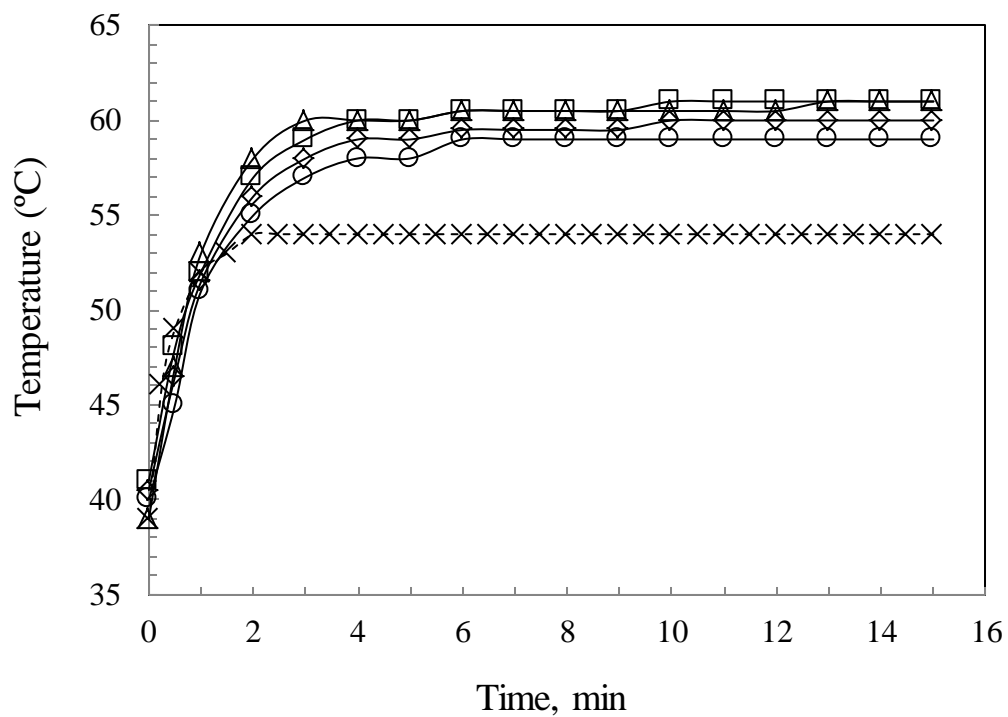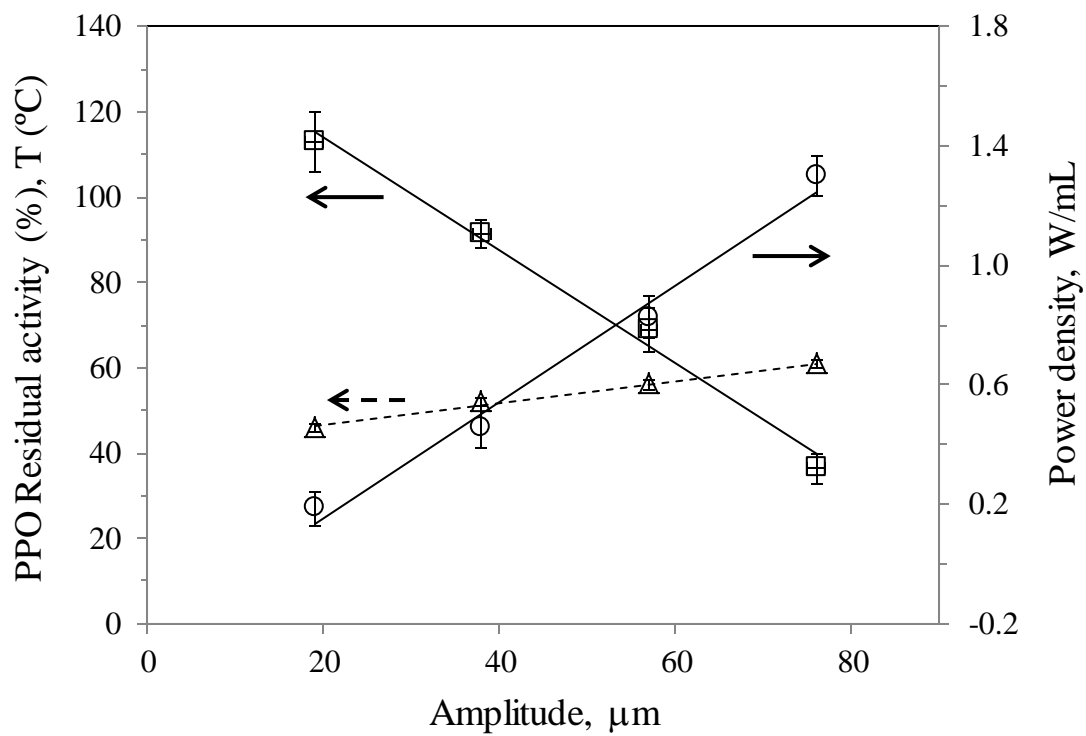
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Figure 2. Effect of the amplitude on PPO residual activity (□), final temperature of the juice (Δ) and power density, W/mL (○), for a treated volume of 80 mL and 40ºC of the jacketed water. Lines represent the linear regression.
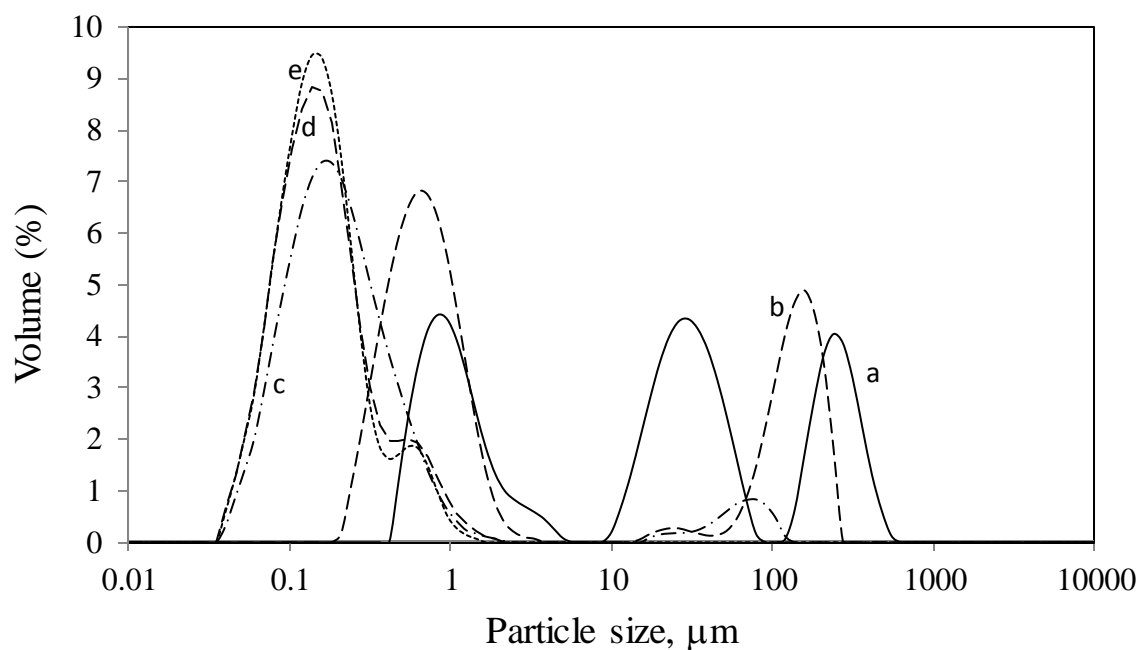
Figure 3. Particle size distribution of fresh cloudy apple juice (a ——) and treated by TS at different amplitudes 19 μm (25% of maximum amplitude, b − − − −), 38 μm (50% of maximum amplitude, c ·—·—·), 57 μm (75% of maximum amplitude, d ━ ━ ━), 76 μm (100% of maximum amplitude, e ······). Treated volume, 80 mL and 40ºC of the jacketed water.
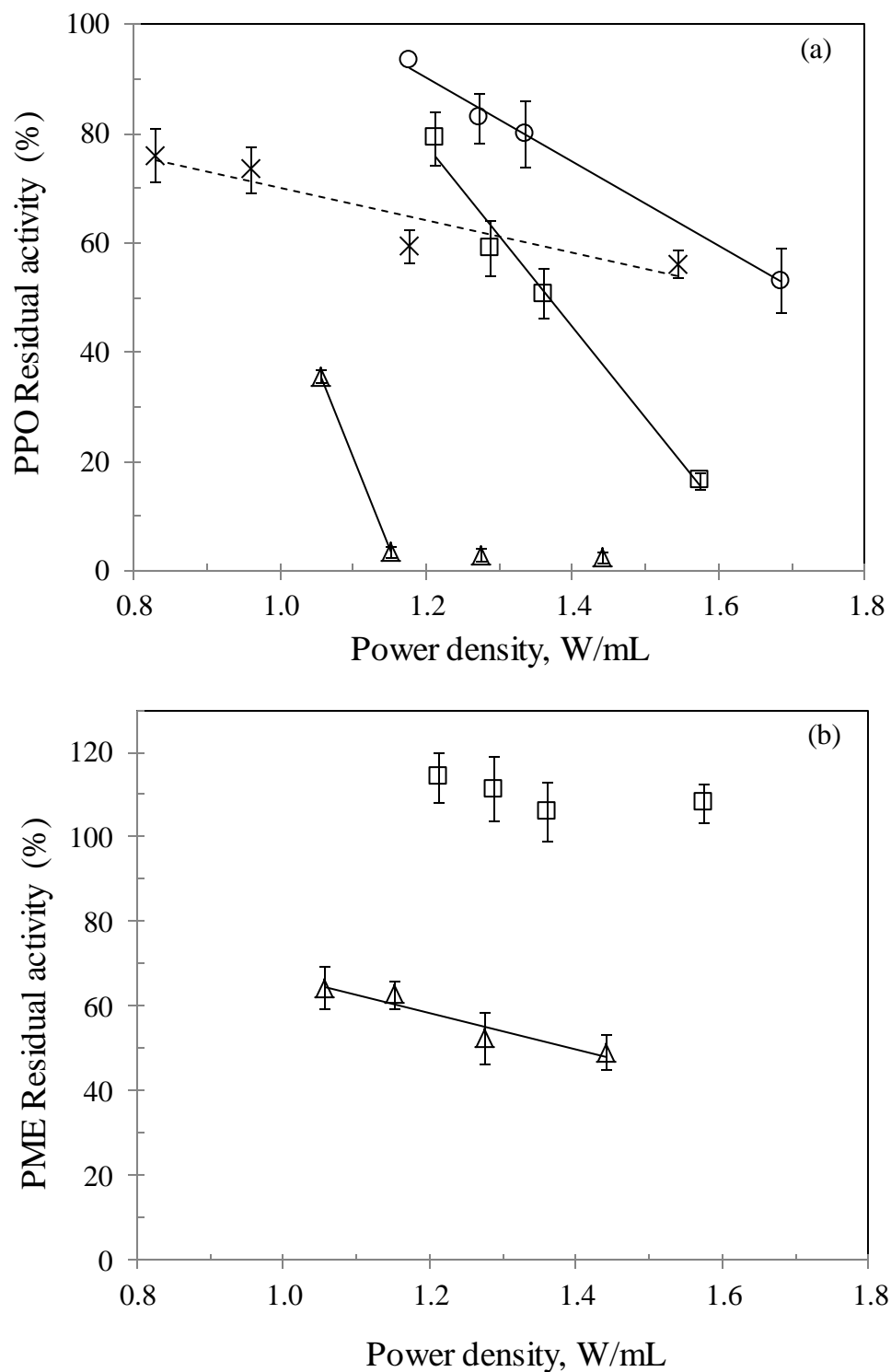
Figure 4. Enzyme residual activity after 15 min of thermosonication at100 % of amplitude (79 µm) and different temperatures (o 52ºC, □ 60ºC, Δ 67ºC, T of the plateau) in a continuous mode and in pulse mode at 54ºC (T of the plateau) (×) as a function of power density, W/mL (a) PPO (b) PME. Lines represent the linear relationship (continuous mode ——, pulse mode - - -).
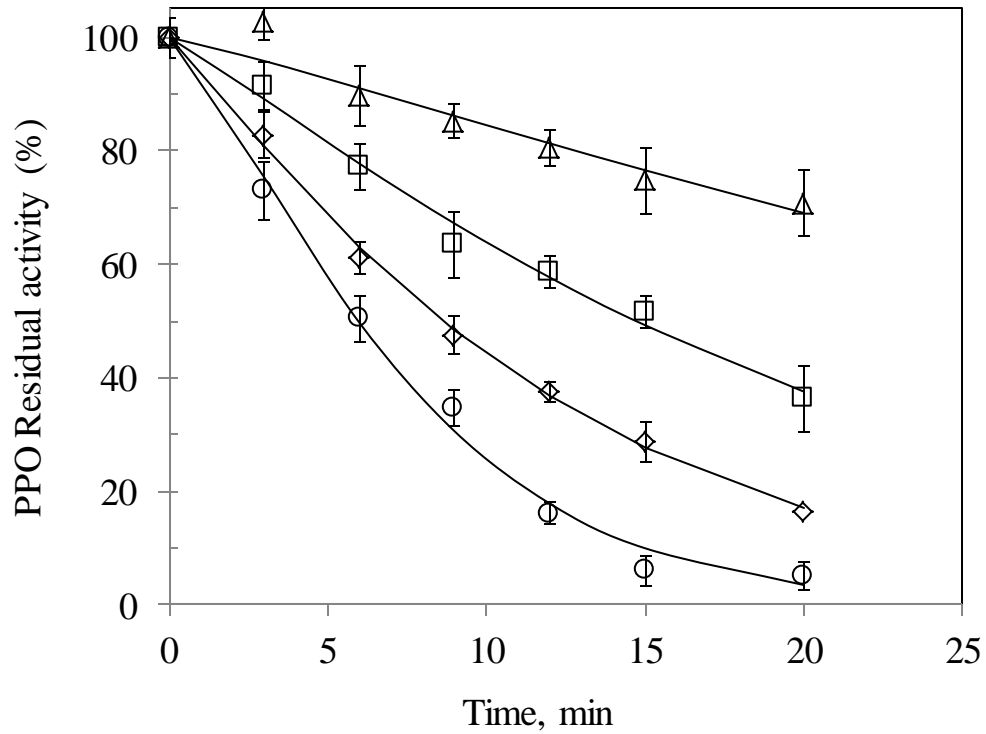
Figure 5. PPO inactivation kinetics during TS treatment at 100 % of amplitude (79 µm) at different operating temperatures (Δ 52ºC, □ 60ºC, ◇ 64ºC, ○ 67ºC, T of the plateau). Treated volume = 80 mL. Continuous lines represent the Weibull model.
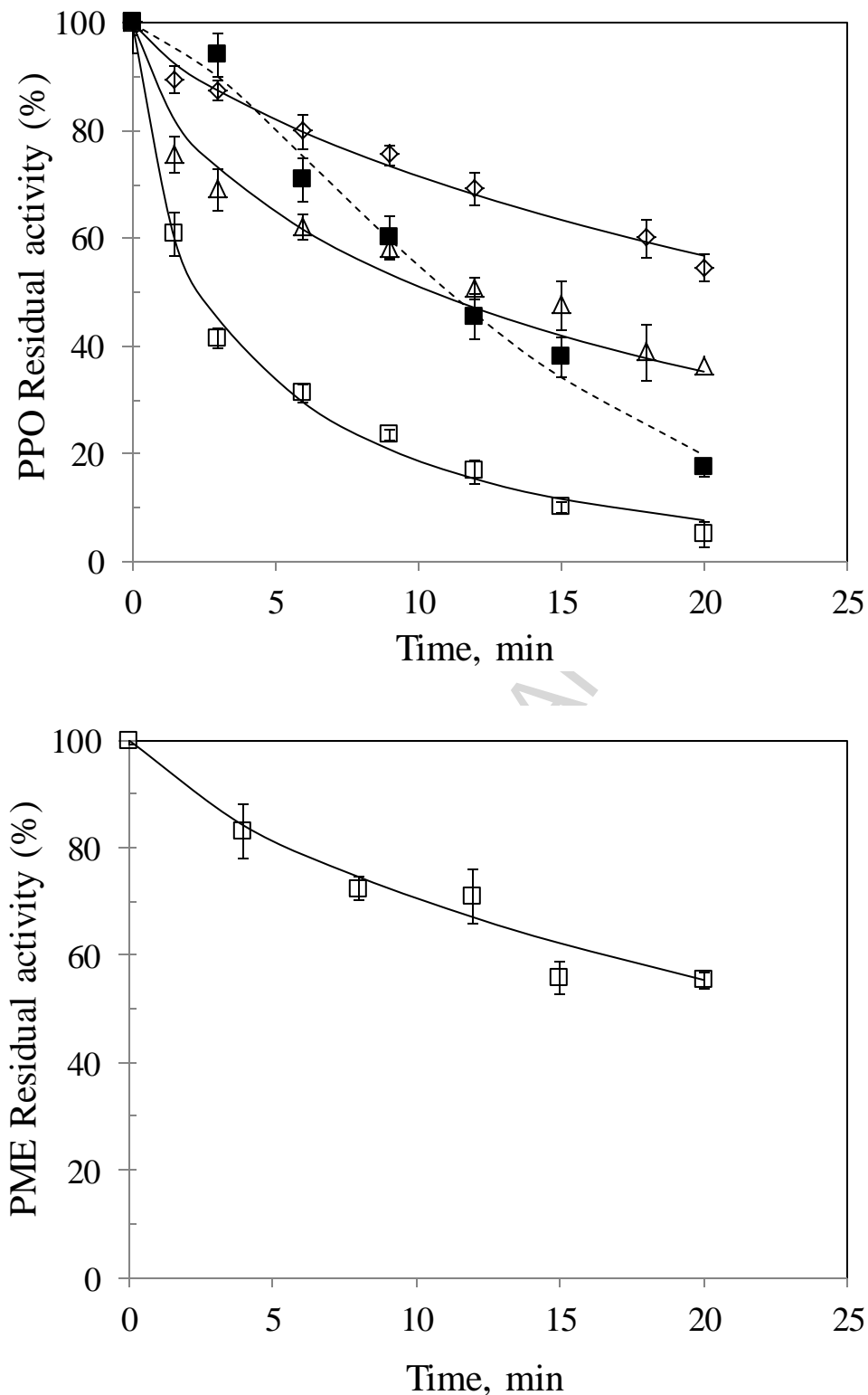
Figure 6. PPO (6a) and PME (6b) inactivation kinetics during TS treatment at 100 % of amplitude (79 μm) at different operating temperatures ($\diamond$ 44ºC; $\triangle$ 52ºC, $\square$, $\blacksquare$ 60ºC (T of the plateau) in presence of nitrogen ($\diamond$, $\triangle$, $\square$) and carbon dioxide ($\blacksquare$). Treated volume = 80 mL. Lines represent the Weibull model (continuous: N_2, dashed line: CO_2).
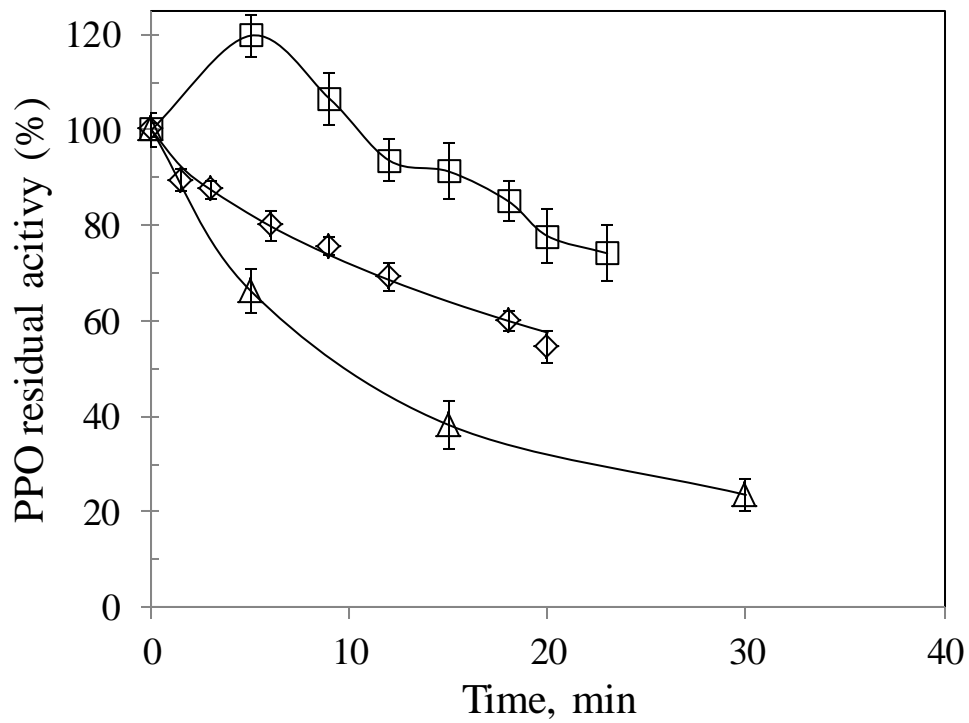
Figure 7. Comparison of PPO inactivation kinetics at 44 - 45 ℃ for different non-thermal treatments: Δ HPCD at 20 MPa, ◇ TS in the presence of N_2 (V=80 mL, 100 % of amplitude), □ TS in the presence of air (V=80 mL, 100 % of amplitude) (Lines are to guide the eye).

Table 1. Effect of amplitude in the thermosonication treatment on the particle size distribution (μm) of cloudy apple juice

Time	D[3,2]	D[4,3]	d(0.1)	d(0.5)	d(0.9)
Cloudy apple juice	2.24 ± 0.04^d	70 ± 2^d	0.690 ± 0.003^d	20.9 ± 0.8^c	249 ± 4^c
19 μm (25 %)	0.82 ± 0.02^c	44 ± 2^c	0.36 ± 0.01^c	0.88 ± 0.01^b	158 ± 4^b
38 μm (50 %)	0.168 ± 0.003^b	4.66 ± 0.06^b	0.086 ± 0.003^b	0.202 ± 0.003^a	0.70 ± 0.06^a
57 μm (75 %)	0.140 ± 0.001^a	0.238 ± 0.001^a	0.077 ± 0.000^a	0.164 ± 0.001^a	0.513 ± 0.001^a
76 μm (100 %)	0.138 ± 0.001^a	0.217 ± 0.001^a	0.078 ± 0.001^a	0.160 ± 0.001^a	0.434 ± 0.001^a

Data: mean $\pm$ SD (n=3). Different letters in a column indicate significant differences by the Tukey's honestly significant difference (HSD) method at p-value $\leq$ 0.05.

Table 2. TS kinetic inactivation parameters at different operating temperatures. Sensititvity temperature parameters, z_T and E_a.

T, ºC	Lineal model			Weibull model			
	k, min^{-1}	R^2	D, min	α, min	β	R^2	t$_{d=1}$, min
52	0.0077 ± 0.0006	0.9542	130	46 ± 10	1.2 ± 0.2	0.9378	92
60	0.0201 ± 0.0008	0.9862	50	20.3 ± 0.9	1.13 ± 0.08	0.9911	42
64	0.036 ± 0.001	0.9943	28	12.0 ± 0.2	1.12 ± 0.04	0.9981	25
67	0.056 ± 0.004	0.9795	18	7.9 ± 0.3	1.31 ± 0.09	0.9944	15
	$z_T = 17.5 \pm 0.8$ ºC (R^2 = 0.9962)			$z'_T = 20 \pm 1$°C (R^2 = 0.9935)			
	$E_a = 123 \pm 4$ kJ/mol (R^2 = 0.9976)			*"Slope ln (1/α) vs 1/T"* = 107 ± 6 kJ/mol (R^2 = 0.9908)			

Table 3. TS kinetic inactivation parameters at different operating temperatures and with different dissolved gases. Sensititvity temperature parameters, Z_T and E_a.

Enzyme	Gas	T, ºC	Lineal model			Weibull model			
			k, min^{-1}	R^2	D, min	α, min	β	R^2	$t_{d=1}$, min
PPO	N_2	44	0.0134 ± 0.0007	0.9591	75	40 ± 3	0.79 ± 0.06	0.9879	115
		52	0.025 ± 0.003	0.8046	40	22 ± 2	0.61 ± 0.06	0.9744	86
		60	0.09 ± 0.01	0.9298	11	4.9 ± 0.5	0.75 ± 0.06	0.9833	15
			$z_T = 20 \pm 4$ºC ($R^2 = 0.9626$)			$z'_T = 18 \pm 4$ ºC ($R^2 = 0.9301$)			
			$E_a = 106 \pm 18$ kJ/mol ($R^2 = 0.9710$)			"$ln(1/\alpha)$ vs $1/T$" $= 117 \pm 24$ kJ/mol ($R^2 = 0.9423$)			
PPO	CO_2	60ºC	0.0279 ± 0.0002	0.9511	36	14.3 ± 0.5	1.4 ± 0.1	0.9901	26
PME	N_2	60ºC	0.015 ± 0.001	0.9355	67	38 ± 8	0.8 ± 0.1	0.9608	108

Table 4. Effect of thermosonication on color parameters, total polyphenolic compounds (TPCs) and hydroxymethyl furfural content

V -PD[1] mL -W/mL	T, ºC	Gas	L*	a*	b*	ΔE	Chroma	TPCs mg GAE/L	HMF mg/L
Untreated apple juice			$37.1 \pm 0.2^{a,b}$	1.7 ± 0.1^{e}	7.8 ± 0.1^{d}		7.9 ± 0.1^{d}	407 ± 20^{a}	$1.9 \pm 0.1^{a,b}$
60 - 1.58	60	Air	38.9 ± 0.2^{d}	0.07 ± 0.05^{c}	7.0 ± 0.2^{c}	2.5 ± 0.3^{b}	7.0 ± 0.2^{c}	419 ± 27^{a}	$2.0 \pm 0.1^{a,b}$
80 - 1.30	60	Air	$37.9 \pm 0.6^{b,c}$	0.47 ± 0.02^{d}	6.9 ± 0.1^{c}	1.7 ± 0.3^{a}	6.9 ± 0.1^{c}	$459 \pm 3^{a,b}$	$1.7 \pm 0.1^{a,b}$
100 - 1.21	60	Air	$39.2 \pm 0.2^{d,e}$	-0.5 ± 0.1^{b}	7.1 ± 0.2^{c}	3.1 ± 0.1^{c}	7.1 ± 0.2^{c}	478 ± 35^{b}	$1.2 \pm 0.1^{a,b}$
80 - 1.43	60	N$_2$	38.0 ± 0.2^{c}	0.48 ± 0.02^{d}	5.9 ± 0.1^{b}	2.3 ± 0.1^{b}	6.0 ± 0.1^{b}	508 ± 13^{b}	2.2 ± 0.1^{b}
60 - 1.51	67	Air	37.1 ± 0.2^{a}	-0.85 ± 0.01^{a}	4.5 ± 0.1^{a}	4.1 ± 0.1^{d}	4.6 ± 0.1^{a}	482 ± 15^{b}	$1.6 \pm 0.2^{a,b}$
80 - 1.28	67	Air	40.3 ± 0.2^{t}	-0.45 ± 0.04^{b}	8.4 ± 0.2^{e}	3.8 ± 0.2^{d}	8.5 ± 0.2^{e}	483 ± 10^{b}	1.1 ± 0.1^{a}
100 - 1.15	67	Air	$39.9 \pm 0.1^{e,f}$	-0.03 ± 0.03^{c}	7.9 ± 0.1^{d}	3.2 ± 0.1^{c}	7.9 ± 0.1^{d}	498 ± 9^{b}	$1.6 \pm 0.1^{a,b}$

[1] Treated volume and power density
Different letters in the same column indicates significant differences by applying the Tukey's honestly procedure.

Highlights

Enzyme inactivation increased with amplitude, power density and temperature

TS treatment decreased particle size of cloudy apple juice

PME was more TS resistant than PPO

Faster enzyme inactivation kinetics in the presence of nitrogen were observed

After TS color change was noticeable and TPCs increased