Article

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Continuous microwave pasteurization of a vegetable smoothie improves its physical quality and hinders detrimental enzyme activity

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Abstract

The effect of a pasteurization treatment at $90 \pm 2\,^{\circ}C$ for 35 s provided by continuous microwave under different doses (low power/long time and high power/short time) or conventional pasteurization on the quality of orange-colored smoothies and their changes throughout 45 days of storage at $5\,^{\circ}C$ was investigated. A better color retention of the microwave pasteurization- treated smoothie using high power/short time than in conventionally processed sample was evidenced by the stability of the hue angle. The continuous microwave heating increased the viscosity of the smoothie more than the conventional pasteurization in comparison with non-treated samples. Lower residual enzyme activities from peroxidase, pectin methylesterase and polygalacturonase were obtained under microwave heating, specifically due to the use of higher power/shorter time. For this kind of smoothie, polygalacturonase was the more thermo-resistant enzyme and could be used as an indicator of pasteurization efficiency. The use of a continuous semi-industrial microwave using higher power and shorter time, such as 1600 W/206 s and 3600 W/93 s, resulted in better quality smoothies and greater enzyme reduction than conventional thermal treatment.

Keywords

Thermal process, viscosity, nutritious beverages, emerging technologies

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INTRODUCTION

The current lifestyle and eating habits have induced consumers to increasingly demand more natural, healthy, safe, and high quality ready-to-eat plant foods. Smoothies are a type of cold beverage that is made with fresh fruit and/or vegetables and have recently arrived to the market as healthy and nutritious new products. Therefore, the development of new vegetable smoothies that are rich in bioactive compounds, and processed under less aggressive heat treatments using emerging technologies for retaining their overall high quality and shelf life, is of great interest.

Industrial pasteurization is used for food preservation and is primarily intended for inactivating naturally found enzymes and destroying pathogenic microorganisms, extending the product's shelf life (Abdullah and Chin, 2014). Peroxidase (POD), pectin methylesterase (PME), and polygalacturonase (PG) are three enzymes that are important for the maintenance of a plant product's physical characteristics and therefore important for the food industry as well. POD is considered to be

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responsible for the development of off-color and off-flavor in most fresh fruit and vegetables, in derived products and during storage time (Ioannou and Ghoul, 2013; Katiyo et al., 2014). POD is one of the most thermally stable enzymes in fruit and vegetables and is therefore considered to be a biological indicator or used as an index of enzyme inactivation of any thermal treatment (Duarte et al., 2002; Elez-Martínez et al., 2006; Matsuia et al., 2007). For this reason, a reduction of POD activity or its complete inactivation is an important aim of the smoothie industry. It has been reported that tomato and carrot juice are not sensitive to enzymatic browning catalyzed by PPO such as apple juice (Aguiló-Aguayo et al., 2008). Pectinolytic enzymes such as PME and PG have the ability to improve clarification of concentrated juices and smoothies (Bastos et al., 2013). Since PME is more heat resistant than spoilage microorganisms such as common bacteria and yeasts in some treated products, it has also been considered to be an indicator of pasteurization efficiency (Ahmed and Ramaswamy, 2007; Sentandreu et al., 2006; Tajchakavit and Ramaswamy 1997).

Color and viscosity are two of the most important physical properties used to distinguish the quality of smoothies (Min and Hang, 2003). The viscosity of tomato products is highly influenced by the composition of pectins, and both PME and PG are involved in the decrease of tomato puree viscosity, as they are responsible for the hydrolysis of the pectins present in the product (Anthon and Barrett, 2012; Igual et al., 2010; Krebbers et al., 2003; Peeters et al., 2004; Vercet et al., 2002). Although thermal food processing commonly leads to a decrease in quality of the final products and their derivatives, microwave (MW) heating has been recognized as an advanced food-processing technology for pasteurization of a variety of liquid food products. This technology is fast, more uniform, more energy efficient, preserving the product's colors and flavors as well as nutrients as compared to conventional pasteurization (CP) (Bejar et al., 2011; Math et al., 2014). MW heating efficiency is highly dependent on the oven's characteristics, the product itself, its geometry and physical properties, and should be specifically optimized for each product. The overall reduction of the product's quality is highly influenced by time-temperature treatments during pasteurization with continuous MW (Math et al., 2014). The inactivation of enzymes such as PME (Schiffmann, 2001) through the use MW can be more efficient, mainly in continuous-flow systems, and is the preferred method for pasteurization over conventional heating as it allows the smoothies to maintain their flavor and nutrients, while increasing their shelf life (Ahmed and Ramaswamy, 2007). Continuous MW technology is not currently used by the beverage industry, but could provide versatility, efficiency, and time savings to those specialized in smoothie production. The different types of treatment (MW vs. CP) will affect the smoothies differently. For these reasons, the aim of this work was to elucidate the effects of different microwave pasteurization (MWP) doses (high power/short time and low power/long time) on POD, PME, and PG inactivation, as well as their effects on the orange-colored smoothie's physical quality (color and viscosity) and stability throughout 45 days of chilled storage.

MATERIALS AND METHODS

Chemical reagents

The following standards were used in the study: Polyvinylpolypyrrolidone (PubChem: CID 6917); hydrogen peroxide (PubChem: 784); O-phenylenediamine (PubChem: CID 7243); pectin (poly-D-galacturonic acid methyl ester); Sodium chloride (PubChem: CID 5234); Tris-hydroxymethyl aminomethane (PubChem: CID 6503); Cyanoacetamide (PubChem: CID 7898); Sodium metabisulfite (PubChem: CID 24346); Galacturonic acid monohydrate (PubChem: CID 10176506) were purchased from Sigma-Aldrich (Spain). Acetone (PubChem: CID 180), di-Sodium Hydrogen Phosphate anhydrous (PubChem CID: 24203) were purchased from Panreac (Spain). Sodium acetate (PubChem CID: 517045) was purchased from Fisher Chemical (UK).

Sample preparation

After several preliminary compositional and sensory tests using a trained 12-person panel (aged 24–67), an orange-colored smoothie was prepared. This smoothie contained tomato (126 g), carrot (61 g), pumpkin (29 g), lemon juice (4 mL, to reach a pH of 4.45), mineral water (50 mL), and 0.3 g marine salt. All the ingredients were blended for 3 min in a Thermomix^{TM} (Vorwerk Elektrowerke, Model TM 31-1, France). The samples were chilled to 5 °C immediately after blending until subjected to subsequent processing at day 0.

Thermal treatments

600 mL of smoothie samples were heated in the above-mentioned Thermomix^{TM} (CP). Alternatively, samples for each MWP treatment were placed in three extra resistant MW glasses (Hostelvia, Vicrila, Leioa, Spain), containing 200 mL of smoothie and treated with a semi-industrial continuous MW oven prototype (Sairem Ibérica S.L. SI-MAQ0101, Barcelona, Spain) with a power control from 0 to 12,000 W (Figure 1). Based on our preliminary studies, several temperature/time

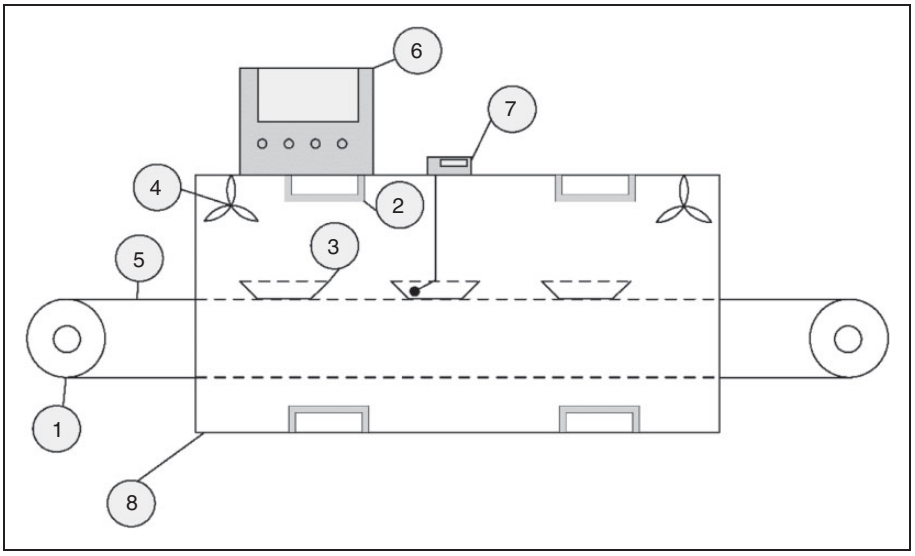

Figure 1. Semi-industrial microwave oven, process diagram. 1: Motor; 2: Magnetron; 3: Sample; 4: Fan; 5: Conveyor belt; 6: Control process; 7: Fiber optical temperature sensor; 8: Microwave chamber.

combinations of MWP, which were deemed appropriate for the inactivation of plant bacteria and enzymes without compromising quality, were selected. These combinations were: low power/long time (210 W/646 s and 260 W/608 s) and high power/short time (1600 W/206 s and 3600 W/93 s). In both methods and in all the treatments, samples were heated from room temperature to a final temperature of $90 \pm 2\,^{\circ}C$, which was maintained for 35 s. The temperature was monitored with an automatic fiber optic thermometer (Neoptix, Quebec, Canada). After the treatments, the samples were packaged aseptically into plastic tubes and rapidly cooled (5 °C) with an ice-water bath. Fresh, unheated samples were used as non-pasteurized controls, evaluated at day 0. For each heating method, the full experiment was conducted independently three times, each one constituting a repetition. Samples were evaluated at day 0 and after 15, 30, and 45 days of 5 °C storage.

Color measurements

The color of samples was determined in triplicate with a colorimeter (Minolta CR-300, Ramsey, NJ, USA). Results were expressed as lightness (L*) and hue angle ($h^{\circ} = \tan^{-1}$ (b*/a*)) values.

Viscosity determination

Viscous flow tests were conducted in triplicate with a controlled shear rate/stress rheometer (AR G-2, TA Instruments, U.K) at 20 °C. Viscous flow tests were performed using a shear rate range between 1 and 100 1/s. The viscous tests were carried out at 20 °C in triplicate.

ENZYME ACTIVITY MEASUREMENTS

POD

POD activity was measured using the method described by Elez-Martínez et al. (2006). POD was assayed by mixing 9 μL enzyme extract, 243 μL of phosphate buffer 0.05 M, 18 μL of phenylenediamine (10 g kg^{-1}), and 9 μL of H_2O_2 (15 g kg^{-1}) in a 96-well polystyrene flat-bottom plate. The absorbance was measured at 509 nm for 10 min at 25 °C with the multiscan plate reader (Tecan Infininte M200, Männedorf, Switzerland). The enzyme activity unit was defined as the change in absorbance per min per mg of protein extracted or the change in absorbance per min per g of tissue (González et al., 2000).

PME

PME activity was determined according to the Ratner et al. (1969) method with slight modifications. A 2.5 mL sample of smoothie was homogenized with 10 mL of 0.2 M sodium chloride solution. After filtering the homogenate with cheesecloth, 2.5 mL was mixed with 15 mL 1% pectin solution. The resulting solution was adjusted to pH 7.0 with 1 N NaOH and the pH was maintained at 7.0 for 10 min using 0.01 N NaOH. One PME U was expressed as the amount of enzyme that produces 1 nmoL of acid per minute at pH 7.0 and 22 °C.

PG

PG activity was measured according to the Aguiló-Aguayo et al. (2008) method with slight modifications. 2 mL of sample were homogenized in 15 mL of cold acetone for 30 min. The supernatant was decanted

and 5 mL of tris-hydroxylmethyl aminomethane buffer (pH 7.0; 0.2 M, including 500 mg L^{-1} of sodium metabisulfite, 1% PVPP and 1 M NaCl) were added. The extraction was carried out for 2 h in an orbital shaker (Stuart, Staffordshire, UK) at $200 \times g$ in darkness inside a polystyrene box with ice at 4 °C. The homogenate was centrifuged at 20,000 × *g* for 15 min at 4 °C. The supernatant was used as the enzyme extract. The PG activity was quantified according to the Gross (1982) method. The substrate consisted of 0.6 mL of a solution containing 0.4% (w/v) polygalacturonic acid in 0.05 M sodium acetate buffer (pH 4.5) and the reaction was carried out by adding 0.15 mL of enzyme extract, followed by incubation at 37 °C for 10 min in a shaker set at 30 r/min. The reaction was stopped with 2 mL of 10 mM borate buffer at pH 9 and 0.4 mL of 1% (w/v) cyanoacetamide. The mixture was put in a boiling water bath (100 °C) for 10 min and then chilled with ice; 200 µL of extraction were placed in a 96-well polystyrene flat-bottom plate. The absorbance was read at 276 nm and 22 °C using the same device as for POD.

Statistical analysis

The experimental design was a randomized design with three replicates per treatment, to determine the influence of CP or MWP heat treatments on the color and enzyme activity of a smoothie. Each dependent variable was subjected to a one-way analysis of variance (ANOVA) at $P \leq 0.05$, with the Statgraphic Plus 5.1 software (Manugistic Inc., Rockville, MD, USA). The mean values were compared with a multiple range least significant difference (LSD) test to identify differences among treatments. A Pearson's correlation analysis was performed to corroborate relationships between the parameters.

RESULTS AND DISCUSSION

Effects of MWP and CP treatments on smoothie color

The effects of both MWP and CP on smoothie L* and h° and the changes throughout 45 days of storage at 5 °C are shown in Tables 1 and 2. L* values increased

Table 1. Lightness (L*) in unheated (control), and two pasteurization treatments: Conventional (CP) and microwave (MWP; low power/long time and high power/short time doses) smoothies throughout storage up to 45 days at 5 °C

L*	Days of storage at 5 °C			
	0	15	30	45
Non-treated	39.26 ± 0.23 e	38.94 ± 0.07 d		
CP	40.29 ± 0.15 d	40.59 ± 0.32 c	41.00 ± 0.59 b	41.60 ± 0.30 ns
MWP: 210 W–646 s	40.86 ± 0.09 c	42.02 ± 0.33 b	42.86 ± 0.67 a	42.71 ± 0.14
MWP: 260 W–608 s	40.94 ± 0.25 c	42.10 ± 0.15 b	42.87 ± 0.33 a	43.26 ± 0.63
MWP: 1600 W–206 s	41.94 ± 0.04 b	42.50 ± 0.17 ab	42.95 ± 0.20 a	43.07 ± 0.34
MWP: 3600 W–93 s	42.89 ± 0.17 a	43.18 ± 0.41 a	43.29 ± 0.37 a	43.40 ± 0.41

CP: conventional pasteurization; MWP: microwave pasteurization.
Values are mean ± standard error (n = 3). For the same sampling day, different letters in the same column denote significant differences among treatments ($P < 0.05$) and ns means there are no significant differences.

Table 2. Hue (h°) in unheated (control), and two pasteurization treatments: conventional (CP) and microwave (MWP; low power/long time and high power/short time doses) smoothies throughout storage up to 45 days at 5 °C

Hue (h°)	Days of storage at 5 °C			
	0	15	30	45
Non-treated	50.36 ± 0.70 c	51.63 ± 0.95 ns		
CP	53.29 ± 0.70 a	54.31 ± 0.51	55.41 ± 0.72 ns	56.07 ± 0.54 ns
MWP: 210 W–646 s	52.48 ± 0.55 ab	53.95 ± 0.63	54.41 ± 0.56	55.01 ± 0.23
MWP: 260 W–608 s	52.33 ± 0.26 ab	53.28 ± 0.02	54.22 ± 0.55	54.82 ± 0.05
MWP: 1600 W–206 s	51.77 ± 0.16 abc	52.65 ± 0.24	53.45 ± 0.22	53.58 ± 0.50
MWP: 3600 W–93 s	51.58 ± 0.47 bc	51.78 ± 0.87	52.83 ± 0.56	53.06 ± 0.64

CP: conventional pasteurization; MWP: microwave pasteurization.
Values are mean ± standard error (n = 3). For the same sampling day, different letters in the same column denote significant differences among treatments ($P < 0.05$) and ns means there are no significant differences.

after both heat treatments. MWP-treated samples showed a slightly higher increase of L* as compared to the CP-processed and untreated smoothies ($P < 0.05$) during the first 30 days of storage. Just after thermal treatment, the L* values increased significantly throughout storage time, with the highest L* value (42.89 ± 0.17) found for MWP samples treated with the highest power/shortest time dose (3600 W, 93 s). On the contrary, L* values of the untreated samples declined after the first 15 days of the shelf life period, with the decrease being related to enzymatic browning reactions, leading to a decrease in its acceptance (Katiyo et al., 2014). Similar results were found by Aguiló-Aguayo et al. (2008) in conventionally heated tomato juice during cold storage. According to the results displayed in Table 2, the h° value of the smoothie significantly increased after thermal treatment in comparison to untreated samples, and no significant changes among any of the treatments in the different evaluation days were found. The shade was similar to the control when a combination of highest power/shortest time (3600 W, 93 s) was used. These color changes were related to an increased lycopene content after all the different heat treatments, specifically under the high power/short time MW treatment (data not shown). The β-carotene content also increased after both the heat treatment methods, but no significant differences among them were found (data not shown). Schiffmann (2001) indicated that the heat transfer is fast in MWP; for that reason, MWP is a method that can be used to better preserve the color of smoothies as compared to CP, and this kind of treatment could be advantageous for this kind of product.

Effects of MWP and CP treatments on viscosity

Significant differences ($P < 0.05$) were found on the smoothie's viscosity when pasteurized by MW or CP as compared to fresh, non-heated smoothies (Figure 2). Emulsions, suspensions, and solutions are non-Newtonian fluids (Mujumdar et al., 2002) and therefore their viscosity is not fixed, but depends on the degree of shear. As the shear rate applied increases, the viscosity decreases. All the smoothies treated with MWP had the highest values of viscosity in comparison to CP and non-treated samples throughout storage time. Just after treatment (day 0), the viscosity ranged from 80 to 59 Pa.s for MWP smoothies compared to 49 Pa.s and 30 Pa.s for CP and unheated samples, respectively. The viscosity significantly decreased down to 7 and 0.5 Pa.s in all treatments, at a shear rate of 100 1/s. An increase in viscosity after the heat treatments could be associated with a change in the pectin fractions (Osorio et al., 2008). Also, the results show that the viscosity of all treated samples decreased with storage time, although MWP-treated smoothies had the highest viscosity levels. These results agree with those shown from research work on tomato puree (Krebbers et al., 2003; Vercet et al., 2002). These authors reported that the viscosity was strongly influenced by PME and PG activities, with the viscosity decreasing according to the different degrees of inactivation of these enzymes during storage, as we also found in this experiment. Also, Aguiló-Aguayo et al. (2008) reported that unheated tomato juice was less viscous than tomato juice that was conventionally heated at 90 °C for 30 s, with this property decreasing with storage time. On the contrary, Min and Hang (2003) detected no significant differences on the viscosity of heated (90 °C, 90 s) tomato juice, indicating non-efficient PME and PG inactivation during processing. Furthermore, the viscosity of tomato products is influenced by other factors such as tomato cultivar and degree of ripening, water-insoluble, and soluble pectins (these compounds reduce soluble solids in suspension and consequently increase the product's viscosity), content of solids and the degree of pectin esterification (Hayes et al., 1998; Sánchez et al., 2002).

Enzyme activity

The effect of MWP and CP on the residual activity of POD during 45 days of storage is displayed in Figure 3. Results indicate that both MWP and CP heating treatments were able to decrease the POD activity in smoothies. These results agree with other authors, who have reported on the reduction of POD activity in conventionally pasteurized tomato juice (Aguiló-Aguayo et al., 2008). The latter authors found that POD was completely inactivated at 90 °C for 60 or 30 s in tomato juice during storage. Our results show that just after MWP treatments at different power/time conditions, POD activity diminished in the smoothies up to 96% (Figure 3). One of the most interesting results found after MWP treatment was elucidating that when the same final temperature (90 ± 2 °C for 35) was used, the best POD inactivation was reached using the high power/short time treatments (1600 W/206 s and 3600 W/93 s). Teixeira (2012) previously reported that high power/short time MW treatments reduced the adverse thermal degradation on food quality while ensuring food safety because the nutritional characteristics of the product were more sensitive to time than to temperature. In our experiment, high power/short time MWP treatments completely stopped POD activity after 45 days of storage. In comparison, the CP treatment reduced the initial POD activity by 70% in the smoothie, reaching 90% inactivation by the end of storage time. Consequently, the fast generation of heat using MWP, more specifically, high power/short

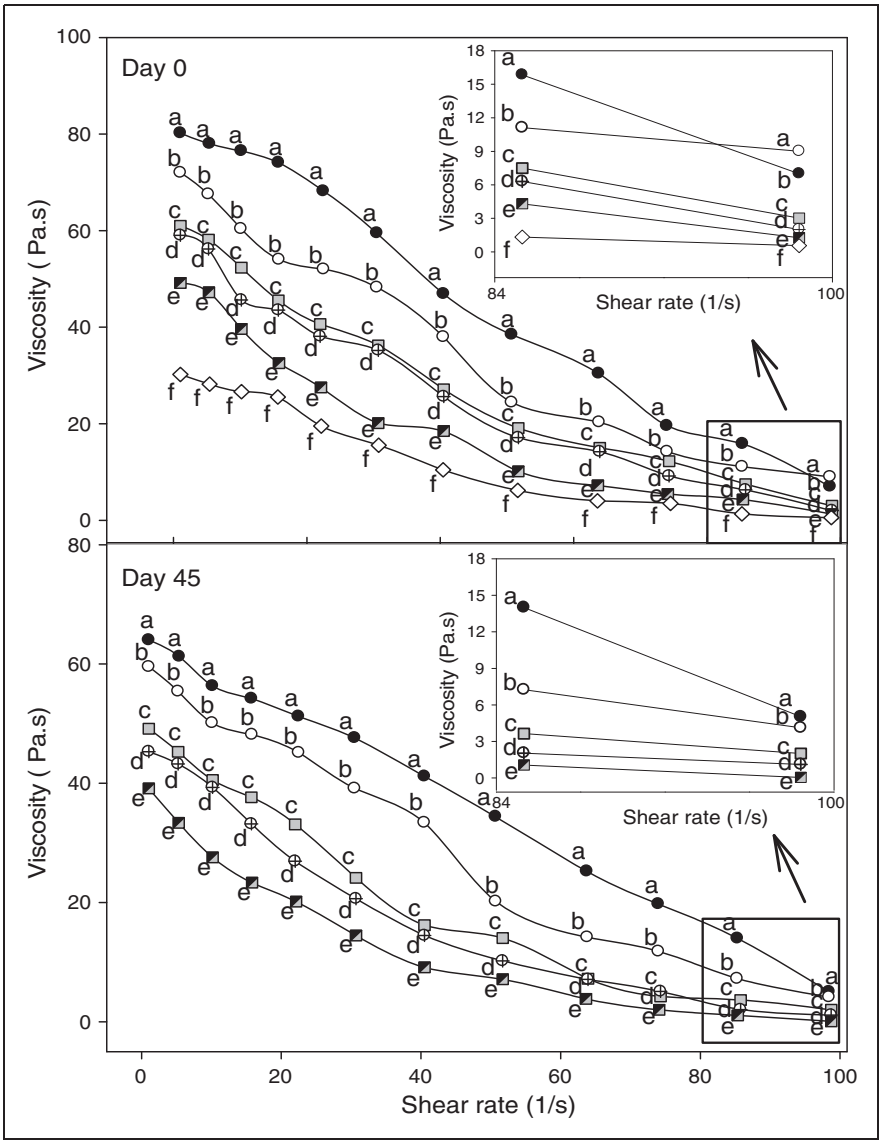

Figure 2. Viscosity (Pa.s) of unheated (control —◇—), conventional (CP —◪—), and microwave pasteurized (MWP; 3600 W, 93 s (—●—), 1600 W, 206 s (—○—), 260 W, 646 s (—□—), 210 W, 608 s—⊕—) smoothies at 0 and 45 days stored at 5 °C. Different letters for the same shear rate denote significant differences among treatments ($P < 0.05$).

time, resulted in the strongest POD inactivation. Similar results have been reported for MW inactivation of POD in green coconut water (Matsui et al., 2008) and asparagus (Zheng and Lu, 2011).

The inactivation of PME in differently treated smoothies is shown in Figure 4. The PME activity results from all the 90 °C treatments confirm previous findings on tomato juice (Peeters et al., 2004; Rodrigo et al., 2006). Also, after the MWP and CP treatments, PME activity was significantly reduced ($P < 0.05$) decreasing with time of storage, with MWP reducing the activity of PME more strongly than CP. These results confirm those reported by Tajchakavit and Ramaswamy (1997), who reported that at the same temperature (60 °C), MW heating largely enhanced PME inactivation as compared to CP (7.37 s for MW and 154 s for CP) in orange juice. Also, the statistical analysis showed a significant effect ($P < 0.05$) of MW doses on the residual enzymatic activity; the maximum inactivation (92%) was reached using the highest power and shortest time, 3600 W/93 s, in comparison with the 81% PME reduction using 210 W for 246 s. Nikdel et al. (1993) and Cinquanta et al. (2010) reported that continuous MWP of orange juice resulted in a

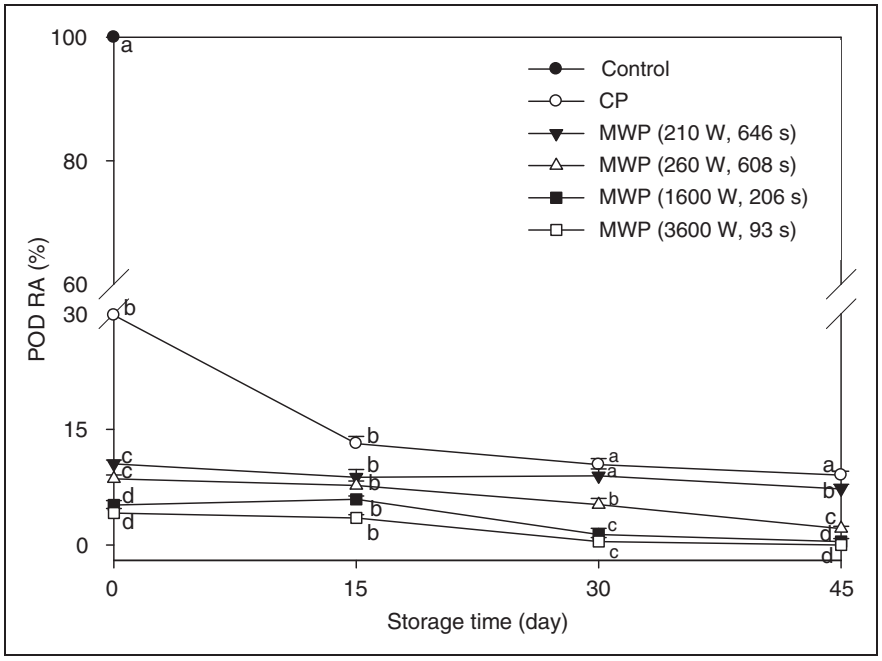

Figure 3. Residual peroxidase (POD) activity in unheated (control), conventional (CP) and microwave pasteurized (MWP; high power/short time and low power/long time doses) smoothies throughout storage up to 45 days at 5 °C. Different letters for the same day of analysis denote significant differences among treatments ($P < 0.05$).

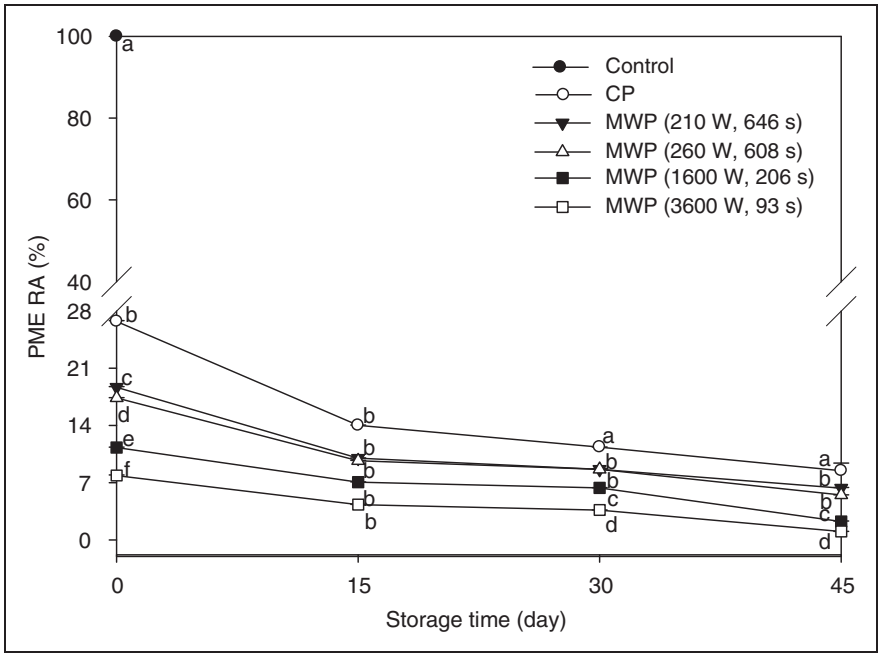

Figure 4. Residual pectin methylesterase (PME) activity in unheated (control), conventional (CP) and microwave pasteurized (MWP; high power/short time and low power/long time doses) smoothies throughout storage up to 45 days at 5 °C. Different letters for the same day of analysis denote significant differences among treatments ($P < 0.05$).

reduction of PME residual activity of 0.5–1.5% at temperatures higher than 75 °C for 10–15 s and 2.5% at 70 °C for 1 min. Our results demonstrated that MW technology is suitable for decreasing enzyme activity using a short processing time, which would indicate that product stability could be preserved effectively. The heat generated by the MW energy provides unique characteristics which helps preserve the quality

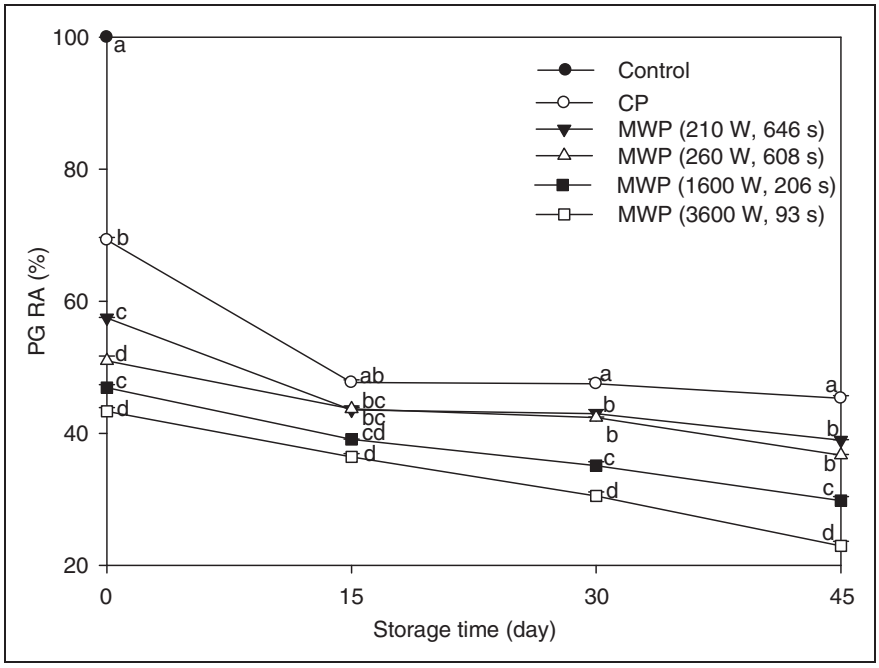

Figure 5. Residual polygalacturonase (PG) activity in unheated (control), conventional (CP) and microwave pasteurized (MWP; high power/short time and low power/long time doses) smoothies throughout storage up to 45 days at 5 °C. Different letters for the same day of analysis denote significant differences among treatments ($P < 0.05$).

of the heated product (Ratti and Kudra, 2006). Tajchakavit and Ramaswamy (1997) found a relationship between time heating and PME inactivation during MW heating (700 W) of orange juice. The time scale was increased showing the enzyme's expanded inactivation kinetics. In our case, when the MW doses were increased, the processing time was reduced, thereby obtaining the best highest power/lowest time combination. Also, Kratchanova et al. (2004) reported that using higher power MW (450, 630, and 930 W) for 10 or 15 min led to a significant inactivation of PME in heated orange peels as compared with the unheated sample.

The effects of MWP and CP treatments on the PG residual activity are shown in Figure 5. Similarly, to the other two enzymes, significant differences ($P < 0.05$) among treatments were found. MWP was able to reduce PG activity to a greater degree than CP. The highest power/shortest time provided the best reduction of enzyme activity. The lowest residual PG activity (50%) was reached when the smoothies were treated with 3600 W/93 s, with the activity gradually decreasing until the end of storage (23%). After CP, the enzyme's residual activity was only reduced to 69%, decreasing to 42% until the end of storage. Our results are similar to those reported by Aguiló-Aguayo et al. (2008), who found that the residual PG activity decreased after a conventional heat treatment, finally reaching 78% and 56%, in tomato juice heated at 90 °C for 30 and 60 s, respectively. Nevertheless, pectins are also broken down by the combined action of PG and PME; therefore, a reduction of PME activity leads to a decrease of PG action (Aguiló-Aguayo et al., 2009; Gross, 1982). The results by Fachin et al. (2003) also showed that at 93 °C for 3 min (conventional heating), PG was inactivated completely in tomato juice. This agrees with our results, where PG was more thermo-resistant than PME and POD (Figures 3 to 5). The results of this research show that at the same final heating temperature, the highest MW power and shortest duration provided a more efficient reduction of enzyme activity as compared to the combination of lower power together with longer time and CP.

Correlation between the appearance parameters measured and the enzyme's activity

Pearson correlation coefficients (r) for the physical characteristics (color and viscosity) and the residual enzymatic activities of MWP and CP-treated smoothies were calculated (Table 3). There was a negative correlation between residual PG and PME enzyme activity and viscosity of treated smoothies (−0.8901 and −0.9028, respectively). This correlation showed that the increases in viscosity during the heat treatments were influenced by the reduction in enzymatic activity, as PME and PG cooperatively regulate the disassembly of pectin polysaccharides. Aguiló-Aguayo et al. (2008) found the same trend for these enzymes after the

Table 3. Pearson correlation coefficients (r) between the appearance parameters and the residual enzyme activity in unheated (control), conventional (CP) and microwave-pasteurized (MWP; low power/long time and high power/short time doses) smoothies

	Hue angle	L*	POD	PME	PG
Hue					
L*	0.7804				
POD	−0.7986	−0.8069			
PME	−0.8006	−0.7838	0.9920		
PG	−0.8310	−0.8412	0.9850	0.9700	
Viscosity	0.8789	0.8470	−0.8902	−0.8901	−0.9028

n = 18, $P = 0.001$. POD: peroxidase; PME: pectin methylesterase; PG: polygalacturonase.

conventional heating of tomato juice. Also, the relationship observed between these two enzyme's activities in this study were confirmed by Fachin et al. (2003), who reported that PME produced a substrate for PG activity and consequently PME inactivation could lead to a decrease of PG activity. On the other hand, a negative correlation was observed between the residual activity of POD and the color parameters (L* and h°). The increase of the L* values in heat-treated smoothies could be explained by the decrease of enzymatic browning reactions by POD (Ioannou and Ghoul, 2013; Katiyo et al., 2014).

CONCLUSION

Continuous MW heating increased the smoothie's viscosity to a greater degree than conventional heating and as compared to non-treated samples. Lower residual POD, PME, and PG activities were obtained under MW heating, specifically when using a higher power/short time dose. The MWP treatments resulted in better color retention as evidenced by the stability of the hue angle. For this kind of smoothie, PG was the most thermo-resistant enzyme and could be used as an indicator of pasteurization efficiency. This study revealed that the use of a continuous semi-industrial MW, using high power/short time doses, could reduce food processing time, increasing the industry's efficiency and leading to better color retention, increased viscosity of smoothies and greater enzyme activity reduction. The enzyme's deactivation was more sensitive to the high power used rather than the time of exposure.

HIGHLIGHTS

1. Microwave pasteurization (MWP) resulted in a better color and viscosity of smoothies.
2. Polygalacturonase was the more thermo-resistant enzyme in this vegetal smoothie.
3. MWP provided versatility, efficiency, and time savings.
4. The degradation of enzymes was more sensitive to high power MWP than time doses.

DECLARATION OF CONFLICTING INTERESTS

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REFERENCES

Abdullah N and Chin NL. (2014). Application of thermosonication treatment in processing and production of high quality and safe to drink fruit juices. *Journal of Agriculture and Agricultural Science Procedia* 2: 320–327.

Aguiló-Aguayo I, Oms-Oliu G, Soliva-Fortuny R and Martín-Belloso O. (2009). Changes in quality attributes throughout storage of strawberry juice processed by high-intensity pulsed electric fields or heat treatments. *LWT – Journal of Food Science and Technology* 42(4): 813–818.

Aguiló-Aguayo I, Soliva-Fortuny R and Martín-Belloso O. (2008). Comparative study on color, viscosity and related enzymes of tomato juice treated by high-intensity pulsed electric fields or heat. *Journal of Food Research Technology* 227(2): 559–606.

Ahmed J and Ramaswamy HS. (2007). Microwave pasteurization and sterilization of foods. In: Shafiur Rahman M (ed.) *Handbook of Food Preservation*. 2nd ed. Boca Raton, FL: CRC Press, Taylor & Francis Group, pp. 691–711.

Anthon GE and Barrett DM. (2012). Pectin methylesterase activity and other factors affecting pH and titratable acidity in processing tomatoes. *Journal of Food Chemistry* 132(2): 915–920.

Bastos SC, Pimenta CJ, Dias DR, Chalfoun SM, Angélico CL and Tavares LS. (2013). Pectinases from a new strain of Cladosporium cladosporioides (Fres.) De Vries isolated from coffee bean. *World Journal of Agricultural Sciences* 9(2): 167–172.

Bejar AK, Kechaou N and Mihoubi NB. (2011). Effect of microwave treatment on physical and functional properties of orange (Citrus Sinensis) peel and leaves. *Journal of Food Processing and Technology* 2(2): 109.

Cinquanta L, Albanese D, Cuccurullo G and Di Matteo M. (2010). Effect on orange juice of batch pasteurization in an improved pilot-scale microwave oven. *Journal of Food Science* 75(1): 46–50.

Duarte ACP, Coelho MAZ and Leite SGF. (2002). Identification of peroxidase and tyrosinase in green coconut water. *CyTA – Journal of Food* 3(5): 266–270.

Elez-Martínez P, Soliva-Fortuny R and Martín-Belloso O. (2006). Comparative study on shelf life of orange juice processed by high intensity pulsed electric fields or heat treatments. *Journal of European Food Research and Technology* 222(3): 321–329.

Fachin D, Van Loey AM, Nguyen BL, Verlent I, Indrawati and Hendrickx ME. (2003). Inactivation kinetics of polygalacturonase in tomato juice. *Journal of Innovative Food Science and Emerging Technologies* 4(2): 135–142.

González EM, De Ancos B and Cano MP. (2000). Partial characterization of peroxidase and polyphenoloxidase activities in blackberry fruits. *Journal of Agriculture Food chemistry* 48(11): 5459–5464.

Gross KC. (1982). A rapid and sensitive spectrophotometric method for assaying polygalacturonase using 2-cyanoacetamide. *Journal of Horticulture Science* 17: 933–934.

Hayes WA, Smith PG and Morris AEJ. (1998). The production and quality of tomato concentrates. *Critical Reviews in Journal of Food Science and Nutrition* 38(7): 537–564.

Igual M, García-Martínez E, Camacho MM and Martínez-Navarrete N. (2010). Effect of thermal treatment and storage on the stability of organic acids and the functional value of grapefruit juice. *Journal of Food Chemistry* 118(2): 291–299.

Ioannou I and Ghoul M. (2013). Prevention of enzymatic browning in fruit and vegetables. *Journal of European Scientific* 9(30): 310–341.

Katiyo W, Yang R, Zhao W, Hua X and Gasmalla MAA. (2014). Optimization of combined pulsed electric fields and mild temperature processing conditions for red apple juice polyphenol oxidase and peroxidase inactivation. *Advance Journal of Food Science and Technology* 6(5): 638–646.

Kratchanova M, Pavlova E and Panchev I. (2004). The effect of microwave heating of fresh orange peels on the fruit tissue and quality of extracted pectin. *Journal of Carbohydrate Polymers* 56(2): 181–185.

Krebbers B, Matser AM, Hoogerwerf SW, Moezelaar R, Tomassen MMM and Van Den Berg RW. (2003). Combined high-pressure and thermal treatments for processing of tomato puree: evaluation of microbial inactivation and quality parameters. *Journal of Innovative Food Science and Emerging Technologies* 4(4): 377–385.

Math R, Nagender A, Nayani S and Satyanarayana A. (2014). Continuous microwave processing and preservation of acidic and non-acidic juice blends. *International Journal of Agriculture and Food Science Technology* 5(2): 81–90.

Matsuia KN, Granadob LM, Oliveirac PV and Tadinia CC. (2007). Peroxidase and polyphenol oxidase thermal inactivation by microwaves in green coconut water simulated solutions. *Journal of Science and Technology* 40(5): 852–859.

Matsui KN, Gut JAW, de Oliveira PV and Tadini CC. (2008). Inactivation kinetics of polyphenol oxidase and peroxidase in green coconut water by microwave processing. *Journal of Food Engineering* 88(2): 169–176.

Min S and Hang QHZ. (2003). Effects of commercial-scale pulsed electric field processing on flavor and color of tomato juice. *Journal of Food Science* 68(5): 1600–1606.

Mujumdar A, Beri AN and Metzner AB. (2002). Transient phenomena in thixotropic systems. *Journal of Non-Newtonian Fluid Mechanics* 102(2): 157–178.

Nikdel S, Chen CS, Parish ME, MacKellar DG and Friedrich LM. (1993). Pasteurization of citrus juice with microwave energy in a continuous-flow unit. *Journal of Agricultural and Food Chemistry* 41(11): 2116–2119.

Osorio O, Martínez-Navarrete N, Moraga G and Carbonell JV. (2008). Effect of thermal treatment on enzymatic activity and rheological and sensory properties of strawberry puree. *Journal of Food Science and Technology* 14(5): 103–108.

Peeters L, Fachin D, Smout C, Van Loey A and Hendrickx ME. (2004). Influence of β-subunit on thermal and high-pressure process stability of tomato polygalacturonase. *Journal of Biotechnology and Bioengineering* 86(5): 543–549.

Ratner A, Goren R and Monseline SP. (1969). Activity of pectin esterase and cellulase in the abscission zone of citrus leaf explants. *Journal of Plant Physiology* 44(12): 1717–1723.

Ratti C and Kudra T. (2006). Drying of foamed biological materials: Opportunities and challenges. *Journal of Drying Technology* 24(9): 1101–1108.

Rodrigo D, Cortes C, Clynen E, Schoofs L, Ann Van Loey AV and Hendrickx M. (2006). Thermal and high-pressure stability of purified polygalacturonase and pectinmethylesterase from four different tomato processing varieties. *Journal of Food Research International* 39(4): 440–448.

Sánchez MC, Valencia C, Gallegos C, Ciruelos A and Latorre A. (2002). Influence of processing on the rheological properties of tomato paste. *Journal of Science Food Agriculture* 82(9): 990–997.

Schiffmann RF. (2001). Microwave processes for the food industry. In: Datta AK, Anantheswaran RC and Marcel D (eds) *Handbook of Microwave Technology for Food Applications*. New York, NY, pp. 299–335.

Sentandreu E, Carbonell L, Rodrigo D and Carbonell JV. (2006). Pulsed electric fields versus thermal treatment: Equivalent processes to obtain equally acceptable citrus juices. *Journal of Food Protection* 69(8): 2016–2018.

Tajchakavit S and Ramaswamy HS. (1997). Thermal vs. microwave inactivation kinetic of pectin methylesterase in orange juice under batch mode heating conditions. *LWT – Journal of Food Science and Technology* 30(1): 85–93.

Teixeira AA. (2012). Simulating thermal food processes using deterministic models. In: Wen-Sun D (ed.) *Thermal Food Processing: New Technologies and Quality Issues*. USA: CRC press, p. 98.

Vercet A, Sanchez C, Burgos J, Montanes L and López Buesa P. (2002). The effects of manothermosonication on tomato pectic enzymes and tomato paste rheological properties. *Journal of Food Engineering* 53(3): 273–278.

Zheng H and Lu H. (2011). Effect of microwave pretreatment on the kinetics of ascorbic acid degradation and peroxidase inactivation in different parts of green asparagus (*Asparagus officinalis* L.) during water blanching. *Journal of Food Chemistry* 128(4): 1087–1093.