High-pressure homogenization of orange juice to inactivate pectinmethylesterase

J. Welti-Chanes a, C.E. Ochoa-Velasco b, J.A. Guerrero-Beltrán c,⁎

a Departamento de Biotecnología e Ingeniería en Alimentos, Instituto Tecnológico y de Estudios Superiores de Monterrey, Av. Eugenio Garza Sada 2501 Sur, Col. Tecnológico, Monterrey, N. L. 64849, Mexico
b Facultad de Ingeniería Química, Benemérita Universidad Autónoma de Puebla, Puebla, Puebla, Mexico
c Depto. Ingeniería Química y Alimentos, Universidad de las América-Puebla, Sta. Catarina Mártir, Cholula 72820, Puebla, Mexico

Abstract

A homogenizer was used to treat orange juice at five pressures (0–250 MPa) and three initial temperatures (22, 35 and 45 °C). A maximum of five passes for the selected conditions were used to process orange juice. Pectinmethylesterase (PME) activity, microbial load, cloudy appearance, and vitamin C were evaluated in just squeezed and homogenized orange juices. A reduction of 50.4, 49.4 and 37.8% of PME activity was observed in juice homogenized by one pass at 250 MPa at the initial temperatures of 22, 35, and 45 °C, respectively. Pectinmethylesterase activity in orange juice was reduced as passes number was increased. The final temperature of the five times homogenized orange juice was not beyond 28 and 37 °C after being treated at 100 and 250 MPa, respectively. More than 30 and 80% of enzyme activity was reduced after five passes at 100 and 250 MPa, respectively. Less that 8.7×10² and 1.85×10³ CFU/mL of mesophiles and yeasts plus molds, respectively, were counted in orange juice treated five times at 100 MPa. The cloudy appearance of the homogenized orange juice was maintained for 12 days under low temperature conditions.

1. Introduction

Emerging technologies are being widely studied to deliver food products with fresh like characteristics that consumers are demanding today. High pressure, static (high hydrostatic pressure, HHP) or dynamic (high-pressure homogenization, HPH), is one of the technologies used mainly to inactivate microorganisms or enzymes which may alter or damage fresh foods products. The advantage of using these novel technologies is that some of them are also called nonthermal technologies. Research related to the effects of high pressure on vegetative microorganisms (Yuste, Capellas, Fung, & Mor-Mur, 2004), microbial spores (Estrada-Girón, Guerrero-Beltrán, Swanson, & Barbosa-Cánovas, 2007; Heinz & Knorr, 2001), activation or inactivation of spores (Estrada-Girón, Guerrero-Beltrán, Swanson, & Barbosa-Cánovas, 2007; Heinz & Knorr, 2001), activation or inactivation of food enzymes (Ludikhuize, Indrawati, Van den Broeck, Weemaes, & Hendrickx, 1998; Ludikhuize, Van Leoy, Indrawati, Denys, & Hendrickx, 2001; Tedjo, Eshtiaghi, & Knorr, 2000; Asaka & Hayashi, 1991, Guerrero-Beltrán, Barbosa-Cánovas, & Swanson, 2004), and chemical reactions influencing quality (Ludikhuize & Hendrickx, 2001) in fruit and vegetable products have been reported elsewhere.

Regarding food enzymes, pectinmethylesterase and polygalacturonase, found in citrus fruit products, react with pectic substances cleaving methyl esters to render poly-o-galacturonic acid (Whistler & Daniel, 1985) which modify the physical appearance of citrus juices. If these enzymes are not inactivated, they eventually will destroy the cloudy stability in citrus fruit juices (Haard, 1985). The ordinary
process to inactivate these enzymes is by pasteurization; however, citrus fruit juices are very sensitive to heat leading to flavor changes in juice during heat processing. Thus, some studies have been conducted by high pressure to study its effect on sensory, microbial and enzyme characteristics of foods. Goodner, Braddock, and Parish (1998) pointed out that high pressure (500–600 MPa) inactivated the heat labile pectinesterase in orange and grapefruit juices. The cloudy stability in orange juice was preserved after 10 min of processing at pressures in the range of 500 to 900 MPa (Goodner, Braddock, Parish, & Sims, 1999). Nienaber and Shellhammer (2001) found Dp-values ranged from 4.6 to 117.5 min to inactivate pectinesterase in orange juice treated at pressures in the range of 400 to 600 MPa. Ogawa, Fukuhisa, Kubo, and Fukumoto (1990) pointed out that pectinesterase was completely inactivated after pressurization at 300 or 400 MPa for 10 min in Satsuma mandarin juice. All of those studies have been carried out using high hydrostatic pressure machines. Nevertheless, a few studies have been conducted using homogenizers for microbial and, or enzyme inactivation purposes. Taylor, Roach, Black, Davidson, and Harte (2007), for instance, observed a significant reduction of Escherichia coli K-12 in sodium chloride solutions (0.9%) in the range of 100 to 250 MPa using a high-pressure homogenizer. Pathanibul, Taylor, Davidson, and Harte (2007) on the other hand, using similar pressure conditions, reported an inactivation of around 5.5 and 6 log10 (CFU/mL) of E. coli K-12 and Listeria innocua, respectively, inoculated in apple and carrot juices. Bríñez, Roig-Sagués, Hernández-Herrero, and Guamis-López (2006) studied the inactivation effect of an HPH system on E. coli inoculated in whole and skim milk treated at 300 MPa; they found an inactivation range of 3.9–4.3 log10 (CFU/mL) units after 2 h of treatment.

The aim of this research was to treat just squeezed orange juice by high-pressure homogenization to inactivate pectinesterase and natural microbial flora.

2. Materials and methods

2.1. Orange juice preparation

Oranges (Citrus sinensis), Valencia variety, were purchased at the Central Market Supplier in Puebla City. Oranges were washed, cut in halves and pressed by hand using a domestic rotary orange juice processor. The orange juice pulp was discarded by sieving.

2.2. High-pressure treatment

The orange juice was high pressure treated using a continuous FPG7400H:350 Hi-Drive homogenizer (Stansted Fluid Power, Ltd., Essex, UK) at a constant flow rate of 7 L/h. Juice was warmed up at three temperatures (22, 35 and 45 °C) just before homogenization by re-circulating juice into the system. Afterward, juice was treated at five pressures (50, 100, 150, and 250 MPa) and selected number of passes (0–5). The homogenizer has a cooling system at the end of the tubing arrangement to cool down any increase of temperature of the processed liquid. The juice temperature did not increase beyond 19, 27, or 37 °C after homogenization at 22, 35, or 45 °C, respectively. The homogenized orange juice was analyzed for pectinesterase activity and natural microbial flora. All processing of orange juice were performed in duplicate.

2.3. Physicochemical analysis

Total soluble solids (TSS, °Bx) were measured using a refractometer (Atago USA, Inc. Bellevue, WA). pH was measured using an Orion pH meter model 420 (Orion Research Inc., Boston, MA). Tritratable acidity (percentage of citric acid) was assessed by the 22.038 AOAC method (1984). Vitamin C was determined by titration with 2,6-dichlorophenolindophenol using the 43.064 AOAC method (1984). Each test was performed in triplicate.

2.4. Pectinmethylesterase assay

Pectinmethylesterase activity was evaluated using the Rouse, Atkins, and Huggart (1954) method with modifications. Ten milliliters of previously warmed up (30 °C) citric pectin solution (CPS) (1%) was placed into a double wall beaker. Afterward, 10 mL of orange juice and 0.175 g of sodium chloride were added to the CPS and mixed for homogenization. The system was heated again until reaching 30 °C by circulating water into the walls of the beaker. Sodium hydroxide solution (0.1 or 0.5 N) was added to the mixture of CPS and juice (CPS–juice) until reaching pH 7.5. After that, titration of the CPS–juice mixture was performed by adding sodium hydroxide solution (0.02 N) to maintain pH at 7.5 as a function of time. The pectinmethylesterase activity (meq/mL min) was calculated using the milliequivalent (meq) of sodium hydroxide (V NaOH × h NaOH), sample volume (mL) and the reaction time (min). A Cole-Parmer (Vernon Hills, Illinois) re-circulation bath was used for controlling temperature. Each test was performed in triplicate.

2.5. Cloudy appearance

Fifty mL of just squeezed or homogenized orange juice was placed into plastic centrifugal tubes and then stored at low temperature (4 °C) during 12 days. The cloudy appearance of the juice was evaluated by simple observation. The percentage of juice separation was evaluated measuring the settling of particles in juice.

2.6. Microbial counts

Mesophiles and yeasts plus molds were counted using the pour plate method. Nutrient and potato dextrose agars were used to evaluate the survivals of mesophiles and yeasts plus molds, respectively, in juice. After pour plating, Petri dishes were placed into ovens at 37 (24–48 h) and 30 °C (5 days), respectively.

2.7. Microbial and enzyme inactivation modeling

A first order modeling was used to analyze the microbial load inactivation:

\[ \log N = -mP + b \]

where \( N \) is the residual microbial load (CFU/mL), \( P \) is pressure (MPa) or number of passes, \( m \) is the slope (1/MPa or 1/number of passes), and \( b \) is the intercept (—). A linear relationship between residual enzyme activity (%) and pressures (MPa) was used to model the effect on pectinmethylesterase.

2.8. Statistical analysis

Experimental data were statistically analyzed performing an analysis of variance (ANOVA) using a Minitab 14 program. A \( p \leq 0.05 \) was used as the criterion value to determine significant differences within treatments.

3. Results and discussion

3.1. Physiochemical characteristics

Table 1 shows the physiochemical characteristics of orange juice before being homogenized. It can be observed that pulp content was the only difference between juice containing and noncontaining pulp. The reason of that was because pulp separation in juice was carried out by sieving before analysis. The reduction of pulp in the juice was important to avoid blocking of the high-pressure system during processing.
On the other hand, pH, TSS, citric acid content and TSS/acid ratio were similar in the two types of juices.

### 3.2. Pressure and initial temperature effect on PME

The initial PME activity [(1.787 ± 0.006) × 10⁻⁴ meq/mL min] in orange juice was reduced to 50.4, 49.4 and 37.8% after one single pass of juice through the HPH system (250 MPa) at the initial temperatures of 22, 35, and 45 °C, respectively. Fig. 1 illustrates the remaining PME activity in orange juice processed by one single pass as a function of pressure. A linear tendency was observed for PME inactivation in orange juice (R²=0.939). It can be also observed that, the higher the temperature to process orange juice, the more inclined the slope going downward; this means that lower PME activity was observed as temperature and pressure increased. A significant difference (p<0.05) was observed among temperatures, being the PME activity more reduced when orange juice was processed at 45 °C. No significant difference (p>0.05) was observed in PME activity when juice was homogenized at 22 and 35 °C. A significant difference (p<0.05) was observed within pressures. Table 2 shows the linear regression coefficients for PME activity, in orange juice, after pressurization at the three initial temperatures. Linear regression coefficients higher that 0.939 were obtained. Therefore, PME inactivation, at the selected temperatures, was inactivated linearly as pressure increased.

### 3.3. Passes number effect on orange juice PME

Fig. 2 illustrates the effect of number of passes and pressure (100 and 250 MPa) on PME activity in orange juice (22 °C). A significant difference (p<0.05) was observed for PME activity in orange juice for both pressure and number of passes. No significant difference (p>0.05) was observed in PME activity in orange juice homogenized from 1 to 5 passes when processing at 100 MPa. It can also be observed that PME activity in juice was dramatically reduced when processing at 250 MPa; however, no significant difference (p>0.05) was observed in PME activity in juice between 1 and 2 passes or among 3, 4 and 5 passes. Therefore, at 250 MPa, the inactivation of PME was higher as the circulation number increased. Therefore, one or two passes may generate orange juice with less than 50% of the initial PME.

### 3.4. Microbial reduction

#### 3.4.1. Pressure and initial temperature effects

Fig. 3 depicts the effect of pressure and initial temperature on microbial load in orange juice. Both, the initial temperature and pressure had an effect on the microbial load in orange juice. The higher reduction of the microbial load was around 1.5 Log cycles. A fist order model was used to examine the microbial load inactivation as pressure increased (Fig. 3, Table 3). Small values of the correlation coefficients were obtained for microbial reductions in the processed juice at an initial temperature of 22 °C; however, no significant difference (p>0.05) was observed for mesophiles and yeasts plus molds in orange juice heated at 22 and 35 °C and treated at the selected pressures. A significant difference (p<0.05) was observed for mesophiles and yeasts plus molds in juice previously heated at 45 °C in comparison with microbial load in juice heated at 22 and 35 °C. No significant difference (p>0.05) was observed within 200 and 250 MPa for the two types of microorganisms in orange juice. Table 3 shows the linear regression parameters after plotting the Log number of survivals (CFU/mL) versus pressure (MPa) data. It is observed, in Table 3, the corresponding amount of pressure (1/slope), at the selected temperatures, to reduce one Log cycle of mesophiles and yeasts plus molds. Therefore, more pressure is required to inactivate one Log cycle of both types of microorganisms (mesophiles and molds plus yeasts) in orange juice when homogenizing at low initial temperatures and vice versa.

![Fig. 1. Pressure and initial temperature (22, 35, 45 °C) effect on PME inactivation in orange juice.](image1)

![Fig. 2. Passes number effect on PME inactivation in orange juice treated at 100 (■) and 250 (▲) MPa.](image2)

![Fig. 3. Initial temperature effect on mesophiles (M) and yeasts plus molds (YM) in orange juice homogenized one time (one pass) at selected pressures.](image3)

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Juice</th>
<th>Containing pulp</th>
<th>Noncontaining pulp</th>
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<td>pH</td>
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<td>4.0±0.27</td>
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<td>TSS (% w/w)</td>
<td>10.2±0.80</td>
<td>10.1±1.00</td>
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<tr>
<td>Citric acid (%)</td>
<td>1.2±0.5</td>
<td>1.1±0.40</td>
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<td>Pulp (%)</td>
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<td>TSS/acid ratio</td>
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<td>8.6±1.0</td>
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Table 2

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Slope (%/MPa)</th>
<th>Intercept (%)</th>
<th>R²</th>
</tr>
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<tbody>
<tr>
<td>22</td>
<td>−0.192</td>
<td>104.3</td>
<td>0.953</td>
</tr>
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<td>−0.195</td>
<td>104.3</td>
<td>0.939</td>
</tr>
<tr>
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<td>98.6</td>
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Table 3

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<tr>
<td>45</td>
<td>−0.262</td>
<td>98.6</td>
<td>0.950</td>
</tr>
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Regression parameters for PME activity in pressurized orange juice at selected initial temperatures.

The initial PME activity [(1.787 ± 0.006) × 10⁻⁴ meq/mL min] in orange juice was reduced to 50.4, 49.4 and 37.8% after one single pass of juice through the HPH system (250 MPa) at the initial temperatures of 22, 35, and 45 °C, respectively. Fig. 1 illustrates the remaining PME activity in orange juice processed by one single pass as a function of pressure. A linear tendency was observed for PME inactivation in orange juice (R²=0.939). It can be also observed that, the higher the temperature to process orange juice, the more inclined the slope going downward; this means that lower PME activity was observed as temperature and pressure increased. A significant difference (p<0.05) was observed among temperatures, being the PME activity more reduced when orange juice was processed at 45 °C. No significant difference (p>0.05) was observed in PME activity when juice was homogenized at 22 and 35 °C. A significant difference (p<0.05) was observed within pressures. Table 2 shows the linear regression coefficients for PME activity, in orange juice, after pressurization at the three initial temperatures. Linear regression coefficients higher that 0.939 were obtained. Therefore, PME inactivation, at the selected temperatures, was inactivated linearly as pressure increased.

Fig. 2 illustrates the effect of number of passes and pressure (100 and 250 MPa) on PME activity in orange juice (22 °C). A significant difference (p<0.05) was observed for PME activity in orange juice for both pressure and number of passes. No significant difference (p>0.05) was observed in PME activity in orange juice homogenized from 1 to 5 passes when processing at 100 MPa. It can also be observed that PME activity in juice was dramatically reduced when processing at 250 MPa; however, no significant difference (p>0.05) was observed in PME activity in juice between 1 and 2 passes or among 3, 4 and 5 passes. Therefore, at 250 MPa, the inactivation of PME was higher as the circulation number increased. Therefore, one or two passes may generate orange juice with less than 50% of the initial PME.

3.3. Passes number effect on orange juice PME

Fig. 3 depicts the effect of pressure and initial temperature on microbial load in orange juice. Both, the initial temperature and pressure had an effect on the microbial load in orange juice. The higher reduction of the microbial load was around 1.5 Log cycles. A fist order model was used to examine the microbial load inactivation as pressure increased (Fig. 3, Table 3). Small values of the correlation coefficients were obtained for microbial reductions in the processed juice at an initial temperature of 22 °C; however, no significant difference (p>0.05) was observed for mesophiles and yeasts plus molds in orange juice heated at 22 and 35 °C and treated at the selected pressures. A significant difference (p<0.05) was observed for mesophiles and yeasts plus molds in juice previously heated at 45 °C in comparison with microbial load in juice heated at 22 and 35 °C. No significant difference (p>0.05) was observed within 200 and 250 MPa for the two types of microorganisms in orange juice. Table 3 shows the linear regression parameters after plotting the Log number of survivals (CFU/mL) versus pressure (MPa) data. It is observed, in Table 3, the corresponding amount of pressure (1/slope), at the selected temperatures, to reduce one Log cycle of mesophiles and yeasts plus molds. Therefore, more pressure is required to inactivate one Log cycle of both types of microorganisms (mesophiles and molds plus yeasts) in orange juice when homogenizing at low initial temperatures and vice versa.

![Fig. 1. Pressure and initial temperature (22, 35, 45 °C) effect on PME inactivation in orange juice.](image1)

![Fig. 2. Passes number effect on PME inactivation in orange juice treated at 100 (■) and 250 (▲) MPa.](image2)

![Fig. 3. Initial temperature effect on mesophiles (M) and yeasts plus molds (YM) in orange juice homogenized one time (one pass) at selected pressures.](image3)
Table 3
Linear regression parameters for mesophiles (M) and yeasts plus molds (YM) inactivation in orange juice homogenized one time (one pass) at different initial temperatures.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Microorganism types</th>
<th>Slope (1/MPa)</th>
<th>Pressure* (MPa)</th>
<th>Intercept</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>M</td>
<td>−0.0021</td>
<td>476</td>
<td>3.20</td>
<td>0.620</td>
</tr>
<tr>
<td>35</td>
<td>YM</td>
<td>−0.0019</td>
<td>526</td>
<td>3.54</td>
<td>0.750</td>
</tr>
<tr>
<td>45</td>
<td>M</td>
<td>−0.0032</td>
<td>312</td>
<td>3.55</td>
<td>0.900</td>
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<td></td>
<td>YM</td>
<td>−0.0023</td>
<td>435</td>
<td>3.89</td>
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<tr>
<td></td>
<td>YM</td>
<td>−0.0050</td>
<td>200</td>
<td>3.81</td>
<td>0.952</td>
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<td></td>
<td>YM</td>
<td>−0.0053</td>
<td>189</td>
<td>3.93</td>
<td>0.926</td>
</tr>
</tbody>
</table>

* Pressure to inactivate one Log cycle.

3.4.2. Passes number effect

Fig. 4 illustrates the passes number effect on the microbial load in the pressurized (100 and 250 MPa) orange juice (22 °C). Both, the passes number and pressure had an effect on the microbial load in the homogenized orange juice. The microbial load decreased as the passes number increased. The microbial counts in orange juice homogenized five times were 870 and 950 mesophiles/mL and 1850 and 700 molds plus yeasts/mL for 100 and 250 MPa of treatment, respectively. A significant difference (p<0.05) was observed, for microbial load inactivation in orange juice, within passes number. However, no significant difference (p>0.05) was observed for microorganisms inactivation in juice among 1, 2, and 3 passes or between 4 and 5 passes. Also, no significant difference (p>0.05) was observed in yeast and molds in the pressurized (100 and 250 MPa) orange juice (22 °C). Both, the passes number and pressure had an effect on the microbial load in the homogenized (one pass) orange juice (22 °C). It can be observed that PME activity, in just squeezed orange juice, was reduced as pressure treatment increased (0 day). Afterward, the PME activity was increased during the storage time. More than 100% of PME activity was achieved in orange juice after twelve days of storage. The increased PME activity could be due to isoenzymes arising (Richardson & Hyslop, 1985) throughout the storing of orange juice. Several researchers have reported increasing of enzyme activity due to high-pressure treatment to fruit products (Asaka & Hayashi, 1991, Guerrero-Beltrán et al., 2004). They pointed out that high pressure may split isoenzymes that could react later on in the food system. Therefore, the same phenomenon could have occurred after orange juice processing by HDP. A significant difference (p<0.05) was observed for PME activity in orange juice within pressures and storage times. Comparable behavior, regarding PME activity, was observed in just squeezed orange juice previously heated at 45 °C (Fig. 6). However, lower PME activation was observed during the storage period in this type of treated juice. This behavior in orange juice, previously heated at 45 °C, concerning PME activity activation could be probably due to the inactivation effect of initial temperature on the enzyme.

The cloudy appearance in orange juice, just after homogenization (0–250 MPa; 22 °C), was opaque and homogenous. No difference was observed by simple inspection between homogenized and non-homogenized orange juices. However, after 6 h of storing at 4 °C, the cloudy appearance of the non-treated orange juice disappeared; therefore, the juice was turning transparent. This means that pectinmethylesterase chemically reacted with pectic substances (pectindemethylation) leading to loss of the cloudy appearance (Haard, 1985) wanted in these types of citrus juices. However, the cloudy appearance of the homogenized (one pass at the selected pressures 50–250 MPa) orange juice stored at refrigeration temperature remained stable for 12 days (Fig. 7). The staying of the cloudy appearance in the orange juice could also be due to the reduction of the particle size of the remaining pulp in juice after squeezing and sieving.

Fig. 5 illustrates the storage (4 °C) time effect on PME activity in homogenized (100 MPa, 1–5 passes, 22 °C) orange juice. A significant difference (p<0.05) was observed for PME activity in orange juice within storage time and passes number. No significant difference (p>0.05) was observed for PME activity in orange juice between zero

Table 4
Linear regression parameters for mesophiles (M) and yeasts plus molds (YM) in orange juice (22 °C) homogenized at 100 and 250 MPa.

<table>
<thead>
<tr>
<th>Pressure (MPa)</th>
<th>Microorganism types</th>
<th>Slope (1/MPa)</th>
<th>Intercept</th>
<th>R²</th>
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<tr>
<td>100</td>
<td>M</td>
<td>−0.220</td>
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<td>M</td>
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<td>YM</td>
<td>−0.218</td>
<td>3.93</td>
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![Fig. 4](Image)

![Fig. 5](Image)

![Fig. 6](Image)
and four days of storing or between eight and 12 days of storing. The same behavior was observed for PME activity in orange juice when homogenization was performed at 250 MPa (Fig. 9). However, using 250 MPa of pressure, to treat orange juice, the PME inactivation was more effective as the passes number increased. The cloudy appearance of the homogenized orange juice remained stable after 12 days of storing at 4 °C (Fig. 7).

3.6. Vitamin C

The vitamin C content (57 ± 7 mg/100 mL in just squeezed juice) in orange juice homogenized (50–250 MPa, 22 °C) by one pass was not affected after pressurization (56 ± 16 mg/100 mL). Vitamin C was barely affected by number of passes (1–5) after processing at 100 (60 ± 3 mg/100 mL) or 250 MPa (54 ± 3 mg/100 mL). As a result, the vitamin C content remained stable after homogenization at different pressures and passes number.

4. Conclusions

A linear behavior was observed in the PME inactivation in orange juice homogenized at the selected pressures and temperatures. No significant difference was observed in PME activity in orange juice warmed up at 22 or 35 °C before processing. A maximum of 50% of PME inactivation was observed after a single pass of the orange juice throughout the homogenizer at 250 MPa; however, its cloudy appearance was maintained for 12 days of storing under refrigeration (4 °C). Thirty one and 80% of PME inactivation in orange juice was observed after five passes at 250 MPa; the cloudy appearance of this processed juice was also maintained for 12 days under refrigeration. The microbial counts in orange juice homogenized five times at 100 and 250 MPa were less that 3.0 and 3.3 Log cycles for mesophiles and yeasts plus molds, respectively.

Acknowledgment

Author C.E. Ochoa-Velazco would like to thank the National Council of Science and Technology (CONACyT) in Mexico for the economical support provided for the completion of his Master’s Degree studies.

References


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The vitamin C content (57 ± 7 mg/100 mL in just squeezed juice) in orange juice homogenized (50–250 MPa, 22 °C) by one pass was not affected after pressurization (56 ± 16 mg/100 mL). Vitamin C was barely affected by number of passes (1–5) after processing at 100 (60 ± 3 mg/100 mL) or 250 MPa (54 ± 3 mg/100 mL). As a result, the vitamin C content remained stable after homogenization at different pressures and passes number.

4. Conclusions

A linear behavior was observed in the PME inactivation in orange juice homogenized at the selected pressures and temperatures. No significant difference was observed in PME activity in orange juice warmed up at 22 or 35 °C before processing. A maximum of 50% of PME inactivation was observed after a single pass of the orange juice throughout the homogenizer at 250 MPa; however, its cloudy appearance was maintained for 12 days of storing under refrigeration (4 °C). Thirty one and 80% of PME inactivation in orange juice was observed after five passes at 250 MPa; the cloudy appearance of this processed juice was also maintained for 12 days under refrigeration. The microbial counts in orange juice homogenized five times at 100 and 250 MPa were less that 3.0 and 3.3 Log cycles for mesophiles and yeasts plus molds, respectively.

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