Effect of high pressure processing on the quality of acidified Granny Smith apple purée product

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\textbf{A B S T R A C T}

The aim of the present study was the evaluation of the physicochemical, nutritional and microbial quality of acidified Granny Smith (GS) apple purée processed on industrial-scale high pressure system during 3 weeks of refrigerated storage (5 °C ± 1 °C). Two commercially feasible pressure treatments (400 and 600 MPa/5 min/20 °C) and a mild conventional pasteurization at 75 °C/10 min, with pasteurization values of $P_{72^\circ C}=8.15$ min., were conducted and their effect on total vitamin C (total Vit C), ascorbic acid (AA) and total phenolic content (TPP), and on instrumental quality parameters (color, viscosity, soluble solids, titratable acidity and pH) were comparatively studied. Inactivation of indigenous microorganisms (total aerobic mesophilic and psychrotrophic counts and moulds and yeasts) of the apple product was also studied and monitored during storage. Total Vit C and AA contents were unaffected by the 400 MPa and the mild pasteurization treatment. TPP content was not changed during processing at 400 MPa, but was affected by the 600 MPa and also slightly by the pasteurization treatment. Experimental data on the loss of total Vit C during storage were described with a first-order reaction kinetic and times of half loss between 9.3 to 10.3 days could be estimated for the three studied processes. Storage provoked loss of TPP content and color deterioration of pressurized GS puree samples, which was attributed to enzymatic browning reactions. Microbial counts were reduced by the different preservation techniques below the detection limit (50 cfu g\textsuperscript{-1}) and storage revealed no further growth.

\textit{Industrial relevance:} This is one of the first studies applying commercial industrial-scale high pressure equipment for the pasteurization of an acidified apple purée product. The pressures of 400 and 600 MPa with 5 min holding time at ambient temperature render economically feasible processes with high throughput and productivity. In the European Union the most important fruits in terms of production are apples. Apple purée is a largely consumed preserve in many households and beside apple juice or cider is one of the most important apple products in the market. In contrast to traditional apple purée preparation, high pressure processing or mild thermal treatments could imply new opportunities for the apple processing industry in developing more fresh-like, value-added apple products with reasonable shelf life.

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1. Introduction

Nowadays, consumers are demanding new ready-to-eat products (e.g. apple desserts), which provide increased convenience, while maintaining fresh-like characteristics of flavour, texture, color, vitamin content, aroma and overall appearance, along with reasonable or extended shelf life. Shelf life and food quality are closely related to microbial quality and other phenomena such as biochemical and enzymatic reactions. To successfully address the new challenges faced by the food industry interests in the research on minimal preservation techniques, like high pressure processing (HPP) as well as mild thermal treatments, on fruit and vegetable produce have been promoted.

The principles of high pressure processing of food have been broadly discussed by several authors since it was first reported by Hite, 1889. High pressure processing (HPP) at room temperature is now known as an alternative nonthermal food preservation method that affects the viability of microbial cells and the structure of proteins/enzymes (Basak & Ramaswamy, 1998; Palou, López-Malo, Barbosa-Cánova, & Swanson, 2007; Palou, Lopez-Malo, Barbosa-Canova, Welti-Chanes, & Swanson, 1999; Rastogi, Raghavarao, Balasubramaniam, Niranjani, & Knorr, 2007), while leaving low molecular weight food compounds, such as vitamins, pigments, flavouring agents and other compounds related to sensory, nutritional and health related qualities of the product, greatly unaffected (Butz et al., 2003, Fernández García, Butz, Bognár, & Tauscher, 2001; Fernández García, Butz, & Tauscher,
2001; Nienaber & Shellhammer, 2001a,b; Oey, Lille, Van Loey, & Hendrickx, 2008). The effect of HPP on the quality characteristics of food has been mainly attributed to the stability of covalent bonds to high pressure (Knoor, 1993). Unlike in thermal treatments, the transmittance of pressure is instantaneous and uniform throughout the entire food, regardless of its size or shape (Palou et al., 2007; Rastogi et al., 2007). Moreover, thermal processes can often lead to quality changes in foods such as the destruction of vitamins, modifications to food texture and color and the development of off-flavours (Norton & Sun, 2008).

Traditional apple purée preparation involves high temperature processing during prolonged time and affects the nutritional quality of the raw material adversely. In industrial apple purée processing, trimmed and cored apples are chopped and precooked for 4–5 min at about 96 °C and then, after finishing and filling, closed containers are pasteurized for 10–15 min in boiling water to ensure destruction of spoilage organisms (Downing, 1989). The precooking process has to assure total inactivation of the endogenous polyphenol oxidase enzyme (PPO), the enzyme mainly responsible for the development of undesirable oxidative browning especially in purée products (Chisari, Barbagallo, & Spagna, 2006; Cocci, Rocculi, Romani, & Dalla Rossa, 2006; Polypedia et al., 2005; Sánchez-Moreno, Plaza, De Ancos, & Cano, 2003). The relative pressure sensitivity of vegetative cells, including yeasts and moulds, in combination with the intrinsic preservation factor of low pH has made the preservation of acid foods (pH ≤4.5), where most spoilage microorganisms are controlled and spore formers cannot grow, the most obvious application of HPP (Palou et al., 2007; Rastogi et al., 2007; Smelt, Hellemons, & Patterson, 2002).

HP treatment can however affect the rheological properties of food products such as crushed fruits and vegetables, purée, pulp and juice. The observed effects are dependent on the conditions of the HP process and the type of fruit and vegetable (Oey et al., 2008).

The industrial relevance for the food processing industry was continuously growing over the last two decades, stimulated also by the numerous studies of different research groups, who pointed out the potential use of this new processing technique. The first pressure-treated products (fruit jams) were introduced in the Japanese market in 1991. Since then, the commercial use of HPP and the total production of pressure–treated food products has been expanding, at the same time as equipment manufacturers have improved, in response to increase equipment productivity (Balasubramaniam, Farkas, & Turek, 2008; Rovere, 2002). To date, the most successful applications now on the market range from the in-pack preservation of guacamole (avocado sauce), sliced meat products (salami, cooked ham, cured ham), chicken or turkey breasts, orange juice, smoothies, and shellfish (oysters). Despite the increased commercial applications, to our knowledge limited data are available on the effect of industrial-scale HPP on the shelf life of fruit and vegetable products, as most research groups focused their work on laboratory scale equipments.

The aim of the present work was to compare the effectiveness of two economically feasible high pressure processes at 400 MPa and 600 MPa/5 min/20 °C with industrial-scale equipment (Hyperbaric Wave 6500/120, N.C. Hyperbaric, S.A., Burgos, Spain) on acidified Granny Smith apple purée product containing ascorbic acid with a desired shelf life of 2–3 weeks at refrigeration conditions. The effect of HPP was evaluated on physicochemical (color, texture, soluble solids, titratable acidity and pH) and nutritional quality parameters (ascorbic acid and total phenolic content, TPP) and compared with a mild conventional pasteurization treatment (P21/5 = 8.15 min) as a function of treatment and storage time (21d/5 °C ± 1 °C). Inactivation of indigenous microorganisms (total aerobic count of mesophilic and psychrotrophic microorganisms, moulds and yeasts) of the apple product was also studied and monitored over the storage period at 5 °C.

2. Materials and methods

2.1. Raw material

Granny Smith (GS) apples were purchased from a local fruit packinghouse (Girona Fruits SCCL, Girona, Spain) and stored at 3 ± 1 °C until processing. The cultivar GS was chosen because of its availability at a regular quality throughout the whole year. GS apple lots were characterized according to fruit firmness (fruit pressure tester, Model FT 327, Effegi, Italy), soluble solids content (SSC), and pH. Initial fruit characterization carried out at least on three fruits in triplicate gave the average characteristics of 8.3 ± 0.6 kg cm−2 firmness, 11.2 ± 0.3 °Brix and pH 3.41 ± 0.06.

2.2. Purée product preparation

Apples were washed, peeled, cut and cored before blending (Thermomix, Vorwerk, Wuppertal, Germany) with acerola C juice powder (0.25% w/w; Obipectin, Bischofzell, Switzerland) and fresh lemon juice (2.5% w/w) as natural antibrowning substances and glucose-fructose syrup (3% w/w; Roquette Laisa España S.A., Barcelona, Spain). Acerola C juice powder served as a strong natural source of vitamin C (16–18% according to Obipectin) in the formulation and
the lemon juice as a natural acidulant. Purée was immediately vacuum packed in composite pouches containing a metallic layer (PET/MET + PE, Sacoliva S.L., Barcelona, Spain) in portions of 100 g. The oxygen permeability of the bags was <1.5 cm² m⁻² 24 h (ASTM D 3985). Three replicates of apple purée were prepared for each combination of treatment and storage time and stored under refrigeration condition (2–4 °C) overnight until HPP and conventional heat pasteurization. Three control samples were prepared in the same manner and stored under the same conditions.

### 2.3. Preservation treatments

#### 2.3.1. High pressure processing

Industrial HPP was performed on commercial-scale high pressure system (Hyperbaric Wave 6500/120, N.C. Hyperbaric, S.A., Burgos, Spain) with a maximum capacity of 120 L and a potential maximum pressure of 650 MPa. Purée samples were pressurized at 400 MPa and 600 MPa for 5 min with water at 20 °C as the pressure-transmitting medium. Pressurization rate for the 400 MPa cycle was 204 MPa/min and 220 MPa/min for the 600 MPa cycle. Time for decompression was ≤10 s. Initial temperature of the purée was between 9–10 °C and the average temperature after pressurization was about 16.0 °C. After treatments apple product was stored at 5 ± 1 °C during 21 days and analyzed as described below.

#### 2.3.2. Conventional pasteurization process

Sample pouches were submersed in a circulated water bath adjusted to 75 °C and held at this temperature during 10 min. The heating regime was validated in preliminary studies by monitoring the temperature profile of the product inside the packages by means of a datalogger (PicoVACQ 1TC, TMI-Orion, Montpellier, France). The pasteurization process was equivalent to a pasteurization value \( P_{120°C} = 8.15 \) min with a maximum temperature of 73.4 °C ± 0.3 °C achieved. After removal from the water bath, samples were immediately placed in refrigeration conditions during 21 days and analyzed as described below.

### 2.4. Physicochemical and physical analysis

Three samples were analyzed periodically for each combination of storage time \( \times \) treatment at day 0, 7, 14 and 21 of cold storage (5 °C) and compared to raw control purée at day 0.

#### 2.4.1. Color measurement

The color change of apple purée was measured with a Konica Minolta chroma meter (Model CR-410 HS, Minolta, Tokyo, Japan). The equipment was set up for illuminate \( D_65 \) (2°observer angle) and calibrated using a standard white reflector plate. Readings were obtained applying the standard CIE \( L^*a^*b^* \) (1976) color system, where \( L^* \) is the lightness value, \( a^* \) indicates hue on a green (−) to red (+) axis, and \( b^* \) indicates hue on a blue (−) to yellow (+) axis. For the measurement, purée was poured into a cylindrical sample cup and measured. Color differences \( \Delta E^* = (\Delta L^*2 + \Delta a^*2 + \Delta b^*2)^{0.5} \) at day 21 of storage was computed.

#### 2.4.2. Soluble solids content and \( \text{pH} \)

Soluble solids content was measured with a portable refractometer (Quick-BrixTM 90; Mettler Toledo GmbH, Giessen, Germany). Apple purée was squeezed through a piece of filter paper (10 μm pore size, Ahlstrom Barcelona, S.A., Barcelona, Spain). One drop was placed on the refractometer glass prism and the SSC was obtained as Brix.

\( \text{pH} \) was measured with a Crison pH meter (Crison PH 25, Crison Instruments S.A., Barcelona, Spain) in combination with a Crison puncture electrode (Crison pH 5053, Crison Instruments S.A., Barcelona, Spain), which was calibrated prior to each measurement with phosphate buffers at pH 4 and 7.

#### 2.4.3. Viscosity

A rotational viscometer (Visco Star plus, Fungilab, S.A., Barcelona, Spain), interfaced to a computer, was used for rheological measurement and data acquisition. Samples were temperature-equilibrated at 25 °C in a water bath and filled in a concentric cylindrical cup. A combination of spindle R6 and velocity 5 rpm was set up for all analysis. Each sample was analyzed in threefold replication and results were expressed as mean values.

### 2.5. Chemical analysis

Purée samples for chemical analysis were frozen stored at −80 °C. All the analyses were carried out in duplicate of each packaged purée sample. All the chemicals used were of analytical reagent grade and water was obtained with a Milli-Q purification system (Branstead, USA).

#### 2.5.1. Determination of ascorbic acid (AA) and total vitamin C (total Vit C)

The method applied to determine total vitamin C (total Vit C = AA + dehydroascorbic acid, DHAA) was based on the procedure of López, Montaño, García, and Garrido (2005) with some modifications. One gram of frozen stored (−80 °C) purée samples was weighed in centrifugation tubes followed by the addition of chilled extraction buffer containing 60 g L⁻¹ metaphosphoric acid (Sigma-Aldrich) and 1 mM EDTA to 10 g of final weight. After gently stirring for 5 min at 4 °C samples were centrifuged for 10 min at 11000× g at 4 °C (Beckman J2–M2, Beckman Coulter, Inc. Fullerton, CA, USA). The supernatant was filtered through a nylon syringe filter (0.45 μm) (Teknokroma, Barcelona, Spain) and analyzed immediately by HPLC giving the AA content. For determination of the total vitamin C, 0.5 mL aliquot of the filtrate was mixed with 0.3 mL of 2 g L⁻¹ dithiothreitol solution in 0.2 M sodium phosphate buffer pH 7 and 0.15 mL 45% (w/v) dipotassium hydrogen phosphate. Samples were incubated 10 min at room temperature in the dark. Reduction was stopped with 0.3 mL 2 M o-phosphoric acid and the samples were filtered as described above. Ten microliters of the samples was injected on an HP 1100 system (Agilent Technologies, Waldbronn, Germany) equipped with a quaternary pump, a DAD and a Zorbax SB-Aq column (150 mm×3.0 mm, 3.5 μm) (Agilent Technologies, Waldbronn, Germany). The mobile phase consisted of ultrapure water adjusted to pH 2.3 with o-phosphoric acid. The flow rate was 0.45 mL/min. The detection was done at 244 nm and AA was quantified according to a standard curve obtained by injecting known amounts of ascorbic acid solutions (0–300 ng) (Sigma, Madrid, Spain). Results were expressed as mg AA (total Vit C) kg⁻¹ fresh weight sample (mg AA kg⁻¹ f.w.). The DHAA content was calculated by subtracting the initial AA content from the total Vit C content.

#### 2.5.2. Total polyphenols (TPP)

Two grams of stored (−80 °C) purée samples was weighed, and 20 mL of solvent (100% methanol) was added and mixed thoroughly. Samples were centrifuged at 12,100×g for 10 min at 4 °C (Beckman J2–M2 Centrifuge, Beckman Coulter, Inc. Fullerton, CA, USA) and aliquots of clear supernatant were collected and stored at −20 °C until analysis. The amount of TPP in extracts was determined according to the Folin–Ciocalteu procedure (Singleton & Rossi, 1965). A total of 0.125 μL of sample extract or gallic acid (GA) standard solutions was mixed with 625 μL Folin–Ciocalteu reagent (diluted 1:10 with H₂O). Samples were incubated 3 min and then 500 μL of 0.4 M sodium carbonate (7.5% w/v) was added. The mixture was incubated 15 min at 45 °C and absorbance was measured at 750 nm using a UV–Vis spectrophotometer (UV-1603, Shimadzu, Kyoto, Japan). Results were expressed as mg gallic acid equivalents (GAE) kg⁻¹ fresh weight sample (mg GAE kg⁻¹ f.w.).
2.5.3. Titratable acidity (TA)

Titratable acidity was determined by titrating 5 g homogenized purée sample diluted in 50 mL water with 0.1 N NaOH to an end point of pH 8.1 using a potentiometric titrator system (785 DMP Titrino, Mettbrohm AG, Herisau, Switzerland) (AOAC, 1990, Part 942.15). Results were expressed as g malic acid equivalents (MAE) kg\(^{-1}\) purée fresh weight (g MAE kg\(^{-1}\) f.w.).

2.6. Microbial determination of indigenous microflora

Total aerobic mesophilic and psychrotrophic count and moulds and yeasts were analyzed before (raw control purée) and after treatments and at regular intervals on samples stored at 5±1 °C throughout the storage period. A sample of purée (10 g) was diluted with 90 mL of saline peptone (SP. 1 g L\(^{-1}\) peptone, 8.5 g L\(^{-1}\) NaCl) and homogenized in a Stomacher (Model 400, Seward, London, UK) at regular speed for 2 min. To determine total aerobic mesophilic (TAM) and psychrotrophic (TCP) counts of purée samples, homogenates were serially diluted and plated on Plate Count Agar (PCA, Biokar Diagnostics, Beauvais, France) followed by incubation at 30±1 °C for 3 days and at 6.5±1 °C for 10 days, respectively. Moulds and yeasts (M&Y) were determined by plating the homogenates in Glucose Chloramphenicol Agar (GCA, Biokar Diagnostics) followed by incubation at 25±1 °C for 5 days. After incubation, plates were counted and the results expressed as logarithm of colony forming units per gram (log cfu g\(^{-1}\)). Determinations were carried out in triplicate (3 packaged samples). Detection limit was 50 cfu g\(^{-1}\) (1.70 log cfu g\(^{-1}\)).

2.7. Statistical analysis

To evaluate the effect of the individual treatments during processing on quality parameters compared to the raw purée, one-way analysis of variance (ANOVA) was performed. General linear model (GLM) with interaction storage time x treatment was applied to assess the effect of treatment and storage time during shelf life. Both analyses were carried out using the SAS Enterprise Guide 3.0.2.414 and means were compared for both analyses according to Tukey (HSD) with a level of significance at P<0.05.

3. Results and discussion

3.1. Physical and general physicochemical analysis

The effects of HPP and conventional pasteurization on physical and physicochemical properties of GS purée product were compared with unprocessed raw control purée (Table 1). The evolution of the parameters was also monitored during the shelf life period of 21 days. The raw purée had a soluble solid content of 14.2±0.1°Brix, pH of 3.20±0.01 and the titratable acidity was 7.8±0.3 g kg\(^{-1}\). Compared with fresh apples (11.2°Brix and pH of 3.41) SSC increased in the purée due to the addition of glucose-fructose syrup and was more acid because of the addition of citric acid. Means of SSC, pH and TA did not significantly change by the different treatments and during refrigerated storage. The consistency of the purée product expressed as viscosity (mPa s) was altered by processing (Table 1). HP processing raised viscosity, but only the 400 MPa treatment was found significantly different compared with control purée. During storage no clear tendency could be found concerning the viscosity stability of the purée product also due to the high variations found within replicate samples. Viscosity was found positively correlated with HPP for many of fruit and vegetable products. Ahmed et al. (2005) reported that the viscosity of mango pulp increased after moderate HP treatments at 100 or 200 MPa (15 or 30 min at 20 °C) while a decreasing trend was found at higher pressure levels (300–400 MPa). HP treated tomato purée (400 MPa/15 min/25 °C) showed the highest consistency compared with conventionally thermal treated purées (Sánchez-Moreno, Plaza, De Ancos, & Cano, 2006).

Navel orange juice treated at 600 MPa for 4 min at 40 °C resulted in a higher viscosity than thermal treated one (Polydera et al., 2005). The viscosity of mango pulp increased after moderate HP treatments at 100 or 200 MPa (15 or 30 min at 20 °C) while a decreasing trend was found at higher pressure levels (300–400 MPa). HP treated tomato purée (400 MPa/15 min/25 °C) showed the highest consistency compared with conventionally thermal treated purées (Sánchez-Moreno, Plaza, De Ancos, & Cano, 2006).

Initial CIE \(L^*a^*b^*\) parameters of the raw purée were \(L^* = 58.3\), \(a^* = -8.0\) and \(b^* = 24.2\) (Table 1). The mild thermal treatment had a significant effect (P<0.05) on the chromaticity coordinates \(a^*\) and \(b^*\), indicating non-enzymatic browning. Parameter \(a^*\) was shifted to more positive (reddish) values and yellowness (value \(b^*\)) decreased. Discoloration and non-enzymatic browning due to thermal treatments can result from several reactions, including Maillard condensation, caramelisation and destruction of pigments (Ibarz, Pagán, & Garza, 2000). Due to the maximum temperatures achieved in our work (≤75 °C), pigment destruction is most likely the responsible factor for discoloration of the purée. HPP increased insignificantly the \(L^*\) values right after processing indicating a lightening of the purée surface. This phenomenon was also observed in tomato based products (Sánchez-Moreno et al., 2006) and might be caused by cell disruption during HP treatment. Chromaticity coordinates \(a^*\) and \(b^*\) remained constant during HP treatment. Ahmed et al. (2005) observed that color quality parameters remained almost constant after HP treatment of mango pulp indicating pigment stability. These findings were also supported by Guerrero-Beltrán and Barbosa-Cánovas (2004) and Guerrero-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th>SSC (°Brix)</th>
<th>pH</th>
<th>Viscosity (mPa s)</th>
<th>(L^*)</th>
<th>(a^*)</th>
<th>(b^*)</th>
<th>TA (g kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>0</td>
<td>14.2 ± 0.1</td>
<td>3.20 ± 0.01</td>
<td>82.922 ± 3940</td>
<td>58.3 ± 0.8</td>
<td>-8.0 ± 0.3</td>
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<td>7.8 ± 0.3</td>
</tr>
<tr>
<td>400 MPa</td>
<td>7</td>
<td>14.4 ± 0.1</td>
<td>3.22 ± 0.01</td>
<td>10.7341 ± 2365</td>
<td>59.5 ± 0.5</td>
<td>-7.9 ± 0.2</td>
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<td>14.1 ± 0.1</td>
<td>3.12 ± 0.01</td>
<td>81.106 ± 2645</td>
<td>54.9 ± 1.2</td>
<td>-2.6 ± 1.0</td>
<td>22.4 ± 0.0</td>
<td>7.6 ± 0.3</td>
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<td>14.3 ± 0.1</td>
<td>2.97 ± 0.00</td>
<td>139.068 ± 8978</td>
<td>47.5 ± 0.8</td>
<td>-4.0 ± 0.6</td>
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<td>600 MPa</td>
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<td>3.15 ± 0.01</td>
<td>93.678 ± 20.21</td>
<td>48.8 ± 0.8</td>
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<td>20.6 ± 0.5</td>
<td>7.6 ± 0.4</td>
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</table>

*Values are means ± standard deviation, n = 3.

Values with abc superscripts within a column and treatment indicate significant differences (P<0.05) as a function of the storage period. xyz superscripts within a column indicate significant differences (P<0.05) between treatments for the same storage day. Values without superscripts are not significantly different.
Beltrán et al. (2005), who demonstrated that peach purée with and without antibrowning agents treated between 103 and 517 MPa (5–25 min) showed a bright yellow color equivalent to the freshly prepared purée. Color compounds of processed fruits can however change during storage due to incomplete inactivation of enzymes and microorganisms, which can result in undesired chemical reactions in the food matrix (Oey et al., 2008).

Table 2 shows the color data during storage represented as color differences (ΔL*, Δa* and Δb*). During storage, discoloration of pressurized samples occurred, which was indicated by the reduction of the lightness variable L* and the increase of the chromaticity variable a*. Color deterioration was of the same magnitude and no statistically significant differences could be observed between the 600 and the 400 MPa treatment. The initial green-yellow color of the GS purée deteriorated between day 7 and 14 of cold storage. From day 14 to day 21, color deterioration slowed down and no significant difference was detected as a function of time concerning the lightness values for any of the treatments. Color coordinate b* (yellowness) remained constant after treatment and during the shelf life period. In contrary, pasteurized samples maintained initial L*a*b* values over time and no significant differences could be detected during refrigerated storage. ΔE* values of the HP processes were 16.4±0.8 and 17.7±0.8 for the 400 and 600 MPa respectively versus 5.3±0.4 as the ΔE* for the mild pasteurization. Similar results were obtained by Guerrero-Beltrán, Barbosa-Cánovas, Moraga-Ballesteros, Moraga-Ballesteros and Swanson (2006) who treated standardized mango purée (pH 3.5 and 500 ppm AA) at 552 MPa during 5 min. The total reduction of PPO (Chisari, Barbagallo and Spagna, 2007; Chutintrasri & Picouet, Landl, Abadias, Castellari, and Viñas (2009) found recently, that for the same purée formulation heated by using microwaves, 100% total Vit C and 71.3% of residual AA content could be retained subsequent treatment. It was generally reported that oxygen and the food matrix influence the AA stability during HPP and subsequent storage. For example, higher Vit C loss was found in fruit juice compared to buffer solutions because of the existence of endogenous pro-oxidants such as metal ions and enzymes (Oey et al., 2008). For HPP (400 MPa/25 °C/15 min) tomato purée it was found 39% and 30% decreases in the initial content of AA and total Vit C respectively (Sánchez-Moreno et al., 2006). In a former study, Sánchez-Moreno et al. (2003) observed that between several combination treatments of HP and heat, HP (100 MPa–400 MPa) with higher temperatures (60 °C) showed a higher decrease in the content of Vit C. During storage the analysis of the Vit C content revealed strong deterioration for all treatments. This deterioration can be explained in a first step by the oxidation of AA to DHAA, which is then further irreversibly converted into 2,3-diketogulonic acid (Davey et al., 2000; Perera, 2007). During shelf life a strong decline of the AA contents concerning all treatments could also be revealed. At day 21, decrease in AA content was in a range of 79% for the pasteurization process compared to 97% and 94.5% in case of the 400 and 600 MPa cycle.

Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ΔL*</th>
<th>Δa*</th>
<th>Δb*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 0</td>
<td>day 7</td>
<td>day 14</td>
</tr>
<tr>
<td>400 MPa</td>
<td>1.2*</td>
<td>−3.4b,y</td>
<td>−7.5y</td>
</tr>
<tr>
<td>600 MPa</td>
<td>1.6*</td>
<td>−2.6b,y</td>
<td>−9.4y</td>
</tr>
<tr>
<td>Past.</td>
<td>−0.3</td>
<td>1.3*</td>
<td>0.1*</td>
</tr>
</tbody>
</table>

Means with abc superscripts within a row are significantly different (P<0.05).
Means with xyz superscripts within column are significantly different (P<0.05).
Means without superscripts are not significantly different.

3.2. Changes in ascorbic acid and total polyphenolic content

Vit C is very susceptible to oxidation under certain environmental conditions like heat, aw, presence of oxygen, heavy metal ions and alkaline pH (Perera, 2007) degrading their biological activity. Hence, Vit C loss provides a useful index of oxidative deterioration and is therefore used to define the shelf life, e.g. of minimally processed fruits and vegetables as well as fruit juices (Perera, 2007; Polydéra et al., 2003, 2005). Initial total Vit C content was 354±34 mg kg−1 whereas 138±100 mg kg−1 was present as AA, resulting in a percentage of 61% DHAA of the raw control purée (Table 3). This initial concentration was mainly attributed to the addition of the acerola juice powder to the purée formulation and to some lesser extent by the fresh lemon juice. After pressurization treatments total Vit C concentration was 331±31 mg kg−1 at 400 MPa and fell significantly (P<0.05) to 278±17 mg kg−1 at 600 MPa. Expressed as the percentage of Vit C retention it was found that 93.5% and 78.5% of the total Vit C retained immediately after treatment. Mild pasteurization had no influence on the total Vit C content, as 100% of the total Vit C was retained after the treatment. AA contents however decreased more substantially during the treatments. The most noticeable decrease of initial AA content was found for the 600 MPa cycle, where only 14.5% of residual content was detected in comparison to 57% of residual AA content found for the 400 MPa process. Retention of initial AA for the pasteurization was 61.5%. Picouet, Landl, Abadias, Castellari, and Viñas (2009) found recently, that for the same purée formulation heated by using microwaves, 100% total Vit C and 71.3% of residual AA content could be retained subsequent treatment. It was generally reported that oxygen and the food matrix influence the AA stability during HPP and subsequent storage. For example, higher Vit C loss was found in fruit juice compared to buffer solutions because of the existence of endogenous pro-oxidants such as metal ions and enzymes (Oey et al., 2008). For HPP (400 MPa/25 °C/15 min) tomato purée it was found 39% and 30% decreases in the initial content of AA and total Vit C respectively (Sánchez-Moreno et al., 2006). In a former study, Sánchez-Moreno et al. (2003) observed that between several combination treatments of HP and heat, HP (100 MPa–400 MPa) with higher temperatures (60 °C) showed a higher decrease in the content of Vit C. During storage the analysis of the Vit C content revealed strong deterioration for all treatments. This deterioration can be explained in a first step by the oxidation of AA to DHAA, which is then further irreversibly converted into 2,3-diketogulonic acid (Davey et al., 2000; Perera, 2007). During shelf life a strong decline of the AA contents concerning all treatments could also be revealed. At day 21, decrease in AA content was in a range of 79% for the pasteurization process compared to 97% and 94.5% in case of the 400 and 600 MPa cycle.
The experimental data on the loss of total Vit C could also be described by using the standard equation of a first-order reaction kinetic given below:

\[
\frac{\Delta C}{\Delta t} = kC
\]

\[
\ln C = \ln C_0 (-kt)
\]

Where \( C \) is the concentration at time \( t \) (days), \( C_0 \) the concentration of the treated purée at time zero, \( k \) the first-order rate constant (day\(^{-1}\)) and \( t \) the storage time (day). Experimental data of the remaining Vit C concentration defined as \( \ln(C/C_0) \) were plotted against storage time (Fig. 1) and the rate constant \( k \) was calculated as the slope of the curves. The calculated \( k \) values together with the correlation coefficient \( R^2 \) are given in Table 4. Lower Vit C loss rates were found for the conventionally pasteurized apple product compared to the respective values for high pressure treated product. Vit C degradation rate \( k \) for the 600 MPa treatment was found slightly lower than for the 400 MPa process despite the high initial loss determined during the 600 MPa pressurization (Table 3). Some authors described that the AA degradation fitted better to a zero order model \( (C = C_0 - kt) \) than to a first-order reaction kinetic. Zulueta, Esteve, and Frigola (2010) reported recently that the AA degradation rate during storage of orange juice-milk beverage was adjusted to a zero order model. Polydera et al. (2003) found two stages for ascorbic acid degradation during storage of HPP reconstituted orange juice with the latter stage fitting more appropriate to a zero order kinetic. However, our experimental data showed good fit to the model given above (Eq. (1)) with correlation coefficients of 0.98–1.00. Shelf life of the acidified apple purée product could be estimated as the time period in which 50% of the Vit C content was lost \( (C = 0.5 \ C_0) \). Shelf life was therefore calculated through Eq. (2) and depicted in Table 4 as times of half loss \( (t_{1/2}) \). According the Vit C loss rates shelf life in a range of 9.3, 10.0 and 10.3 days were estimated for the 400 and 600 MPa process and for the mild pasteurization process respectively. However, even if vitamin C was lost during storage, its content in purée samples was higher than that of fresh apple flesh, which is reported to be different between cultivars, seasons, harvest date and some other factors. Values between 29 and 120 mg/kg have been reported in different cultivars (Molina-Delgado, Larragaudiere, & Recasens, 2006; Planchon, Lateur, Dupont, & Lognay, 2004; Vilaplana, Valentines, Toivonen, & Larragaudiere, 2006).

Total polyphenolic compounds were affected significantly \( (P<0.05) \) by pasteurization and pressurization at 600 MPa and remained unchanged during processing at 400 MPa in comparison with the raw apple product (Table 3). The retention of TPP content after 600 MPa process was 75%, whereas in case of the pasteurization 87% of the phenolics were retained in the purée product. The data show that there was no loss of polyphenolic compounds found for the 400 MPa. Storage revealed that mild pasteurization preserved higher levels of phenolics than pressure-treated samples. There was no major loss of TPP content concerning the pasteurized purée samples during storage. Comparing the 400 MPa and the 600 MPa treatments, it was found that despite the adverse affect on the initial TPP content of the product, towards the end of the storage period \( (\text{days 14 and 21}) \) the 600 MPa cycle retained significantly higher contents of TPP \( (P<0.05) \) than the 400 MPa. Regarding the 400 MPa treatment, levels of TPP content decreased sharply between day 7 and day 14. Data suggest that levels of phenolic compounds are related to residual PPO activity after treatment, reflecting the efficacy of the treatments in preserving the nutritional quality of the purée.

### Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th>Total Vit C (mg kg(^{-1}))</th>
<th>Ascorbic acid (mg kg(^{-1}))</th>
<th>DHAA (mg kg(^{-1}))</th>
<th>TPP (mg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>0</td>
<td>354 ± 34(^a)</td>
<td>138 ± 100(^k)</td>
<td>216 ± 116</td>
<td>1180 ± 23(^m)</td>
</tr>
<tr>
<td>400 MPa</td>
<td>0</td>
<td>331 ± 31(^k)</td>
<td>79 ± 19(^y)</td>
<td>265 ± 47(^m)</td>
<td>1179 ± 46(^k)</td>
</tr>
<tr>
<td>400 MPa</td>
<td>7</td>
<td>201 ± 2(^x)</td>
<td>17 ± 6</td>
<td>184 ± 7(^h)</td>
<td>966 ± 48(^h)</td>
</tr>
<tr>
<td>400 MPa</td>
<td>21</td>
<td>69 ± 1(^l)</td>
<td>4 ± 1</td>
<td>66 ± 2(^b)</td>
<td>462 ± 11(^c)</td>
</tr>
<tr>
<td>600 MPa</td>
<td>0</td>
<td>278 ± 17(^y)</td>
<td>20 ± 13(^y)</td>
<td>265 ± 19(^c)</td>
<td>888 ± 143(^x)</td>
</tr>
<tr>
<td>600 MPa</td>
<td>7</td>
<td>136 ± 17(^y)</td>
<td>15 ± 2</td>
<td>111 ± 20(^b)</td>
<td>816 ± 100(^y)</td>
</tr>
<tr>
<td>600 MPa</td>
<td>21</td>
<td>97 ± 9(^e)</td>
<td>8 ± 0</td>
<td>93 ± 3(^d)</td>
<td>685 ± 89(^e)</td>
</tr>
<tr>
<td>Past.</td>
<td>0</td>
<td>335 ± 44(^x)</td>
<td>85 ± 17(^y)</td>
<td>289 ± 91(^a)</td>
<td>1028 ± 49(^y)</td>
</tr>
<tr>
<td>Past.</td>
<td>7</td>
<td>188 ± 20(^x)</td>
<td>61 ± 26</td>
<td>127 ± 9(^b)</td>
<td>1063 ± 28(^ab)</td>
</tr>
<tr>
<td>Past.</td>
<td>21</td>
<td>128 ± 7(^x)</td>
<td>40 ± 9</td>
<td>88 ± 7(^b)</td>
<td>1029 ± 20(^a)</td>
</tr>
</tbody>
</table>

\(^a\) Values are means ± standard deviation, \( n = 3 \).

### Table 4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vit C loss rate ( k ) (days(^{-1}))</th>
<th>Time of half loss ( t_{1/2} ) (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 MPa</td>
<td>−0.075 ± 0.002(^a)</td>
<td>1.00</td>
</tr>
<tr>
<td>600 MPa</td>
<td>−0.070 ± 0.004(^b)</td>
<td>0.98</td>
</tr>
<tr>
<td>Past.</td>
<td>−0.068 ± 0.003(^c)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

\(^a\) Standard error.
517 MPa for 5 min, no microbial growth of total count or yeasts and molds (<10 cfu g−1) was observed after storage at 3 °C (Guerrero-Beltrán et al., 2005). Lavinas et al. (2008) investigated the effect of HPP on the survival of natural microbiota of cashew apple juice treated by HPP and they found that after treatment at 350 MPa for 7 min or 400 MPa for 3 min, aerobic mesopholic bacteria decreased from a range of 4.6 to 5.9 cfu/ml to undetectable levels (10 cfu/ml). They also did not observe microbial growth after 8 weeks of storage at 4 °C. Krebbers et al. (2003) showed that HPP treatment of tomato puree at 700 MPa for 2 min reduced the natural microbiota (3.6 log cfu total aerobic plate count at 30 °C/Ml) to a level below the detection limit (<1.5 log cfu/ml) and no outgrowth of the microorganisms occurred during storage of 8 weeks. In a recent study, Picouet et al. (2009), observed a slight increase of TAM count during storage at 5 °C for the same puree formulation treated by microwaves, but under different packaging conditions.

4. Conclusions

This is one of the first studies dealing with the evident potential of non-thermal high pressure pasteurization of acidified GS apple purée with industrial-scale equipment. It was evidenced that unlike the 600 MPa treatment, the 400 MPa pressurization and the mild conventional pasteurization did not affect adversely the initial instrumental and nutritional quality of the acidified Granny Smith purée. During storage however none of the treatments prevented fully undesirable changes during a shelf life of 3 weeks. The time of half loss of total Vit C at 5 °C was in a range of 9.3 to 10.3 days. Color deterioration occurred especially for the pressurized purées between day 7 and 14 of cold storage. Quality constraints could be overcome by using another apple cultivar with lower browning tendency or by combining other more powerful antibrowning agents. In this sense, ‘Shampion’ apple cultivar was suggested for the production of light-colored purées, due to its lower chlorogenic acid content and PPO characteristics. In conclusion, a commercial HP-processed peach purée with antibrowning agents was stable when stored at 5 °C and comparable with the mild conventionally pasteurized purée. In a recent study, Picouet et al. (2009), observed a slight increase of TAM count during storage at 5 °C for the same purée formulation treated by microwaves, but under different packaging conditions.

Acknowledgements

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References


Norton, T., & Sun, D. W. (2008). Recent advances in the use of high pressure as an effective processing technique in the food industry. Food and Bioprocess Technology, 1(1), 2–34.


