Effect of thermal treatment and storage on the stability of organic acids and the functional value of grapefruit juice

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A B S T R A C T
The effect of conventional and microwave pasteurisation on the main bioactive compounds of grapefruit juice and their stability during 2 months’ refrigerated and frozen storage was evaluated. Ascorbic acid (AA), vitamin C and organic acids were analysed by HPLC, whereas total phenols and antioxidant capacity (%DPPH) were measured by spectrophotometry. The results showed that conventional treatment led to a significant decrease in citric acid (from 1538 to 1478 mg/100 g) and AA (from 36 to 34.3 mg/100 g), whilst microwave pasteurisation preserved these compounds. Frozen storage maintained AA and vitamin C, especially in treated samples. Frozen non-treated samples and conventional pasteurised ones preserved about a 75% and 20% of the total phenols and antioxidant capacity, respectively, whilst in frozen microwave pasteurised juices this preservation was of 82% and 33%. From these results, the use of microwave energy may be proposed as an alternative to traditional heat pasteurisation in order to preserve the natural organoleptic characteristics and essential thermolabile nutrients of grapefruit juice.

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1. Introduction

Evidence from a large number of epidemiological, in vitro and in vivo studies has shown that the consumption of citrus fruit is generally good for the health and contributes to the prevention of degenerative processes, particularly lowering the incidence and mortality rate of cancer and cardio and cerebro-vascular diseases (Poulose, Harris, & Patil, 2005). Citrus juice is an important dietary source of bioactive compounds, whose beneficial health effects are ascribed, in part, to its high content of ascorbic acid. Vitamin C is a natural antioxidant that may inhibit the development of major oxidative human reactions. In addition to the well-known vitamin C, citrus juice also contains phenolic compounds which contribute to their antioxidant capacity and that may produce beneficial effects by scavenging free radicals (Xu et al., 2008). Vinson and Bose (1988) emphasised the importance of ascorbic acid as a natural component in citrus juice where other natural compounds present in the juice, such as flavonoids, increase the bioavailability of this acid. On the other hand, organic acids, including citric, tannic and malic acids in citrus juice are important components which contribute to flavour attributes and are usually used as “fingerprints” to detect the quality of the juice and accomplish its authentication (Cen, Bao, He, & Sun, 2007). High concentrations of organic acids and low pH in most fruits are critical for the preservation of derivative products. They also help to stabilise ascorbic acid and anthocyanins (Wang, Chuang, & Ku, 2007).

Nowadays, consumers demand the maximum preservation of the endogenous sensory, nutritional and health related qualities of fruit products. Traditional heat pasteurisation of citrus juices is necessary in order not only to destroy microorganisms and reduce pectin methylesterase (PME) enzymatic activity, but it also leads to detrimental changes in the quality (Elez-Martínez, Aguiló-Aguayo, & Martín-Belloso, 2006). The colour and flavour are different from those of freshly squeezed juice and there is also a decrease in the number of biochemical compounds. PME inactivation is important because this enzyme catalyses pectin degradation and alters the colloidal stabilising power of the pectin, which imparts the favourable appearance and mouth feel to orange juice. As PME is more resistant to heat than microorganisms, thermal treatments are focused on the inactivation of this enzyme. The search for new technologies that cause minimum damage to the organoleptic and nutritional characteristics may be considered as an alternative to conventional thermal juice pasteurisation. In this sense, the use of microwave energy seems to cause smaller changes in the fruit quality attributes (Nikdel, Chen, Parish, MacKellar, & Friedrich, 1993). Several studies have successfully been carried out into the microwave pasteurisation of fruit juices, as it preserves the natural organoleptic characteristics of the juice and reduces the time of exposure to energy, with the subsequently lower risk of losing
essential thermolabile nutrients (Cañumir, Celis, Brujin, & Vidal, 2002).

The aim of this work was to characterise the main bioactive compounds (vitamin C, total phenol, organic acids) and their relative contribution to the antioxidant capacity of freshly squeezed grapefruit juice and assess the effect of conventional and microwave pasteurisation on these compounds and their antioxidant capacity. Their stability during 2 months’ refrigeration and frozen storage was also evaluated.

2. Materials and methods

2.1. Raw material

For this work, grapefruits (Citrus paradise var. Star Ruby) from the city of Murcia were purchased from a local supermarket. Grapefruits were selected on the basis of a similar degree of ripeness (ratio ‘Brix/acidity ≈ 4) and apparent fruit quality (firmness, size, colour and absence of physical damages). Fruit was processed in the laboratory immediately after being purchased.

2.2. Treatments

Freshly squeezed (FS) grapefruit juice was extracted through a domestic squeezer (Braun Citromatic Pulp Control MP26), filtered using a sieve (light of mesh diameter 1 mm, Cisa 029077, 1 series) and immediately processed. To obtain conventional pasteurised juice (CP) samples of 40 ml were heated in glass tubes at 900 W for 30 s using a microwave (Moulinex 5141 AFW2, Spain). At this temperature, the juice took 80 s to reach 80 ± 2.5°C and it remained at this temperature for 11 s. In the case of microwave pasteurised juice (MP), samples of 20 ml were heated in 25 ml glass tubes at 900 W for 30 s using a microwave (Moulineix 5141 AFW2, Spain). Treated samples were immediately cooled in ice-water till juice reached 30°C. Both processes were previously optimised to reach ≈10% of fresh juice pectinmethylesterase (PME) residual activity.

2.3. Enzymatic determinations

2.3.1. Pectin methylesterase (PME) activity measurement

PME activity in grapefruit juice was measured using the Kimball (1995) method. Briefly 10 ml of grapefruit juice and 40 ml of 1% peel citrus pectin dissolution (60% degree of esterification, Fluka Biochemika, Switzerland) containing 0.02 M NaCl, previously tempered to 30°C in a thermostat bath, were mixed and kept in continuous agitation. NaOH was used to adjust the resulting solution to pH 7.7 (Consort C830 pH meter, Belgium) and then 100 μl of 0.05 N NaOH were immediately added. The exact time needed to lower the pH back to 7.7 by enzyme’s action was then measured. As it is a first order reaction, the enzyme activity (A) can be calculated according to the concentration of acid produced using Eq. (1).

\[
A = \frac{(V_{\text{NaOH}}) \times (N_{\text{NaOH}})}{(t_f) \times (W_{\text{sample}})}
\]

(1)

where \(V_{\text{NaOH}}\) is the NaOH volume used in the titration (ml), \(N_{\text{NaOH}}\) is the normality of the NaOH solution used (mEq ml\(^{-1}\)), \(t_f\) is the reaction time (min) and \(W_{\text{sample}}\) is the weight of the sample (g).

The percentage of residual enzyme activity (RA) was defined as indicated by Eq. (2):

\[
RA = 100 \times \frac{A_t}{A_0}
\]

(2)

where \(A_t\) and \(A_0\) were the enzyme activities of treated and untreated samples, respectively. \(A_t\) and \(A_0\) were determined immediately after processing to avoid the effects of storage time.

2.3.2. Polyphenoloxidase (PPO) activity measurement

PPO activity was measured by spectrophotometry. The enzyme was extracted from grapefruit juice using the method of Valero, Varón, and García-Carmona (1988) modified by Rapeanu, Van Loey, Smout, and Hendrickx (2006). Briefly 100 μl of clarified juice were added to 1 ml substrate (0.1 M cathecol in McIlvaine buffer, pH 5) and the increase in absorbance at 400 nm at 25°C was recorded automatically for 30 min (Thermo Electron Corporation, USA). One unit of PPO activity was defined as a change in absorbance at 400 nm min\(^{-1}\) ml\(^{-1}\) of enzymatic extract. Enzyme activity was calculated from the linear part of the curve. The percentage of residual enzyme activity was calculated using Eq. (2).

2.3.3. Peroxidase (POD) activity measurement

POD activity in grapefruit juice was measured using the method described by Cano, Hernández, and De Ancos (1997) with some modifications made by Elez-Martínez et al. (2006). Briefly 10 ml of sample were homogenised with 20 ml 0.2 M sodium phosphate buffer (pH 6.5) and centrifuged (15,000 rpm, 20 min) at 4°C (P-Selecta Medifrigar BL-S, Spain) to obtain the enzymatic extract. POD activity was assayed spectrophotometrically by placing 2.7 ml 0.2 M sodium phosphate buffer (pH 6.5) and 0.2 ml p-phenylenediamine (10 g kg\(^{-1}\), 0.1 ml hydrogen peroxide (15 g kg\(^{-1}\)) and 0.1 ml of enzymatic extract in a 1 cm path cuvette. The oxidation of p-phenylenediamine was measured at 485 nm and 25°C using a Thermo Electron Corporation spectrophotometer (USA). POD activity was determined by measuring the initial rate of the reaction, which was computed from the linear portion of the plotted curve. One unit of POD activity was defined as a change in absorbance at 485 nm min\(^{-1}\) ml\(^{-1}\) of enzymatic extract. The percentage of residual enzyme activity was calculated using Eq. (2).

2.4. Analytical determinations

2.4.1. Soluble solids

Total soluble solids were estimated as ‘Brix with a refractometer (Abbe Atago 89553 by Zeiss, Japan) at 20°C.

2.4.2. pH

To determine the pH, a Consort C830 pH meter (Belgium) with a penetration electrode was used.

2.4.3. Organic acids

HPLC (Jasco, Italy) was applied to the quantitative determination of citric (CA), malic (MA) and tartaric acid (TA) according to Cen et al. (2007). Samples were centrifuged at 10,000 rpm for 15 min and filtered by 0.22 μm membrane. HPLC method and instrumentation was: Ultrabase-C18, 5 l/250 mm) column (Spain); mobile phase 0.01 mol/l potassium dihydrogen phosphate solution, volume injection 20 μl, flow rate 1 ml/min, detection at 215 nm at 25°C. Standard curves of each reference acid (Panreac, Spain) were used to quantify the acids.

2.4.4. Ascorbic acid and total vitamin C

Ascorbic acid (AA) and total vitamin C (ascorbic acid + dehydroascorbic acid) were determined by HPLC (Jasco, Italy). To determine the ascorbic acid (Xu et al., 2008), 1 ml sample was extracted with 9 ml 0.1% oxalic acid for 3 min and immediately filtered before injection. The procedure employed to determine total vitamin C was the reduction of dehydroascorbic acid to ascorbic acid, using DL-dithiothreitol as the reductant reagent (Sanchez-Mata, Cámara-Hurtado, Díez-Marques, & Torija-Isasa, 2000; Sanchez-Moreno, Plaza, De Ancos, & Cano, 2006). A 0.5 ml aliquot sample was taken to react with 2 ml of a 20 g/l dithiothreitol solution for 2 h at room temperature and in darkness. Afterwards, the same procedure as that used for the ascorbic acid method was performed. The HPLC
method and instrumentation was: Ultrabase-C18, 5 μm (4.6 × 250 mm) column (Spain); mobile phase 0.1% oxalic acid, volume injection 20 μl, flow rate 1 ml/min, detection at 243 nm and at 25 °C. AA standard solution (Panreac, Spain) was prepared.

2.4.5. Total phenols
The extraction of total phenols (Tomás-Barberán et al., 2001) consisted of homogenising 35 g of the sample (T25 Janke and Kunkel turrax) for 5 min with 40 ml of methanol, 10 ml of HCl and NaF to inactivate polyphenol oxidases and prevent phenolic degradation. The homogenate was centrifuged (10,000 rpm, 10 min, 4 °C) to obtain the supernatant. Total phenols (TF) were quantified by using the method reported by Selvendran and Ryden (1990) and Benzie and Strain (1999) based on the Folin–Ciocalteu method. Absorbance was measured at 765 nm in a UV–visible spectrophotometer (Thermo Electron Corporation, USA). The total phenolic content was expressed as mg of gallic acid equivalents (GAE) (Sigma–Aldrich, Germany) per gram of sample, using a standard curve range of 0–800 mg of gallic acid/ml.

2.4.6. Antioxidant capacity
Antioxidant Capacity was assessed using the free radical scavenging activity of the samples evaluated with the stable radical DPPH, as described by Sánchez-Moreno, Plaza, and De Ancos, Cano (2003). Briefly, 0.1 ml of grapefruit juice sample was added to 3.9 ml of DPPH (0.030 g/l, Sigma–Aldrich, Germany) in methanol. A Thermo Electron Corporation spectrophotometer (USA) was used to measure the absorbance at 515 nm at 0.25 min intervals until the reaction reached a plateau (at the time of steady state). The changes in absorbance were measured at 25 °C. Appropriately diluted juice samples were used on the day of preparation. The percentage of DPPH (%DPPH) was calculated as Eq. (3):

$$\%\text{DPPH} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}$$

where $A_{\text{control}}$ is the absorbance of the control and $A_{\text{sample}}$ the absorbance of the sample.

2.5. Storage conditions
Samples (FS, CP and MP) were stored immediately after treatment in sterile polypropylene packages and kept in darkness at 4 and −18 °C during a period of 60 days.

2.6. Statistical analysis
Significant differences between treatments and storage time were calculated by means of the analysis of variance (ANOVA). Differences of $p < 0.05$ were considered to be significant. Furthermore, a correlation analysis between antioxidant activity and all the studied components with a 95% significance level was carried out. All statistical analyses were performed using Statgraphics Plus 5.1.

3. Results and discussion
Pectinmethylesterase (PME) residual activity detected in samples after thermal treatments was 12.04% ± 3.86% and 10.07% ± 0.63% in CP and MP, respectively. These are intermediate values in the 0–18% range found by Snir, Koehler, Sims, and Wicker (1996), who carried out the heat treatment at 70 °C for 5 min. Nevertheless, they are high enough to obtain good quality products with a convenient cloud stabilisation, which will be kept under refrigeration conditions with low level bacteria growth. According to studies performed by Sentandreu, Carbonell, Rodrigo, and Carbonell (2006), PME had a greater heat resistance than microorganisms. As in some studies of fresh orange juice carried out by other authors (Cano et al., 1997), analyses of freshly squeezed and pasteurised grapefruit juice did not show PPO activity. According to Dziezak (1993), citric acid, which is an important component of grapefruit juice, provokes the copper quelation present in this enzyme, disabling the activity of the PPO. With regard to the POD activity of fresh grapefruit juice, the obtained result (5.2 ± 0.2) was similar to values found in the bibliography for citrus juices (Cano et al., 1997). In CP, an inactivation of 94.3% ± 0.7% was reached, which in the case of MP was 88.1% ± 0.3%, showing the significant differences that exist between them.

Table 1 shows the physicochemical and compositional parameters of freshly squeezed grapefruit juice, conventional pasteurised juice and that which has been microwave treated. In general, FS obtained for this work presented the characteristic physicochemical parameters shown in the bibliography for grapefruit juice (Moraga, Moraga, Fito, & Martínez-Navarrete, 2009). As was observed, neither pasteurisation process affected ‘Brix, at a range between 9.9–10.1, or pH (2.92–3). Similar results were found by Kim and Tadini (1999), who showed that temperature and holding time had no effect on pH and ‘Brix of conventional pasteurised juice. These quality parameters are important as they are closely related with the stability of the bioactive compounds in fruit products (Sánchez-Moreno et al., 2006). A significant ($p < 0.05$) decrease in CA and TA content was observed in the pasteurisation treatments of the juice; the citric acid content was less affected when microwaves were applied to pasteurised juice. In no case did the pasteurisation treatment influence the malic acid content. Cañamur et al. (2002) studied the effect of microwaves comparing them with conventional pasteurisation in apple juice and they observed that total acidity tended to increase when microwave pasteurisation was used, whereas the pH tended to be lower.

Vitamin C is used as reference in different industrial processes since its presence ensures a high nutritional quality of the final product due to its easy degradation (Klimczak, Malecka, Szlachta, & Gliszczynska, 2007). The initial values of ascorbic acid and vitamin C in the fresh juice were similar, 36.0 ± 0.1 mg/100 ml and 34.0 ± 1 mg/100 ml, respectively. This is the AA grapefruit value obtained by Leong and Shui (2002). No significant differences between AA and vitamin C content were observed, as reported by other authors (Plaza et al., 2006). Vitamin C shows great thermal stability at the low pH of citrus fruits (Sánchez-Moreno et al., 2003) and in fact it was not affected by the treatments applied in this case. The conventionally pasteurised juice presented the lowest, statistically significant ($p < 0.05$) ascorbic acid content. In this

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
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<tbody>
<tr>
<td><strong>Mean values (with standard deviation) of ‘Brix, pH, CA, MA, TA, AA, vitamin C, TP and %DPPH in freshly squeezed (FS), conventional pasteurised (CP) and microwave pasteurised (MP) juice.</strong></td>
</tr>
<tr>
<td>FS</td>
</tr>
<tr>
<td>‘Brix</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>CA</td>
</tr>
<tr>
<td>MA</td>
</tr>
<tr>
<td>TA</td>
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<tr>
<td>AA</td>
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<tr>
<td>Vitamin C</td>
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<tr>
<td>TP</td>
</tr>
<tr>
<td>%DPPH</td>
</tr>
</tbody>
</table>

The same letter in superscript indicates homogeneous groups established by the ANOVA ($p < 0.05$). In columns: FS, freshly squeezed juice; CP, conventional pasteurised juice; MP, microwave pasteurised juice. In rows: CA, citric acid; MA, malic acid; TA, tartaric acid; AA, ascorbic acid; TP, total phenols.
p The same letter in superscript indicates homogeneous groups in the same physico-chemical property and treatment during storage (temperature, T and time, t) established by the ANOVA (p < 0.05).

In columns: FS, freshly squeezed juice; CP, conventional pasteurised juice; MP, microwave pasteurised juice.

Table 2
Mean values (with standard deviation) of °Brix and pH evolution of grapefruit juices stored at 4 °C (A) and −18 °C (B) for 2 months.

<table>
<thead>
<tr>
<th>Storage</th>
<th>°Brix</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (°C)</td>
<td>FS</td>
<td>CP</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9.9 (0.1)ab</td>
<td>10.0 (0.1)a</td>
</tr>
<tr>
<td>4</td>
<td>9.9 (0.1)ab</td>
<td>10.1 (0.1)ab</td>
</tr>
<tr>
<td>12</td>
<td>9.7 (0.1)a</td>
<td>9.6 (0.1)b</td>
</tr>
<tr>
<td>25</td>
<td>10.0 (0.1)ab</td>
<td>10.1 (0.1)ab</td>
</tr>
<tr>
<td>35</td>
<td>10.0 (0.1)ab</td>
<td>10.1 (0.1)ab</td>
</tr>
<tr>
<td>45</td>
<td>10.1 (0.1)ab</td>
<td>10.1 (0.1)ab</td>
</tr>
<tr>
<td>60</td>
<td>10.0 (0.1)ab</td>
<td>10.2 (0.1)ab</td>
</tr>
<tr>
<td>−18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9.9 (0.1)ab</td>
<td>10.1 (0.1)ab</td>
</tr>
<tr>
<td>12</td>
<td>9.7 (0.1)a</td>
<td>9.5 (0.1)b</td>
</tr>
<tr>
<td>25</td>
<td>10.0 (0.1)ab</td>
<td>10.2 (0.1)ab</td>
</tr>
<tr>
<td>35</td>
<td>10.1 (0.1)ab</td>
<td>10.2 (0.1)ab</td>
</tr>
<tr>
<td>45</td>
<td>10.1 (0.1)ab</td>
<td>10.1 (0.1)ab</td>
</tr>
<tr>
<td>60</td>
<td>10.1 (0.1)ab</td>
<td>10.2 (0.1)ab</td>
</tr>
</tbody>
</table>

Fig. 1. Evolution of citric acid (CA) of FS (A), CP (B) and MP (C) grapefruit juices stored at 4 and −18 °C for 2 months. Letters indicate homogeneous groups established by the ANOVA (p < 0.05).

As Fig. 1 shows, the storage conditions (refrigeration and freezing) affected the malic acid content of all the samples in the same way: the MA content remained constant for the first 24 h, but sharply and significantly (p < 0.05) decreased during the next 3 days. After 12 days, the CA continued to decrease significantly in FS but remained constant in MP and CP. No significant decrease in CA was observed throughout storage at −18 °C. As Fig. 2 shows, the storage conditions (refrigeration and freezing) affected the malic acid content of all the samples in the same way: the MA content remained constant for the first 25 storage days. Then, there was a significant (p < 0.05) drop in the content activity seems to be more related to total phenols than to ascorbic acid.
which, once again, stabilised till the end of the storage. With regards to the TA content (Fig. 3), a significant ($p < 0.05$) decrease took place during the first 4 days, with a subsequent recovery. There were no clear differences observed between the TA stability of the different samples and under differing storage conditions.

The evolution of the ascorbic acid content of grapefruit juices stored at 4 and $-18$ °C for 2 months is presented in Fig. 4. In general, the AA content of all juice samples studied behaved in a similar way whether under refrigeration or freezing conditions and no significant ($p < 0.05$) changes were observed till 12 days of storage. From this moment on, the samples stored under frozen conditions seem to maintain the AA content till the end of storage, whilst in the refrigerated juice the proportion of this component decreased significantly ($p < 0.05$). In this respect, from an industrial point of view, it would be advisable to freeze the pasteurised juice, for example, in the case of overproduction (Gil-Izquierdo, Gil, & Ferreres, 2002). According to the published data, the content of AA in different juices decreases during storage, depending on temperature, oxygen and light access (Klimczak et al., 2007). The degradation of AA follows both aerobic and anaerobic pathways. The oxidation of ascorbic acid occurs mainly during the processing of citrus juices, whereas anaerobic degradation, which is particularly observed in thermally preserved citrus juices, mainly appears during storage (Burdulu, Koca, & Karadeniz, 2006). For instance, Polydera, Galanou, Stoforos, and Taoukis (2004) reported that thermally pasteurised juice (80 °C, 60 s) showed 72% AA retention after 1 month at 5 °C. With regards to our CP and MP samples, 75% and 78% AA retention was reached after 25 days refrigeration, respectively, whereas in freezing conditions the retention was 84% in CP and 85% in MP. The loss of ascorbic acid during storage might be a quality indicator and a critical factor for the shelf life of some products, such as citrus juices (Plaza et al., 2006).

Fig. 5 shows the vitamin C evolution of grapefruit juices stored for 2 months at 4 °C and $-18$ °C. In the CP and MP juice samples,
the vitamin C content behaved in a similar way whether under refrigeration or freezing conditions and no significant \((p < 0.05)\) changes occurred till 12 days in the case of CP samples and 25 days in the case of MP samples. From this moment on, under frozen conditions, the vitamin C content of CP samples suffered a significantly \((p < 0.05)\) smaller decrease than that of refrigerated juice. In the case of frozen MP samples, this component remained stable whereas it dropped under refrigerated conditions.

At the end of the refrigerated storage, there were some significant \((p < 0.05)\) differences observed between the AA and vitamin C content in pasteurised samples. As other authors suggest, the changes observed in the ascorbic acid concentration of the samples stored under refrigeration, suggest the continuation of the oxidative degradation reactions of ascorbic acid to other oxidised forms such as dehydroascorbic acid, which also presents biological activity as vitamin C (Russell, 2004). The mechanism for enzyme degradation could be direct, by ascorbic acid oxidase, or indirect through polyphenoloxidase, cytochrome oxidase or peroxidase (Belizet & Grosch, 1997). This could be the reason why the values of vitamin C were higher than those of AA at the end of storage of treated samples.

During the storage time studied (Fig. 6), storage temperature seems not to affect FS phenol content since it evolved in a similar way whether stored under refrigeration or frozen, whereas PT significantly \((p < 0.05)\) diminished till 25 days, after which it remained constant. In this way, Tavirini, D’Innocenti, Remorini, Massai, and Guidi (2008) reported that phenols did not change in kiwifruits stored for 2 months at 0 °C, but they observed a significant rise after a long storage (6 months at 0 °C) which further increased after a week at ambient temperature. In CP and MP refrigerated samples, the phenol content significantly \((p < 0.05)\) diminished after day four, whilst under freezing conditions, the evolution of TP was constant.
Fig. 6. Evolution of total phenols (mg GAE / 100 ml) of FS (A), CP (B) and MP (C) grapefruit juices stored at 4 and –18 °C for 2 months. Letters indicate homogeneous groups established by the ANOVA (p < 0.05).

Fig. 7. Evolution of antioxidant activity (%DPPH) of FS (A), CP (B) and MP (C) grapefruit juices stored at 4 and –18 °C for 2 months.

Table 3
Mean values (with standard deviation) of variation of components (%) due to treatment and after 60 days of storage.

<table>
<thead>
<tr>
<th></th>
<th>Refrigeration</th>
<th>Frozen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FS</td>
<td>CP</td>
</tr>
<tr>
<td>Brix</td>
<td>1.21 (0.01)a</td>
<td>2.21 (0.01)b</td>
</tr>
<tr>
<td>pH</td>
<td>0.33 (0.01)a</td>
<td>0.33 (0.01)b</td>
</tr>
<tr>
<td>CA</td>
<td>12.64 (2.87)a</td>
<td>5.59 (2.68)b</td>
</tr>
<tr>
<td>MA</td>
<td>–20.63 (8.17)a</td>
<td>–20.29 (2.81)a</td>
</tr>
<tr>
<td>TA</td>
<td>–5.96 (3.56)a</td>
<td>0.89 (5.25)b</td>
</tr>
<tr>
<td>AA</td>
<td>–31.80 (0.13)b</td>
<td>–93.85 (1.26)a</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>–28.41 (5.03)b</td>
<td>–63.75 (2.82)a</td>
</tr>
<tr>
<td>TP</td>
<td>–27.37 (1.03)a</td>
<td>–33.62 (2.44)a</td>
</tr>
<tr>
<td>TP</td>
<td>–82.67 (4.86)a</td>
<td>–79.24 (0.51)a</td>
</tr>
</tbody>
</table>

The same letter in superscript indicates homogeneous groups established by the ANOVA (p < 0.05).

In columns: FS, freshly squeezed juice; CP, conventional pasteurised juice and MP, microwave pasteurised juice.
In rows: CA, citric acid; MA, malic acid; TA, tartaric acid; AA, ascorbic acid; TP, total phenols.
As can be observed in Fig. 7, the antioxidant capacity of both thermally treated grapefruit juices was affected by the storage conditions in a similar way. On the other hand, the antioxidant capacity of both the chilled and frozen fresh juice decreased during the first 24 h of storage. From 24 h of storage on, the FS sample evolved in a similar way to pasteurised juices, regardless of storage conditions and till the end of the study. In general, the %DPPH of all the samples decreased throughout the storage. Frozen stored MP samples had a significantly (p < 0.05) greater antioxidant capacity at the end of the period.

In Table 3, the variation of components due to treatment and 60 days of storage can be observed. These values were calculated as the difference of each compound in fresh or treated juice at the end of storage related to fresh juice and referred to 100 g of fresh juice. In general, frozen juices showed the smallest losses. The greatest losses were produced in FS refrigerated samples, except in the cases of AA and vitamin C, which were in greater proportions in CP refrigerated samples. When frozen, the vitamin C and AA content of the pasteurised samples remained the highest. Nevertheless, the studied bioactive compounds in the frozen MP juices maintained a greater stability and the smaller observed losses in antioxidant capacity point to this fact.

As regards to organic acids, refrigerated FS samples showed the greatest significant (p < 0.05) loss in CA (12.64%). No significant (p < 0.05) differences were observed for the other acids in the rest of the samples and storage conditions (mean value of the loss is 3.58%, 18.18% and 3.06% for CA, MA, and TA, respectively). AA and vitamin C were more stable when the samples were frozen, especially in the case of microwave-treated samples. Nevertheless, in refrigerated samples, FS juice contained the greatest amount of these compounds. In both cases, the greatest loss was observed in CP samples. Neither treatment nor storage temperature affected total phenols and antioxidant activity significantly (p > 0.05), except in the case of frozen MP samples which showed the lowest significant (p < 0.05) loss of TP (18.35%) and %DPPH (67.1%)

In order to explain the influence of the different compounds quantified in this study on the antioxidant capacity of the samples, correlation statistical analyses were performed. Only TA showed a negative Pearson’s correlation coefficient with %DPPH (−0.5258, p < 0.05). Total phenols played a major role in the antioxidant capacity of grapefruit juices (0.8389, p < 0.05), followed by the vitamin C (0.7216, p < 0.05), ascorbic acid (0.5563, p < 0.05), malic acid (0.5458, p < 0.05), citric acid (0.4785, p < 0.05). Other studies (Bahorun, Luximon-Ramma, Crozier, & Aruoma, 2004) confirm the existence of a positive relationship between the phenolic content of a fruit and its antioxidant capacity. Fruits with high antioxidant activity generally contain a great quantity of antioxidant substances, especially phenolic compounds and specifically flavonoids (Tavirini et al., 2008).

4. Conclusion
Contrary to conventional treatment which leads to a significant decrease in CA and AA in grapefruit juice, microwave treatment preserved these compounds. Moreover, frozen microwave pasteurised juices better preserved total phenols and antioxidant capacity when compared with fresh or conventional pasteurised ones and maintained the amount of AA and vitamin C, especially in pasteurised samples. Therefore, the use of microwave energy offers a good alternative to conventional pasteurisation.

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References


