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

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25

26 **1. Introduction**

27 Evidence from a large number of epidemiological, in vitro and in vivo studies has shown
28 that the consumption of citrus fruit is generally good for the health and contributes to the
29 prevention of degenerative processes, particularly lowering the incidence and mortality
30 rate of cancer and cardio and cerebro-vascular diseases (Poulose, Harris & Patil, 2005).
31 Citrus juice is an important dietary source of bioactive compounds, whose beneficial health
32 effects are ascribed, in part, to its high content of ascorbic acid. Vitamin C is a natural
33 antioxidant that may inhibit the development of major oxidative human reactions. In
34 addition to the well-known vitamin C, citrus juice also contains phenolic compounds which
35 contribute to their antioxidant capacity and that may produce beneficial effects by
36 scavenging free radicals (Xu, Liu, Chen, Ye, Ma & Shi, 2008). Vinson and Bose (1988)
37 emphasized the importance of ascorbic acid as a natural component in citrus juice where
38 other natural compounds present in the juice, such as flavonoids, increase the
39 bioavailability of this acid. On the other hand, organic acids, including citric, tartaric and
40 malic acids in citrus juice are important components which contribute to flavour attributes
41 and are usually used as “fingerprints” to detect the quality of the juice and accomplish its
42 authentication (Cen, Bao, He & Sun, 2007). High concentrations of organic acids and low
43 pH in most fruits are critical for the preservation of derivative products. They also help to
44 stabilize ascorbic acid and anthocyanins (Wang, Chuang & Ku, 2007).
45 Nowadays, consumers demand the maximum preservation of the endogenous sensory,
46 nutritional and health related qualities of fruit products. Traditional heat pasteurization of
47 citrus juices is necessary in order not only to destroy microorganisms and reduce pectin
48 methylesterase (PME) enzymatic activity, but it also leads to detrimental changes in the
49 quality (Elez-Martínez, Aguiló-Aguayo & Martín-Belloso, 2006). The colour and flavour are
50 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 catalyzes pectin degradation and alters the colloid stabilizing 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 focussed 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 pasteurization. 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 pasteurization 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 characterize 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 pasteurization on these compounds and their antioxidant capacity. Their stability during 2 months' refrigerated 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 ºBrix/acidity ≈ 4) and apparent fruit quality (firmness, size, colour and absence of physical damages). Fruit was processed in the laboratory immediately after being purchased.

77

78 *2.2.Treatments*

79 Freshly squeezed (FS) grapefruit juice was extracted through a domestic squeezer (Braun
80 Citromatic Pulp Control MPZ6), filtered using a sieve (light of mesh diameter 1 mm, Cisa
81 029077,1 series) and immediately processed. To obtain conventional pasteurized juice
82 (CP) samples of 40 mL were heated in glass tubes in a thermostatic water bath
83 (Precisterm, Selecta, Spain) operating at 95 ºC. In this way, the juice took 80 s to reach 80
84 ºC $\pm$ 2.5 and it remained at this temperature for 11 s. In the case of microwave pasteurized
85 juice (MP), samples of 20 mL were heated in 25 mL glass tubes at 900 W for 30 s using a
86 microwave (Moulinex 5141 AFW2, Spain). Treated samples were immediately cooled in
87 ice-water till juice reached 30ºC. Both processes were previously optimized to reach $\approx$10
88 % of fresh juice pectimethylesterase (PME) residual activity.

89

90 *2.3. Enzymatic determinations*

91 2.3.1. Pectin Methylesterase (PME) activity measurement

92 PME activity in grapefruit juice was measured using the Kimball (1999) method. Briefly 10
93 mL of grapefruit juice and 40 mL of 1% peel citrus pectin dissolution (60% degree of
94 esterification, Fluka Biochemika, Switzerland) containing 0.02 M NaCl, previously
95 tempered to 30 ºC in a thermostat bath, were mixed and kept in continuous agitation.
96 NaOH was used to adjust the resulting solution to pH 7.7 (Consort C830 pH meter,
97 Belgium) and then 100 µL of NaOH 0.05N were immediately added. The exact time
98 needed to lower the pH back to 7.7 by enzyme's action was then measured. As it is a first
99 order reaction, the enzyme activity (A) can be calculated according to the concentration of
100 acid produced using equation (1).

101

$$A = \frac{(V_{NaOH})\,x\,(N_{NaOH})}{(t_R)\,x\,(W_{sample})} \quad (1)$$

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

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

$$RA = 100 \times \frac{A_t}{A_0} \quad (2)$$

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 ºC 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 equation 2.

2.3.3. Peroxidase (POD) activity measurement

127 POD activity in grapefruit juice was measured using the method described by Cano,
128 Hernández and De Ancos (1997) with some modifications made by Elez-Martínez et al.,
129 (2006). Briefly 10 mL of sample were homogenized with 20 mL 0.2M sodium phosphate
130 buffer (pH=6.5) and centrifuged (15.000 rpm, 20 min) at 4 ºC (P-Selecta Medifrigar BL-S,
131 Spain) to obtain the enzymatic extract. POD activity was assayed spectrophotometrically
132 by placing 2.7 mL 0.2 M sodium phosphate buffer (pH=6.5), 0.2 mL *p*-phenylenediamine
133 (10 g kg^{-1}), 0.1 mL hydrogen peroxide (15 g kg^{-1}) and 0.1 mL of enzymatic extract in a 1
134 cm oath cuvette. The oxidation of *p*-phenylenediamine was measured at 485 nm and 25
135 ºC using a Thermo Electron Corporation spectrophotometer (USA). POD activity was
136 determined by measuring the initial rate of the reaction, which was computed from the
137 linear portion of the plotted curve. One unit of POD activity was defined as a change in
138 absorbance at 485 nm min^{-1} mL^{-1} of enzymatic extract. The percentage of residual enzyme
139 activity was calculated using equation 2.
140
141 *2.4. Analytical determinations*
142
143 2.4.1. Soluble solids
144 Total soluble solids were estimated as ºBrix with a refractometer (Abbe Atago 89553 by
145 Zeiss, Japan) at 20 ºC.
146
147 2.4.2. pH
148 To determine the pH, a Consort C830 pH meter (Belgium) with a penetration electrode
149 was used.
150
151 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 μm (4.6x250 mm) column (Spain); mobile phase 0.01mol/L potassium dihydrogen phosphate solution, volume injection 20 μL, flow rate 1mL/min, detection at 215 nm at 25 ºC. 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 reductant reagent (Sanchez-Mata, Cámara-Hurtado, Diez-Marques, & Torija-Isasa, 2000 and Sánchez-Moreno, Plaza, De Ancos & Cano, 2003). A 0.5 mL aliquot sample was taken to react with 2 mL of a 20 g/L dithiothreitol solution for 2 hours 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 (4.6x250 mm) column (Spain); mobile phase 0.1 % oxalic acid, volume injection 20 μL, flow rate 1mL/min, detection at 243 nm and at 25 ºC. AA standard solution (Panreac, Spain) was prepared.

2.4.5. Total phenols

The extraction of total phenols (Tomás-Barberán, Gil, Cremin, Waterhouse Hess-Pierce & Kader, 2001) consisted of homogenizing 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 ºC) 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 Sanchez-Moreno et al. (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 (time at the steady state). The changes in absorbance were measured at 25 ºC. Appropriately diluted juice samples were used on the day of preparation. The percentage of DPPH• (%DPPH•) was calculated as equation (3):

$$\%\mathrm{DPPH}\bullet = \frac{(\mathrm{A}_{\mathrm{control}} - A_{sample})}{\mathrm{A}_{\mathrm{control}}} \quad (3)$$

where $A_{control}$ is the absorbance of the control and A_{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 4ºC and -18ºC during a period of 60 days.

203

204 *2.6. Statistical analysis*

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

210

211 **3. Results and Discussion**

212 Pectimethylesterase (PME) residual activity detected in samples after thermal treatments
213 was 12.04 % ± 3.86 and 10.07 % ± 0.63 in CP and MP, respectively. These are
214 intermediate values in the 0-18 % range found by Snir, Koehler, Sims and Wicker (1996),
215 who carried out the heat treatment at 70 ºC for 5 min. Nevertheless, they are high enough
216 to obtain good quality products with a convenient cloud stabilization, which will be kept
217 under refrigeration conditions with low level bacteria growth. According to studies
218 performed by Sentandreu, Carbonell, Rodrigo and Carbonell (2006), PME had a greater
219 heat resistance than microorganisms.

220 As in some studies of fresh orange juice carried out by other authors (Cano et al., 1997),
221 analyses of freshly squeezed and pasteurized grapefruit juice did not show PPO activity.
222 According to Dziezak (1993), citric acid, which is an important component of grapefruit
223 juice, provokes the copper quelation present in this enzyme, disabling the activity of the
224 PPO.

225 With regard to the POD activity of fresh grapefruit juice, the obtained result (5.2 ± 0.2) was
226 similar to values found in the bibliography for citric juices (Cano et al., 1997). In CP, an

227 inactivation of 94.3 % ± 0.7 was reached, which in the case of MP was 88.1 % ± 0.3,
228 showing the significant differences that exist between them.
229 Table 1 shows the physicochemical and compositional parameters of freshly squeezed
230 grapefruit juice, conventional pasteurized juice and that which has been microwave
231 treated. In general, FS obtained for this work presented the characteristic physicochemical
232 parameters shown in the bibliography for grapefruit juice (Moraga, Moraga, Fito & Martínez-
233 Navarrete, 2009). As can be observed, neither pasteurization process affected ºBrix, that
234 ranged between 9.9 -10.1, or pH (2.92-3). Similar results were found by Kim and Tadini
235 (1999), who showed that temperature and holding time had no effect on pH and ºBrix of
236 conventional pasteurized juice. These quality parameters are important as they are closely
237 related with the stability of the bioactive compounds in fruit products (Sánchez-Moreno,
238 Plaza, De Ancos, & Cano, 2006). A significant ($p<0.05$) decrease in CA and TA content
239 was observed due to the pasteurization treatments applied to the juice; the citric acid
240 content was less affected when microwaves were applied to pasteurized juice. In no case
241 did the pasteurization treatment influence the malic acid content. Cañumir et al. (2002)
242 studied the effect of microwaves comparing them with conventional pasteurization in apple
243 juice and they observed that total acidity tended to increase when microwave
244 pasteurization was used, whereas the pH tended to be lower.
245 Vitamin C is used as reference in different industrial processes since its presence ensures
246 a high nutritional quality of the final product due to its easy degradation (Klimczak,
247 Malecka, Szlachta, & Gliszcynska, 2007). The initial values of the ascorbic acid and
248 vitamin C of the fresh juice were similar, 36.0 ± 0.1 mg/100mL and 34.0 ± 1 mg/100mL,
249 respectively. This is the AA grapefruit value obtained by Leong and Shui (2002). No
250 significant differences between AA and vitamin C content were observed, as reported by
251 other authors (Plaza, Sánchez-Moreno, Elez-Martínez, Ancos, Martín-Belloso, & Cano,
252 2006). Vitamin C shows great thermal stability at the low pH of citrus fruits (Sánchez-

253 Moreno et al., 2003) and in fact it was not affected by the treatments applied in this case.
254 The conventionally pasteurised juice presented the lowest, statistically significant ($p<0.05$)
255 ascorbic acid content. In this sense, the obtained results were the expected ones, since
256 according to Vadivambal and Jayas (2007), ascorbic acid retention is superior in the
257 microwave treatment than in the traditional one. In this respect, in order to diminish the
258 impact on quality provoked by processing the juice, it would be preferable to apply
259 microwave pasteurization since a greater proportion of this compound is preserved.
260 Total phenols and %DPPH of FS were similar to the values found for orange juice by other
261 authors (Klimczak et al., 2007). Pasteurization provoked a significant ($p<0.05$) decrease of
262 the total phenol content and %DPPH. This decrease was similar in both treatments,
263 producing a total phenol and %DPPH loss of 14.64 and 40 %, respectively. Studies
264 performed on other fruits, for example strawberry (Klopotek , Otto & Bohm, 2005),
265 demonstrate that there is a relationship between the decrease of antioxidant capacity,
266 ascorbic acid, phenol content and anthocyanins and the processing necessary to obtain
267 pasteurized juice. In general, the obtained results are comparable to those observed for
268 citrus juices by other authors (Klimczak et al., 2007; Xu et al., 2008). In this case,
269 antioxidant activity seems to be more related to total phenols than to ascorbic acid.
270 Table 2 shows the ºBrix and pH obtained for fresh and pasteurized samples stored in
271 refrigeration and freezing conditions for 2 months. From the statistical analysis and the
272 evolution of the values, it can be stated that ºBrix remained stable for all the samples,
273 affected neither by storage conditions nor by storage time. Nevertheless, there is a general
274 increase in pH, with no observed differences between the samples stored at 4 ºC and
275 those kept at -18 ºC.
276 Figure 1 shows that, in refrigeration conditions, the citric acid content of all the samples
277 remained stable for the first 24 hours, but sharply and significantly ($p<0.05$) decreased in
278 the next three days. After 12 days, the CA continued to decrease significantly in FS but

279 remained constant in MP and CP. No significant decrease in CA was observed throughout
280 storage at -18 ºC.
281 As Figure 2 shows, the storage conditions (refrigeration and freezing) affected the malic
282 acid content of all the samples in the same way: the MA content remained constant for the
283 first 25 storage days. Then, there was a significant ($p<0.05$) drop in the content which,
284 once again, stabilised till the end of the storage. As regards the TA content (Figure 3), a
285 significant ($p<0.05$) decrease took place during the first four days, with a subsequent
286 recovery. There were no clear differences observed between the TA stability of the
287 different samples and under differing storage conditions.
288 The evolution of the ascorbic acid content of grapefruit juices stored at 4 ºC and -18 ºC for
289 2 months is presented in Figure 4. In general, the AA content of all juice samples studied
290 behaved in a similar way whether under refrigeration or freezing conditions and no
291 significant ($p<0.05$) changes were observed till 12 days of storage. From this moment on,
292 the samples stored under frozen conditions seem to maintain the AA content till the end of
293 storage, while in the refrigerated juice the proportion of this component decreased
294 significantly ($p<0.05$). In this respect, from an industrial point of view, it would be advisable
295 to freeze the pasteurized juice, for example, in the case of overproduction (Gil-Izquierdo,
296 Gil & Ferreres, 2002). According to the published data, the content of AA in different juices
297 decreases during storage, depending on temperature, oxygen and light access (Klimczak
298 et al., 2007). The degradation of AA follows both aerobic and anaerobic pathways. The
299 oxidation of ascorbic acid occurs mainly during the processing of citrus juices, whereas
300 anaerobic degradation, which is particularly observed in thermally preserved citrus juices,
301 mainly appears during storage (Burdulu, Koca & Karadeniz, 2006). For instance, Polydera,
302 Galanou, Stoforos and Taoukis (2004) reported that thermally pasteurized juice (80 ºC, 60
303 s) showed 72% AA retention after 1 month at 5 ºC. As regards our CP and MP samples,
304 75% and 78% AA retention was reached after 25 days in refrigeration, respectively,

305 whereas in freezing conditions the retention was 84% in CP and 85% in MP. The loss of
306 ascorbic acid during storage might be a quality indicator and a critical factor for the shelf
307 life of some products, such as citrus juices (Plaza et al., 2006).
308 Figure 5 shows the vitamin C evolution of grapefruit juices stored for 2 months at 4 ºC and
309 -18 ºC. In the CP and MP juice samples, the vitamin C content behaved in a similar way
310 whether under refrigeration or freezing conditions and no significant ($p<0.05$) changes
311 occurred till 12 days in the case of CP samples and 25 days in the case of MP samples.
312 From this moment on, under frozen conditions, the vitamin C content of CP samples
313 suffered a significantly ($p<0.05$) smaller decrease than that of refrigerated juice. In the
314 case of frozen MP samples, this component remained stable whereas it dropped under
315 refrigerated conditions.
316 At the end of the refrigerated storage, there were some significant ($p<0.05$) differences
317 observed between the AA and vitamin C content in pasteurized samples. As other authors
318 suggest, the changes observed in the ascorbic acid concentration of the samples stored
319 under refrigeration, suggest the continuation of oxidative degradation reactions of ascorbic
320 acid to other oxidized forms such as dehydroascorbic acid, which also presents biological
321 activity as vitamin C (Russell, 2004). The mechanism for enzyme degradation could be
322 direct, by ascorbic acid oxidase, or indirect through polyphenoloxidase, cytochrome
323 oxidase or peroxidase (Belizt, H.D. & Grosch, W., 1997). This could be the reason why the
324 values of vitamin C were higher than those of AA at the end of storage of treated samples.
325 During the storage time studied (Figure 6), storage temperature seems not to affect FS
326 phenol content since it evolved in a similar way whether under refrigeration or frozen
327 stored, whereas PT significantly ($p<0.05$) diminished till 25 days, after which it remained
328 constant. In this way, Tavirini, D'Innocenti, Remorini, Massai and Guidi (2008) reported
329 that phenols did not change in kiwifruits stored for 2 months at 0 ºC, but they observed a
330 significant rise after a long storage (six months at 0 ºC) which further increased after a

331 week at ambient temperature. In CP and MP refrigerated samples, the phenol content
332 significantly ($p<0.05$) diminished after day four, while under freezing conditions, the
333 evolution of TP was constant.
334 As can be observed in Figure 7, the antioxidant capacity of both thermally treated
335 grapefruit juices was affected by the storage conditions in a similar way. On the other
336 hand, the antioxidant capacity of both the chilled and frozen fresh juice decreased during
337 the first 24 hours of storage. From 24 h of storage on, the FS sample evolved in a similar
338 way to pasteurized juices, regardless of storage conditions and till the end of the study. In
339 general, % DPPH of all the samples decreased throughout the storage. Frozen stored MP
340 samples had a significantly ($p<0.05$) greater antioxidant capacity at the end of the period.
341 In Table 3, the variation of components due to treatment and 60 days of storage can be
342 observed. These values were calculated as the difference of each compound in fresh or
343 treated juice at the end of storage related to fresh juice and referred to 100 g of fresh juice.
344 In general, frozen juices showed the smallest losses. The greatest losses were produced
345 in FS refrigerated samples, except in the cases of AA and vitamin C, which were in greater
346 proportions in CP refrigerated samples. When frozen, the vitamin C and AA content of the
347 pasteurized samples remained the highest. Nevertheless, the studied bioactive
348 compounds in the frozen MP juices maintained a greater stability and the smaller observed
349 losses in antioxidant capacity point to this fact.
350 As regards organic acids, refrigerated FS samples showed the greatest significant
351 ($p<0.05$) loss in CA (-12.64 %). No significant ($p<0.05$) differences were observed for the
352 other acids in the rest of the samples and storage conditions (mean value of the loss is
353 3.58, 18.18 and 3.06 % for CA, MA and TA, respectively). AA and Vitamin C were more
354 stable when samples were frozen, especially in the case of microwave-treated samples.
355 Nevertheless, in refrigerated samples, FS juice contained the greatest amount of these
356 compounds. In both cases, the greatest loss was observed in CP samples. Neither

357 treatment nor storage temperature affected total phenols and antioxidant activity
358 significantly ($p<0.05$), except in the case of frozen MP samples which showed the lowest
359 significant ($p<0.05$) loss of TP (-18.35 %) and %DPPH (-67.1 %).
360 In order to explain the influence of the different compounds quantified in this study on the
361 antioxidant capacity of the samples, correlation statistical analyses were performed. Only
362 TA showed a negative Pearson's correlation coefficient with % DPPH (-0.5258, $p<0.05$).
363 Total phenols played a mayor role in the antioxidant capacity of grapefruit juices (0.8389,
364 $p<0.05$), followed by the vitamin C (0.7216, $p<0.05$), ascorbic acid (0.5563, $p<0.05$), malic
365 acid (0.5548, $p<0.05$), citric acid (0.4785, $p<0.05$). Other studies (Bahorun, Luximon-
366 Ramma, Crozier & Aruoma, 2004) confirm the existence of a positive relationship between
367 the phenolic content of a fruit and its antioxidant capacity. Fruits with high antioxidant
368 activity generally contain a great quantity of antioxidant substances, especially phenolic
369 compounds and specifically flavonoids (Tavarini et al., 2008).
370
371 **Conclusion**
372 Contrary to conventional treatment which leads to a significant decrease in CA and AA in
373 grapefruit juice, microwave treatment preserved these compounds. Moreover, frozen
374 microwave pasteurized juices better preserved total phenols and antioxidant capacity
375 when compared with fresh or conventional pasteurized ones and maintained the amount of
376 AA and vitamin C, especially in pasteurized samples. Therefore, the use of microwave
377 energy offers a good alternative to conventional pasteurization.
378
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382

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513 **Table 1**. Mean values (with standard deviation) of ºBrix, pH, CA, MA, TA, AA, vitamin C,
514 TP and % DPPH in freshly squeezed (FS), conventional pasteurized (CP) and microwave
515 pasteurized (MP) juice.
516 **Table 2**. Mean values (with standard deviation) of ºBrix and pH evolution of grapefruit
517 juices stored at 4 ºC (A) and -18 ºC (B) for 2 months.
518 **Table 3**. Mean values (with standard deviation) of variation of components (%) due to
519 treatment and after 60 days of storage**.**

520 **FIGURE CAPTIONS**

521 **Figure 1**. Evolution of citric acid (CA) of FS (A), CP (B) and MP (C) grapefruit juices
522 stored at 4 ºC and -18ºC for 2 months. Letters indicate homogeneous groups established
523 by the ANOVA ($p<0.05$).
524 **Figure 2**. Evolution of malic acid (MA) of FS (A), CP (B) and MP (C) grapefruit juices
525 stored at 4 ºC and -18ºC for 2 months. Letters indicate homogeneous groups established
526 by the ANOVA ($p<0.05$).
527 **Figure 3**. Evolution of tartaric acid (TA) of FS (A), CP (B) and MP (C) grapefruit juices
528 stored at 4 ºC and -18ºC for 2 months. Letters indicate homogeneous groups established
529 by the ANOVA ($p<0.05$).
530 **Figure 4**. Evolution of ascorbic acid (AA) of FS (A), CP (B) and MP (C) grapefruit juices
531 stored at 4 ºC and -18ºC for 2 months. Letters indicate homogeneous groups established
532 by the ANOVA ($p<0.05$).
533 **Figure 5**. Evolution of vitamin C of FS (A), CP (B) and MP (C) grapefruit juices stored at 4
534 ºC and -18ºC for 2 months. Letters indicate homogeneous groups established by the
535 ANOVA ($p<0.05$).
536 **Figure 6**. Evolution of total phenols (mg GAE / 100 mL) of FS (A), CP (B) and MP (C)
537 grapefruit juices stored at 4 ºC and -18ºC for 2 months. Letters indicate homogeneous
538 groups established by the ANOVA ($p<0.05$).

539 **Figure 7**. Evolution of antioxidant activity (%DPPH) of FS (A), CP (B) and MP (C)
540 grapefruit juices stored at 4 ºC and -18ºC for 2 months.
541
542

Figure 1

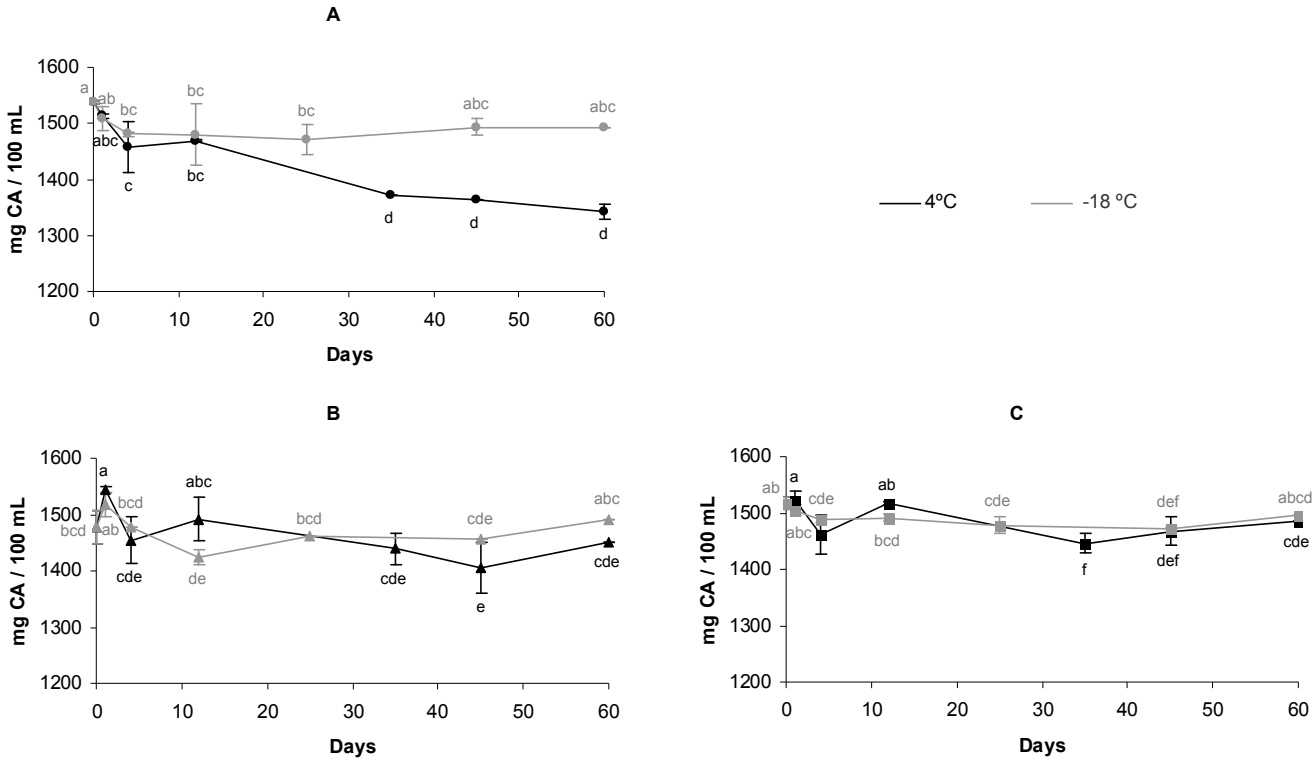

Figure 1.

Figure 2

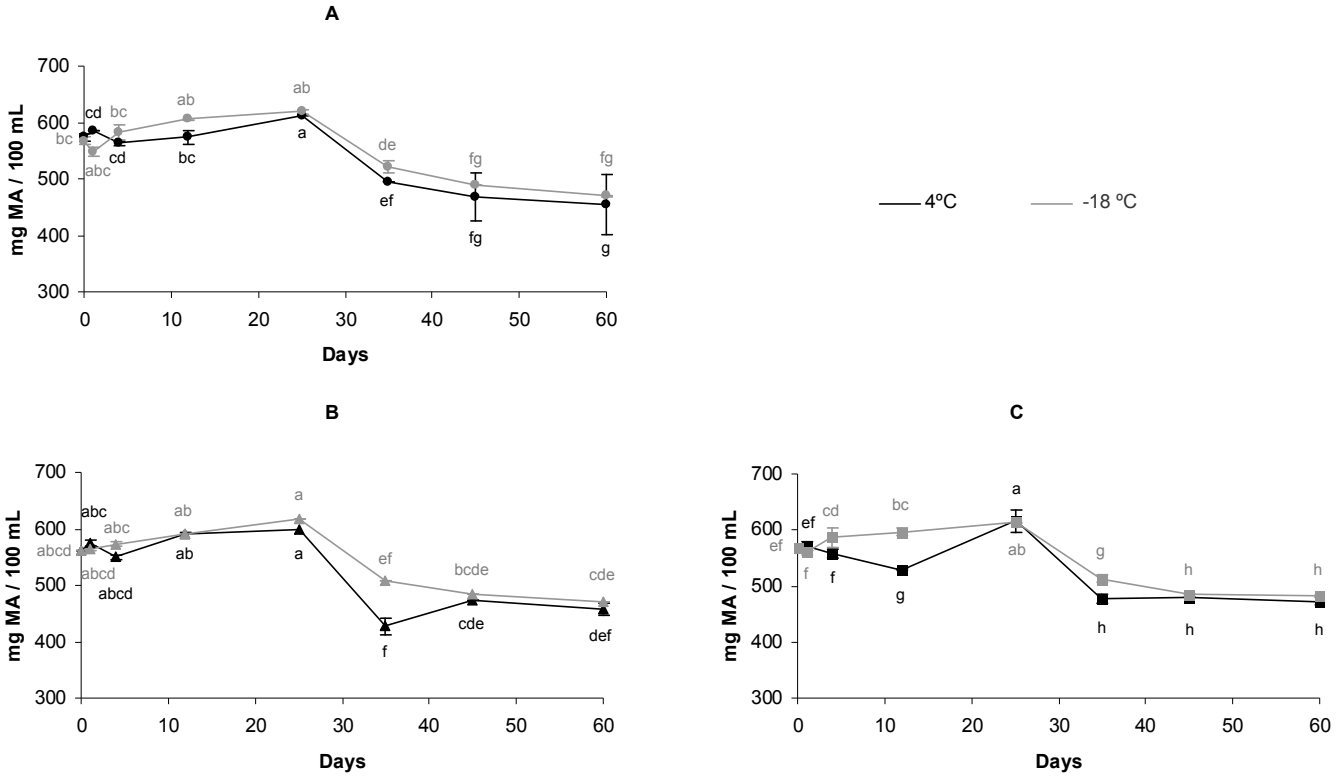

Figure 2.

Figure 3

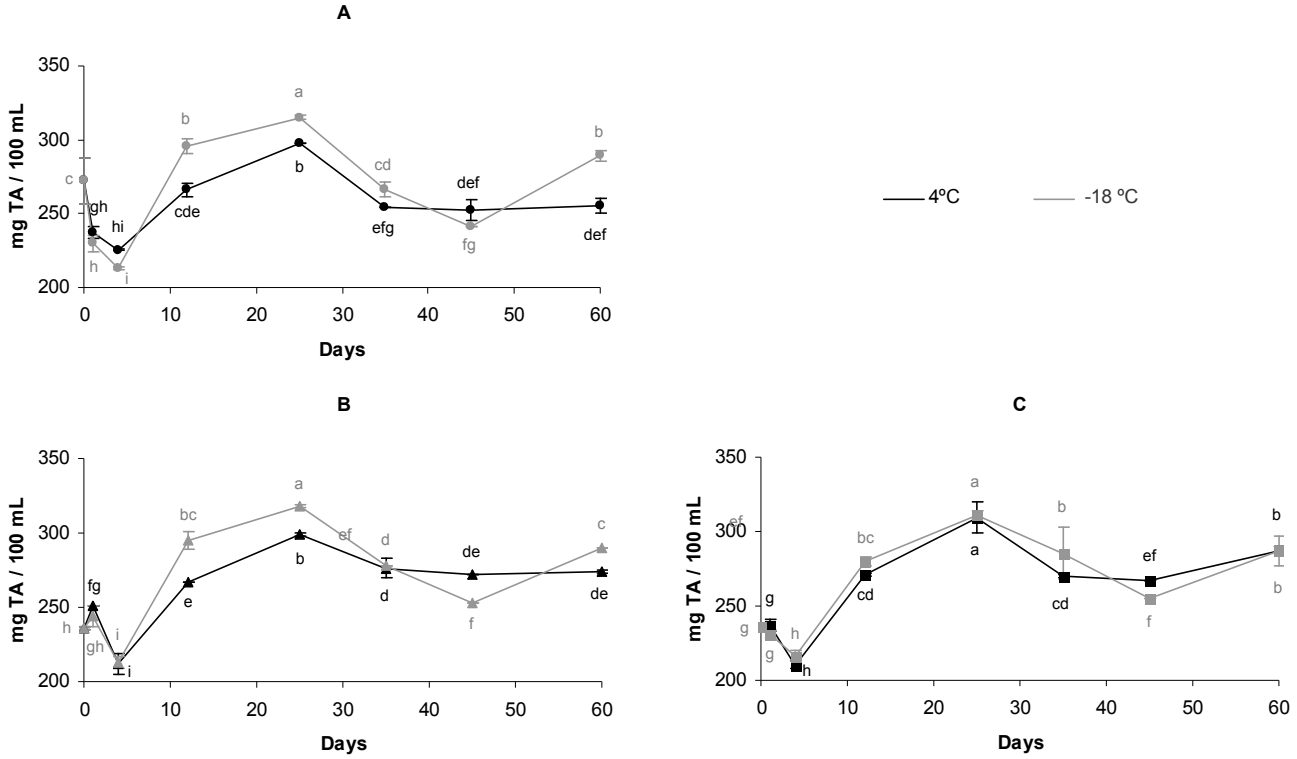

Figure 3.

Figure 4

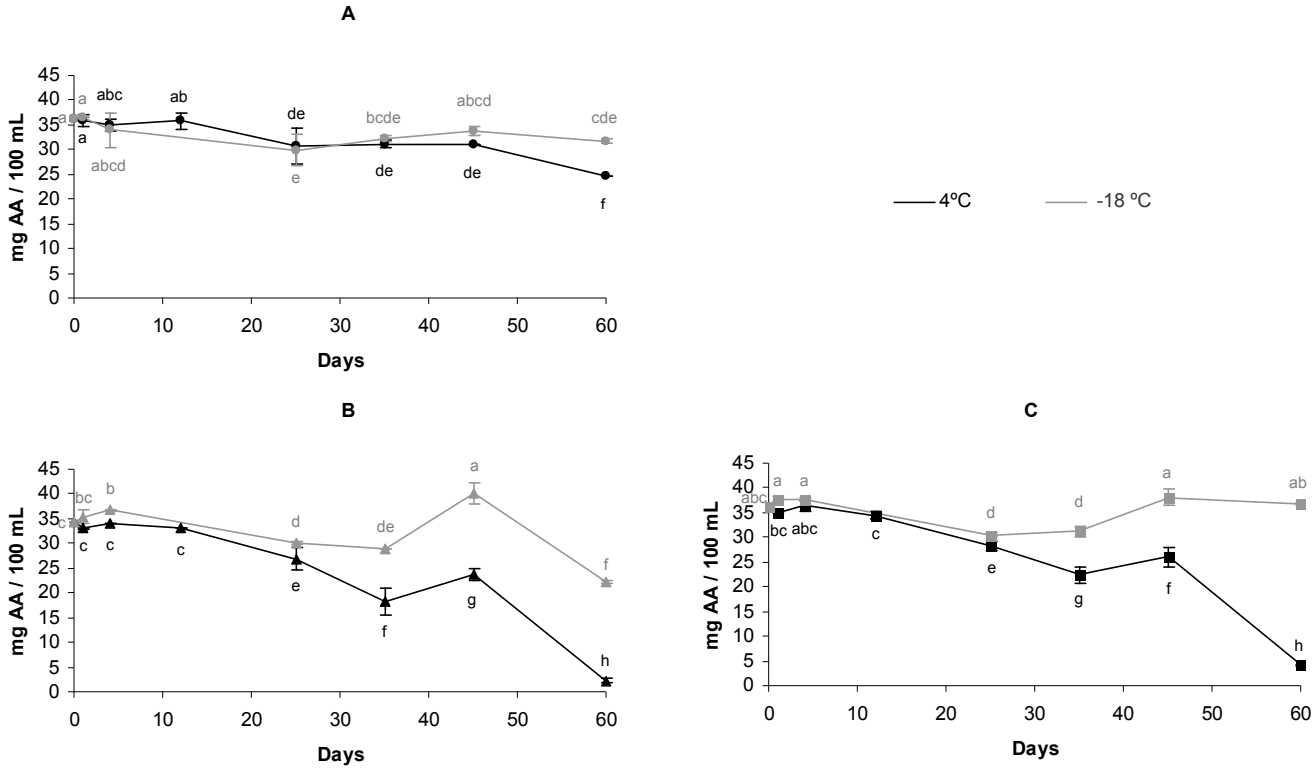

Figure 4.

Figure 5

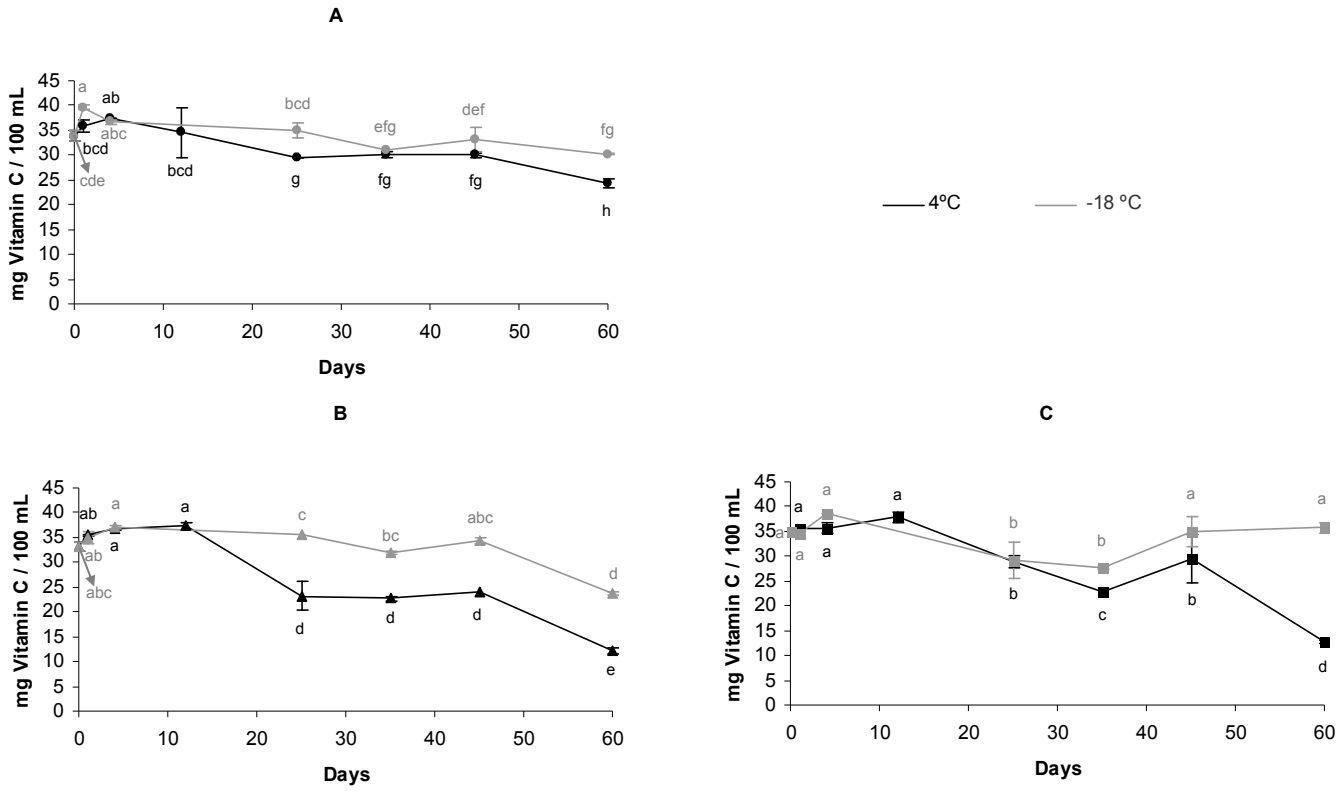

Figure 5.

Figure 6

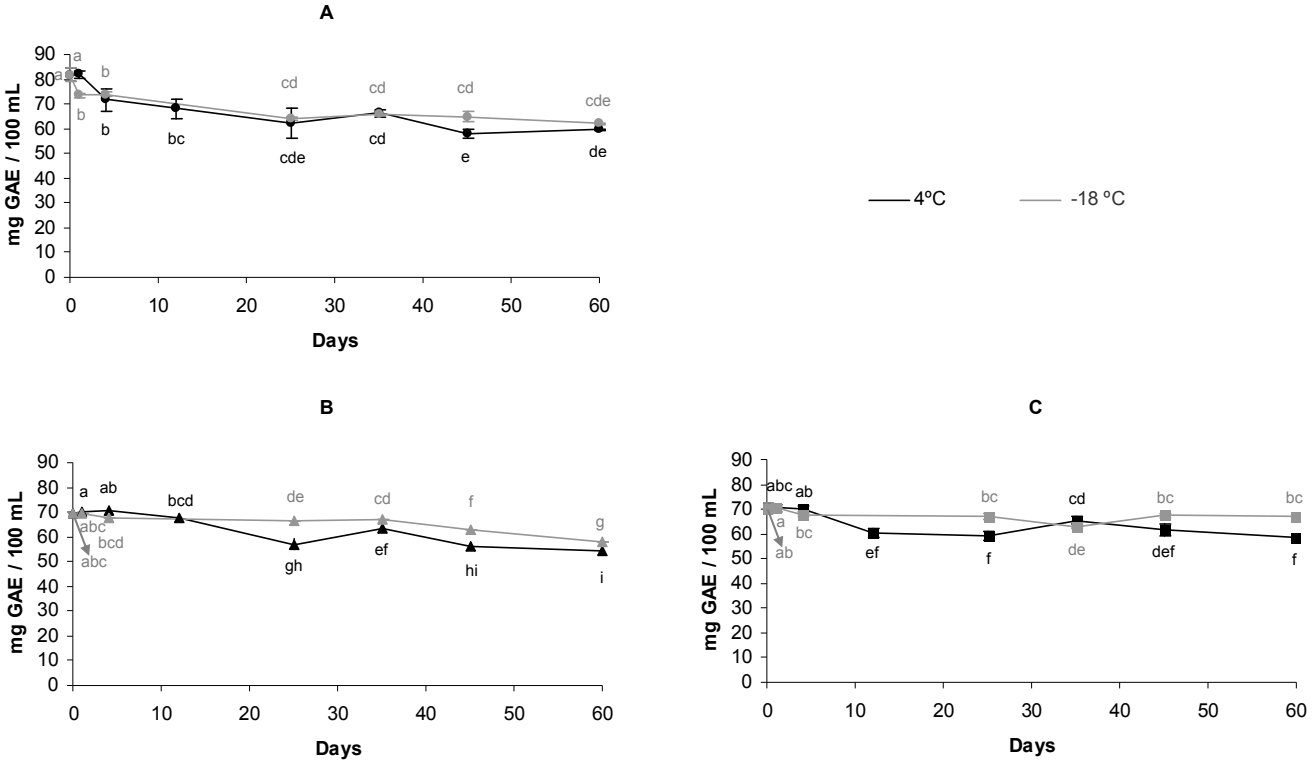

Figure 6.

Figure 7

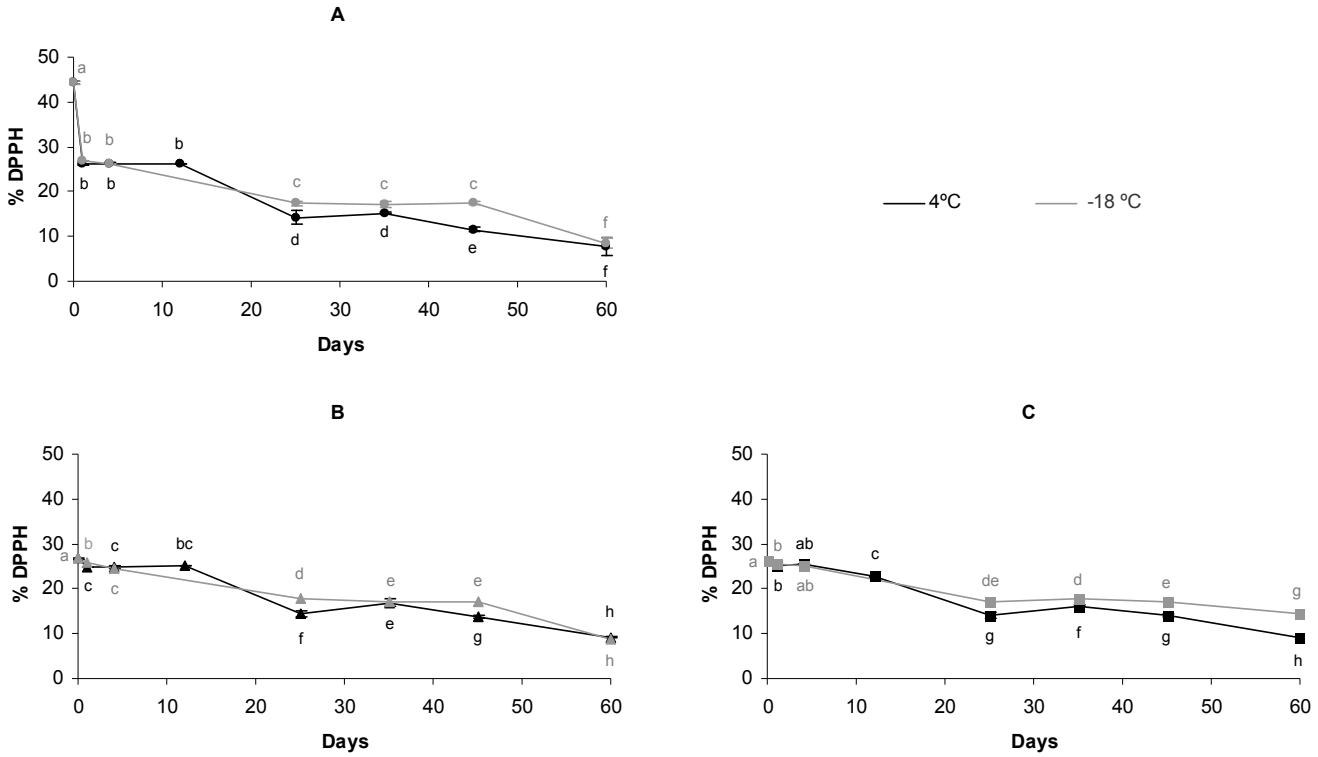

Figure 7.

Table 1.

	FS	CP	MP
ºBrix	9.9 (0.1)[a]	10.1 (0.1)[a]	10.1 (0.1)[a]
pH	3.00 (0.01)[a]	2.98 (0.01)[a]	2.92 (0.01)[b]
CA	1538 (3)[a]	1478 (30)[b]	1518 (11)[ab]
MA	574 (8)[a]	562 (1)[a]	570 (5)[a]
TA	272 (16)[a]	236 (1)[b]	237 (1)[b]
AA	36.0 (0.1)[a]	34.3 (0.1)[b]	36 (0.3)[a]
Vitamin C	34 (1)[a]	33 (1)[a]	35 (1)[a]
TP	82 (3)[a]	69 (1)[b]	70 (2)[b]
%DPPH	44.4 (0.3)[a]	26.7 (0.1)[b]	26.1 (0.1)[b]

The same letter in superscript indicates homogeneous groups established by the ANOVA ($p<0.05$)
In columns: FS: freshly squeezed juice; CP: conventional pasteurized juice and MP: microwave pasteurized juice.
In rows: CA: citric acid; MA: malic acid; TA: tartaric acid; AA: ascorbic acid; TP: total phenols.

Table 2

Table 2.

Storage		º Brix			pH		
T (ºC)	t (d)	FS	CP	MP	FS	CP	MP
5	1	9.9 (0.1)^{ab}	10.0 (0.1)^{a}	10.0 (0.1)^{a}	3.09 (0.01)^{a}	3.12 (0.01)^{a}	3.08 (0.01)^{a}
	4	9.9 (0.1)^{ab}	10.1(0.1)^{ab}	10.0 (0.1)^{a}	3.15 (0.01)^{b}	3.12 (0.01)^{a}	3.13 (0.01)^{b}
	12	9.7 (0.1)^{a}	9.6 (0.1)^{c}	9.6 (0.1)^{b}	2.92 (0.01)^{cd}	2.89 (0.01)^{b}	2.91 (0.01)^{c}
	25	10.0 (0.1)^{ab}	10.1 (0.1)^{ab}	9.6 (0.1)^{b}	2.95 (0.01)^{de}	2.90 (0.01)^{b}	2.86 (0.01)^{d}
	35	10.0 (0.1)^{ab}	10.0 (0.1)^{a}	10.1 (0.1)^{a}	2.95 (0.01)^{de}	2.95 (0.01)^{c}	2.93 (0.01)^{cef}
	45	10.1 (0.1)^{b}	10.1 (0.1)^{ab}	10.1 (0.1)^{a}	3.00 (0.01)^{f}	3.00 (0.01)^{d}	3.00 (0.01)^{h}
	60	10.1 (0.1)^{b}	10.2 (0.1)^{ab}	10.1 (0.1)^{a}	3.01 (0.01)^{f}	2.99 (0.01)^{d}	2.99 (0.01)^{h}
-18	1	10.0 (0.1)^{ab}	10.4 (0.1)^{b}	10.3 (0.1)^{a}	3.20 (0.01)^{g}	3.12 (0.01)^{a}	3.15 (0.01)^{b}
	4	9.9 (0.1)^{ab}	10.1 (0.1)^{ab}	10.1 (0.1)^{a}	3.11 (0.01)^{a}	3.16 (0.01)^{f}	3.15 (0.01)^{b}
	12	9.7 (0.1)^{a}	9.5 (0.1)^{c}	9.6 (0.1)^{b}	2.92 (0.01)^{cd}	3.00 (0.01)^{d}	2.92 (0.01)^{ce}
	25	10.1 (0.1)^{b}	10.2 (0.1)^{ab}	9.6 (0.1)^{b}	2.90 (0.01)^{c}	2.85 (0.01)^{e}	2.87 (0.01)^{d}
	35	10.1 (0.1)^{b}	10.2 (0.1)^{ab}	10.2 (0.1)^{a}	2.93 (0.01)^{cd}	2.95 (0.01)^{c}	2.95 (0.01)^{efg}
	45	10.1 (0.1)^{b}	10.1 (0.1)^{ab}	10.1 (0.1)^{a}	2.98 (0.01)^{ef}	2.95 (0.01)^{c}	2.98 (0.01)^{gh}
	60	10.1 (0.1)^{b}	10.2 (0.1)^{ab}	10.2 (0.1)^{a}	3.00 (0.01)^{f}	3.01 (0.01)^{d}	2.96 (0.01)^{fgh}

The same letter in superscript indicates homogeneous groups in the same physico-quemical 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 pasteurized juice and MP: microwave pasteurized juice.

Table 3

Table 3

	Refrigeration			Frozen		
	FS	**CP**	**MP**	**FS**	**CP**	**MP**
ºBrix	1.21 (0.01)^{a}	2.21 (0.01)^{b}	1.31 (0.14)^{a}	1.21 (0.01)^{a}	2.37 (0.22)^{b}	2.32 (0.14)^{b}
pH	0.33 (0.01)^{c}	-0.33 (0.01)^{b}	-0.33 (0.01)^{b}	-0.33 (0.47)^{b}	0.50 (0.23)^{c}	-1.33 (0.01)^{a}
CA	-12.64 (2.87)^{a}	-5.59 (2.68)^{b}	- 3.38 (0.27)^{b}	-3.02 (0.14)^{b}	-2.83 (0.24)^{b}	-3.09 (0.13)^{b}
MA	-20.63 (8.17)^{a}	-20.29 (2.81)^{a}	-17.51 (2.11)^{a}	- 18.21 (1.36)^{a}	-16.83 (0.10)^{a}	-15.60 (0.90)^{a}
TA	-5.96 (3.56)^{a}	0.89 (5.25)^{ab}	5.85 (5.27)^{ab}	6.57 (4.80)^{ab}	7.56 (7.19)^{b}	3.46 (5.40)^{ab}
AA	- 31.80 (0.13)^{d}	-93.85 (1.26)^{a}	-88.53 (0.43)^{b}	-11.89 (2.77)^{e}	-38.45 (1.02)^{c}	-0.85 (0.39)^{f}
Vitamin C	-28.41 (5.03)^{b}	-63.75 (2.82)^{a}	-62.27 (0.18)^{a}	-10.72 (3.18)^{c}	-29.84 (1.72)^{b}	6.05 (6.63)^{d}
TP	-27.37 (1.03)^{a}	-33.62 (2.44)^{a}	-28.23 (0.40)^{a}	-24.61 (0.77)^{a}	-28.11 (3.15)^{a}	-18.35 (0.89)^{b}
%DPPH	-82.67 (4.86)^{a}	-79.24 (0.51)^{a}	-79.67 (0.70)^{a}	- 80.73 (2.69)^{a}	-80.45 (0.91)^{a}	-67.10 (1.26)^{b}

The same letter in superscript indicates homogeneous groups established by the ANOVA ($p<0.05$).
In columns: FS: freshly squeezed juice; CP: conventional pasteurized juice and MP: microwave pasteurized juice.
In rows: CA: citric acid; MA: malic acid; TA: tartaric acid; AA: ascorbic acid; TP: total phenols.