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Changes in flavonoid content of grapefruit juice caused by thermal treatment and storage

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Abstract

The effect of conventional and microwave pasteurisation on the main flavonoids present in grapefruit juice and their stability throughout 2 months of refrigerated and frozen storage was evaluated. Individual flavonoids were analysed by HPLC. The results showed that naringin, narirutin, quercetin and naringenin were the most abundant flavonoids in grapefruit juice. In general, although every pasteurization treatment caused a significant reduction in the content of all the studied flavonoids, the treated samples were more stable during storage. Whilst fresh squeezed juice (FS) and conventional pasteurized juice (CP) were better preserved under refrigeration conditions, microwave pasteurized juice (MP) conserved better when frozen stored. In fact, after 2 months, frozen MP samples showed the greatest flavonoid retention. From this point of view, microwave treatment can be considered as a good alternative to conventional pasteurization.

Keywords: grapefruit, juice, pasteurization, microwaves, high performance liquid chromatography, flavonoids, refrigerated and frozen storage.

Industrial relevance

Flavonoids are polyphenolic compounds present in fruits and vegetables relevant not only in terms of quality, as they influence the visual appearance and taste, but also from a therapeutical point of view, as they appear to be associated with the prevention of degenerative diseases. The consumption of grapefruit juice is fairly widespread among the population. Traditionally, juices have been pasteurized by heat treatment to prolong their shelf life. However, this process may cause irreversible losses of nutritional quality and antioxidant activity and, in consequence, may affect their health-related properties. In this sense, the use of microwaves can be considered an alternative to conventional thermal pasteurization. Microwave energy was applied as alternative to conventional heating for grapefruit juice pasteurization. The results obtained in this study showed that when the effect of pasteurization process and storage is considered together, the use of microwave energy led to a greater retention of all the analysed flavonoids, thereby representing a good alternative to conventional pasteurisation. In this case, frozen storage of processed product would be recommended to better preserve these compounds.

1. Introduction

There is widespread interest in the health properties of citrus fruits. This is due to their content in natural antioxidant substances which appear to be associated with the prevention of degenerative processes and the reduced risk of certain chronic diseases such as cancer (Liu et al., 2001; Poulose et al., 2005; Vanamala et al., 2006), osteoporosis (Deyhim et al., 2006) and cerebrocardiovascular diseases (Sanchez-Moreno et al., 2003; Yu et al., 2005). Among the natural nutritional antioxidants present in these fruits, phenolic compounds stand out as they have a wide range of therapeutical properties for medical and clinical applications such as anti-inflammatory, antihypertensive, diuretic, analgesic and hypolipidemic activities (Rapisarda et al., 1998; Gil-Izquierdo et al., 2001).

53 Furthermore, citrus phenolic compounds are relevant in terms of quality, as they influence
54 the visual appearance (pigmentation and browning) and taste (astringency, bitterness) (Yu
55 et al., 2005). Flavonoids are polyphenolic compounds that can be categorized into six
56 groups: flavonols, flavones, flavanones, isoflavones, anthocyanins and flavans (Peterson
57 & Dwyer, 1998). Flavanones are found abundantly in citrus fruit, conferring its typical taste
58 (Macheix et al.,1990). They are present in the forms of glycoside or aglycone. Among the
59 glycoside forms, two types are classified: neohesperidosides and rutinosides (Macheix et
60 al., 1990; Gionfriddo et al., 1996,). Neohesperidosides (naringin, neohesperidin, poncirin
61 and neoeriocitrin) consist of a flavanone with neohesperidose (rhamnosyl-α-1,2 glucose)
62 and they have a bitter taste, while rutinosides (hesperidin, narirutin and didymin) have a
63 flavanone and a rutinose disaccharide residue (rhamnosyl- α-1,6 glucose) and they are
64 tasteless (Tripoli et al., 2007). Moreover, other important flavonoid compounds in citrus are
65 flavone aglycone naringenin and flavonol aglycone quercetin.
66 Of all the citrus products, the juices constitute a good alternative to fresh fruit consumption
67 because they are quick and easy to take. Traditionally, juices have been pasteurized by
68 heat treatment to prolong their shelf life. However, this process may cause irreversible
69 losses of nutritional quality and antioxidant activity and, in consequence, may affect their
70 health-related properties. In this sense, the use of microwaves can be considered an
71 alternative to conventional thermal pasteurization. This technique permits a reduction in
72 the warm up time and it can help to preserve the natural organoleptic and nutritional
73 characteristics of the juice (Cañumir et al., 2002).
74 Knowing the flavonoid content of citrus juices is paramount in order to understand their
75 role in human health. In the literature, little information is available on the changes in
76 individual flavonoid constituents of grapefruit during storage. For fresh and pasteurized
77 grapefruit juices, influence of storage time and temperature (refrigeration and frozen) on
78 the total phenol content was previously investigated (Igual et al., 2010). In that study, the

79 authors found that the total phenol content of freshly squeezed juice was not affected by
80 storage temperature, although it was affected by storage time. Moreover, in refrigerated
81 pasteurized juices, the phenol content significantly diminished during storage, whilst under
82 freezing conditions no evolution of the total phenols was observed. The objective of the
83 present study was to evaluate the effect of conventional and microwave pasteurization on
84 the individual flavonoid content of grapefruit juice. The effect of storage time and
85 temperature on these components and their relationship with antioxidant capacity was also
86 studied.
87
88 **2. Materials and Methods**
89 *2.1. Raw material*
90 For this work, grapefruits (*Citrus paradise* var. Star Ruby) from the city of Murcia were
91 purchased from a local supermarket. Grapefruits were selected on the basis of a similar
92 degree of ripeness (ratio ºBrix/acidity ≈ 4) and apparent fruit quality (firmness, size, colour
93 and absence of physical damages). Fruit was processed in the laboratory immediately
94 after being purchased.
95
96 *2.2. Treatments*
97 Freshly squeezed (FS) grapefruit juice was extracted through a domestic squeezer (Braun
98 Citromatic Pulp Control MPZ6), filtered using a sieve (light of mesh diameter 1 mm, Cisa
99 029077,1 series) and immediately processed. To obtain conventional pasteurized juice
100 (CP) samples of 40 mL were heated in glass tubes in a thermostatic water bath
101 (Precisterm, Selecta, Spain) operating at 95 ºC. In these conditions, the juice took 80 s to
102 reach 80 ºC ± 2.5 and it remained at this temperature for 11 s more. In the case of
103 microwave pasteurized juice (MP), samples of 20 mL were heated in 25 mL glass tubes at
104 900 W for 30 s using a microwave (Moulinex 5141 AFW2, Spain), reaching 80ºC. Both

105 processes were previously selected to ensure ≈10 % of fresh juice pectimethylesterase
(PME) residual activity (Igual et al., 2010). The tubes with the treated samples were
immediately cooled in ice-water till the juice reached 30 ºC.
108
109 *2.3. Flavonoid determination*
110 The extraction of flavonoids was carried out following the procedure proposed by Tomás-
111 Barberán et al., (2001). It consisted of homogenizing 35 g of the sample (T25 Janke and
112 Kunkel turrax) for 5 min with 40 mL of methanol, 10 mL of bidistilled water and NaF to
113 inactivate polyphenol oxidases and prevent phenolic degradation. The homogenate was
114 centrifuged (Selecta Medifriger-BL,10,000 rpm, 10 min, 4 ºC) to obtain the supernatant
115 which was filtered through a 0.45 µm membrane filter. The flavonoids were determined
116 and quantified by high performance liquid chromatography (HPLC). The HPLC (Jasco,
117 Italy) equipment consisted of a ternary pump (Jasco PU-1580 HPLC pump), a gradient
118 generator (LG-1580-02 Ternary Gradient Unit), Ultrabase-C18 column (5 µm, 4.6x250
119 mm) and a UV-visible detector (MD-1510) with a range of measurement wavelength of 190
120 to 650 nm. The mobile phase was composed of (A) methanol and (B) water and a linear
121 gradient elution was performed starting at 30:70 to reach 100:0 at 70 min, volume injection
122 25 µL and flow rate 1mL/min. Chromatograms were recorded at 286, 284 and 254 nm and
123 at 25 ºC. The standard curves of the reference flavonoids, narirutin (NAT), naringin (NAR),
124 hesperidin (HES), neohesperidin (NEOH), didymin (DID), poncirin (PON), naringenin
125 (NAG) and quercetin (QUER) (Extrasyntesis, France) were used to quantify the flavonoids.
126 Naphthalene was used as internal standard (Peiró, 2007).
127
128 *2.4. Storage conditions*
129 Samples (FS, CP and MP) were stored immediately after treatment in sterile
130 polypropylene packages and kept in the dark at 4ºC and -18ºC for a period of 60 days.

131

132 *2.5. Statistical analysis*

133 Significant differences among treatments and storage time were evaluated by means of
134 the analysis of variance (ANOVA). Differences of $p < 0.05$ were considered to be
135 significant. Furthermore, a Pearson correlation analysis was carried out between the total
136 flavonoid content and each flavonoid with a 95 % significance level. All the statistical
137 analyses were performed using Statgraphics Plus 5.1. A Principal Component Analysis
138 (PCA) with varimax rotation was applied to the values of the flavonoid content, using
139 SPSS program version 16.0.

140

141 **3. Results and Discussion**

142 Flavanones constitute 98% of the total flavonoids present in grapefruits (Gorinstein et al.,
143 2006; Peterson et al., 2006; Vanamala et al., 2006). In this work, the specific flavonoid
144 groups analyzed were flavanones (naringin, narirutin, hesperidin, neohesperidin, didymin
145 and poncirin), flavones (naringenin) and flavonols (quercetin). The chemical structures of
146 these compounds can be seen in Figure 1. Table 1 shows the flavonoid composition of a
147 freshly squeezed grapefruit juice and conventional and microwave pasteurised ones. The
148 total flavonoid content analysed in the whole juices was in good agreement with previous
149 findings of the total phenol content (82 mg of gallic acid/100 mL for FS and 70 mg of gallic
150 acid/100 mL for CP and MP; Igual et al., 2010). Flavanones constitute, in this case, 87 %
151 of the analyzed flavonoids. NAR was the most abundant flavonoid in grapefruit juice,
152 followed by NAT, QUER and NAG, results which coincided closely with other studies
153 (Ross et al., 2000, Gorinstein et al., 2006, Peterson et al., 2006, Vanamala et al., 2006).
154 These four components represent about 90% of the analysed flavonoids. The values found
155 for every compound were in the same range as those reported in other publications (Ross
156 et al., 2000, Xu et al., 2008). In general, the application of every pasteurization treatment

157 caused a significant ($p < 0.05$) reduction in the content of all the studied flavonoids.
158 Flavonoids of conventional and microwave pasteurizated juices did not present significant
159 ($p > 0.05$) differences. CP and MP processing caused losses of 6 % in the total content of
160 the studied flavonoids.
161 The evolution in the amount of NAT, NAR and QUER of grapefruit juices stored at 4 ºC
162 and -18ºC for 2 months is shown in Figures 2, 3 and 4, respectively. In general, these
flavonoids significantly diminished ($p < 0.05$) till 12 days of storage. From this time on, the
content of NAT and NAR presented a slight increase up to 25 days, after which they
remained the same.This increase for QUER was observed at the end of the storage
166 period considered.Table 2 shows the corresponding percentage of loss after 12 and 25
167 storage days. For the three studied treatments, the NAT content was higher in refrigerated
168 juice than in frozen till day 12, after which it was of the same order in both storage
169 conditions. In general, pasteurized samples kept a greater amount of this flavonoid than
170 FS, especially microwave treated ones. In the case of NAR and QUER, refrigerated FS
171 and CP samples also showed a better retention of these compounds than frozen ones,
172 while the loss in MP refrigerated juices was greater than in frozen stored samples. The
173 conventional treatment was the one that best preserved NAR during storage at both
174 temperatures, while refrigerated microwave treated juices were the worst in this regard.
175 The lowest QUER stability was obtained in non-treated samples.
176 Evolution in the amount of HES, NEOH and NAG of grapefruit juices stored at 4 ºC and -
177 18ºC for 2 months is shown in Figures 5, 6 and 7, respectively. The content of these
178 flavonoids diminished in every sample during all the storage, but especially at shorter
179 times. In general, NAG is the flavanone with the greatest losses in every sample, followed
180 by HES. Table 3 shows the percentage of loss at 24 h and 60 days. In general, no
181 significant differences were observed due to storage temperature. Non-treated juice

182 presented greater losses of these flavonoids than pasteurized ones. Only in the case of
183 NEOH, microwave treatment clearly supposed a better preservation.
184 As regards DID (Figure 8), this component was the minority flavonoid found in all the
185 analyzed grapefruit juices. In general, no significant ($p < 0.05$) loss of this component was
186 detected at the end of 2 months of storage in comparison with the initial DID content,
187 despite the fluctuations which occurred during the storage period.
188 Figure 9 shows PON evolution throughout 2 months of both refrigerated and frozen
189 storage. During storage only the refrigerated FS samples showed a decrease in PON
190 content (15 % after 60 days). In the rest of the samples, this was the only flavonoid that
191 significantly ($p < 0.05$) increased during the storage period in CP and MP juices with no
192 significant effect of the storage temperature. After 60 days of storage, an increase of about
193 30 % was observed.
194 The increase observed in PON could be explained from remarks made by other authors.
195 Lin et al. (2003) mentioned that the absence of methoxy groups in A and B- rings of the
196 flavanone NAG (Figure 1) may be the cause of its instability, as these groups act to protect
197 the structure from degradation. They also described the NAG degradation in acid media.
198 Furthermore, Hou et al., (2001) noted that the presence of sugars, especially fructose,
199 favoured the hydrolysis of naringenin. The enzymatic methylation of NAG with O-
200 methyltransferase, present in grapefruit, converts it into ponciretin. The ponciretin can be
201 transformed into poncirenin and this into its glycosylated form PON (Kim et al., 2005). As
202 regards the increase in PON, other reactions described were modifications of the aromatic
203 ring structure of NAR, such as substitutions on the B-ring to obtain NEOH or PON
204 (Jourdan et al., 1985).
205 Figure 10 shows the evolution of the total analyzed flavonoid content during the
206 refrigerated and frozen storage period under study. Regardless of the storage conditions,
207 FS samples experienced major losses in all juice samples. After 24 h, the loss was of 15

208 and 20 % for refrigerated and frozen juices, respectively, while after 60 days it was of 21
209 and 25 %. As to the CP samples, the storage temperature provoked no significant
210 differences in the loss of total flavonoids. After 24 h, total flavonoids were better preserved
211 in refrigerated MP samples (loss of 5 %) but, after 60 days, frozen MP samples showed
212 the greatest flavonoid retention (loss of about 12 %).
213 If the changes provoked by the type of treatment and storage are considered together
214 (Table 4), after 60 days it can be seen that the frozen pasteurized samples retained the
215 greatest total flavonoid content, especially the microwave treated ones. Under freezing
216 conditions, FS samples preserved 75 % of total flavonoid retention at the end of the
217 storage period, whilst CP and MP juices preserved 87 and 89 %, respectively. As can be
218 observed in this table, a significantly greater retention of all the analyzed compounds
219 occurs in MP frozen samples. When a Pearson correlation (Table 5) was carried out with
220 individual and total flavonoids, NAR, NAT, HES and QUER were found to be mainly
221 responsible for the variation in the content of total flavonoids in grapefruit juice, as the
222 Pearson correlation coefficients were 0.92, 0.84, 0.82 and 0.81, respectively.
223 On applying a PCA analysis (Figure 11) to the values of the flavonoid content
224 corresponding to all the juice samples at different storage times and temperatures, the first
225 two factors showed eigenvalues higher than 1. The consideration of both factors
226 accounted for 75.16 % of the total variability. The first factor (F1), explaining 46.98% of the
227 variability, was associated with NAT (r=0.80), NAR (r=0.76), HES (r=0.75), NEOH (r=0.68),
228 DID (r=0.66) and QUER (r=0.89) values. The second factor (F2) accounted for 28.18 % of
229 the variability and it was mainly associated with PON (r=0.80) and NAG (r=0.75) values. At
230 the beginning of the storage period, although all the grapefruit juices showed a high
231 content of the flavonoids associated with F1, in the case of F2, there was a high content of
232 NAG but low of PON. During the storage of samples, a decrease of both F1 and F2 factors
233 was observed. A more marked decrease in NAT, NAR, HES, NEOH, DID and QUER was

observed after 24 h of storage, while changes in PON (increase) and NAG (decrease) were more intense after 60 days. Applying a multifactor ANOVA to the values of F1 and F2 corresponding to all the juice samples, only the advance of storage time showed significant difference in the F1.

4. Conclusion

NAR was the most abundant flavonoid in grapefruit juice, followed by NAT, QUER and NAG. Of all the analysed flavonoids, NAR, NAT, HES and QUER were the ones that were mainly responsible for the variation in the content of total flavonoids in grapefruit juice. In general, the content of all the studied flavonoids diminished significantly when applying any of the different pasteurization treatments, with no significant differences between either thermal technique. Nevertheless, pasteurized samples lost fewer flavonoids during storage than fresh ones. The analyzed compounds were better, or just as well, preserved under refrigerated as under frozen conditions, except in the case of MP samples that were better preserved under frozen storage. When the effect of pasteurization and storage was considered together, the use of microwave energy led to a greater retention of all the analysed flavonoids, thereby representing a good alternative to conventional pasteurisation.

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351

352 **Table 1**. Mean values in mg/100 mL (with standard deviation) of flavonoids (narirutin
353 naringin, hesperidin, neohesperidin, didymin, poncirin, naringenin and quercetin) analysed
354 in freshly squeezed (FS), conventional pasteurized (CP) and microwave pasteurized (MP)
355 juice.
356 **Table 2**. Percentage of variation of narirutin, naringin and quercetin (mean values and
357 standard deviation) after 12 and 25 days of storage of freshly squeezed (FS), conventional
358 pasteurized (CP) and microwave pasteurized (MP) juice.
359 **Table 3**. Percentage of variation of hesperidin, neohesperidin and naringenin (mean
360 values and standard deviation) after 24 hours and 60 days of storage of freshly squeezed
361 (FS), conventional pasteurized (CP) and microwave pasteurized (MP) juice.
362 **Table 4**. Percentage of variation of components (mean values and standard deviation)
363 according to type of treatment and after 60 days of storage of freshly squeezed (FS),
conventional pasteurized (CP) and microwave pasteurized (MP) juice.

Table 5. Pearson correlation coefficients among individual and total flavonoids.

366

367 **FIGURE CAPTIONS**

368 **Figure 1**. Chemical structures of studied flavonoids (narirutin naringin, hesperidin,
369 neohesperidin, didymin, poncirin, naringenin and quercetin).
370 **Figure 2**. Evolution of narirutine of FS, CP and MP grapefruit juices stored at 4 ºC and -
371 18ºC for 2 months. Letters indicate homogeneous groups established by the ANOVA
372 ($p<0.05$) when analyzing the effect of storage time and temperature.
373 **Figure 3**. Evolution of naringin of FS, CP and MP grapefruit juices stored at 4 ºC and -
374 18ºC for 2 months. Letters indicate homogeneous groups established by the ANOVA ($p <$
375 0.05) when analyzing the effect of storage time and temperature.

Figure 4. Evolution of quercetin of FS, CP and MP grapefruit juices stored at 4 ºC and -18ºC for 2 months. Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$) when analyzing the effect of storage time and temperature.

Figure 5. Evolution of hesperidin of FS, CP and MP grapefruit juices stored at 4 ºC and -18ºC for 2 months. Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$) when analyzing the effect of storage time and temperature.

Figure 6. Evolution of neohesperidin of FS, CP and MP grapefruit juices stored at 4 ºC and -18ºC for 2 months. Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$).

Figure 7. Evolution of naringenin of FS, CP and MP grapefruit juices stored at 4 ºC and -18ºC for 2 months. Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$).

Figure 8. Evolution of didymin of FS, CP and MP grapefruit juices stored at 4 ºC and -18ºC for 2 months. Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$).

Figure 9. Evolution of poncirin of FS, CP and MP grapefruit juices stored at 4 ºC and -18ºC for 2 months. Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$).

Figure 10. Evolution of total flavonoids of FS, CP and MP grapefruit juices stored at 4 ºC and -18ºC for 2 months. Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$).

Figure 11. Principal Component Analysis (PCA) with varimax rotation of the values of flavonoid content corresponding to all the grapefruit juice samples.

Table 1.

	FS	CP	MP
NAT	15.0 (0.2)[a]	14.5 (0.2)[b]	14.2 (0.2)[b]
NAR	41.6 (0.2)[a]	40.0 (0.4)[b]	40.3 (0.2)[b]
HES	1.85 (0.06)[a]	1.6 (0.2)[a]	1.68 (0.04)[a]
NEOH	1.87 (0.05)[a]	1.61 (0.03)[b]	1.59 (0.02)[b]
DID	0.90 (0.02)[a]	0.83 (0.04)[a]	0.9 (0.2)[a]
PON	1.95 (0.07)[a]	1.63 (0.02)[b]	1.71 (0.01)[b]
NAG	3.23 (0.06)[a]	2.60 (0.05)[b]	2.6 (0.2)[b]
QUER	7.2 (0.2)[a]	6.2 (0.2)[b]	6.3 (0.3)[b]
TOTAL	73.62 (0.6)[a]	69.0 (0.2)[b]	69.4 (0.7)[b]

The same letter in superscript within rows indicates homogeneous groups established by the ANOVA ($p<0.05$)
NAT: Narirutine, NAR: Naringin, HES: Hesperidin, NEOH: Neohesperidin, DID: Didymin, PON: Poncirin, NAG: Naringenin and QUER: Quercetin.

Table 2

Table 2.

After 12 d of storage

Treatment	NAT		NAR		QUER	
	Refrigeration	Frozen	Refrigeration	Frozen	Refrigeration	Frozen
FS	-25 (2)^{aA}	-26 (1)^{aAB}	-23 (3)^{aAB}	-22 (1)^{aA}	-42 (2)^{bA}	-56 (2)^{aA}
CP	-18 (3)^{bAB}	-30 (1)^{aA}	-17 (6)^{aB}	-28 (3)^{aA}	-28 (2)^{aB}	-38 (6)^{aB}
MP	-16 (1)^{aB}	-17 (6)^{aB}	-32 (1)^{aA}	-19 (4)^{bA}	-35 (3)^{aAB}	-31 (4)^{aB}

After 25 d of storage

Treatment	NAT		NAR		QUER	
	Refrigeration	Frozen	Refrigeration	Frozen	Refrigeration	Frozen
FS	-8 (1)^{aA}	-16 (11)^{aA}	-9.5 (0.5)^{aB}	-16 (3)^{aA}	-44 (2)^{aA}	-49.8 (0.5)^{aA}
CP	-7.9 (0.4)^{aA}	-11 (8)^{aA}	-7 (2)^{aB}	-7.3 (0.4)^{aB}	-29 (1)^{aB}	-37 (8)^{aAB}
MP	0.3 (0.4)^{bB}	-13 (3)^{aA}	-21 (1)^{aA}	-15 (2)^{aA}	-37 (4)^{aAB}	-29 (6)^{aB}

The same capital letter in superscript within columns indicates homogeneous groups established by the ANOVA ($p<0.05$) comparing treatments.
For each flavonoid, the same small letter in superscript within rows indicates homogeneous groups established by the ANOVA ($p<0.05$) comparing storage conditions.
NAT: Narirutine, NAR: Naringin and QUER: Quercetin.

Table 3

Table 3.

After 24 h of storage

Treatment	HES		NEOH		NAG	
	Refrigeration	Frozen	Refrigeration	Frozen	Refrigeration	Frozen
FS	-46 (2.)^{aA}	-37 (3)^{aA}	-20 (2)^{aA}	-23 (1)^{aA}	-31 (5)^{aA}	-48 (2)^{aA}
CP	-23 (13)^{aB}	-21 (6)^{aB}	-8.1 (0.7)^{aB}	-1.51 (2.84)^{aB}	-20 (8)^{aA}	-19 (2)^{aC}
MP	-25.2 (0.2)^{bB}	-32.2 (0.2)^{aAB}	-11 (2)^{aB}	-0.20 (0.33)^{bB}	-26 (10)^{aA}	-34 (4)^{aB}

After 60 d of storage

Treatment	HES		NEOH		NAG	
	Refrigeration	Frozen	Refrigeration	Frozen	Refrigeration	Frozen
FS	-65 (2)^{aA}	-65 (1)^{aA}	-42 (6)^{aA}	-36 (2)^{aA}	-81 (4)^{aA}	-89 (2)^{aA}
CP	-52 (5)^{aA}	-49 (6)^{aB}	-25.3 (0.8)^{aB}	-25 (3)^{aB}	-59 (2)^{aB}	-69 (5)^{aB}
MP	-55 (6)^{aA}	-47 (1)^{aB}	-19.3 (0.2)^{aB}	-15 (3)^{aC}	-66 (1)^{aB}	-66 (3)^{aB}

The same capital letter in superscript within columns indicates homogeneous groups established by the ANOVA ($p<0.05$) comparing treatments.
For each flavonoid, the same small letter in superscript within rows indicates homogeneous groups established by the ANOVA ($p<0.05$) comparing storage conditions.
HES: Hesperidin, NEOH: Neohesperidin and NAG: naringenin

Table 4.

	Refrigeration			Frozen		
	FS	CP	MP	FS	CP	MP
NAT	-17.3 (0.2)^{c}	-19 (2)^{bc}	-23.0 (0.7)^{a}	-21 (1)^{ab}	-16.77 (0.05)^{c}	-12 (1)^{d}
NAR	-14 (4)^{ab}	-18 (2)^{ab}	-21.17 (0.13)^{a}	-18 (2)^{ab}	-13.7 (0.5)^{b}	-13 (4)^{b}
HES	-65 (2)^{a}	-65.28 (0.02)^{a}	-63 (1)^{a}	-65.0 (0.9)^{a}	-62.8 (0.9)^{a}	-57 (4)^{b}
NEOH	-42 (6)^{a}	-39.0 (0.6)^{ab}	-34 (3)^{ab}	-36 (2)^{ab}	-38 (4)^{ab}	-30 (6)^{b}
DID	3.1 (0.3)^{c}	-19 (5)^{a}	-15 (1)^{ab}	-7 (3)^{ab}	-16 (9)^{ab}	-2 (1)^{c}
PON	-15 (5)^{a}	18 (10)^{b}	16 (1)^{b}	15 (4)^{b}	15 (5)^{b}	17 (6)^{b}
QUER	-34 (7)^{a}	-28 (4)^{ab}	-35 (4)^{a}	-38 (8)^{a}	-18 (3)^{b}	-19.2 (0.9)^{b}
NAG	-81 (4)^{ab}	-78 (5)^{b}	-85 (4)^{ab}	-89 (2)^{a}	-88 (2)^{a}	-85 (2)^{ab}
TOTAL	-21 (3)^{bc}	-22 (1)^{abc}	-25.9 (0.4)^{a}	-25 (2)^{ab}	-19.21 (0.12)^{cd}	-17 (2)^{c}

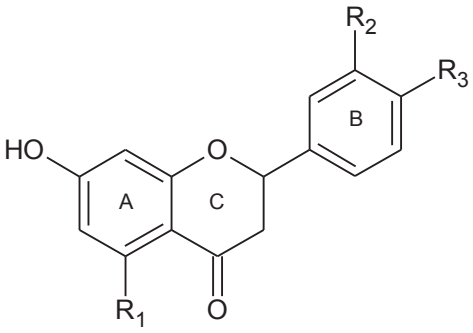
The same letter in superscript within rows indicates homogeneous groups established by the ANOVA ($p<0.05$)
NAT: Narirutine, NAR: Naringin, HES: Hesperidin, NEOH: Neohesperidin, DID: Didymin, PON: Poncirin,
NAG: Naringenin and QUER: Quercetin.

Table 5.

	NAT	NAR	HES	NEOH	DID	PON	NAG	QUER
Total Flavonoids	0.8385*	0.9214*	0.8249*	0.6708*	0.3510*	-0.1092	0.5541*	0.8124*
NAT		0.6890*	0.6604*	0.5332*	0.3320*	-0.0594	0.4370*	0.5939*
NAR			0.6469*	0.4357*	0.2379*	-0.1384	0.3072*	0.6306*
HES				0.8201*	0.2162*	-0.3117*	0.7767*	0.7319*
NEOH					0.2051*	-0.1869	0.8336*	0.6461*
DID						0.0972	0.1050	0.4042*
PON							-0.3632*	0.0606
NAG								0.4975*

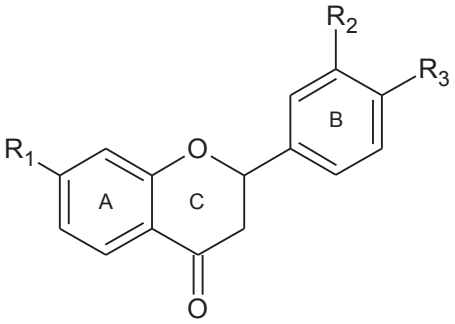
* Correlation is significant at the 0.05 level
NAT: Narirutine, NAR: Naringin, HES: Hesperidin, NEOH: Neohesperidin, DID: Didymin, PON: Poncirin, NAG: Naringenin and QUER: Quercetin.

Figure 1



Flavone Aglycone

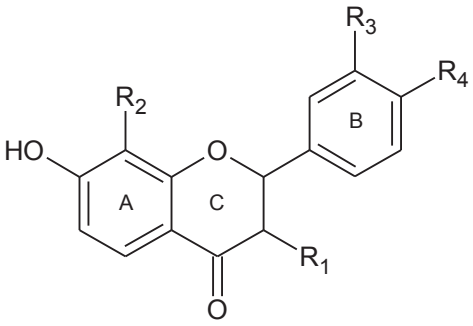
Flavonoid	R_1	R_2	R_3
Naringenin	OH	H	OH



Flavanone O-glycoside

Flavonoid	R_1	R_2	R_3
Narirutine	O-Ru	H	OH
Naringin	O-Nh	H	OH
Hesperidin	O-Ru	OH	O-CH_3
Neohesperidin	O-Nh	OH	O-CH_3
Didymin	O-Ru	H	O-CH_3
Poncirin	O-Nh	H	O-CH_3

Ru: rutinoside
Nh: neohesperidoside



Flavonol Aglycone

Flavonoid	R_1	R_2	R_3	R_4
Quercetin	OH	H	OH	OH

Figure 1.

Figure 2

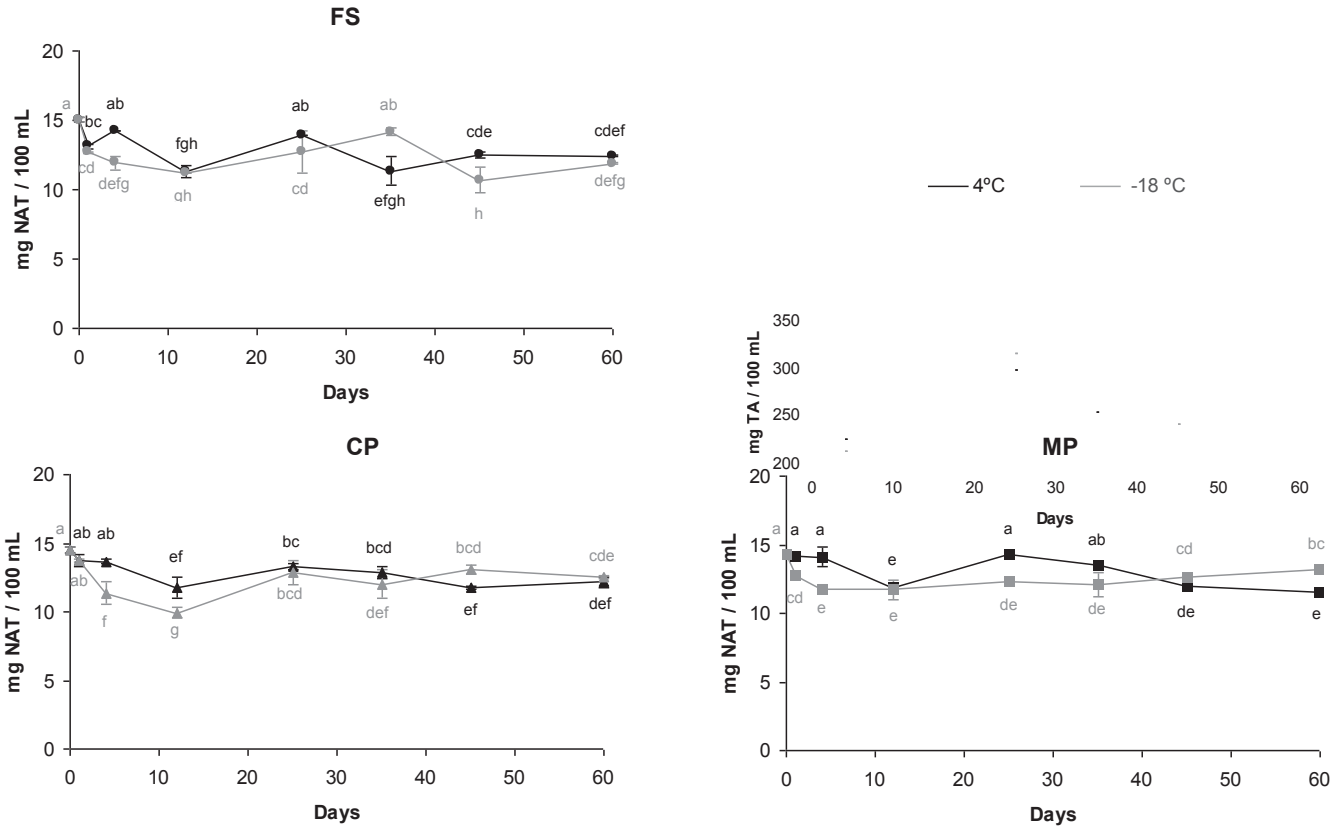


Figure 2.

Figure 3

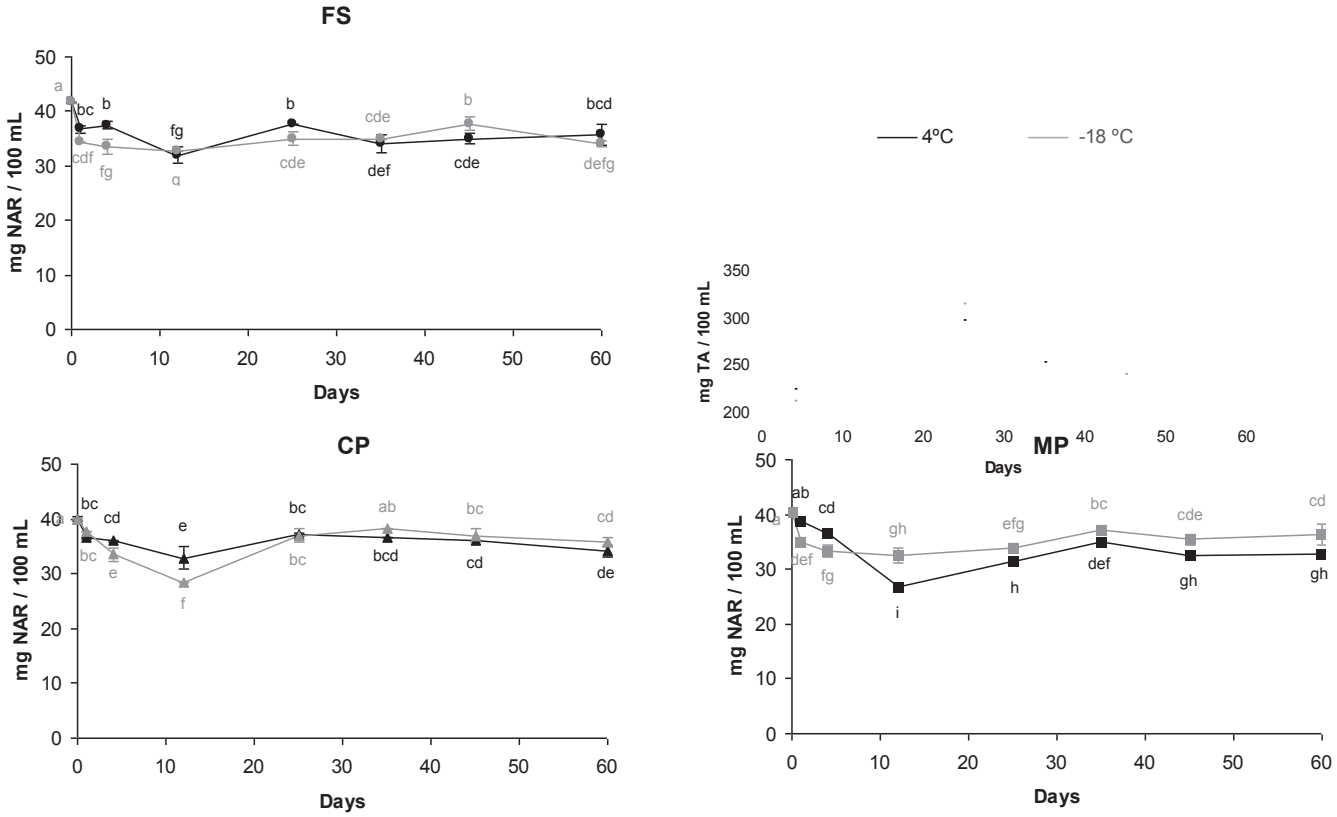


Figure 3.

Figure 4

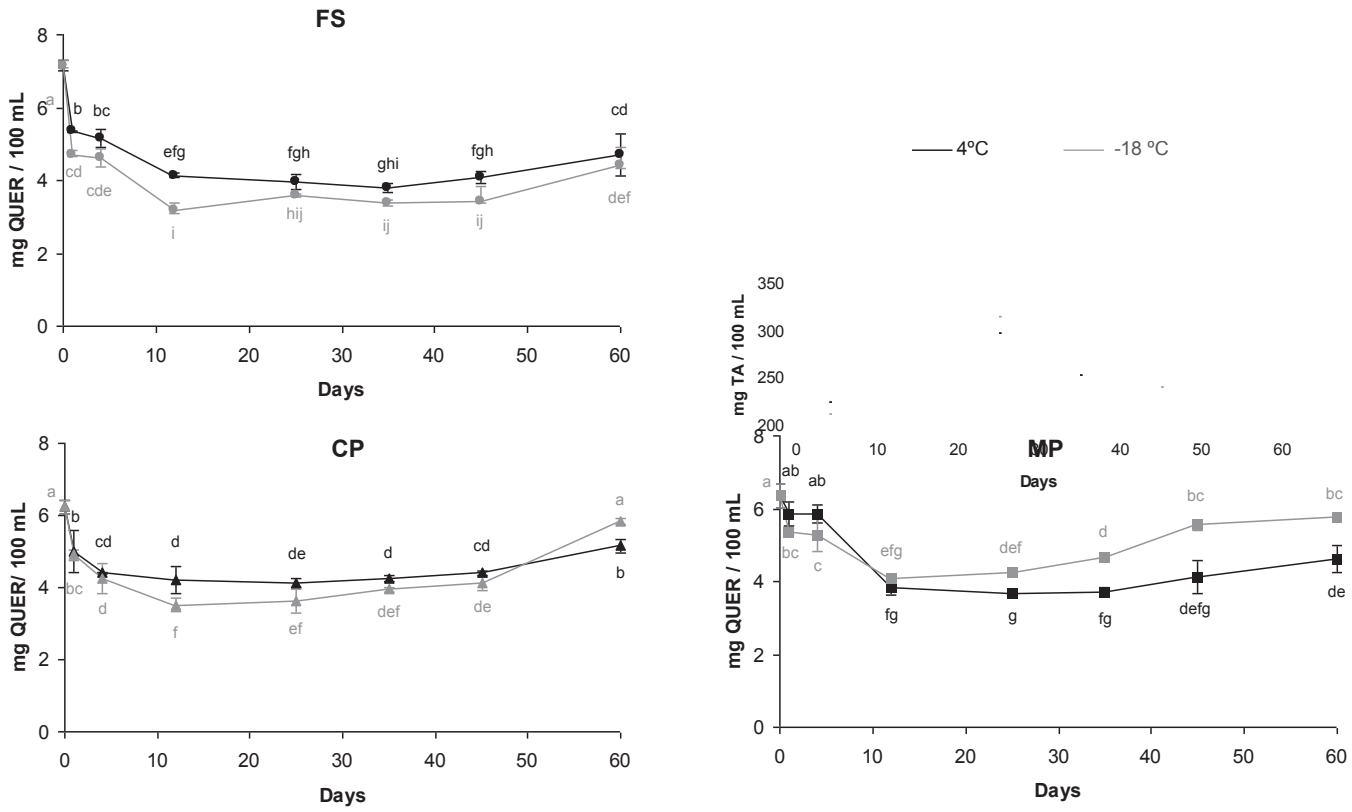


Figure 4.

Figure 5

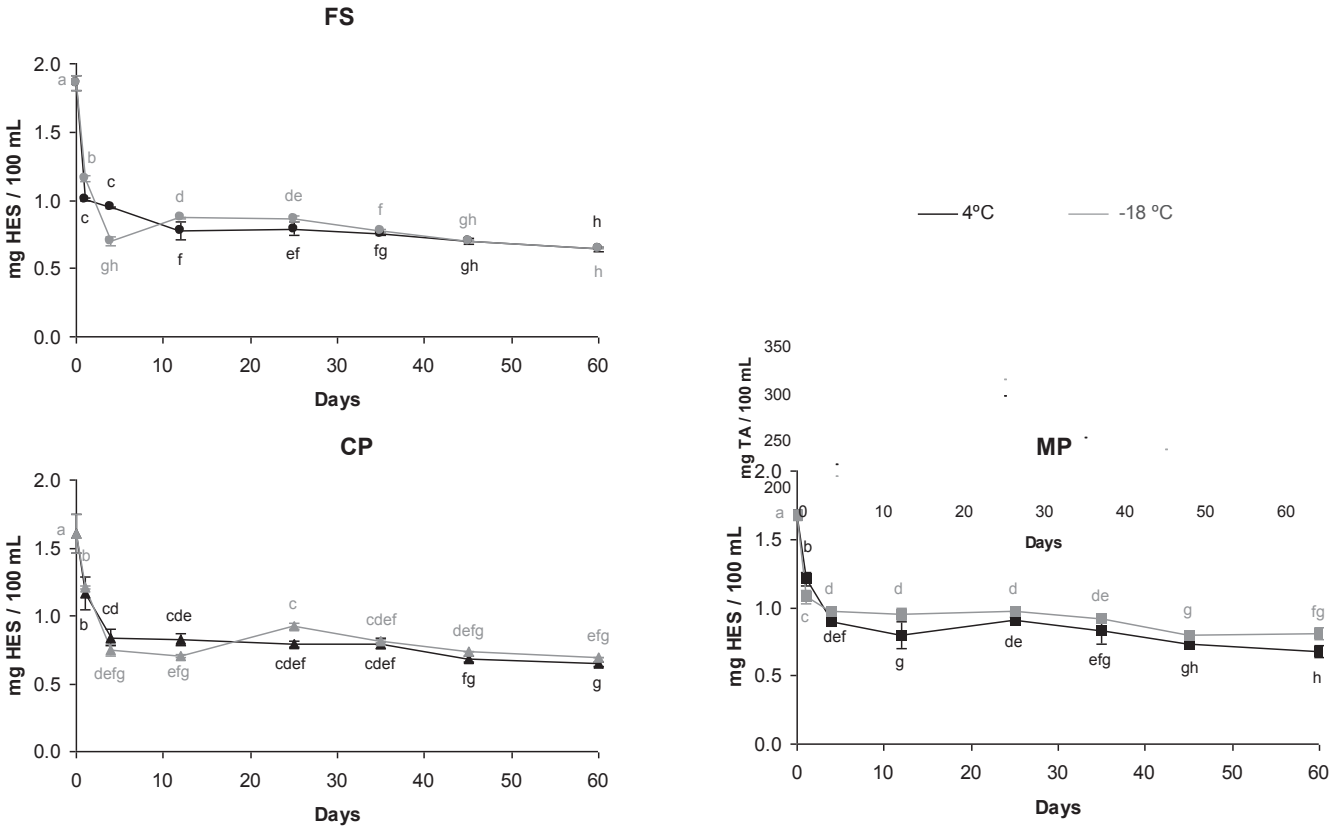


Figure 5.

Figure 6

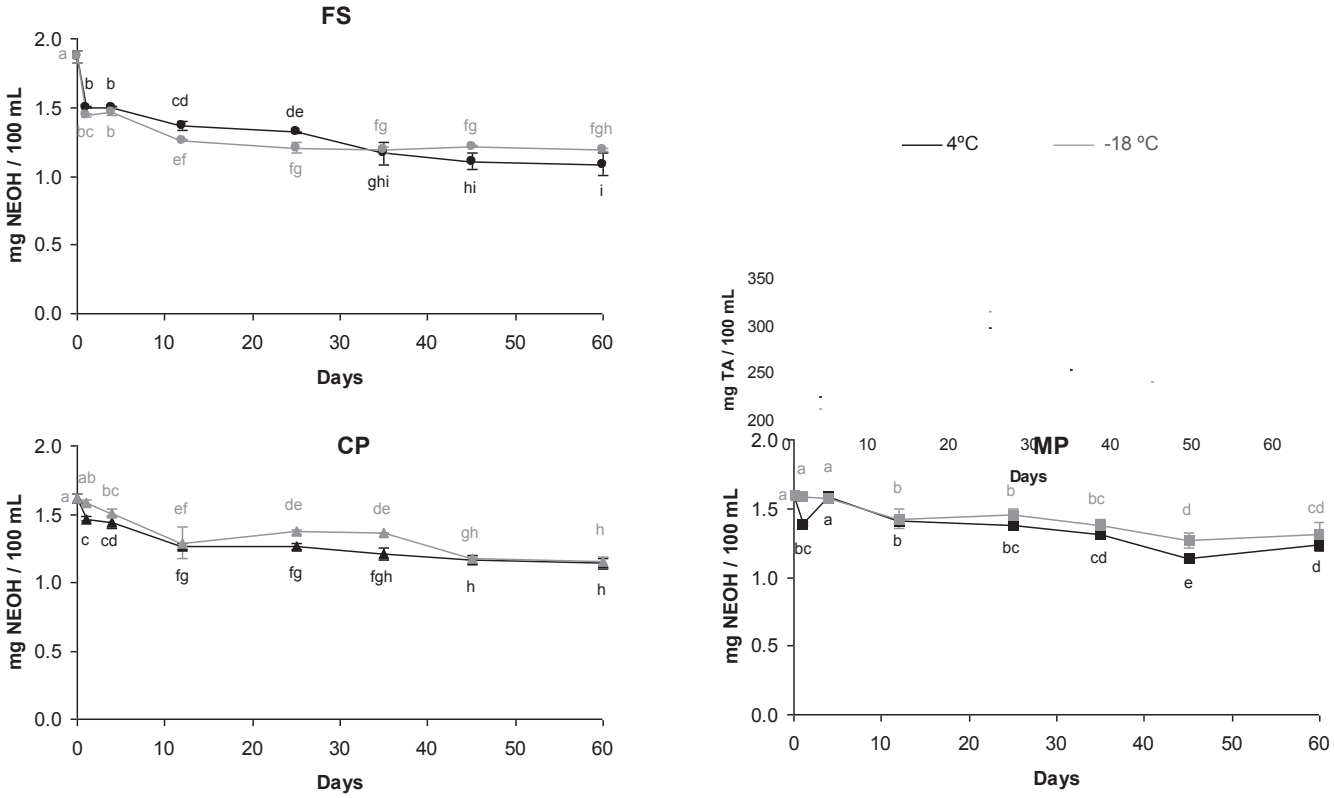


Figure 6.

Figure 7

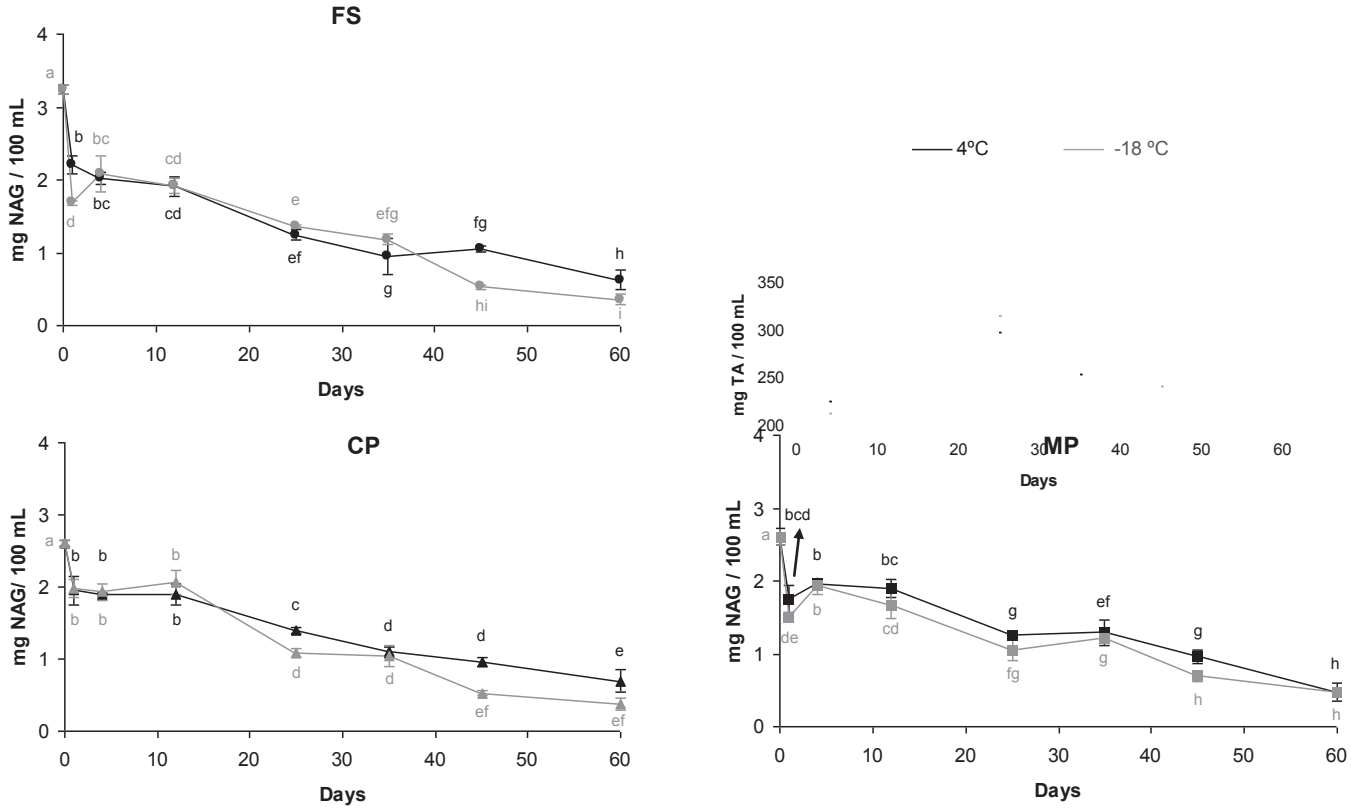


Figure 7.

Figure 8

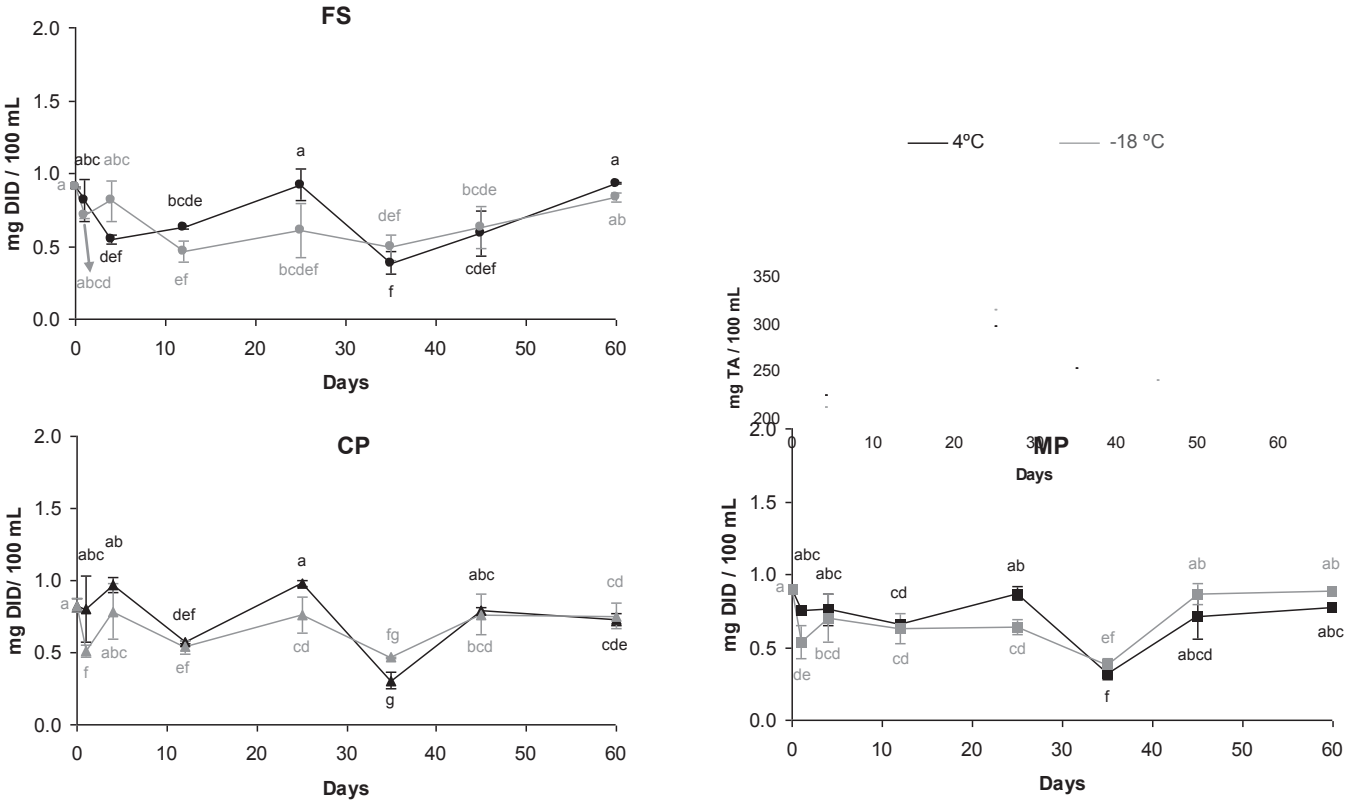


Figure 8.

Figure 9

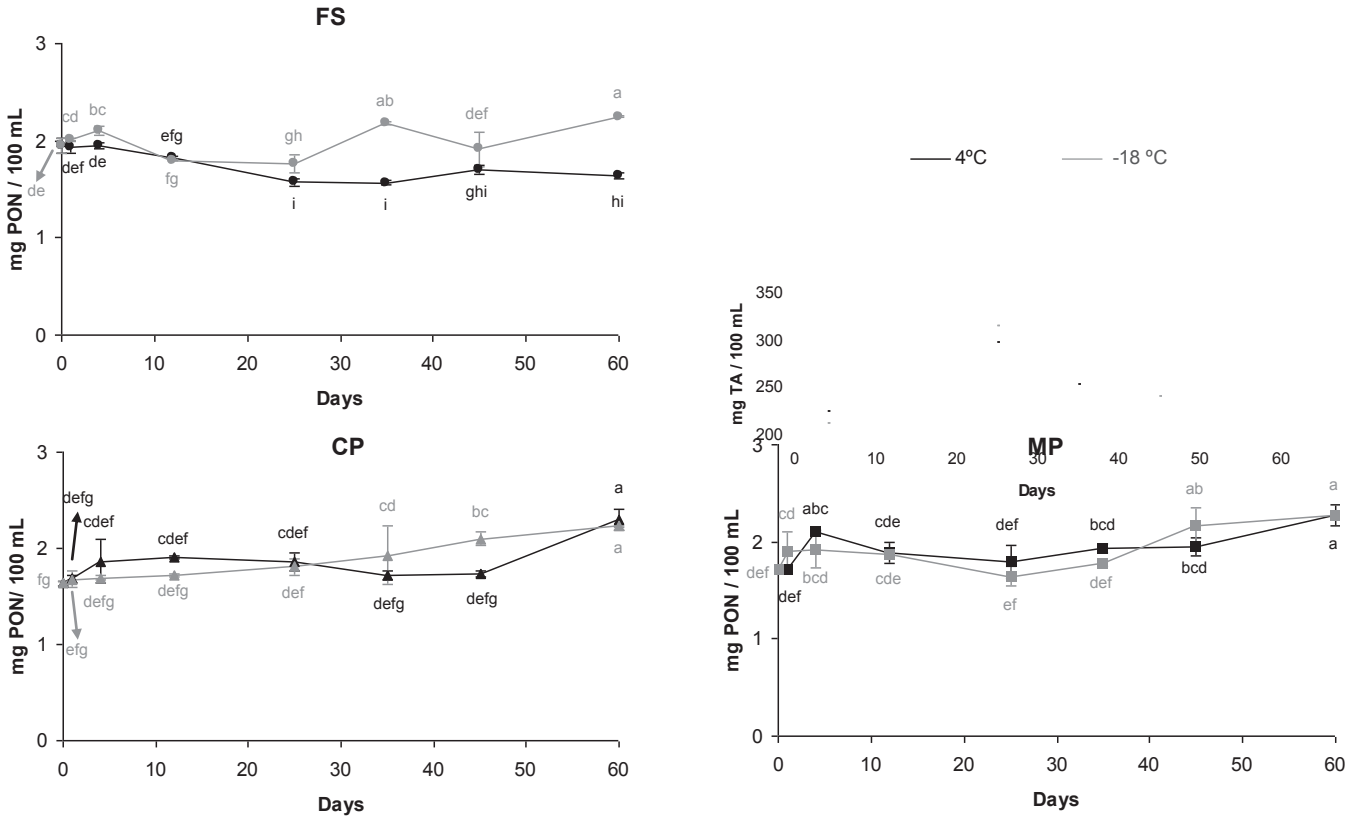


Figure 9.

Figure 10

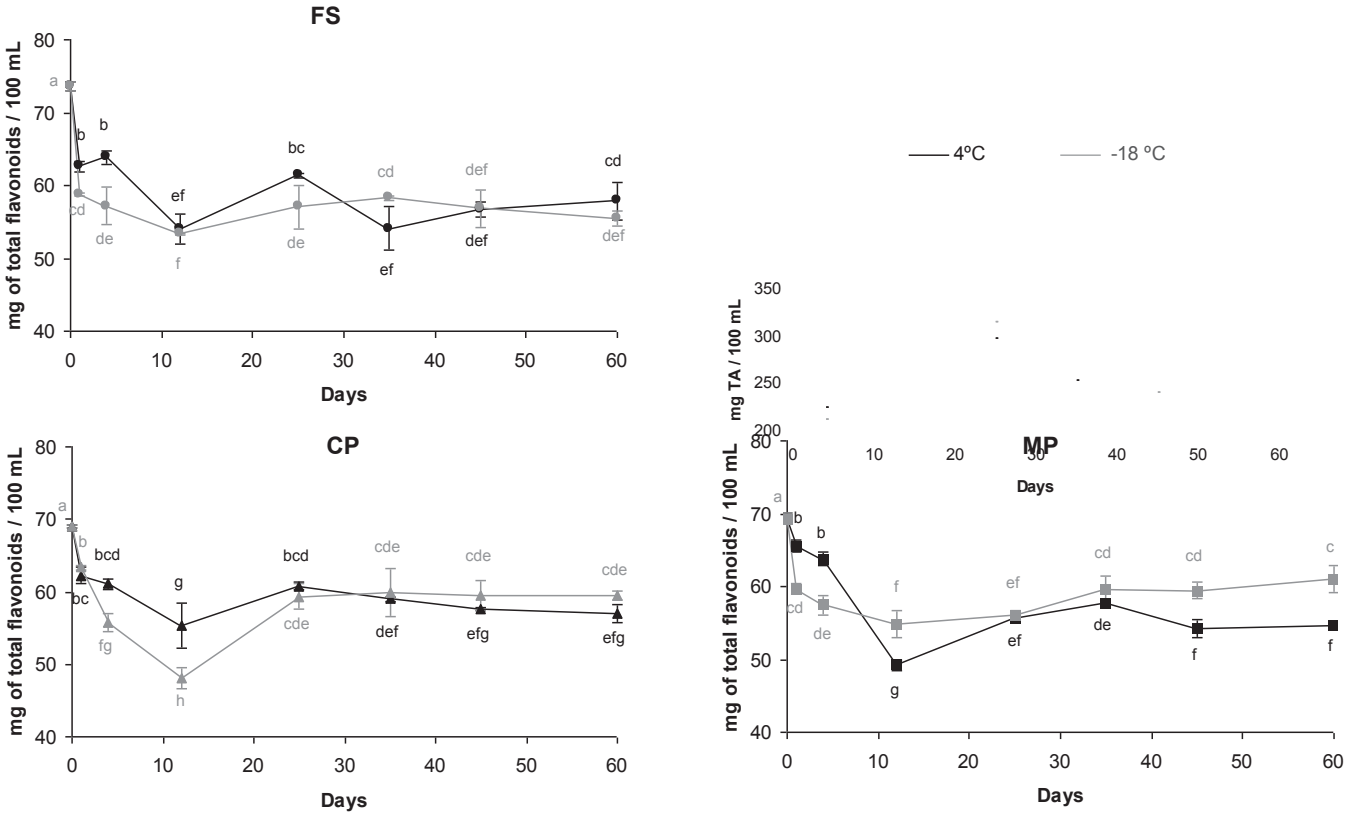


Figure 10.

Figure 11

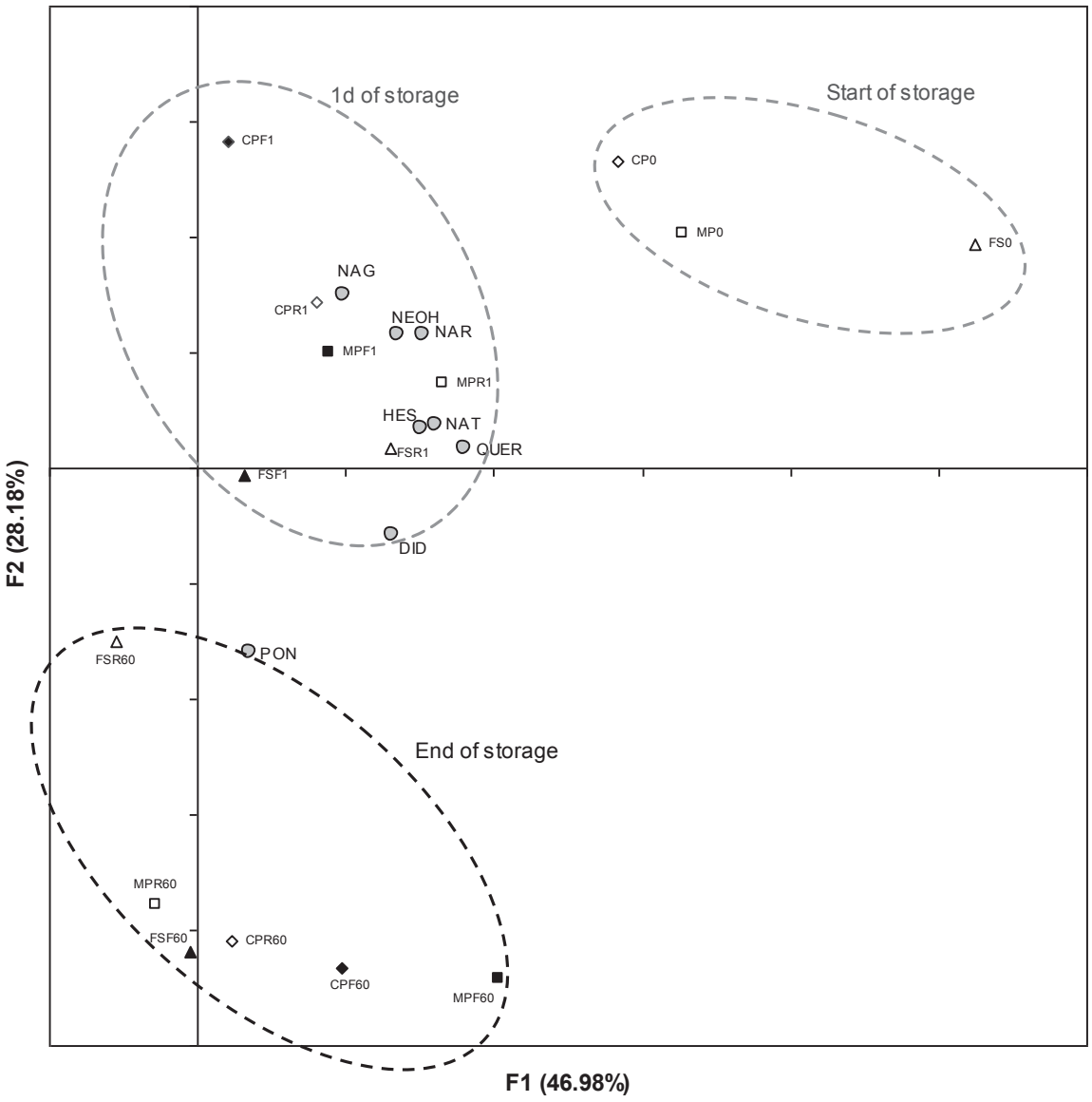


Figure 11.