Impact of ozone processing on microbiological, physicochemical and bioactive characteristics of refrigerated stored *Cantaloupe* melon juice

Sengly Sroy, Joana F. Fundo, Fátima A. Miller, Teresa R.S. Brandão, Cristina L. M. Silva*

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Arquiteto Lobão Vital, 172, 4200-374 Porto, Portugal

Email: clsilva@porto.ucp.pt
Phone: +351 22 5580058
Fax: +351 22 5090351
*Corresponding author

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Abstract

The objective of this study was to evaluate the impact of ozone treatment on microbiological decontamination (intrinsic microflora and inoculated *Listeria innocua*) and some physicochemical characteristics and bioactive compounds of *Cantaloupe* melon juice, also during refrigerated storage.

To determine an adequate ozone exposure, the survival curve of *L. innocua* was previously assessed. A thermal treatment was also performed seeking comparison with ozone treatment impact.

After ozone exposure, *L. innocua* was not detected in juice samples, while thermal pasteurization allowed a reduction of 5.2±0.2 log-cycles. Although ozone reduced the intrinsic microflora loads, this reduction was higher for heat treated samples.

Vitamin C was highly retained in ozone treated juices (68%), when compared with the pasteurized ones (39%). After 13 days of storage, ozone allowed retention of the most quality parameters analysed and, therefore, it can be considered as a promising alternative to traditional pasteurization of *Cantaloupe* melon juice.

Practical application

The actual consumers demand for high-quality food standards have launched research to alternative and milder non-thermal processes, which have gained increasing attention and importance in the fruit juice industries. Ideally, preservation and/or processing of foods should involve technologies that prevent undesirable microbial survival and minimize quality attributes changes and nutrient losses. Thermal treatments are conventionally used to attain such targets, however, the content and the biological activity of the most health-related compounds are dramatically reduced. In this context, and particularly for the beverages industries, ozone has been exploited due to their potential for inactivating spoilage and pathogenic microorganisms while being effective in overall quality retention of the products.

Keywords (6 maximum): fruit juices, non-thermal technologies, thermal pasteurization, storage, *Listeria innocua*, bioactive compounds

1. Introduction

Cantaloupe melon is a commonly consumed fruit worldwide due to its nutritional and organoleptic properties, being recognized as a good source of bioactive compounds, with a desirable flavor, appreciated texture and low caloric value (Mukhopadhyay et al., 2016). Due to these healthy and sensorial properties, together with high water content and sugar concentration, *Cantaloupe* melon is extensively used as raw material in fruit juices industry. Most of fresh juices hold a population of 4 to 6 log microorganisms per gram (Ukuku et al., 2016) and particularly *Cantaloupe* melon has been associated to several outbreaks, since its composition is favorable to the growth of pathogenic and spoilage microorganisms (Kaya et al., 2015). From 1973 to 2011, the Centre Disease Control and Prevention reported 34 foodborne disease outbreaks that were caused by the consumption of contaminated melons, in which 19 were caused by *Cantaloupe* melons (Nyarko et al., 2016). Among all the foodborne pathogens, *Listeria monocytogenes* is the most dangerous due to its high mortality rate of 20 to 25% (Todd and Notermans, 2011). In 2011 occurred one of the deadliest outbreak caused by *Listeria monocytogenes* in USA, which was also linked to *Cantaloupe* melon consumption (McCollum et al., 2013). Food and Drug Administration (FDA) stablished that fruit juices industry should apply treatments that will allow a reduction of 5 $\log_{10}$ of target spoilage and/or pathogenic microorganisms (Tiwari et al., 2009c). Thermal processes are traditionally applied and their effectiveness on fruit juices decontamination has been widely studied (Torlak, 2014). However, high temperatures dramatically impacts sensorial attributes (e.g. color, flavor) and the concentration of important bioactive and nutritional compounds, such as vitamins and phenolics, are also negatively affected (Fonteles et al., 2012; Sung et al., 2014). To avoid these problems, several non-thermal technologies are being exploited in order to guarantee fruit juices safety and retention of quality and nutritional characteristics (Miller et al., 2013). Among the non-thermal technologies, ozone is a promising one with potential application to fruits sanitation (Silveira et al., 2018) and preservation (Patil et al., 2010; Jaramillo Sánchez et al., 2018). In 2001, FDA approved ozone application as a direct food additive for the

85 treatment, storage, and processing of foods, due to its classification as “Generally Recognized
86 as Safe” (GRAS status) (Torres et al., 2011). Ozone technology allows to achieve 5-log cycles
87 microbial reduction by oxidation of microorganisms’ cellular components (Patil et al., 2010;
88 Torlak et al., 2013) and it is also considered a green technology, environmentally friendly (Patil
89 et al., 2009). Several studies reported microbial reductions in fruit juices treated with gaseous
90 ozone. More than 5 $\log_{10}$ reductions of *E.coli* were achieved by Tiwari et al. (2009c), Patil et
91 al. (2009) and Torlak (2014), for strawberry, orange and apple juices, respectively. Garcia
92 Loredo et al. (2015) reported almost 5 $\log_{10}$ reduction of *L. innocua* in ozone treated peach
93 juice. Fundo et al. (2018a) evaluated the effectiveness of ozone treatments on *A.*
94 *acidoterrestris* spores inactivation in melon juice, and concluded that this technology can be
95 an adequate decontamination process.
96 All these facts contributed for the potential of ozone application and encouraged several
97 commercial fruit juices industries in USA and Europe to start using ozone as an alternative
98 non-thermal treatment (Torres et al., 2011).
99 The objective of this study was to assess the impact of ozone exposure on the quality and
100 safety attributes of *Cantaloupe* melon juice by comparison with thermal pasteurization. *L.*
101 *innocua* was used to assess effective ozone exposure time. Some physicochemical
102 characteristics (color, pH and soluble solids content), bioactive compounds (total phenolics
103 and vitamin C), total antioxidant activity, and microbial indicators (total mesophylls, and yeasts
104 and molds). These characteristics were evaluated before and after treatments and during 13
105 days of refrigerated storage.
106
107 **2 Material and methods**
108
109 2.1 Sample preparation
110 *Cantaloupe* melons (*Cucumis melo L.* var. *reticulatus)* were acquired in a local supermarket.
111 Juice was obtained using a domestic centrifuge (Centrifugal juicer Excel JE850, UK).

112 Part of the juice (600.0 mL) was inoculated with *L. innocua* culture. *L. innocua* 2030c cultures
113 of 10^7 CFU/mL at stationary phase were obtained as described by Miller, Gil, Brandão,
114 Teixeira, and Silva (2009).
115
116 2.2 Ozone treatments
117 Ozone gas was generated with a corona discharge equipment model (OZ5, SPO3-Sociedade
118 Portuguesa de Ozono, Lda. Porto, Portugal) producing ozone at 5 g/h. The methodology was
119 the one described by Fundo et al. (2018a). Pure oxygen was supplied via an oxygen cylinder
120 at 0.5 bar. Ozone at a concentration of 7.7 ± 2.4 g/L was pumped directly into an Erlenmeyer
121 flask containing 300.0 mL of juice continuously agitated. Ozone concentration was monitored
122 by potential difference (Redox probe; SZ 275, B & C Electronics, Carnate, Italy). This redox
123 probe was connected to a controller with a set point, which allowed the monitoring of ozone
124 production and the consequence maintenance of ozone concentration in the juice samples
125 during the treatment time. The ozone concentration was determined by the iodometric titration
126 method (IOA, 1996), also described by Torlak et al. (2013). For juices inoculated with *L.*
127 *innocua*, samples of 1.0 mL were taken every 2 min till a maximum of 10 min of exposure. For
128 uncontaminated samples, ozone was applied for 10 min. Three replicates of each treatment
129 were performed.
130
131 2.3 Thermal pasteurization
132 For both contaminated and non-contaminated samples, the treatment was performed using a
133 thermostatic water bath with stirring capacity (Julabo® FP40, Seelbach, Germany) and
134 temperature control (Julabo® HC-E07, Seelbach, Germany). Juice (300.0 mL) was placed in
135 Erlenmeyer flasks and heated at 72 °C. The juice temperature was measured with a
136 thermometer (± 0.05 °C) and when 72 °C was attained, *L. innocua* suspension was inoculated
137 and both contaminated and non-contaminated samples were maintained at this temperature
138 for 15 seconds. Three replicates of each treatment were performed.
139

140 2.4 Refrigerated storage

141 After the different treatments, 40.0 mL of juice samples were placed into 100.0 mL sterile
142 plastic containers and stored at refrigerated conditions (5±2 °C) till a maximum of 13 days.
143 Analyses were carried out before and after treatments and throughout 13 days of storage.
144 Samples were collected at days 1, 3, 6, 8, 10 and 13 and one plastic container was used for
145 each sampling day.

146

147 2.5 Microbiological analysis

148 For total mesophylls and yeast and molds enumeration, juice decimal dilutions were carried
149 out in buffered peptone water (BPW). Total mesophylls were assessed, in duplicate, using
150 Plate Count Agar – PCA (Lab M, Lancashire, UK). Samples were incubated at 37 °C during
151 48 hours. Yeasts and molds were determined, also in duplicate, using Rose Bengal Agar -
152 RBA (Lab M, Lancashire, UK). Samples were incubated at 25 °C for 60 h. Analyses were
153 carried out for untreated and treated samples, and during storage period.
154 *L. innocua* was quantified in duplicate through decimal dilutions and using Palcam agar
155 containing selective supplement (Merck, Darmstadt, Germany). Samples were incubated at
156 30 °C for 3 days. Analyses were carried out for untreated and treated samples.
157 Three replicates were carried out and the obtained results were expressed as $\log_{10}$ CFU per
158 mL.

159

160 2.6 Physicochemical analysis

161 2.6.1 Color properties

162 Juice color coordinates (*L**, *a**, *b**) were measured using a Minolta CR-400 colorimeter
163 (Konica-Minolta, Osaka, Japan). Two readings of three different replicates were performed for
164 each sample.
165 Total color difference (TCD) was calculated according to Eq. 1 in order to evaluate the color
166 changes of samples (Alibas, 2009; Ihns et al., 2011). Higher TCD values indicate more
167 pronounced color deterioration.

$$TCD = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (1)$$

Where the index “0” indicates the reference sample.

2.6.2 Soluble solids content and pH

Juice soluble solids content (SSC), expressed as °Brix, was directly measured using a Palette PR-32 digital refractometer (Atago, Tokyo, Japan). Juice pH was measured by using a pH meter (GLP 22, Crison Instruments, Spain). For both pH and SSC, duplicate measurements were performed for each replicate.

2.7 Bioactive compounds

2.7.1 Total phenolics content

25.0 mL of juice were blended in 50.0 mL of 100% methanol (Merck), using an ultra-turrax homogenizer (Ika digital T25, IKA®-Werke GmbH & Co. KG, Staufen, Germany). The remaining method is in accordance with the one described by Fundo et al. (2018b) and samples phenolics content were reported as µg gallic acid equivalent per mL of juice.

2.7.2 Vitamin C

Vitamin C (ascorbic acid (AA) plus dehydroascorbic acid (DHA)) were determined using a HPLC system (Jasco, Japan) equipped with a PU-1580 pump and an AS-1555 injector. Separation was carried out using a reverse phase C18-silica analytical column (Waters Spherisorb ODS2 5 µm 4.6 x 250 mm). Mobile phase, standard solutions and juice samples were prepared according to Fundo et al. (2018b), following the method .previously described by Zapata and Dufour (1992). The peaks were detected using a Refractive Index detector (RI-1530, Jasco) and analyzed using Jasco-Borwin software v.1.50 (JMBS Developments, Fontaine, France). Results were expressed in mg of vitamin C per 100 mL of juice.

2.8 Total antioxidant capacity

196 Extractions were carried out as described for total phenolics determination, and the ABTS
197 assay was performed according to Fundo et al. (2018b). Total antioxidant activity results were
198 expressed as µg of ascorbic acid per mL of juice.
199
200 2.9 Statistical analysis
201 Differences between samples, concerning all characteristics analysed, were detected by one-
202 way ANOVA. Tukey's test was performed for post-hoc pairwise differences of means.
203 Shapiro-Wilk and Levene's tests were used to assess data normality and homoscedasticity,
204 respectively. Kruskal-Wallis test was carried out alternatively to one-way ANOVA when data
205 normality was not proved. In such cases, Mann-Whitney non-parametric test was posteriorly
206 performed to detect differences. In all analyses performed, 5% of significance level was
207 assumed. Results were expressed as mean ± margin of confidence interval at 95%. Data
208 analyses were carried out using IBM SPSS Statistics 24 for Windows® (SPSS Inc., Chicago,
209 USA).
210
211 3 **Results and discussion**
212
213 3.1 *L. innocua* inactivation curve
214 In order to determine an adequate ozone exposure, the inactivation curve of *L. innocua* was
215 previously assessed. After 6 min of ozone treatment, *L. innocua* reduced 5.3 log cycles; after
216 10 min of treatment *L. innocua* was no longer detected (Fig. 1). In this way, 10 min of exposure
217 was the selected time for the ozone treatment.
218 In agreement with these results, several studies also reported microbial reductions in fruit
219 juices, such as strawberry, orange, apple and peach, treated with gaseous ozone (Garcia
220 Loredo et al., 2015; Patil et al., 2009; Tiwari et al., 2009c; Torlak, 2014).
221
222 3.2 Comparison of the impact of thermal pasteurization and ozone treatment
223 3.2.1. Microbial indicators

L. innocua (non-pathogenic surrogate of *L. monocytogenes*) was the selected target microorganism to determine ozone exposure time. As previously mentioned, after 10 min of ozone exposure, *L. innocua* was no longer detected in juice samples. For comparison, a traditional thermal pasteurization (72 °C for 15 seconds) was also applied. As can be observed in Figure 1, a 5.2 log cycles decay was achieved with thermal pasteurization. This result is in accordance with FDA requirements for an efficient juice processing. Mena et al. (2013) also obtained 5.0 log reductions in pathogenic bacteria (*Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes*) in fruit juices using milder heat treatments (71.1 °C for 3 seconds).

The effectiveness of both ozone exposure and heat treatments was also evaluated in terms of juice intrinsic microflora. Therefore, total mesophylls and yeasts and molds were determined before and after treatments. Results are presented in Table 1. As it can be observed, 10 min of ozone exposure did not significantly reduce juice total mesophylls, while traditional pasteurization significantly inactivated this group of microorganisms. Although both treatments significantly decreased yeasts and molds counts, this effect was much more evident in pasteurized juice. Mena et al. (2013) also observed the effect of thermal processing on mesophylls inactivation in pomegranate juice, and obtained 3.9 and 4.1 log cycles reduction after 30 and 60 seconds respectively, at 65 °C. Habibi Najafi and Haddad Khodaparast (2009) stated that these groups of microorganisms may be more resistant to inactivation treatments, since they naturally occur in fruit juices. The same authors reported that 1 hour of ozone exposure of date fruits at a concentration considerable lower that the one used in this experimental work (0.005 g/L), could be successfully used for reducing coliforms. However, longer exposure times or higher ozone concentrations may be required for the elimination of the total mesophilic bacteria, yeasts and molds.

3.2.2. Physicochemical characteristics

The effects of thermal pasteurization and 10 min of ozone treatment on some physicochemical parameters of melon juice are shown in Table 2. For a global perception of color alterations of treated juices, TCD was calculated (the reference juice samples were the untreated ones).

252 In accordance with the scale reported by Cserhalmi et al. (2006) the perceivable differences
253 obtained for ozone treated samples were more visible than the ones attained for heat treated
254 samples. The negative impact of ozone exposure on fruit juices color has also been reported
255 for other fruits, such as apple (Patil et al., 2010), grape (Tiwari et al., 2009b), orange (Tiwari
256 et al., 2008), strawberry (Tiwari et al., 2009c) and tomato juices (Tiwari et al., 2009a). Different
257 compounds are responsible for color changes, depending on the fruit (Miller et al., 2013). In
258 this specific case, a possible explanation for the color changes that occurred after both
259 treatments could be the carotenoid pigments degradation.
260 Regarding pH, no significant differences were found in thermal and ozone treated juices. This
261 is in agreement with several authors that also reported no significant changes in pH of
262 thermally treated orange juice (Rabie et al., 2015) and grape, and orange and tomato ozonized
263 juices (Tiwari et al., 2008; Tiwari et al., 2009a; Tiwari et al., 2009b). pH can be considered an
264 important fruit juice physicochemical characteristic, since it can be associated with the stability
265 of bioactive compounds (Rabie et al., 2015).
266 Soluble solids content (SSC) were not affected by both thermal and ozone treatments, similar
267 to what has been reported in the majority of published works (Mariana et al., 2014; Miller et
268 al., 2013; Mukhopadhyay et al., 2016).
269
270 3.2.3. Bioactive parameters and total antioxidant activity
271 Total phenolics content was one of the bioactive indicators used for the assessment of the
272 impact of thermal and ozone treatments on *Cantaloupe* melon juice. This group of compounds
273 provides antioxidant potential and health-promoting properties and contributes to the flavor
274 and color attributes of fruits and vegetables (Rabie et al., 2015). Results include in Table 3
275 show that no significant differences were attained either between fresh and treated samples,
276 or between treatments.
277 Vitamin C is also considered an indicator of the nutritional quality of fruit juices (Rabie et al.,
278 2015). Although results showed a significant decrease of this parameter after both treatments
279 applied, this decrease was higher for heat treated samples (Table 3). Ozonized juices retained

68% of vitamin C, while pasteurized samples only preserved 39%. Ascorbic acid is unstable during processing and its degradation is induced by factors such as high temperatures and oxygen availability (Wibowo et al., 2015b). Although ascorbic acid might be oxidised by direct interaction with ozone (Tiwari et al., 2009c), its degradation is higher when subject to heat treatment. Higher temperatures lead to higher vitamin C degradation (Zahid et al., 2008). This may be related to the fact that exposure to ozone may cause cells oxidative stress, which probably induce defense mechanisms, allowing the maintenance of some antioxidants compounds, such as ascorbic acid (Miller et al. 2013).

The antioxidant activity of fruits is due to the presence of vitamin C, carotenoids, and polyphenols. Both processes significantly affected this parameter (Table 3). However, an increase was achieved after pasteurization, while with ozone treatment a decrease of antioxidant activity was observed. According to Wibowo et al. (2015b), pasteurization could have oxidized ascorbic acid, which may act as an antioxidant compound. Ozone causes the loss of antioxidant compounds due to its strong oxidizing activity (Miller et al., 2013).

In conclusion, it is possible to observe that ozone exposure and heat pasteurization are comparable in terms of *L. innocua* inactivation and impact on the majority of physicochemical and bioactive parameters of melon juice. However, heat treatments allowed a higher reduction of the juice intrinsic microflora and had a milder impact on color alterations.

3.3. Effect of treatments on melon juice shelf-life

3.3.1. Microbial parameters

Since more than 5 $\log_{10}$ cycle's reduction of artificially inoculated *L. innocua* were obtained with thermal treatment and 10 min of ozone exposure, mesophylls and yeasts and molds were the microorganisms monitored during the storage period (Fig. 2). As can be observed in Fig. 2(a), total mesophylls behavior was significantly different between untreated and treated samples. Untreated samples showed a significant increase of total mesophylls during the storage time. Regarding ozonized samples, mesophylls remained almost constant during 13 days, while in pasteurized juice a slight increase occurred. However, both treatments

appeared to be effective on mesophylls control.

Regarding yeasts and molds, thermal treatment inactivated completely this group of microorganisms, which were not detected throughout all storage period. Concerning untreated and ozonized samples, no significant differences were observed. In both cases, a slight but not significant increase was observed during the 13 days of refrigerated storage.

3.3.2. Physicochemical indicators

TCD was the parameter used to assess juice color changes (the reference value was the one obtained before storage for a given treatment) during the storage period (Figure 3(a)). For all untreated and treated samples, this parameter significantly increased from day 0 to day 13 of storage. Wibowo et al. (2015c) explained this behavior with the possible formation of colored compounds throughout the polymerization of intermediate degradation products from ascorbic acid or sugars. Although, at the end of storage period, ozonized juices presented the lowest TCD values, these results were not significantly different from the ones obtained for untreated and pasteurized samples.

Regarding pH behavior (Fig. 3(b)), a significant increase was observed for untreated samples while in treated juices this parameter was maintained almost constant during all storage period. Although contradictory results were found in the literature (Rabie et al., 2015; Zahid et al., 2008), some works also reported no significant differences in the pH of ozonized and pasteurized fruit juices along storage (Mena et al., 2013; Vegara et al., 2013; Wibowo et al., 2015a).

SSC (results not shown) was constant during all storage period for both untreated and treated samples. In general, no significant changes in fruits SSC have been reported when short-term gaseous ozone treatments were applied (Miller et al., 2013). Concerning heat treatments, also some contradictory study results were found in literature. According to Wibowo et al. (2015b) pasteurization did not change total sugar content during the storage of orange and lemon juices. However, Zahid et al. (2008) observed a significant increase on SSC of apple juice during the storage time.

336

337 3.3.3. Bioactive compounds and total antioxidant activity

338 Results on the effect of thermal pasteurization and ozone treatment on total phenolics, during
339 refrigerated storage are presented in Fig. 4(a). No significant differences were attained either
340 between treatments or during the storage period. This is in accordance with the majority of
341 reported studies, which stated no significant changes in this parameter after ozone processing
342 (Nicole et al., 2015) and thermal treatment (Klimczak et al., 2007; Odriozola-Serrano et al.,
343 2009).

344 Regarding vitamin C (Fig. 4(b)), a significant decrease was observed during the storage period
345 and for both untreated and treated samples. The vitamin C loss at the end of storage period
346 was 50, 37 and 45% for untreated, pasteurized and ozonized juices, respectively. Wibowo et
347 al. (2015a) also observed a decrease in ascorbic acid concentration during storage of
348 ozonized mango juice. This degradation can be attributed to the dissolved oxygen in the juice
349 and the one present in the headspace on the early stages of storage (Torres et al., 2011).
350 Throughout the aerobic degradation pathway, ascorbic acid is easily oxidized and
351 decomposed in the presence of oxygen (Yuki et al., 2005).

352 Total antioxidant activity (Fig. 4(c)) of ozonized samples was maintained constant during the
353 storage period. In contrast, on pasteurized juice, a significant decrease was observed for this
354 parameter at the end of storage time. Despite the loss of antioxidant compounds, immediately
355 after ozone exposure due to its strong oxidizing activity, its oxidative stress may also induce
356 some defence mechanisms in fruits, allowing the maintenance of this parameter (Miller et al.,
357 2013).

358 Generally, comparing the effects of both treatments on juice shelf-life it is possible to conclude
359 that ozone was more effective on the control of intrinsic microflora and on the maintenance of
360 physicochemical characteristics, phenolic compounds and antioxidant activity.

361

362 **4. Conclusions**

363 Ozone can be successfully applied for melon juice decontamination, since 10 min of treatment

364 was able to reduce more than 5 $\log_{10}$ cycles of artificially inoculated *L. innocua*.
365 Regarding juice intrinsic microflora, heat treatment was more effective on the reduction of the
366 microorganisms. However, during the storage period, ozone also allowed the maintenance of
367 intrinsic microflora loads.
368 Although no significant differences between ozonized and pasteurized juices were observed
369 in the majority of quality parameters analysed, ozone was more effective on vitamin C
370 preservation. During the storage period, ozone treated samples also retained better all
371 physicochemical characteristics, phenolic compounds and antioxidant activity.
372 Despite ozone effectiveness is dependent on a wide range of process conditions, such as flow
373 rate, concentration or temperature, also intrinsic characteristics (pH, soluble solids content,
374 and composition) of the juices may influence conclusions. However, the results obtained
375 demonstrated that ozone can be considered as a promised alternative to thermal
376 pasteurization of melon juices.
377

Conflict of interest

There are no conflicts of interest regarding this paper.

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Figure Captions

Figure 1. Inactivation of *L. innocua* in inoculated melon juice without treatment (♦), pasteurized ( ) and with ozone exposure (○). Data represents mean values and bars the limits of confidence intervals at 95%.

Figure 2. Total mesophylls (a) and yeasts and molds (b) for melon juice without treatment (♦), pasteurized ( ) and ozonized (○), along refrigerated storage. Data represents mean values and bars the limits of confidence intervals at 95%.

Figure 3. Total color difference (a) and pH (b) of melon juice without treatment (♦), pasteurized ( ) and ozonized (○), along refrigerated storage. Data represents mean values and bars the limits of confidence intervals at 95%.

Figure 4. Total phenolics (a), total vitamin C (b) and total antioxidant activity (c) of *Cantaloupe* melon juice without treatment (♦), pasteurized ( ) and ozonized ( ), along refrigerated storage. Data represents mean values and bars the limits of confidence intervals at 95%.