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Comparison of traditional hot water and vacuum assisted blanching methods on the physico-chemical quality parameters and antioxidant activity of zucchini (*Cucurbita pepo* L.) slices

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(Article begins on next page)

1 **Comparison of traditional and vacuum assisted blanching methods on the color,**  
2 **functional components and antioxidant activity of zucchini rings**

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12  
13 **Abstract**

14 This study aimed to compare two blanching methods in order to determine the effect of treatment  
15 on loss of phytochemical content, color characteristics and antioxidant activity. Fresh and treated  
16 zucchini were investigated for their pH, total soluble solids, moisture, color, total phenols and  
17 flavonoids, and antioxidant activities by using a multi-target approach. The color, total soluble  
18 solids, pH and water activity of zucchini samples were not affected by the blanching process. The  
19 obtained data demonstrated that the vacuum treatment protected the antioxidant activity of zucchini  
20 rings extending the shelf-life of the food compared to the traditional blanching method and the fresh  
21 sample. All samples showed values of phenolic compounds comparable to the fresh product,  
22 although the samples treated with vacuum blanching for 8 minutes had the highest values. Hot water  
23 blanching for 8 min caused a significant increase in the total phenolic content of blanched zucchini,  
24 which had the greatest increase compared to blanching for 2 and 5 min, for the entire storage period.  
25 During storage, a significant decrease was observed in total phenol and flavonoid content,  
26 antioxidant activity and color values in all samples, independently of the applied process. PCA

27 showed that the factorial axis associated with PC-1 samples and had the highest content of bioactive  
28 compounds.

29

30 **Keywords:** *Cucurbita pepo*; blanching; color; phenolic compounds; antioxidant activity.

31

## 32 **1. Introduction**

33 Vegetables contain a large number of bioactive compounds, which significantly contribute to their  
34 functional properties including free radical scavenging activity, inhibition of hydrolytic and oxidative  
35 enzymes and anti-inflammatory activity. The consumption of vegetables is important to prevent  
36 several chronic disease such as hypertension, stroke, cancer etc. (Jaiswal *et al.*, 2012). However, it is  
37 known that the quality attributes of untreated vegetables, such as nutrients, texture, color and flavor  
38 can be affected by the changes occurring during postharvest storage (Chemat *et al.*, 2017). In  
39 particular, zucchini (*Cucurbita pepo* L.) provide beneficial effects on human health in the daily diet  
40 for their high content of chemical constituents, such as carotenoids, tocopherols, phenols, terpenoids,  
41 saponins, sterols and fatty acids (Mu-kherjee, and Chattopadhyay, 2007; Jacobo-Valenzuela *et al.*,  
42 2011). Nevertheless, for their seasonal and short-lived nature, they are subject to rapid deterioration  
43 by microorganisms and enzymes (Wang *et al.*, 2020).

44 Furthermore, trimming, peeling, washing and cutting damage the quality of the fresh product  
45 (Martínez-Valdivieso *et al.*, 2017). Based on this, the food industry has improved processing and  
46 preservation treatments in order to maintain the freshness of these products (Neves *et al.*, 2019).

47 It is well documented that peroxidase and polyphenol oxidase enzymes, when present in processed  
48 vegetables, are responsible for undesirable quality changes as well as nutrient=degradation. A  
49 combination of thermal treatment and appropriate packaging are a suitable approach to inactivate the  
50 biochemical reactions and reduce microbial load (Xiao *et al.*, 2017).

51 Hot-water blanching slows down the enzyme deterioration and the Maillard reaction ensuring the  
52 nutritional and biological stability of vegetables, increasing the consumer acceptability of products  
53 (Patras *et al.*, 2016). However, literature is available on the negative effects of blanching, such as  
54 pigment modifications, tissue softening or nutrient reduction (Aguilar *et al.*, 2004). The loss of total  
55 polyphenol and antioxidant components could occur as an effect of thermal degradation, diffusion  
56 and leaching. It is crucial to select blanching treatment conditions (time and temperature) in order to  
57 reduce the exposure of the product to heat (Aguilar *et al.*, 2004).

58 Therefore, the main object of this work was to compare the effect of traditional hot water blanching  
59 and innovative vacuum blanching method on nutritional and quality characteristics of zucchini during  
60 storage.

61

## 62 **2. Materials and Methods**

### 63 2.1 Sampling and experimental design

64 Zucchini (*Cucurbita pepo*), obtained from a farm located in Reggio Calabria (Italy), were  
65 selected of uniform size and color, and free of defects, and subsequently processed at the Food  
66 Technologies laboratory of the University Mediterranea of Reggio Calabria (Italy).

67 After the zucchini were washed and cut into circular slices with a thickness of 4 mm, they were  
68 divided in two groups and subjected to two different blanching methods: hot water blanching and  
69 innovative vacuum blanching. For hot water blanching (ZB) samples were immersed in a water  
70 bath at 95° C for three different blanching times: 2, 5 or 8 minutes (ZB2, ZB5 and ZB8). After  
71 thermal treatment, the samples were vacuum-packed and stored at 4°C. For vacuum blanching  
72 treatment (ZS), the samples were wrapped in heat resistant vacuum storage bags (Royal Pack  
73 iVacuum, Italy) and subjected to the same blanching conditions described above, and are indicated  
74 as follows: ZS2, ZS5, ZS8. Each sample was analyzed on the day of production and thereafter every  
75 seven days for a total of 28 days storage.

76

## 77 2.2 Quality parameters

78 Physical characteristics of treated and untreated samples as pH and Brix value, moisture content,  
79 water activity, and color were monitored. Aqueous extracts were prepared by adding 10 mL of  
80 distilled water to 1 g of vegetables. Samples were then homogenized using an Ultra-Turrax T-25  
81 (Janke & Kunkel, IKA-Labortechnik). The pH values of extracts were measured at room temperature  
82 using a pH-meter (Crison Basic 20) and Brix degrees (°Brix) were determined by the measurement  
83 of the refractive index with a refractometer (ATAGO 8269 Japan) at 25 °C.

84 For the determination of moisture content, about 30 g of sample was tested in an oven at 105 °C  
85 while water activity ( $a_w$ ) of vegetables was measured by Aqualab LITE hygrometer (Decagon  
86 devices Inc., Washington USA).

87 The color coordinates of the CIELAB space ( $L^*$ ,  $a^*$  and  $b^*$ ) were monitored during storage by a  
88 tristimulus colorimeter (Konica Minolta CM-700d, Osaka, Japan). Measurements were performed in  
89 three replicates.

90

## 91 2.3 Extraction procedure

92 Ultrasound procedure represented a key-technology in achieving the objective of sustainable  
93 “green” extraction with a significant effect on the rate of various processes in the chemical and food  
94 industry. This procedure resulted the most promising procedure to obtain extracts characterized by  
95 the highest of bioactivity in terms of antioxidant and enzymes inhibition. In addition, the extractions  
96 can be completed in minutes with high reproducibility, simplifying manipulation, reducing the  
97 consumption of solvent, and giving higher purity of the final product (Chemat *et al.*, 2017).

98 For this reason, for the extraction of bioactive phytochemicals and in order to test antioxidant  
99 activity treated, the fresh zucchini were subjected to ultrasound-assisted maceration process using  
100 EtOH as solvent (200 mL, 3 × 1 h), or EtOH/H<sub>2</sub>O (80:20 v/v, 300 ml, 3 × 1 h). For this extraction  
101 procedure three extraction cycles with an ultrasonic frequency of 40 kHz at a temperature of 30 °C  
102 for 30 min were conducted for each sample in a Branson model 3800-CPXH water bath (Branson,

103 Milan, Italy). After each extraction cycle, the mixture was filtered through Whatman filter Paper 4  
104 under vacuum, and the solvent was removed using a rotary vacuum evaporator at 30 °C. Each  
105 extraction was performed in triplicate.

106

#### 107 2.4 Phytochemicals content

108 Total phenol content (TPC) was investigated using the Folin-Ciocalteu method (Leporini *et al.*,  
109 2020a). A mixture of sample (1.5 mg/mL), Folin-Ciocalteu solution (0.5 mL), water and 20% sodium  
110 carbonate were prepared. The mixture was incubated at room temperature for 2 hours and the  
111 absorbance was read at 765 nm employed a UV-Vis Jenway 6003 (Carlo Erba, Milan, Italy). Results  
112 were expressed as mg of chlorogenic acid equivalents (CAE)/g of extract.

113 Total flavonoid content (TFC) was determined as previously reported by Leporini *et al.* (2020a).  
114 A solution of aluminium chloride was mixed with sample (1.5 mg/mL). After 15 min of incubation  
115 at room temperature the absorbance was read at 510 nm using UV-Vis Jenway 6003  
116 spectrophotometer (Carlo Erba, Milan, Italy). Results were expressed as mg quercetin equivalents  
117 (QE)/g of extract.

118

#### 119 2.5 Antioxidant activity

120 The evaluation of antioxidant activity is context-dependent. Many different methods have been  
121 used for the evaluation of antioxidant activity and no single concentration can completely evaluate  
122 the antioxidant potential of vegetable extracts (Pinchuk *et al.*, 2012). In addition, plant extracts are  
123 rich in phenolic compounds that exhibited antioxidant activity through different mechanisms. Most  
124 of them are based on the measurement of the relative abilities of antioxidants to scavenge radicals in  
125 comparison with the antioxidant potency of a standard antioxidant compound (Leporini *et al.*, 2020a).  
126 For this complexity, more than one test was carried out *in vitro* (ABTS, DPPH, and  $\beta$ -carotene  
127 bleaching assays) in order to evaluated antioxidant activities of zucchini extracts. The radical

128 scavenging activity was investigated by DPPH and ABTS assay as previously described (Loizzo *et*  
129 *al.*, 2020).

130 In DPPH test, the samples (1 mg/mL) were added to DPPH solution and after 30 min, the  
131 absorbance was read at 517 nm using UV-Vis Jenway 6003 spectrophotometer (Carlo Erba, Milan,  
132 Italy).

133 In ABTS test, a mixture of ABTS radical cation solution and sample (400-1 µg/mL) was prepared  
134 and after 6 min of incubation, the absorbance at 734 nm was measured using the same apparatus  
135 previously indicated. Ascorbic acid was used as positive control in both assays.

136 The potential of samples to inhibit lipid peroxidation was assessed using the β-carotene bleaching  
137 test as previously reported (Leporini *et al.*, 2020b). A solution of β-carotene, linoleic acid and Tween  
138 20 was added a 96-well microplate containing the samples (100-5 µg/mL). The microplates were  
139 placed in a water bath for 30 and 60 min at 45 °C. The absorbance was read at 470 nm employing a  
140 using UV-Vis Jenway 6003 spectrophotometer (Carlo Erba, Milan, Italy). Propyl gallate was used as  
141 positive control.

142

## 143 2.6 Statistical analysis

144 Results were expressed as means of three different experiments ± standard deviation (S.D.). All  
145 data were analyzed using one-way analysis of variance (ANOVA) with SPSS 17.0 (SPSS Inc.,  
146 Chicago, IL, USA) statistical software. Significant differences were calculated according to Tukey's  
147 multiple range tests. Differences at  $P < 0.05$  were considered to be statistically significant while at  
148  $P < 0.01$  were considered to be highly significant. Principal Component Analysis (PCA) were applied  
149 using SPSS software for Windows, version 17.0 (Chicago, IL, USA).

150 The concentration-response curve and the inhibitory concentration 50% (IC<sub>50</sub>) was calculated by  
151 using Prism GraphPad Prism version 4.0 for Windows, GraphPad Software (San Diego, CA, USA).

152 One-way analysis of variance test (ANOVA) followed by a multicomparison Dunnett's test ( $p =$   
153 0.05) was used to compare differences within and between groups with the positive control in all  
154 bioassays.

155

### 156 **3. Results and discussion**

#### 157 3.1. Quality parameters

158 The preservation of vegetable freshness over time depends on different factors, such as water  
159 activity ( $a_w$ ), moisture content, TSS and pH values. Particularly, the  $a_w$  is an important variable for  
160 evaluating the food stability seeing as it is responsible for optimizing microbiological and physical  
161 properties of the product, such as texture, flavor, odor and color (Coupland *et al.*, 2000). A value of  
162  $a_w$   $0.99 \pm 0.00$  was obtained for the fresh samples and ANOVA data elaboration showed that no  
163 significant variations ( $p > 0.05$ ) were observed during storage (Table 1). The different treatments  
164 applied to the samples did not affect the  $a_w$  parameter (Owureku-Asare *et al.*, 2018). Regarding the  
165 moisture content, at the beginning of storage ( $t_0$ ), all samples showed values of relative humidity  
166 comparable to the fresh product ( $68.71 \pm 1.22$  %), although samples processed with blanching in hot  
167 water for 2, 5 and 8 minutes (ZS2, ZS5 and ZS8) had the highest values. Other authors have  
168 investigated the effect of blanching on relative humidity, proving that the increase of moisture content  
169 is linked to the absorption and the adhesion of water to surface of products (Mondragón-Portocarrero  
170 *et al.*, 2006). However, a significant decrease of  $a_w$  was observed for all samples during the storage  
171 as observed in Table 1.

172 The change in pH values after treatments and during storage is reported in Table 1. In fresh  
173 products the pH value is  $6.54 \pm 0.42$  while, for treated samples values ranged between 6.67 and 6.37  
174 at time 0. A slight increase in pH values was observed for the samples ZB2, ZB5 and ZB8. Literature  
175 data (Martinez *et al.*, 2013) reported that during blanching treatments the loss of soluble compounds  
176 and organic acids could produce an increase in pH values. This effect is more noticeable for the long  
177 processing time confirming that the duration of treatment also affects this parameter. A significant

178 decrease ( $p<0.01$ ) in pH was detected over time, probably due to the release of  $H^+$  caused by the  
179 reaction between the tissue water and the  $CO_2$  produced (Rocha *et al.*, 2007).

180 In correlation with the pH results, the samples treated with vacuum blanching showed a higher  
181 amount of total suspended solids (TSS) expressed as °Brix value (Table 1). In particular, the samples  
182 ZS2, ZS5 and ZS8 showed the highest TSS content at the end of storage. These results demonstrate  
183 that vacuum blanching has a higher capacity to protect the soluble components of vegetables  
184 compared to traditional treatment.

185 As is well known, browning is very common in the processing and storage of fruits and vegetables.  
186 For this reason, the effectiveness of treatments was evaluated in terms of color variations. The color  
187 parameters are reported in Table 2 and Fig. 1. In general, the obtained profiles are qualitatively  
188 similar. Significant variations in color parameters ( $p<0.01$ ) were detected during storage. Exposure  
189 time and different treatments seemed to affect significantly ( $p<0.01$ ) the color attributes of samples.  
190 On the day of production, zucchini processed with vacuum blanching for 2 minutes (ZS2) are  
191 characterized by similar colorimetric parameters to the fresh product ( $L^*_{ZS2} 76.86 \pm 4.23$ ,  $a^*_{ZS2} -$   
192  $0.9 \pm 0.00$ ,  $b^*_{ZS2} 23.04 \pm 2.22$ ,  $C^*_{ZS2} 22.10 \pm 1.56$ ;  $L^*_{FP} 79.971 \pm 3.56$ ,  $a^*_{FP} -0.88 \pm 0.00$ ,  $b^*_{FP} 21.97 \pm 1.25$ ,  
193  $C^*_{FP} 21.99 \pm 2.02$ ).

194 The increase of treatment time makes the samples become less bright ( $L^*$  decreasing) and greener  
195 ( $a^*$  decreasing). The same trend was observed following the direct immersion in hot water, except  
196 for the sample ZB8 that showed a higher  $L^*$  value. No clear trend was observed during storage;  
197 nevertheless, absolute  $L^*$  values indicate oscillations within a very narrow interval for ZS2 and ZS5  
198 from the start to the end of storage. For all samples, lower  $C^*$  values were detected compared to the  
199 fresh product, and a significant variation was observed over time. A previous study reported that the  
200 reduction in  $C^*$  could be linked to degradation of chlorophyll and a migration of chromophore  
201 compounds into the blanching water (Jaiswal *et al.*, 2012). Taken together, our data suggests that  
202 vacuum blanching could delay the browning process and consequently undesirable changes in color  
203 parameters (Liu *et al.*, 2019).

204

### 205 3.2 Extraction yield, total phenols and total flavonoids

206 Literature data demonstrated that the application of ultrasound procedures increase the extraction  
207 yield of the bioactive compounds due to the acceleration of mass transfer from the solid to the liquid-  
208 phase. Indeed, the passage of ultrasound determines a greater penetration of the solvent within the  
209 material increasing the surface area (Yolmeh *et al.*, 2014).

210 Zucchini extraction yields (w/w) are reported in Fig. 2. Fresh samples showed the highest  
211 extraction yield (10.01%), followed by the samples subject to blanching methods with innovative  
212 vacuum blanching (8.85-5.01%). A lower extractive yield was observed for samples subjected to hot  
213 water blanching.

214 The loss of nutrients during hot water blanching is caused by leaching or diffusion (Mu-kherjee and  
215 Chattopadhyay, 2007). All water-soluble nutrients can leach out from plant tissues to the blanching  
216 water. The quality of blanched products depends significantly on the time-temperature combinations  
217 of blanching, and also on the process type. The daily intake of polyphenols has received much  
218 attention due to the health benefits of their antioxidant/anti-radical, anti-inflammatory, anti-  
219 carcinogenic, antiviral and antimicrobial activities. Zucchini has a high nutritional value and a low  
220 calorie content.

221 A number of studies have investigated the effect of blanching on the TPC of zucchini (Iswaldi *et*  
222 *al.*, 2013; Seleim *et al.*, 2015; Baljeet *et al.*, 2016). The results of blanching on the TPC is showed in  
223 Table 3. Samples had on average 39.4 mg of CAE/g of extract, and neither hot-water blanching nor  
224 vacuum blanching caused any damaging effects. All samples showed values of phenolic compounds  
225 comparable to the fresh product (39.4±1.6 mg of CAE/g of extract), although the samples ZB2, ZB4  
226 and ZB8 had the highest values. However, a gradual decrease in the total polyphenol content was  
227 observed throughout the storage period. Hot water blanching (ZB) for 8 min caused a significant  
228 increase ( $p < 0.05$ ) in the TPC of zucchini, and showed a greater increase compared to blanching for  
229 2 and 5 min, throughout storage. In fact, ZB8 sample at time 0 showed a higher TPC equal to 39.9

230 mg of CAE/g of extract. Similar values were also observed for ZB8 after 7, 14, 21 and 28 days with  
231 37.1, 34.7, 33.5 and 31.4 mg of CAE/g of extract, respectively. Increase in the TPC of the zucchini  
232 may be ascribed to the reduction in the enzyme polyphenol oxidase. Furthermore, the greater quantity  
233 of TPC could be due to the solubilization of phenolic acids after the destruction of cellular  
234 components (Francisco *et al.*, 2010). Maximum flavonoid content was recorded in control samples  
235 (22.8 mg/g), because during blanching the flavonoid content was lost (Danesi and Bordonni, 2008).

236 As shown in Table 3, The TFC of the vacuum-blanching zucchini ranged from 18.8 (t0) to 14.3  
237 (t28) mg QE/g of extract for ZS2, 13.05 (t0) to 9.4 (t28) mg/g for ZS5 and 13.7 (t0) to 9.1 (t28) for  
238 ZS8. While for the traditionally-blanching zucchini, it ranged from 17.4 (t0) to 11.1 (t28) mg QE/g of  
239 extract for ZB2, from 19.9 (t0) to 8.2 (t28) for ZB5 and 15.5 (t0) to 6.4 (t28) mg QE/g of extract for  
240 ZB8. The samples ZB2, ZB5 and ZB8 showed the highest TFC. In particular, the ZB8 sample at time  
241 0 showed a higher content of flavonoids equal to 19.9 mg QE/g of extract. In any case, a significant  
242 reduction in the TFC of the zucchini after blanching was observed in all samples analyzed throughout  
243 the entire storage time.

244

### 245 3.3 Antioxidant activity

246 The antioxidant activity of zucchini extracts derived from treated and non-treated vegetables was  
247 investigated. All samples showed an antioxidant activity in a concentration-dependent manner.

248 In DPPH assay (Table 4), the samples subjected to vacuum blanching showed higher values than  
249 the untreated and traditional blanching samples. In particular, the ZB8 sample at time 0 displayed a  
250 better radical scavenging potential with a percentage of 49.58%, an activity 1.34-times greater than  
251 traditional blanching. However, over the 28 days the antioxidant ability decreased more markedly for  
252 traditional blanching. The *Pearson's* correlation coefficient was positive between TPC and DPPH,  
253 with  $r = 0.85$ .

254 The same considerations can be made for the ABTS test (Table 4). Generally, ZB8 throughout the  
255 whole 28 days showed the best results. Indeed, this sample at time 0 showed the highest radical

256 scavenging activity with IC<sub>50</sub> values of 14.9 µg/mL. Similar values were also observed for ZB8 after  
257 7 and 14 days with IC<sub>50</sub> of 15.04 and 15.96 µg/mL, respectively.

258 The potential of samples to inhibit lipid peroxidation was assessed using the β-carotene bleaching  
259 test (Table 5). Generally, vacuum treatment after 5 and 8 minutes increases protection against lipid  
260 peroxidation. In particular, ZB8 at time 0 showed the highest values with IC<sub>50</sub> of 13.74 and 15.79  
261 µg/mL, respectively after 30 and 60 minutes of incubation. For ZB8 after 7 days a similar activity  
262 was reported (IC<sub>50</sub>=14.08 and 16.09 µg/mL, respectively after 30 and 60 minutes of incubation). Also  
263 interesting were the results obtained for ZB5 and ZS8 at 0- and 7-days in β-carotene bleaching test  
264 after 30 min of incubation with IC<sub>50</sub> values of 14.68, 15.20, 16.24 and 17.60 µg/mL, respectively.  
265 Moreover, after 60 min of incubation IC<sub>50</sub> values of 18.35 and 19.06 µg/mL, respectively for ZB5 at  
266 0- and 7-days were observed.

267 The obtained data demonstrated that the vacuum treatment protected the antioxidant activity of  
268 *Cucurbita pepo* extending the shelf-life of the food compared to traditional blanching and the fresh  
269 sample. Additionally, the best results can be observed after 8 min of treatment probably related to the  
270 formation of substances having greater antioxidant activity. According to [Tiwari and Cummins](#)  
271 [\(2013\)](#), the processing of vegetables can result in a significant reduction in their phytochemical  
272 content. Indeed, in traditional blanching a reduction in antioxidant activity was observed. However,  
273 the vacuum preserved the integrity of the food and consequently its antioxidant properties. Similarly,  
274 [Liu et al. \(2019\)](#) compared an innovative non-contacted blanching (vacuum) method with traditional  
275 hot water blanching methods.

276 Data showed that vacuum blanching represented the most effective method and preserved the  
277 antioxidant capacity of food as well as the content of bioactive compounds. The effect of vacuum  
278 blanching compared to the traditional method was also investigated by [Tanongkankit et al. \(2015\)](#).  
279 The authors confirmed that vacuum treatment contained higher amounts of antioxidants and  
280 antioxidant activity than hot air treatment.

281

### 282 3.4 Principal component analysis

283 Principal component analysis (PCA), used to study the dimensionality of a data set is reduced by  
284 defining several mathematical factors (principal components) which are a linear combination of the  
285 original variables (D'Agostino *et al.*, 2014). PCA eigenvalues are used as a measurement of the  
286 amount of variance explained by each of these factors, while PCA loadings afford information on the  
287 associated variables (volatile compounds) and its importance for each principal component.  
288 Parameters were assessed through data generated according to a factorial design using PCA. PCA  
289 (Fig. 3) showed that the two principal components accounted for 76.67 % of total variance, with PC1  
290 for 42.12 % and PC2 for 34.55% of total variance. Thus, these components can be used to represent  
291 the set of variables measured in the packages tested, since they incorporate over 76 % of the variance.  
292 The first principal component (Fig. 3) shows strong positive correlation with TPC (t0, t7, t14, t21,  
293 t28), TFC (t0, t7, t14) and DPPH (t0, t7, t14, t21, t28). The significant correlations obtained support  
294 the hypothesis that total phenolic content contributes significantly to the antioxidant activity,  
295 especially for DPPH assay (Sicari *et al.*, 2016).

296 In addition, from the analysis of variable loads, it was seen that the PC1 has a negative correlation  
297 with pH (t0, t7, t14, t21, t28), TSS (t0, t7, t14, t21, t28) and ABTS (t0, t7, t14, t21, t28).

298 The second principal component is correlated with pH-t21, ABTS (t0, t7, t14, t21, t28),  $\beta$ -carotene  
299 bleaching test t30 (t0, t7, t14, t21, t28) and  $\beta$ -carotene bleaching test t 60 (t0, t7, t14, t21, t28).

300 Fig. 3 also showed that PC1 positive correlation with ZB2, ZB5 and ZB8 obtained from hot water  
301 blanching and was characterized by the presence of TPC, TFC and DPPH. In addition, the different  
302 relationships between the antioxidant activity and the total phenolic content may be due to many  
303 factors; in fact, the total phenolic content does not incorporate all the antioxidants.

304 PCA showed that the factorial axis associated with PC-1 is the axis which of all the imaginable axes  
305 best represents the similarities and differences between the observations, and distinguishes samples  
306 with the highest content of bioactive compounds (TPC and TFC).

307

## 308 **5. Conclusions**

309 The different blanching treatments have an effect on the chemical-physical characteristics,  
310 functional components and antioxidant activity of zucchini rings. In addition, also the duration of the  
311 treatment has a great effect on total phenolic content, total flavonoid content and antioxidant  
312 activities.

313 Results suggest that a blanching time of 8 minutes allows the bioactive compounds to be preserved,  
314 thereby maintaining the antioxidant activity of fresh product. In particular, the application of a  
315 vacuum minimized the total soluble solids, the antioxidant capacity and the total phenolic losses  
316 increasing protection against lipid peroxidation and delaying the browning process. The combination  
317 of vacuum and heat treatments appears to be a suitable technology to preserve the nutritional and  
318 sensorial attributes of products leading to a product with a higher quality retention of up to 28 days  
319 of storage.

320

321 **Ethical Guidelines:** Ethics approval was not required for this research.

322 **Conflicts of Interest:** The authors declare no conflict of interest.

323

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450 **Table 1.** Changes in moisture, pH and TSS of different blanching treatments during storage  
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		t0	t7	t14	t21	t28	Sign.
<b>MOISTURE</b>	Fresh	68.71±1.12 <sup>b</sup>					
	ZS2	69.44±3.22 <sup>aA</sup>	66.07±4.85 <sup>aB</sup>	65.53±5.25 <sup>cC</sup>	65.74±4.85 <sup>dD</sup>	65.91±5.3 <sup>eE</sup>	**
	ZS5	68.64±4.12 <sup>cA</sup>	66.2±5.36 <sup>bB</sup>	65.86±4.85 <sup>bB</sup>	65.93±6.03 <sup>cC</sup>	65.88±4.23 <sup>bD</sup>	**
	ZS8	68.41±5.01 <sup>dA</sup>	65.98±6.03 <sup>cC</sup>	64.79±4.78 <sup>aB</sup>	65.93±6.25 <sup>bB</sup>	65.56±4.58 <sup>aD</sup>	**
	ZB2	67.62±3.78 <sup>fB</sup>	65.3±7.21 <sup>dC</sup>	66.16±6.56 <sup>eD</sup>	65.64±5.61 <sup>aA</sup>	65.58±6.23 <sup>cE</sup>	**
	ZB5	67.63±6.02 <sup>fA</sup>	66.29±4.58 <sup>eC</sup>	66.36±4.89 <sup>cB</sup>	66.08±4.78 <sup>fD</sup>	66.25±5.23 <sup>dE</sup>	**
	ZB8	68.12±5.12 <sup>eA</sup>	66.04±4.52 <sup>dB</sup>	65.83±5.36 <sup>dB</sup>	65.92±5.55 <sup>eC</sup>	66.57±4.62 <sup>cdD</sup>	**
	Sign.	**	**	**	**	**	
<b>pH</b>	Fresh	6.54±0.42 <sup>b</sup>					
	ZS2	6.41±1.63 <sup>aB</sup>	6.24±1.37 <sup>dC</sup>	6.17±1.01 <sup>eD</sup>	6.69±1.06 <sup>aA</sup>	6.06±0.88 <sup>cE</sup>	**
	ZS5	6.44±1.33 <sup>aA</sup>	6.15±0.88 <sup>cC</sup>	6.32±0.77 <sup>cB</sup>	6.09±1.01 <sup>fE</sup>	6.00±1.22 <sup>dD</sup>	**
	ZS8	6.37±1.03 <sup>aA</sup>	6.25±1.06 <sup>dB</sup>	6.25±1.22 <sup>dB</sup>	6.13±0.88 <sup>eC</sup>	6.04±1.45 <sup>cdD</sup>	**
	ZB2	6.67±0.56 <sup>cA</sup>	6.46±0.89 <sup>aB</sup>	6.34±0.67 <sup>cC</sup>	6.24±0.36 <sup>dD</sup>	5.86±0.69 <sup>eE</sup>	**

	ZB5	6.64±1.06 <sup>cA</sup>	6.40±1.03 <sup>bbB</sup>	6.37±1.02 <sup>bbB</sup>	6.32±0.69 <sup>cC</sup>	6.23±1.08 <sup>bdD</sup>	**
	ZB8	6.69±2.01 <sup>dA</sup>	6.35±1.56 <sup>cC</sup>	6.55±1.54 <sup>aB</sup>	6.36±1.02 <sup>bcC</sup>	6.33±1.77 <sup>aD</sup>	**
	Sign.	**	**	**	**	**	
<b>TSS</b>	Fresh	1.94±0.02 <sup>c</sup>					
	ZS2	2.18±0.23 <sup>aB</sup>	2.45±0.31 <sup>bA</sup>	1.96±0.05 <sup>bcC</sup>	1.98±0.28 <sup>bcC</sup>	1.98±0.45 <sup>bcC</sup>	**
	ZS5	2.15±0.47 <sup>aB</sup>	2.47±0.25 <sup>aA</sup>	1.99±0.86 <sup>bcC</sup>	1.98±0.02 <sup>aC</sup>	1.98±0.42 <sup>bcC</sup>	**
	ZS8	1.99±0.56 <sup>bdD</sup>	2.95±0.62 <sup>bbB</sup>	3.49±0.66 <sup>aA</sup>	2.98±0.47 <sup>bbB</sup>	2.46±0.82 <sup>aC</sup>	**
	ZB2	1.48±0.23 <sup>dD</sup>	2.48±0.02 <sup>bA</sup>	1.73±0.06 <sup>cbB</sup>	1.98±0.04 <sup>bcC</sup>	1.97±0.06 <sup>bbB</sup>	**
	ZB5	1.47±0.02 <sup>dA</sup>	1.49±0.02 <sup>dA</sup>	1.48±0.02 <sup>dB</sup>	1.25±0.03 <sup>dA</sup>	1.49±0.04 <sup>cA</sup>	**
	ZB8	1.21±0.02 <sup>eD</sup>	1.72±0.02 <sup>cbB</sup>	1.97±0.02 <sup>bA</sup>	1.49±0.02 <sup>cC</sup>	1.24±0.02 <sup>dD</sup>	**
	Sign.	**	**	**	**	**	

452 Data are presented as means ± standard deviations. Means within a column and a row with different letters are significantly  
453 different by Tukey's post hoc test. \*\* Significance at  $p < 0.01$ .

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456 **Table 2.** Change in the color parameters of treated zucchini rings during storage  
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Parameters	time	ZS2	ZS5	ZS8	ZB2	ZB5	ZB8	Sign.
<b>L*</b>	t0	76.86±4.23 <sup>aA</sup>	71.53±8.69 <sup>bA</sup>	66.53±6.35 <sup>dD</sup>	67.13±6.88 <sup>eC</sup>	67.20±4.11 <sup>dC</sup>	68.92±6.23 <sup>cA</sup>	**
	t7	61.90±6.23 <sup>fE</sup>	66.68±8.36 <sup>cC</sup>	63.75±7.26 <sup>eE</sup>	64.42±6.69 <sup>dD</sup>	67.99±6.21 <sup>aB</sup>	67.54±7.59 <sup>bC</sup>	**
	t14	63.19±7.23 <sup>cD</sup>	62.79 ±7.26 <sup>fE</sup>	74.80±8.88 <sup>aA</sup>	63.52±7.83 <sup>dE</sup>	65.93±8.87 <sup>bE</sup>	65.43±4.55 <sup>cE</sup>	**
	t21	63.54±6.39 <sup>cC</sup>	63.11±9.36 <sup>dD</sup>	73.26±7.74 <sup>aB</sup>	67.97±7.56 <sup>cA</sup>	66.95±6.56 <sup>dD</sup>	68.36±5.21 <sup>bB</sup>	**
	t28	63.90±5.36 <sup>eB</sup>	66.84±7.23 <sup>dB</sup>	69.30±4.25 <sup>aC</sup>	67.27±6.28 <sup>cB</sup>	68.68±3.88 <sup>bA</sup>	66.81±6.10 <sup>dD</sup>	**
	Sign.	**	**	**	**	**	**	**
<b>a*</b>	t0	0.9±0.00 <sup>aA</sup>	2.32±0.01 <sup>cC</sup>	1.82±0.01 <sup>dB</sup>	1.55±0.01 <sup>cC</sup>	1.33±0.01 <sup>bB</sup>	1.87±0.05 <sup>dD</sup>	**
	t7	2.02±0.01 <sup>bD</sup>	2.36±0.01 <sup>cD</sup>	2.52±0.03 <sup>eD</sup>	2.39±0.02 <sup>dD</sup>	1.97±0.03 <sup>aE</sup>	2.04±0.02 <sup>cE</sup>	**
	t14	1.52±0.00 <sup>cC</sup>	1.54±0.01 <sup>cB</sup>	2.08±0.03 <sup>dC</sup>	1.2±0.02 <sup>aB</sup>	1.43±0.03 <sup>bC</sup>	1.56±0.02 <sup>cC</sup>	**
	t21	1.35±0.00 <sup>bB</sup>	1.34±0.00 <sup>bA</sup>	2.09±0.40 <sup>dC</sup>	1.2±0.02 <sup>aB</sup>	1.24±0.03 <sup>aA</sup>	1.47±0.01 <sup>cB</sup>	**
	t28	2.21±0.02 <sup>eE</sup>	1.53±0.00 <sup>cB</sup>	1.57±0.01 <sup>cA</sup>	1.1±0.01 <sup>aA</sup>	1.85±0.03 <sup>dD</sup>	1.31±0.01 <sup>bA</sup>	**
	Sign.	**	**	**	**	**	**	**
<b>b*</b>	t0	23.04±2.22 <sup>aA</sup>	20.26±3.56 <sup>cA</sup>	18.52±2.58 <sup>dD</sup>	20.17±1.47 <sup>cA</sup>	21.03±3.22 <sup>bB</sup>	20.08±4.03 <sup>cA</sup>	**
	t7	15.16±2.36 <sup>eC</sup>	14.97±4.36 <sup>dD</sup>	15.92±2.08 <sup>dE</sup>	17.34±3.22 <sup>dE</sup>	19.36±3.21 <sup>aC</sup>	18.44±2.33 <sup>bC</sup>	**
	t14	14.9±1.59 <sup>eD</sup>	13.59±2.36 <sup>fC</sup>	25.2±5.31 <sup>aA</sup>	19.20±2.36 <sup>dE</sup>	17.79±2.55 <sup>dE</sup>	18.92±2.33 <sup>bB</sup>	**
	t21	17.16±1.25 <sup>eB</sup>	13.92±2.36 <sup>fE</sup>	22.28±3.26 <sup>aB</sup>	18.05±2.39 <sup>cD</sup>	18.79±5.02 <sup>bD</sup>	17.50±2.54 <sup>cD</sup>	**
	t28	13.76±2.02 <sup>fE</sup>	17.58±4.23 <sup>eB</sup>	19.60±2.23 <sup>bC</sup>	19.74±2.23 <sup>aB</sup>	18.19±2.30 <sup>dA</sup>	18.87±2.11 <sup>cB</sup>	**
	Sign.	**	**	**	**	**	**	**

458 The data are presented as means ± standard deviations. Means followed by different capital letters, for the variation over  
 459 time, and different lowercase letters, for the variation among different treatments, are significantly different by Tukey  
 460 HSDa test. \*\* Significance at  $p < 0.01$ .  
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**Table 3.** Total phenolic (TPC) and total flavonoids (TFC) content in zucchini rings extracts

		t0	t7	t14	t21	t28	Sign.
<b>TPP</b>	Fresh	39.4 ± 1.6 <sup>b</sup>					
	ZS2	32.1±1.7 <sup>dA</sup>	29.2±1.3 <sup>bB</sup>	27.0±1.4 <sup>cC</sup>	25.2±1.1 <sup>deD</sup>	23.6±1.4 <sup>cE</sup>	**
	ZS5	29.9±1.2 <sup>eA</sup>	27.6±1.7 <sup>cB</sup>	26.2±1.0 <sup>dC</sup>	25.3±1.0 <sup>dD</sup>	24.5±1.4 <sup>cE</sup>	**
	ZS8	26.1±1.4 <sup>fA</sup>	24.5±1.4 <sup>dB</sup>	24.4±1.1 <sup>eB</sup>	24.1±1.2 <sup>eB</sup>	22.6±0.9 <sup>dC</sup>	**
	ZB2	37.5±1.5 <sup>eA</sup>	36.8±1.1 <sup>aB</sup>	32.6±1.2 <sup>bC</sup>	31.9±1.4 <sup>bC</sup>	29.4±1.1 <sup>bD</sup>	**
	ZB5	37.8±1.1 <sup>eA</sup>	37.5±1.4 <sup>aA</sup>	34.3±1.7 <sup>aB</sup>	29.1±1.2 <sup>cC</sup>	28.4±1.3 <sup>bD</sup>	**
	ZB8	39.9±1.4 <sup>aA</sup>	37.1±1.0 <sup>aB</sup>	34.7±1.4 <sup>aC</sup>	33.5±1.1 <sup>aD</sup>	31.4±1.2 <sup>aE</sup>	**
	Sign.	**	**	**	**	**	
<b>TFC</b>	Fresh	22.8 ± 0.9 <sup>a</sup>					
	ZS2	18.8±0.4 <sup>cA</sup>	17.3±0.3 <sup>bB</sup>	16.6±0.4 <sup>aC</sup>	15.3±0.8 <sup>aD</sup>	14.3±0.5 <sup>aE</sup>	**
	ZS5	13.5±0.5 <sup>fA</sup>	12.4±0.4 <sup>fB</sup>	12.1±0.3 <sup>eC</sup>	11.8±0.6 <sup>bD</sup>	9.4±0.3 <sup>cE</sup>	**
	ZS8	13.7±0.4 <sup>fA</sup>	13.2±0.3 <sup>eB</sup>	11.9±0.4 <sup>eC</sup>	10.7±0.2 <sup>dD</sup>	9.1±0.4 <sup>dE</sup>	**
	ZB2	17.4±0.8 <sup>dA</sup>	16.5±0.2 <sup>cB</sup>	14.8±0.2 <sup>cC</sup>	11.3±0.5 <sup>cD</sup>	11.1±0.3 <sup>bE</sup>	**
	ZB5	19.9±0.3 <sup>bA</sup>	18.5±0.4 <sup>aB</sup>	15.4±1.0 <sup>bC</sup>	11.9±0.5 <sup>bD</sup>	8.2±0.4 <sup>eE</sup>	**
	ZB8	15.5±0.7 <sup>eA</sup>	14.4±0.5 <sup>dB</sup>	12.8±0.5 <sup>dC</sup>	10.8±0.6 <sup>dD</sup>	6.4±0.2 <sup>fE</sup>	**
	Sign.	**	**	**	**	**	

465 Data are presented as means ± standard deviations. Means followed by different capital letters in a row and different  
466 lowercase letters in a column are significantly different by Tukey HSDa test. \*\* Significance at  $p < 0.01$ .

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**Table 4.** Radical scavenging activities evaluated by DPPH and ABTS test of zucchini rings extracts

	Days				
	t0	t7	t14	t21	t28
<b>DPPH test (% at 1 mg/mL)</b>					
<b>Fresh</b>	41.82 %				
<b>ZS2</b>	39.93 %	39.33 %	38.57 %	37.17 %	33.36 %
<b>ZS5</b>	39.71 %	38.84 %	37.24 %	36.41 %	36.21 %
<b>ZS8</b>	36.84 %	35.81 %	35.36 %	35.26 %	34.93 %
<b>ZB2</b>	41.26 %	41.18 %	41.02 %	39.75 %	39.39 %
<b>ZB5</b>	44.74 %	44.33 %	44.19 %	43.73 %	43.24 %
<b>ZB8</b>	49.58 %	48.64 %	48.13 %	44.94 %	44.16 %
<b>ABTS test (IC<sub>50</sub> µg/mL)</b>					
<b>Fresh</b>	20.45 ± 1.81****				
<b>ZS2</b>	23.05 ± 2.03****	27.21 ± 2.31****	29.86 ± 2.41****	30.34 ± 3.01****	34.58 ± 3.23****
<b>ZS5</b>	37.31 ± 3.34****	39.48 ± 3.81****	41.81 ± 3.93****	55.93 ± 4.11****	61.87 ± 4.32****
<b>ZS8</b>	17.55 ± 1.82****	21.74 ± 1.93****	25.03 ± 2.04****	28.41 ± 2.34****	29.16 ± 2.44****
<b>ZB2</b>	29.16 ± 2.86****	29.81 ± 2.89****	31.17 ± 2.98****	31.86 ± 2.97****	36.48 ± 3.23****
<b>ZB5</b>	22.67 ± 1.84****	22.83 ± 1.85****	23.94 ± 1.99****	34.22 ± 3.06****	34.78 ± 3.10****
<b>ZB8</b>	14.98 ± 1.25****	15.46 ± 1.36****	15.96 ± 1.32****	16.64 ± 1.43****	17.96 ± 1.55****

499 Data are expressed as means ± S.D. (*n* = 3). Differences within and between groups were evaluated by one-way ANOVA  
500 followed by a multicomparison Dunnett's test: \*\*\*\**p* < 0.0001, compared with the positive control ascorbic acid (IC<sub>50</sub> of  
501 5.0 ± 0.07 and 1.7 ± 0.06 in DPPH and ABTS test, respectively).  
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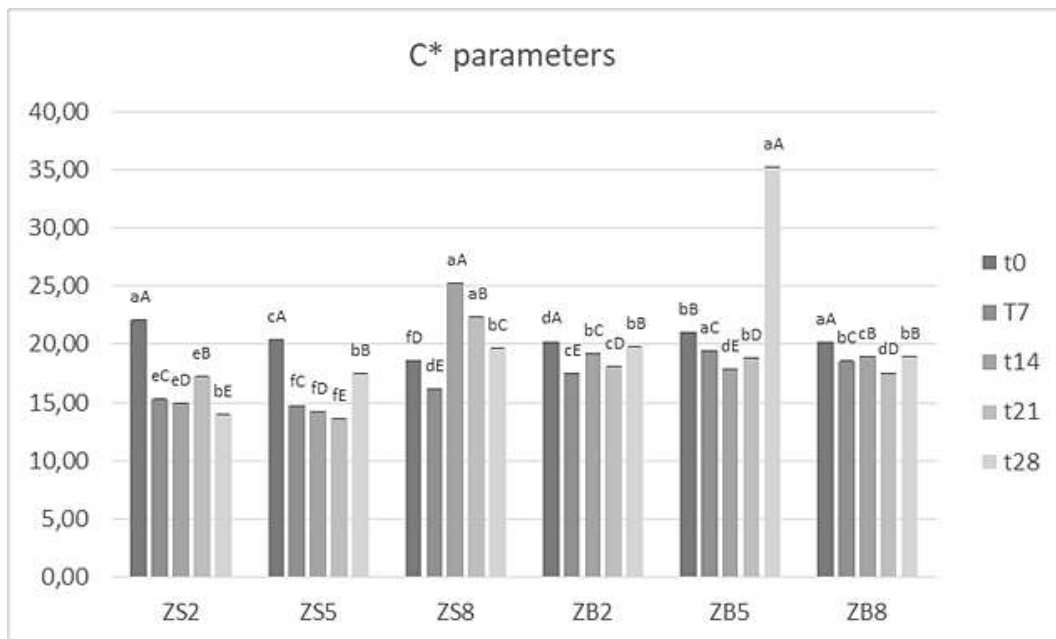
**Table 5.** IC<sub>50</sub> values of β-carotene bleaching test after 30 and 60 min of incubation of zucchini rings extracts

30 min incubation	Days				
	t0	t7	t14	t21	t28
	<b>Fresh</b>	14.37 ± 1.21****			
<b>ZS2</b>	17.25 ± 1.68****	54.34 ± 4.16****	41.95 % <sup>a</sup>	41.46 % <sup>a</sup>	38.46 % <sup>a</sup>
<b>ZS5</b>	39.14 ± 2.58****	53.15 ± 4.05****	49.10 % <sup>a</sup>	47.24 % <sup>a</sup>	46.16 % <sup>a</sup>
<b>ZS8</b>	15.22 ± 1.16****	17.66 ± 1.62****	29.12 ± 2.43****	48.05 ± 0.81****	50.11 ± 3.86****
<b>ZB2</b>	52.27 ± 3.82****	55.52 ± 3.95****	56.46 ± 3.82****	56.96 ± 3.86****	58.76 ± 4.01****
<b>ZB5</b>	14.68 ± 1.27****	16.24 ± 1.34****	42.32 ± 3.10****	43.94 ± 3.26****	47.06 ± 3.38****
<b>ZB8</b>	13.74 ± 1.15****	14.86 ± 1.24****	19.48 ± 1.63****	29.23 ± 2.00****	35.73 ± 3.12****
<b>60 min incubation</b>					
<b>Fresh</b>	26.11 ± 2.33****				
<b>ZS2</b>	27.31 ± 2.47****	55.91 ± 3.84****	35.64 % <sup>a</sup>	34.56 % <sup>a</sup>	32.35 % <sup>a</sup>
<b>ZS5</b>	44.21 ± 2.87****	55.74 ± 3.83****	47.09 % <sup>a</sup>	45.25 % <sup>a</sup>	43.18 % <sup>a</sup>
<b>ZS8</b>	22.36 ± 2.36****	25.76 ± 2.57****	32.41 ± 2.8****	52.33 ± 3.71****	53.53 ± 3.88****
<b>ZB2</b>	54.77 ± 3.93****	57.88 ± 4.06****	58.46 ± 4.0****	58.94 ± 4.12****	60.00 ± 4.36****
<b>ZB5</b>	18.35 ± 1.87****	19.06 ± 1.95****	45.44 ± 3.7****	45.91 ± 3.85****	50.11 ± 4.09****
<b>ZB8</b>	15.79 ± 1.41****	16.92 ± 1.53****	21.23 ± 1.8****	31.44 ± 2.33****	37.82 ± 2.96****
<b>Positive control</b>					
<b>Propyl gallate</b>	0.09 ± 0.004				

527 Data are expressed as means ± S.D. (n = 3). Differences within and between groups were evaluated by one-way ANOVA  
528 followed by a multicomparison Dunnett's test: \*\*\*\*p < 0.0001, compared with the positive control. <sup>a</sup> sample at  
529 concentration of 100 µg/mL.

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531 **Fig. 1.** Comparison of chroma values during zucchini shelf-life (t0-t28)  
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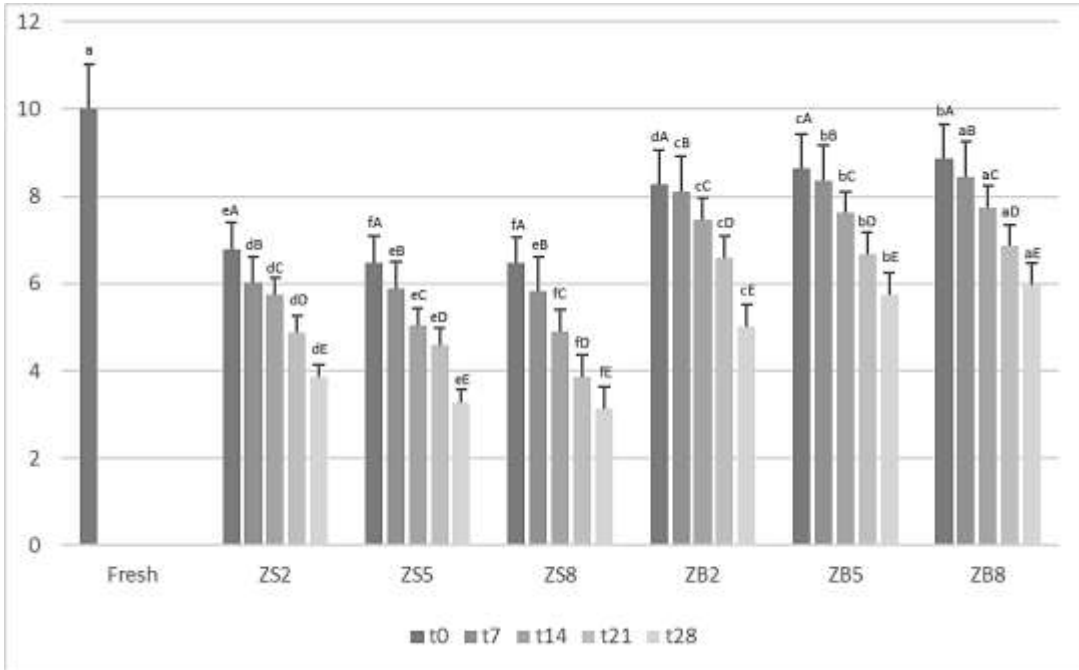
534 The data are presented as means ± standard deviations. Means followed by different capital letters,  
535 for the variation over time, and different lowercase letters, for the variation among different treatments,  
536 are significantly different by Tukey HSDa test. \*\* Significance at P < 0.01.  
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540 **Fig. 2.** Extraction yield

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543 The data are presented as means  $\pm$  standard deviations. Means followed by different capital letters, for  
544 the variation over time, and different lowercase letters, for the variation among different treatments, are  
545 significantly different by Tukey HSDa test. \*\* Significance at  $P < 0.01$ .

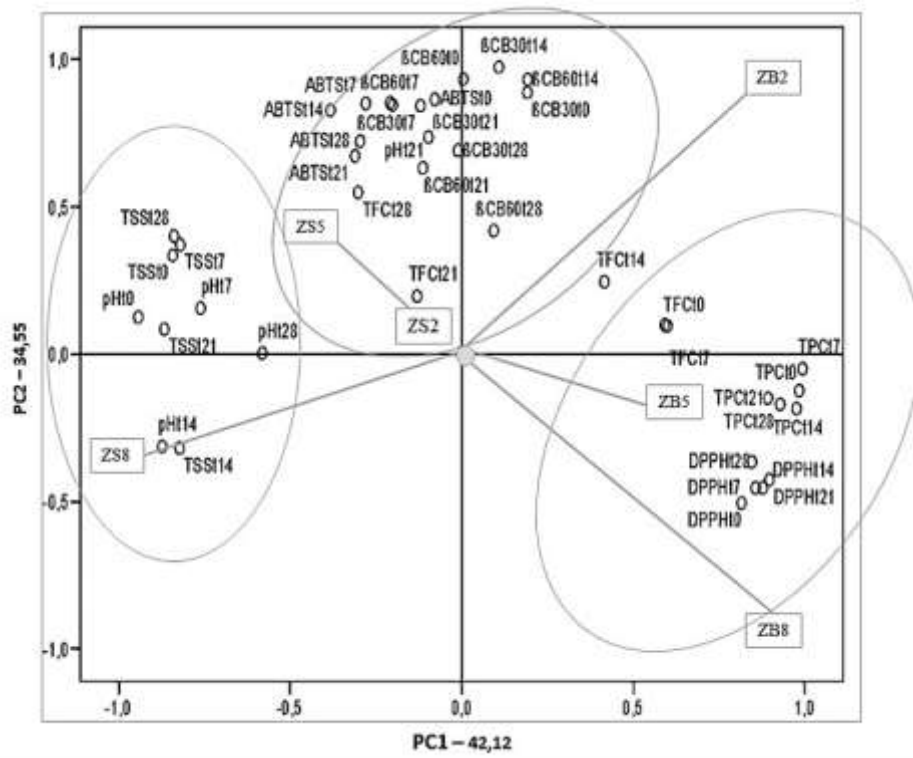
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550 **Fig. 3.** Relationship between the two principal components as for the physical-chemical  
551 parameters, bioactive compounds and antioxidant activity of the treatments between storage  
552 days 0 and 28.  
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