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

Comparison of traditional and vacuum assisted blanching methods on thecolor, functional components and antioxidant activity of zucchini rings

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Abstract

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

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

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

1. Introduction

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

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

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

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

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

2. Materials and Methods

2.1 Sampling and experimental design

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

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

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 than 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 (aw) 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_2O (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 β-carotene
127 bleaching assays) in order to evaluated antioxidant activities of zucchini extracts. The radical

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

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

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

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

2.6 Statistical analysis

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

The concentration-response curve and the inhibitory concentration 50% (IC_{50}) was calculated by using Prism GraphPad Prism version 4.0 for Windows, GraphPad Software (San Diego, CA, USA).

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

3. Results and discussion

3.1. Quality parameters

The preservation of vegetable freshness over time depends on different factors, such as water activity (a_w), moisture content, TSS and pH values. Particularly, the a_w is an important variable for evaluating the food stability seeing as it is responsible for optimizing microbiological and physical properties of the product, such as texture, flavor, odor and color (Coupland *et al.*, 2000). A value of a_w 0-99 ± 0.00 was obtained for the fresh samples and ANOVA data elaboration showed that no significant variations (p >0.05) were observed during storage (Table 1). The different treatments applied to the samples did not affect the a_w parameter (Owureku-Asare *et al.*, 2018). Regarding the moisture content, at the beginning of storage (t0), all samples showed values of relative humidity comparable to the fresh product (68.71±1.22 %), although samples processed with blanching in hot water for 2, 5 and 8 minutes (ZS2, ZS5 and ZS8) had the highest values. Other authors have investigated the effect of blanching on relative humidity, proving that the increase of moisture content is linked to the absorption and the adhesion of water to surface of products (Mondragón-Portocarrero *et al.*, 2006). However, a significant decrease of a_w was observed for all samples during the storage as observed in Table 1.

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

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

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

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

The increase of treatment time makes the samples become less bright (L* decreasing) and greener (a* decreasing). The same trend was observed following the direct immersion in hot water, except for the sample ZB8 that showed a higher L* value. No clear trend was observed during storage; nevertheless, absolute L* values indicate oscillations within a very narrow interval for ZS2 and ZS5 from the start to the end of storage. For all samples, lower C* values were detected compared to the fresh product, and a significant variation was observed over time. A previous study reported that the reduction in C* could be linked to degradation of chlorophyll and a migration of chromophore compounds into the blanching water (Jaiswal *et al.*, 2012). Taken together, our data suggests that vacuum blanching could delay the browning process and consequently undesirable changes in color 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 bleaching 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 Bordoni, 2008).
236 As shown in Table 3, The TFC of the vacuum-blanched 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-blanched 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

scavenging activity with IC_{50} values of 14.9 µg/mL. Similar values were also observed for ZB8 after 7 and 14 days with IC_{50} of 15.04 and 15.96 µg/mL, respectively.

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

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

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

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), β-carotene
299 bleaching test t30 (t0, t7, t14, t21, t28) and β-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

5. Conclusions

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

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

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

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

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Table 1. Changes in moisture, pH and TSS of different blanching treatments during storage

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

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

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

Table 2. Change in the color parameters of treated zucchini rings during storage

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

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

Table 3. Total phenolic (TPC) and total flavonoids (TFC) content in zucchini rings extracts

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

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

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

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

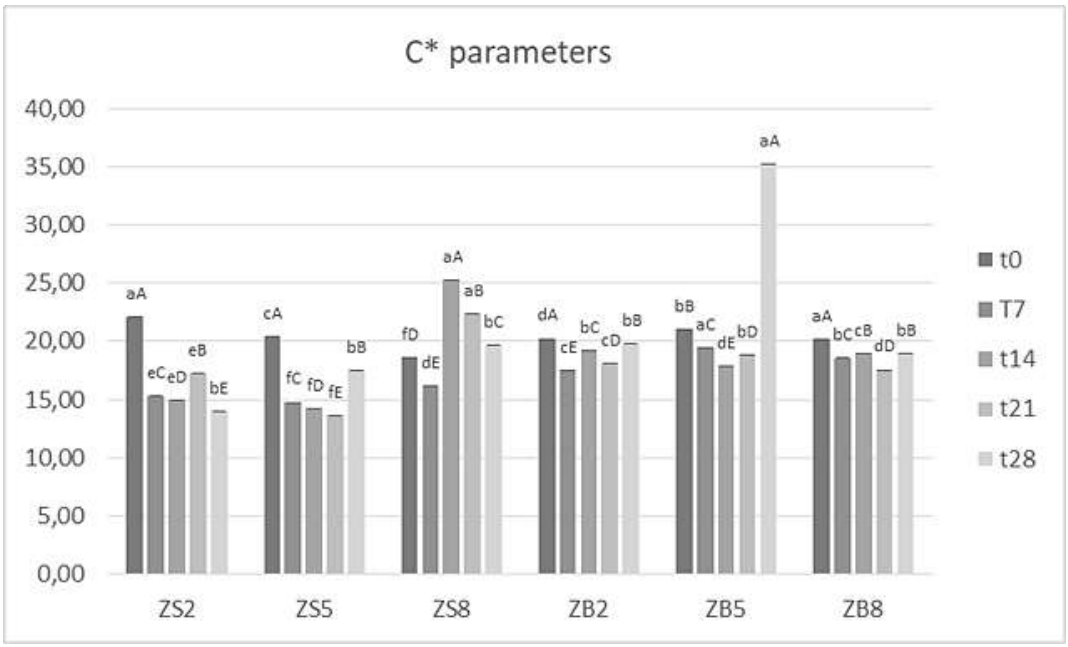
Data are expressed as means ± S.D. ($n = 3$). Differences within and between groups were evaluated by one-way ANOVA followed by a multicomparison Dunnett's test: ****$p < 0.0001$, compared with the positive control ascorbic acid (IC_{50} of 5.0 ± 0.07 and 1.7 ± 0.06 in DPPH and ABTS test, respectively).

Table 5. IC_{50} values of β-carotene bleaching test after 30 and 60 min of incubation of zucchini rings extracts

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

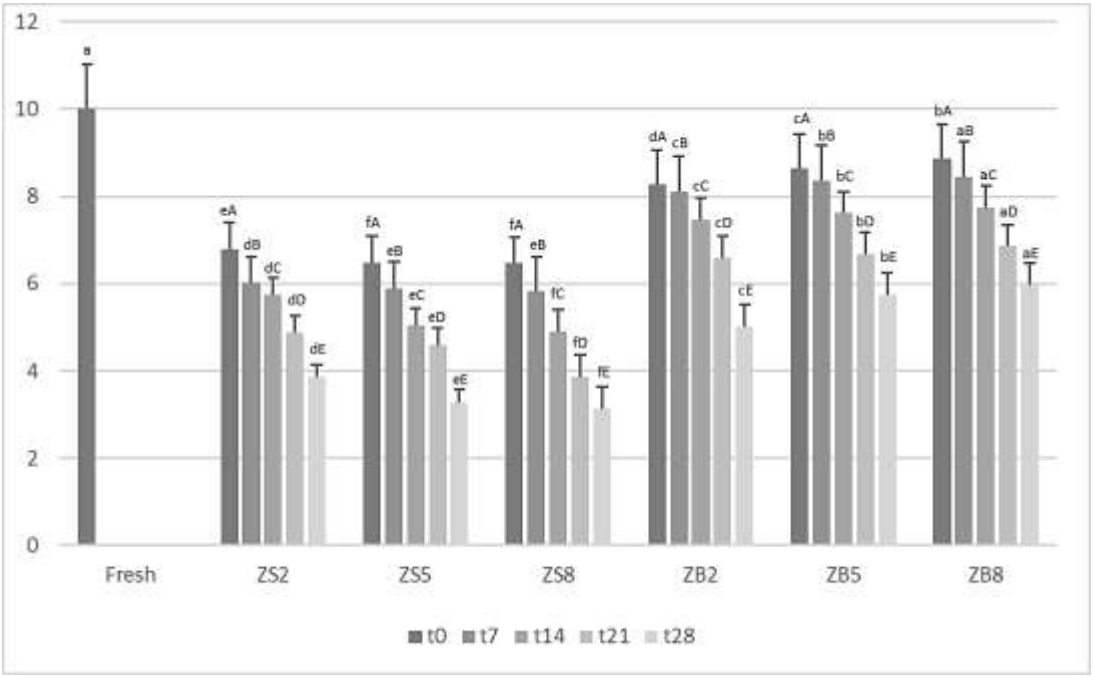
Data are expressed as means ± S.D. (n = 3). Differences within and between groups were evaluated by one-way ANOVA followed by a multicomparison Dunnett's test: ****p < 0.0001, compared with the positive control. [a] sample at concentration of 100 μg/mL.

Fig. 1. Comparition of croma values during zucchini *shelf-life* (t0-t28)



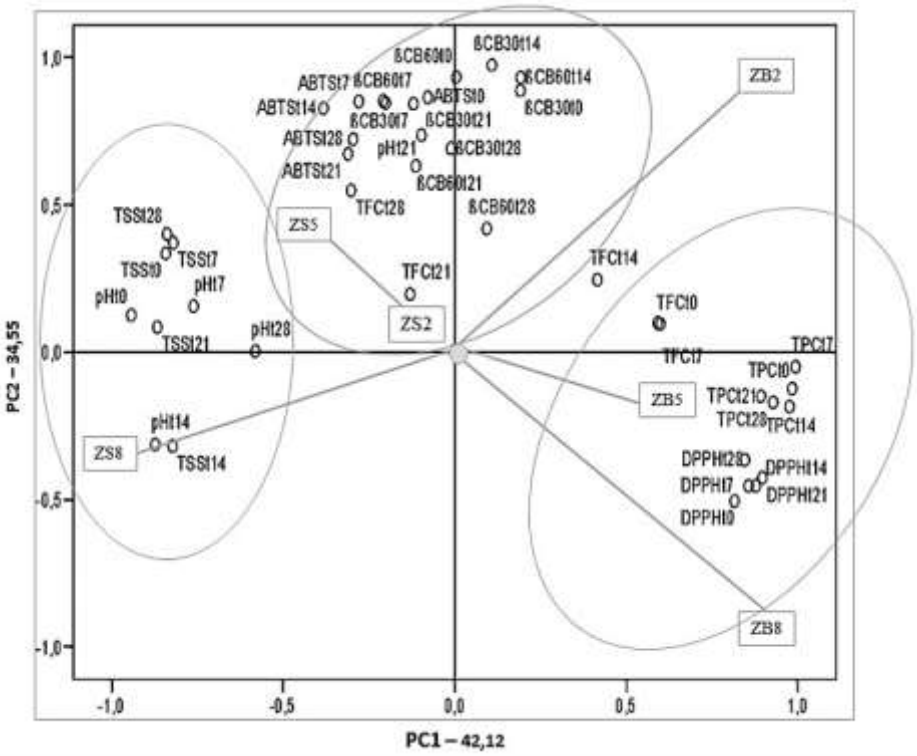
The data are presented as means ± standard deviations. Means followed by different capital letters, for the variation over time, and different lowercase letters, for the variation among different treatments, are significantly different by Tukey HSDa test. ** Significance at P < 0.01.

Fig. 2. Extraction yield



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

Fig. 3. Relationship between the two principal components as for the physical-chemical parameters, bioactive compounds and antioxidantactivity of the treatments between storage days 0 and 28.


ZB2
ZS5
ZS2
ZB5
ZS8
ZB8
ABTSt7
ABTSt14
ABTSt28
ABTSt21
ABTSt0
pHt21
pHt7
pHt0
pHt28
pHt14
TSSt28
TSSt7
TSSt0
TSSt21
TSSt14
TFCt21
TFCt28
TFCt14
TFCt0
TFCt7
TPCt7
TPCt0
TPCt21
TPCt28
TPCt14
DPPHt28
DPPHt14
DPPHt7
DPPHt21
DPPHt0
PC2 – 34,55
PC1 – 42,12
1,0
0,5
0,0
-0,5
-1,0