Increase of antioxidant activity of brined olives (Carolea cv.) thermally treated in different packaging types

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Running title: Different packaging types for pasteurised olives

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Abstract

This work was aimed to study the effect of a thermal processing on brined olives (and their covering liquids) packaged in different types of containers (glass jar with two volumes and plastic pouches made of two materials). The results show an increase of antioxidant activity of all pasteurised olives because of the melanoidin formation in pulp after processing with no differences among materials and sizes of packaging.
In this study, glass packaging involved lower depletion of total polyphenol content and better preservation of the α-tocopherol content in olive pulp than plastic pouches during the processing. The olives packaged in PA/PE showed higher content of antioxidant compounds than the olives processed in OPA/PP pouches.

Practical application:

Practical application for the study entitled “Increase of antioxidant activity on brined olives (Carolea cv.) thermally treated in different packaging types” regards the study of chemical changes on olives in different containers. Therefore in food industry the positive effect of heat and the right packaging form could be useful for preservation of olives and also enhancing of their functional properties.
The results of this work showed an increased antioxidant activity in brined olives thermally treated, due to the melanoidin formation. Therefore in food industry the positive effect of heat and the right packaging form could be suitable for preservation of olives and also enhancing of their functional properties.

1. Introduction

Brined olive drupes generally undergo fermentation with the production of lactic acid and a decrease in pH, which stabilizes the product. Sometimes the fermentation does not occur and brined olives become edible by diffusion processes. In fact, when the olives are put into brine solution, it is hard to predict the trend of fermentation because of several chemical, physical and microbiological variables. It is important to control some processing parameters, as pH, acidity, salt concentration, temperature, aerobic/anaerobic metabolism, use of starter bacteria (selected clones of L. plantarum). A concentrated brine of 6-7 % NaCl is essential for an optimal beginning and the salt percentage must be controlled and gradually increased to 10 %. It is necessary to compensate for the salt absorption into the drupes and to favour the optimal conditions for the growth of the bacteria useful for the olive fermentation. The bacteria growth depends also on drupe conditions linked to ripening, harvesting, and preliminary treatments. If the cited processing conditions do not develop the fermentation does not completely occur and the drupes bitterness is only partially leached into the brine. Because of this, the olives should be preserved by conditioning treatments, as sterilization, pasteurisation and preservative addition.

At the end of the brining process, the olives that have proper physicochemical characteristics can be packed and sold for consumption. In the past, the stability of packed olives was mainly influenced by their physicochemical characteristics, such as high free acidity values, sodium chloride content and low pH. Today a proper preservation of the product is achieved by the pasteurisation treatment. The microorganisms of reference for this process are the propionic bacteria. The minimum

value of lethality units established for a suitable preservation of the product is 15 [1]. In general, fresh brine is used in the final packaging of table olives; but the re-use of fermentation brine is also possible for this operation [2]. Although glass jars are the most widespread, plastic pouches are also frequently used as packaging material for several types of food preparations, especially for local brands and for a more functional use. However, their pasteurisation can be more difficult because heat accelerates the product browning caused by the polymerization of *o*-diphenols. Moreover the thermal process might cause some alterations of food, for example the degradation of colour and texture of pickled green olives as studied by Sánchez et al. [3]. Montano et al. [4] observed that processing and pasteurisation had no effect on the α-tocopherol and γ-tocopherol contents immediately after treatment, but significant losses of both tocopherols were found after 12 months of storage in olives previously pasteurised. Otherwise thermal treatments could have some positive effects on the properties of the treated foods, such as the improvement of the antioxidant activity [5, 6]. Normally, thermal treatments promote the Maillard reaction that, in the advanced stages, leads to melanoidin formation. Melanoidins are polymers of high molecular weight with biological and health implications like antioxidant activity [7], besides colour and taste modification on food. Some foodstuffs were studied for melanoidin content: cacao, roasted meat, bread, coffee beans, apricots, dried olives [8-13]. Piscopo et al. [13] evidenced that the changes in thermally treated olives (Carolea *cv*.) regarded the general diminution of polyphenolic compounds and a parallel increase of new formed dark products (melanoidins) that improve the total radical scavenging of vegetables.

The aim of this study is the evaluation of the thermal effect on the qualitative characteristics of brined olives packaged in containers that differed in materials and size. An investigation on variation of physical and chemical properties of olives after heat processing was conducted, with particular attention to the original antioxidant components and the new-formed ones, as the melanoidins. For this purpose, a sequential extraction was applied on samples to separate melanoidin fractions that are differently soluble to several solvents. The determination of antioxidant activity was achieved by using the DPPH methods and the ABTS assays that evaluate the radical scavenging of lipophilic and hydrophilic fraction on olives.

2. Material and Methods

2.1 Preparation of samples

Carolea variety olives were harvested at the completely green state, at maturation index of 0, in a specialized olive grove in Calabria. Calibration by weight was performed in order to have uniform fruit calipers (weight of whole drupe, pulp, kernel, pulp and kernel ratio). Carpological analyses were carried out on 50 fruits randomly sampled from the whole lot.The olives were put into 15 L- plastic containers filled with freshly prepared 7 % NaCl brine. Olives were brined with a fruit/brine ratio of 1.5 (10 kg of olives and 7 L of brine) approximately and maintained at ambient temperature. During the period of brining, salt concentration, pH and free acidity were monitored. After 6 months of brining, the olives were packed and thermally treated. The brines used to fill up the packages were the original ones, simply filtered. The used packages differed in material, shape and volume as follows:

a) Glass jar, 370 mL of volume (6.5 cm of diameter), filled with approximately 190 g of olives and 150 mL of brine, named P1;
b) Glass jar, 580 mL of volume (8.5 cm of diameter), filled with approximately 300 g of olives and 220 mL of brine, named P2;
c) PA/PE (Polyamide/Polyethylene) pouches, 450 mL of volume (20x15 cm), 90 μm of thickness, filled with approximately 190 g of olives and 150 mL of brine, named P3;
d) OPA/PP (oriented polyamide /polypropylene) pouches, 450 mL of volume (20 x 15 cm), 80 μm of thickness, filled with approximately 190 g of olives and 150 mL of brine, named P4.

After packaging, table olives were put in a thermostatic bath and the core of the samples was kept at 70°C for about 18 minutes. These conditions were chosen to stress the thermally assisted reactions and compare them with the table olive processing applied in small factories. Then the samples were rapidly cooled in an ice bath. The treatments were monitored by a Data Logger (Escort Junior, Astori tecnica, Italy) and were carried out in triplicate.

2.2 Chemicals

The following reagents were obtained commercially: acetic acid, acetonitrile, 2-propanol, chloroform, ethanol, *n*-hexane, 2-propanol, (Merck, Darmstadt, Germany); Folin Ciocalteu reagent, α-tocopherol, caffeic acid, cinnamic acid, ferulic acid, *o*-coumaric acid, quercetin 7- glucoside dehydrate, syringic acid, vanillic acid , 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) (Sigma-Aldrich, Switzerland); chlorogenic acid, gallic acid, tyrosol (Fluka, Steinheim, Germany);

hydroxytyrosol (TCI America, Portland OR, United States) oleuropein (Extrasynthèse, France). For the antioxidant assays the DPPH·(2,2-diphenyl-1-picrylhydrazyl) was provided from Carlo Erba (Milan, Italy) and the ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) was purchased from Sigma Aldrich (Switzerland)

2.3 Physicochemical analyses

The pH and free acidity of the olives were determined by the following extraction procedure: 10 g of each sample were homogenised with 30 mL of distilled water three times by an Ultraturrax. The filtrated solution was collected and filled up to 100 mL in a graduated flask with distilled water. This solution was used to measure pH and free acidity of flesh olives [14].

The water activity (a_w) was measured by an Aqua lab (3TE, Decagon devices Inc., Washington) apparatus which uses the chilled-mirror dew point technique to measure the a_w of the homogenized pulp samples.

The dry matter content was determined by oven drying at 105 °C up to constant weight.

The color of the olives was measured using a reflection colorimeter (Minolta CR 300, Osaka, Japan). The CIE L*a*b* coordinates were measured using D65 illuminant. Chroma (C*) was calculated as $(a^2 + b^2)^{1/2}$ and ΔE as $(\Delta L^2+\Delta a^2+\Delta b^2)^{1/2}$. Ten olives from each sample were randomly chosen for the color measurement that was carried out on two different points of the drupe surface. The brine color was measured on the filtered liquid put into appropriate vessels and repeated for 5 times.

The total polyphenols of the olives were extracted according to the method reported by Amiot et al. [15] and measured spectrophotometrically at 725 nm after reaction with the

Folin-Ciocalteu's reagent and expressed as mg of gallic acid equivalent (GAE) kg^{-1} of dry weight (d.w.). The total polyphenols in brines were quantified on 2 mL for each sample.

2.3.1 HPLC analysis of the phenol fraction

The preparation of olive extract, phenolic standards and HPLC analysis of phenols were carried out according to McDonald et al.[16]. An aliquot of olives were mixed with Methanol:water solution, then the filtrate was washed with hexane to remove oil. The extract was moreover filtered and diluted in the methanolic before the injection to the HPLC apparatus. The HPLC analysis was conducted using a HPLC Smartline Pump 1000 (Knauer, Berlin, Germany), equipped with Smartline UV detector 2600 (Knauer, Berlin, Germany) set at 280 nm. A C18 Eurospher 100-5 (5 μm particle size, 4.6 x 150 mm) column (Knauer, Berlin, Germany) fitted with guard column was used.

The injection volume was 20 μL, the solvent flow rate was 1.0 mL min^{-1} and the analysis was performed at 37 °C. The mobile phases consisted in water acidified with acetic acid 98:2, v/v (solvent A) and acetonitrile (solvent B). After 33 min of isocratic conditions in 95% A – and 5% B, the elution gradient changed to 70% A and 30% B in 10 min. After this period the gradient reached 65% A and 35% B in 10 min, and 50% A and 50% B in 10 min. Moreover it arrived to 5% A and 95% B after 10 min. This condition was maintained for 10 min, and then the gradient returned to 95% A and 5% B in 3 min and was maintained for other 9 min. The phenolic compounds were identified by comparing the retention times of with those of pure standards, gallic acid was used as internal standard. The results were expressed as mg kg^{-1} d.w.

The phenols in the brine were analyzed by HPLC after the acidification of 2 mL of brine with acetic acid (40 μL) and the addition of gallic acid as internal standard. The results were expressed as mg L^{-1}.

2.3.2 Tocopherol HPLC analysis

The lipid fraction was extracted by the method by Folch et al. [17] and the quantification was made by HPLC as reported by Lynch et al [18]. Briefly, after extraction 2 g of lipid fraction were dissolved in 10 mL of *n*-hexane and filtered on PVDF (Polivinyldenfluoride) filters (Sigma-Aldrich, Switzerland). The HPLC analysis was conducted using a Knauer HPLC Smartline Pump 1000, equipped with Knauer Smartline UV detector 2600 set at 294 nm. A C18 Knauer Eurospher 100-5 (5 μm particle size, 3.4 x 250 mm) column fitted with guard column was used. The mobile phase consisted in 0.5 % (v/v) of 2-propanol in *n*-hexane (solvent A) and 10 % of 2-propanol in *n*-hexane (solvent B). After 4 min of isocratic conditions in 100% A, the elution gradient moved to 60% A and 40% B in 18 min, to 40% A and 60% B in 4 min. Then the gradient returned to 100% A in 4 min and was maintained for other 5 min. The solvent flow rate was 1.0 mL min^{-1} and the analysis was performed at 37 °C. The results were expressed as mg of α-tocopherol kg^{-1} d.w. The identification and quantification were performed using pure α-tocopherol as a standard and a calibration line was obtained between the concentration of 10 mg kg^{-1} and 250 mg kg^{-1}.

2.3.3 Total antioxidant capacity: DPPH· and ABTS assays

To determine the antioxidant capacity by DPPH·, the olive extract was prepared according to Nakatani et al. [19] with some modifications. 25 mL of distilled water were added to 3 g of sample, homogenized with a vortex apparatus for 1 min and

centrifuged at 6000 g at ambient temperature for 5 min. The supernatant was filtered through a Whatman n. 4 filter and then through a 0.45 µm filter, before the spectrophotometric reading. The total antioxidant activity determination was performed following Brand-Williams et al. [20], based on the reaction mechanism between the free radical DPPH· (2,2-diphenyl-1-picrylhydrazyl) and the antioxidants present in the samples.

25 µl of the sample extract were left for 2 hours and 30 minutes in a cuvette containing 3 ml of a 6 x 10^{-5} M methanol solution of DPPH· in order to obtain a decrease in absorbance. The spectrophotometric reading was conducted at dark in a UV-Vis spectrometer (Agilent mod. 8453, Santa Clara, United States) at 515 nm of wavelength and a temperature of 20 °C to eliminate the risk of thermal degradation of the molecules tested [21]. A graph of absorbance versus time showed that decoloration curve of the radical decrease followed a fourth order kinetic (R^2 = 0.99). The results were expressed as -OD^{-3} min^{-1} g^{-1} d.w., as in the following formula:

A^{-3}- A_0^{-3} = -3kt

were OD is the optical density, A_0 is the initial OD, A is the OD at rising time t.

The antioxidant activity of the brine was obtained after dilution of 1 mL of sample into 10 mL of distilled water and was expressed as -OD^{-3} min^{-1} mL^1.

The methods reported by Othman et al. [22] and Re et al. [23] were used for extraction and antioxidant capacity determination by ABTS assay, respectively. This analysis evaluates the capacity of the sample to inhibit the ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical oxidation, as compared to a standard antioxidant (0-15 µM of Trolox). After the preparation of the ABTS

radical, the spectrophotometric analysis was performed at 734 nm and the results were calculated as inhibition percentage. The following formula was applied:

Inhibition % = $((OD_0\text{-}OD)\ OD_0^{-1}) \times 100$

where OD is the optical density, OD_0 at the initial time and OD at the final time. Inhibition % was plotted as a function of the concentration of the extracts and of Trolox for the standard reference data. The antioxidant capacity was expressed as TEAC (Trolox Equivalent Antioxidant Capacity) values, μmol Trolox g^{-1} d.w.).

2.3.4 Extraction and Fractionation of melanoidins

The extraction of melanoidins was carried out following the literature [12, 24]. Three fractions were obtained after extraction with water, ethanol/water and propanol/water and were named F1, F2 and F3, respectively. The remaining solid fraction, named F4, consisted of pieces of fruit. The yields of the individual fractions (g of dry matter per 100 g of dried fruit) were recorded.

2.4. Statistical analysis

One-way and two-way analyses of variance (ANOVA) were applied to the data to determine the presence of significant differences (Tukey's test, significant level $P<0.05$). Moreover Pearson's correlation was determined between the DPPH· assay and the total phenol content. SPSS software (Version 11.0, SPSS Inc., Chicago, IL, USA) was used for data processing.

3. Results and Discussion

The carpological analysis of the olives showed that the drupes are of good quality, with a flesh to stone ratio (4.75±1.39) that confirms they are suitable for table consumption. Their whole weight being 4.8 g, these olives fall in a medium size type standard (data not shown).

The color parameters were measured in olive samples to compare the thermal treatment effects. Significant differences were observed between samples packed in the two materials but no one between different volumes of glass jars and moreover between the two types of plastic materials, as denoted by post-hoc test (Table 1). P1 and P2, olives packaged into glass jars, generally had the same chromatisms as the starting material (B sample), with the exception of a light browning, expressed in the increased a* values. In P3 and P4, olives packaged in plastic pouches, the darkening was accentuated also by decrease in luminosity (L*), probably due to the stronger effect of heat on plastic pouches. The results for blueness-yellowness (b*) and Chroma had a similar trend in all the samples, with a decrease in P3 and P4 and no statistical differences between types of plastic pouches. The ΔE* parameter, that measures the colour differences, confirmed the results above discussed. In fact, low changes respect to the unpasteurised samples were observed in the colour of olives packaged in both glass containers and also in P3, represented by olives packaged in PA/PE plastic pouches. P4 showed the highest colour variation (8.03±3.95).

The chlorophyll breakdown, the enzymatic reactions, the chemical oxidation of natural *o*-diphenols of olives, the new formation of dark colored compounds derived by the Maillard reaction can explain these changes of the olive appearance.

No significant differences (P>0.05) were observed concerning the a_w values measured on samples. The thermally treated samples did not show any significant difference in terms of free acidity with respect to the olives before treatment, but a decrease in pH was observed, particularly in olives packed in pouches. The highest decrease of pH in P3 and P4 could be explained by a stronger heat transfer to the drupes through the plastic packages respect the drupes preserved in glass materials. This could involve a modified acid-base equilibrium of some components. The total phenol content significantly decreased after thermal treatment (P<0.01), in particular in olives packed in plastic pouches. Several authors reported this trend in olives after heating [25, 26]. Heating promoted the loss of drupe solution, including hydrophilic substances like phenols, which probably diffused through the drupe fractures to the brine. Moreover, the polyphenol depletion could also be due to the enzymatic reaction that maintains the polyphenol oxidase (PPO) active at some given conditions (temperatures below 50°C) [27]. As demonstrated by the recorded thermal profiles (data not shown), the pasteurisation process highlighted a 20-minutes period below 50 °C. These conditions could promote the polyphenol oxidase activity and the following chemical change. Moreover, the highest polyphenol degradation was observed in OPA/PP pouches that probably transferred the heat more strongly than the other studied plastic material. The α–tocopherol content in food depends on many variables: species, variety, harvest, processing, storage time and conditions, sample preparation and variation in analytical methods [28]. The initial content of 532 mg of α–tocopherol kg^{-1} d.w of olives submitted a significant decrease after brining and thermal process. The data in Table 1 show that heating leads to an increase in α–tocopherol amounts in all treated olive

pulps. These results are confirmed in the literature [29] that shows higher levels of α–tocopherol in some processed fruits and vegetables with respect to the raw products. Moreover some marked differences were observed between different packaging materials, i.e. glass and plastic, but no variations were observed when comparing the different volumes of glass jars and the two plastic materials. The main polyphenols identified and quantified by HPLC in olive pulp are reported in Table 2. As an effect of the brining process, oleuropein, the most representative phenol in olives, underwent hydrolysis resulting in hydroxytyrosol. Actually, this compound was present in brined olives and especially in treated ones in higher amounts than in fresh olives. As confirmed by literature [30], tyrosol is usually present in olives in lower content amounts than hydroxytyrosol. After the applied thermal treatment the tyrosol content in the different tested samples did not vary significantly. Both hydroxytyrosol and tyrosol are anyway responsible for antioxidant properties, as previously demonstrated by Owen et al. [31]. Among the others phenols quantified, caffeic acid is important for its chelating activity on metals [32] and antioxidant activity on the lipid fraction [16]. Its content did not significantly change after pasteurisation, as oleuropein and quercetin-7-glucoside. The thermal treatment induced a decrease in chlorogenic and ferulic acids, while the syringic acid content tended to increase without differences between differently packaged olives. Pasteurisation, sterilization and thermal processes in general can improve the antioxidant property of food [33]. The treated olives had an increased antioxidant activity as measured by DPPH· moving from 26.25 -$OD^{-3}min^{-1}$ g^{-1} d.w. to about 40 -$OD^{-3}min^{-1}$ g^{-1} d.w (Table 3). A similar trend was observed in measurements by ABTS moving from an initial value of 6.25 µmol Trolox g^{-1} d.w to 8-

9 µmol Trolox g^{-1} d.w after heating. No difference was observed between the various packaging alternatives by post-hoc test, showing a similar effect of heat on the Maillard reaction. However these results confirm the effect of heat and the formation of polymers which affect the biological activity [34]. The correlation between the total phenols and the antioxidant activity (DPPH· assay) was revealed by a Pearson test ($r=0.769$, $P<0.05$). A not exclusive contribution of phenols was observed, but also new compounds seemed to influence the overall antioxidant activity of the olive preparations studied, like the Maillard reaction products. Concerning the melanoidin yield obtained after sequential solvent extraction, the most abundant fraction was F1 with an amount ranging from 2.79 g d.w. 100 g^{-1} of dried fruit in P2 to 6.01 g d.w. 100 g^{-1} of dried fruit in P4. It proved the presence of polar monomers or polymers with high solubility in water, probably derived from Maillard reaction [35]. Figure 1 illustrates the antioxidant activities of melanoidin fractions isolated from olive samples. As confirmed by the literature for other food matrix [24], the dark brown ethanol soluble fraction (F2) of olive samples showed great activity, in particular in olives packed in plastic pouches (P2). As expected, F4, which represents the residual matter, was the least active isolated melanoidin. Table 4 shows that the water-soluble fraction (F1) was the biggest contributor to the overall antioxidant power of olives, as highlighted previously [12], with the only exception in P2. The different size of glass containers thus influenced the heat transfer to the olives during pasteurization and so the formation of the different melanoidin fractions. It was reflected also in the explicated antioxidant activity of these. The sum of radical scavenging activities of the single fractions was higher than the

measured power of the whole set of treated olives. This confirms the observations conducted on other types of heated olives [13].

The evaluation of brine characteristics after thermal treatment was considered as important from the visual point of view, considering also the impact on consumer acceptance. Regarding the brine color, significant differences were revealed by the statistical analysis of different samples, in particular a big difference was observed between the two types of pouches. A general blanching effect was observed in all the treated olives, and in particular in P4, which had the lowest value of a* and the highest amounts of L* and b* (Table 5).

The thermal process produced a decrease in pH in the brines of olives treated in pouches, reflecting also an increase in free acidity. The differences in acidity between brines contained in glass and plastic materials could probably be linked to a bigger extraction effect in plastic pouches (P3 and P4) because of olive shaking movement inside the pouches and the further diffusion in brines. P1 and P2 olive samples did not suffer this phenomenon due to the rather stationary position into the glass jars. However, all the brines had a pH value above the hygienic limit of 4.5 with the only exception of P3.

The most important identified phenols in brines were hydroxytyrosol, tyrosol and oleuropein. A significant increase in the hydroxytyrosol amount and a corresponding decay of the oleuropein content were observed. As previously highlighted in olive flesh, no difference was observed in tyrosol concentration in brines before and after heating. A greater total content of polyphenols was quantified in brines after pasteurisation, particularly in glass jars with no differences between packaging sizes. This event is

ascribed to the previously discussed diffusion of these compounds from flesh to brine. Finally, concerning the brine antioxidant capacity expressed by DPPH· assay, a similar increasing trend to olives was observed in brines after thermal treatment. Nevertheless the different packaging types did not statistically influenced the obtained results.

4. Conclusions

The pasteurised brined olives studied were shown to have different physico-chemical characteristics. A positive effect of thermal treatment, in terms of increased antioxidant activity, was observed in all the packages tested as proven by melanoidin formation. Glass jars performed better than plastic pouches as to these properties with no great differences about the type of container. The olives packaged in OPA/PP pouches were affected by several negative changes, so the PA/PE pouches were more useful to the studied processed food. It can be concluded that the packaging material affects the quality of olives, promoting or reducing some chemical changes as the presence of constituents having a radical scavenging activity. A stronger contribution of water-soluble melanoidins to the overall antioxidant activity of olives was observed. Further studies of the shelf life would be worth doing to identify the best packaging for these products.

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Table 1 Physico-chemical analyses of olive samples before and after thermal treatment

Samples[§]	B	P1	P2	P3	P4	Sign.
L*	50.86±4.14^{a}	50.42±3.78^{a}	50.57±4.37^{a}	46.04±3.57^{b}	44.84±4.42^{b}	<0.001
a*	5.44±1.74^{b}	6.36±1.09^{ab}	6.47±0.95^{ab}	6.86±0.68^{a}	7.07±1.22^{a}	<0.001
b*	38.81±4.57^{a}	36.93±3.89^{a}	37.30±4.90^{a}	31.86±4.66^{b}	29.73±6.17^{b}	<0.001
Chroma	39.24±4.40^{a}	37.51±3.65^{a}	37.89±4.70^{a}	32.62±4.50^{b}	30.64±5.85^{b}	<0.001
ΔE	//	3.05±1.25^{b}	2.83±1.06^{b}	2.29±0.54^{b}	8.03±3.95^{a}	<0.001
a_w	0.97±0.00	0.96±0.01	0.95±0.00	0.96±0.00	0.96±0.01	0.144
Dry matter (%)	35.60±1.45^{b}	36,58±0.50^{b}	38,26±0.63^{ab}	38,50±0.99^{ab}	39,04±0.84^{a}	<0.001
Total acidity (% of citric acid)	0.51±0.07	0.51±0.05	0.57±0.02	0.60±0.07	0.51±0.07	0.288.
pH	5.22±0.07^{a}	5.13±0.02^{ab}	5.11±0.01^{ab}	5.00±0.03^{c}	5.07±0.04^{bc}	0.001
Total polyphenols (mg kg^{-1})	10307±342^{a}	7470±1026^{bc}	8217±665^{b}	6842±286^{c}	4969±228^{d}	<0.001
Total tocopherols (mg kg^{-1})	231 ±10^{c}	371±36^{a}	372±22^{a}	309 ±5^{b}	287.36±67^{b}	<0.001

Results are expressed as mean ±SD of three sample replicates. § B (Brined olives); P1 (Olives pasteurised in 370 mL-glass jars); P2 (Olives pasteurised in 580 mL-glass jars); P3 (Olives pasteurised in PA/PE pouches); P4 (Olives pasteurised in OPA/PP pouches). Results followed by different letters are significantly different by Tukey's multiple range test.

Table 2 Phenolic compounds determined by HPLC on olives before and after thermal treatment.

Samples	B	P1	P2	P3	P4	Sign
Hydroxytyrosol	279.58±0.19^{b}	326.65±6.22^{ab}	370.13±36.69^{a}	394.96±0.84^{a}	373.05±14.13^{a}	0.007
Tyrosol	18.85±2.27	19.88±0.1	21.99±1.46	20.66±0.92	18.73±0.71	0.216
Chlorogenic acid	10.31±1.05^{a}	3.57±0.08^{b}	3.85±0.06^{b}	2.11±0.24^{b}	2.58±0.31^{b}	<0.001
Caffeic acid	20.11±5.29	25.01±0.52	25.86±1.01	26.67±3.78	25.64±0.01	0.314
Syringic acid	15.27±0.44^{b}	24.05±1.69^{a}	26.81±2.36^{a}	25.61±2.12^{a}	24.14±0.04^{a}	0.005
Ferulic acid	26.88±1.08^{a}	3.21±0.68^{b}	2.86±0.36^{b}	0.01±0.00^{c}	0.01±0.02^{c}	<0.001
***o*-Cumaric acid**	9.62±1.53^{b}	12.28±0.7^{ab}	13.35±0.17^{a}	11.28±0.22^{ab}	11.72±1.1^{ab}	0.063
Oleuropein	1.36±1.92	2.57±0.39	2.75±0.09	3.24±0.49	3.37±0.24	0.305.
Quercetin-7 glucoside	38.84±6.12	31.03±1.16	30.19±0.05	31.53±0.34	30.03±0.57	0.103

Results are expressed as mean ±SD of three sample replicates. § B, P1, P2, P3, P4, a,b,c see Table 1

Table 3 Antioxidant activity of olives samples before and after thermal treatment

Samples	B	P1	P2	P3	P4	Sign.
DPPH˙assay¥	26.25 ±1.47^{b}	43.77±3.01^{a}	43.76 ±3.04^{a}	39.26 ±2.20^{a}	39.18 ±1.81^{a}	<0.001
ABTS assay§	6.25±0.10^{b}	9.70 ±0.92^{a}	8.81±0.40^{a}	8.39±1.03^{a}	8.35±0.89^{a}	0.003

Results are expressed as mean ±SD of three sample replicates. data expressed as: ¥-$OD^{-3}min^{-1}$ g^{-1} d.w.; § µmol Trolox $d.w.^{-1}$. B, P1, P2, P3, P4; a,b,c see Table 1

Table 4 Relative and overall contribute of melanoidin fractions (F1, F2 and F3) to the olive antioxidant activity (-OD^{-3} min^{-1} g^{-1} d.w.)

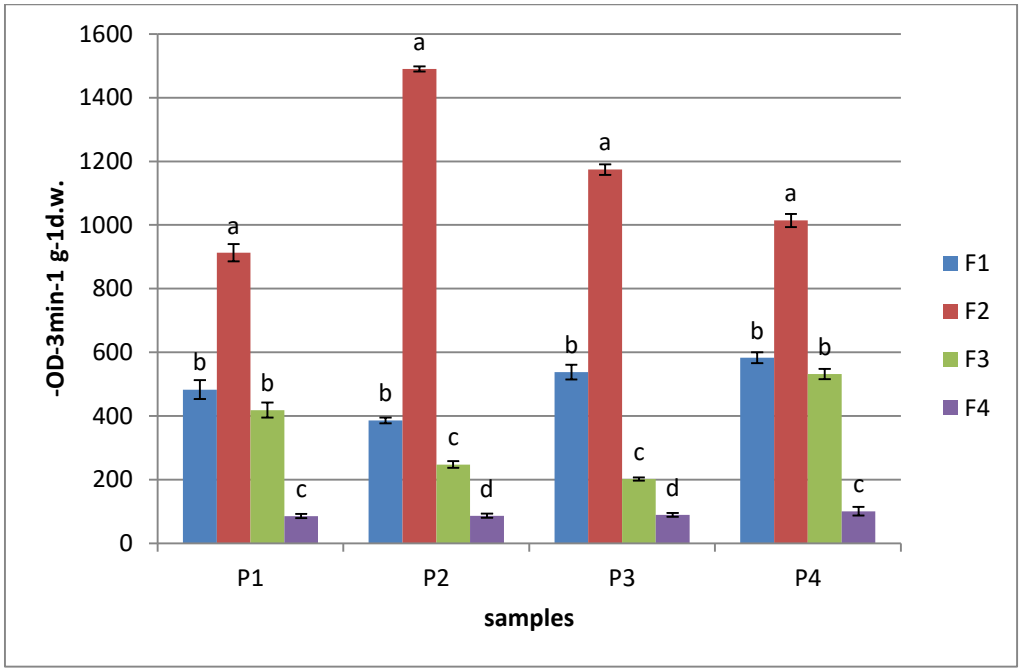
Samples	F1	F2	F3	Sign.	∑	Antioxidant activity of olives
P1	49.31±3.05^{a}	14.48±0.43^{b}	8.38±0.48^{b}	<0.001	72.16	43.77
P2	25.8±0.61^{b}	30.48±0.16^{a}	3.41±0.15^{c}	<0.001	59.68	43.76
P3	53.64±2.32^{a}	18.18±0.26^{b}	3.95±0.11^{c}	<0.001	75.77	39.26
P4	52.11±1.57^{a}	11.56±0.24^{b}	4.63±0.14^{c}	<0.001	68.30	39.18

Results are expressed as mean ±SD of three sample replicates. P1, P2, P3, P4; a,b,c see Table 1

Table 5 Physico-chemical analyses of brines before and after thermal treatment

Samples	B	P1	P2	P3	P4	Sign.
L*	24.57±0.03^{b}	24.59±0.03^{ab}	24.51±0.04^{b}	24.43±0.04^{b}	24.82±0.03^{a}	0.001
a*	-0.36±0.01^{a}	-0.47±0.01^{bc}	-0.46±0.02^{ab}	-0.40±0.02^{ab}	-0.56±0.01^{c}	<0.001
b*	0.18±0.01^{c}	0.26±0.03^{b}	0.27±0.03^{b}	0.69±0.03^{a}	0.68±0.08^{a}	<0.001
Chroma	0.41±0.01^{c}	0.54±0.02^{b}	0.53±0.02^{b}	0.85±0.03^{a}	0.88±0.08^{a}	<0.001
ΔE	//	0.14±0.03^{b}	0.15±0.03^{b}	0.59±0.00^{a}	0.62±0.09^{a}	<0.001
Acidity(g % of citric acid)	0.17±0.09^{b}	0.08±0.18^{c}	0.07±0.02^{c}	0.34±0.38^{a}	0.33±0.10^{a}	<0.001
pH	4.73±0.03^{a}	4.67±0.01^{a}	4.68±0.01^{a}	4.50±0.02^{b}	4.57±0.07^{b}	<0.001
Hydroxytyrosol (mg L^{-1})	140.78±24.76^{b}	359,88±20.28^{a}	365,16±28,32^{a}	302,10±83,93^{ab}	271,80±22,79^{ab}	0.017
Tyrosol (mg L^{-1})	55,87±30,92	52,03±0,89	45,77±0,88	37,27±4,78	39,08±0,34	0.647
Oleuropein(mg L^{-1})	32,85±1,52^{a}	10,63±0,02^{b}	7,36±1,03^{bc}	6,01±1,29^{bc}	4,27±2,07^{c}	<0.001
Total polyphenols(mg of gallic acid L^{-1})	119,41±6,80^{c}	1297,40±19,97^{a}	1262,63±40,12^{ab}	1192,35±79,85^{ab}	1155,85±7,91^{b}	<0.001
DPPH˙assay (-OD^{-3} $min^{-1}mL^{-1}$)	91,03±1,42^{b}	190,97±21,46^{a}	151,07±37,01^{a}	146,27±4,42^{a}	149,93±13,05^{a}	0.002

Results are expressed as mean ±SD of three sample replicates; B, P1, P2, P3, P4; a,b,c see Table 1



P1, P2, P3, P4; a,b,c see Table 1

Figure 1 Antioxidant activity by DPPH assay of the melanoidin fractions