# ANTIOXIDANT ACTIVITY OF DRIED GREEN OLIVES (CAROLEA CV.)

Amalia Piscopo *, Alessandra De Bruno [a], Angela Zappia [b], Marco Poiana [c]

Department of AGRARIA, University Mediterranea of Reggio Calabria, 89124 Vito (Reggio Calabria-Italy)

*corresponding author. Tel. +39 0965814998, fax +39 0965311092. E-mail address: amalia.piscopo@unirc.it,

[a] E-mail address: alessandra.debruno@unirc.it

[b] E-mail address: angela.zappia@unirc.it

[c] E-mail address: mpoiana@unirc.it

**Abstract**

The main objective of this paper was to evaluate the variation of qualitative parameters in dried olives and brined dried olives. Physical and chemical analyses and an investigation of new formed antioxidants were carried out on samples before and after treatments. The results of analyses reported a decreased content of total phenolic compounds after drying in both typologies of olives, with the biggest reduction at medium temperature. On the other hand, higher drying temperature increased the total antioxidant capacity of olives and this is probably related to the new formation of melanoidins. A further fractionation of these compounds confirmed its contribution to the overall reducing property of the extracts of dried olives. Brined olives were instead characterized by a lower amount of antioxidant constituents, particularly phenolics. They decreased in olive pulp due to its osmotic release in the brine during fermentation and successively by the oxidative reaction after drying.

**Key words:** ABTS, Antioxidant capacity, DPPH, Drying, Melanoidins, Olives

## 1. Introduction

According to the International Olive Council (2012) total world production of table olives in 2011/2012 was approximately 2.5 million tonnes with an attested increase compared to previous years. Generally, in Italy the use of olive varieties for oil production is more widespread than for table olives. The consumption of table olives, in particular green olives, is increasing which tend to be preferred by Italian consumers. The principal aim of table olive processing is to hydrolyze the oleuropein, the bitter phenolic agent naturally present in the drupe which depends on local methods and customs. Before the fermentation process, the olives can be treated in sodium hydroxide, put into brine and successively rinsed in water, or they can also be directly fermented in brine (natural method).

Southern Italian businesses are often characterized by low production capacity, small size and manual practices. A recent work (Romeo, Piscopo, Mincione, and Poiana, 2012a) evidenced a great heterogeneity among commercial olive preparations (Nocellara del Belice *cv)* for the same kind of treatment and company. This fact reflected a low level of standardization achieved in these productions typical of the Southern Italy. Because of the economic importance of these foodstuffs and the variability depending on the cultivar characteristics, recently several studies have also been based on table olive processing (Aponte et al., 2010; Romeo, Piscopo, and Poiana, 2010). Product preservation is generally determined by final brine conditions such as low pH and high acidity and by the bacteriostatic activity of sodium chloride. Therefore product thermal stabilization may not be necessary but several doubts have recently been risen about the hygienic conditions of non-pasteurized olives. The use of heat treatment for prolonging shelf-life principally by destroying pathogens, reducing total bacterial count and deactivating enzymes, increases food stability and lessens the oxidizing processes. In the Mediterranean area, dehydration is a traditional method to preserve olives which is often used locally in non-industrial ovens. This technology can pose concerns about the quality and safety of the end product so careful control of process parameters is

necessary. Several studies have focused on the effects of some pre-treatments or the olive varietal characteristics on the final products (Gambella, Piga, Agabbio, Vacca, and D'hallewin, 2000; Öngen, Sargin, Tetik, and Köse, 2005; Romeo, Piscopo, and Poiana, 2012b). Convection drying is the most widely used technique for the production of dehydrated fruits and vegetables whereas heat treatments have a negative influence on product quality since they affect appearance (Brasiello, Adiletta, Russo, Crescitelli, Albanese, and Di Matteo, 2013), polyphenolic, lipid, protein and vitamin fractions. Indeed Romeo, De Luca, Piscopo, Perri, and Poiana (2009) observed a decrease of *o*-diphenolic compounds in green table olives after pasteurization. On the other hand, literature has reported an increase of antioxidant activity in other drupes after application of processing temperatures because of the formation of new products derived by Maillard reaction (Madrau, Sanguinetti, Del Caro, Fadda, and Piga, 2010) such as the intermediate hydroxymethylfurfural (HMF).

Melanoidins are polymeric and brown compounds formed at the advanced stages of Maillard reaction. The chemical structures of melanoidins are not yet widely known but it is assumed that they differ in molecular weight due to the interaction of their surface with other compounds. Several studies have reported that in fat moiety, heat treatment stimulates the formation of brown polymers by a reaction between the fat carbonyl and amino groups (Oliviero, Capuano, Cammerer, and Fogliano, 2009; Hidalgo and Zamora, 2008). It has been reported that melanoidin fractions in food and model systems can scavenge a variety of reactive oxygen species (Wang, Qian, and Yao, 2011). In this respect the application of heat treatments on table olives appears to be significant in developing new alternative products and tastes which are also provided with functional properties.

The aim of this work is to evaluate the effects of drying treatment on olives (Carolea *cv*) before and after brining, with particular reference to antioxidant compounds. No scientific data appear on literature about the thermal effect on the Carolea olives, in terms of antioxidants and their *in vitro* activity. The Carolea *cv* is widespread in the South of Italy and this study could be useful to

78 evaluate future applications of drying process to obtain "ready-to-eat" functional foods and
79 ingredients for food industry, e.g., pizza topping, and bakery products.
80
81 **2. Materials and methods**
82 **2.1 Sampling**
83 Carolea green olives were harvested in October 2012 in an olive grove in Rizziconi (province of
84 Reggio Calabria, Italy) and immediately transported to the laboratory where only fruits without peel
85 defects were selected. Calibration by weight was performed in order to have uniform fruit sizes.
86 Carpological analyses were carried out on 50 fruits randomly sampled within the lot.
87
88 **2.2 Materials**
89 Olives (named "O") were separated into two aliquots: a part of these was immediately dried ("DO":
90 Dried Olives) in a tangential air-flow cabinet ("Scirocco" model, Società Italiana Essiccatoi, Milan,
91 Italy), equipped with automatic temperature and air moisture control devices Air flows tangentially
92 to fruits (1840 $m^3$/h), while a recycling system allows for mixing the exhaust with fresh air. The
93 drying process was applied until the olives, placed on 56 cm diameter steel food trays, reached a dry
94 matter value of 70% (of final olive weight) estimated by weight loss calculation. The air
95 temperatures were set at 50 °C and 70 °C throughout the process, so the dried samples were named
96 "DO 50", "DO 70".
97 The other aliquot of olives was put in triplicate into 15 L plastic containers, filled with freshly
98 prepared 7% NaCl brine. Olives were brined with a fruit/brine ratio of 1.5 approximately (10 kg/7
99 L) and maintained at a controlled temperature of 20-25 °C. During the brining period the following
100 indexes were monitored by sampling the brine at several layers: pH by electrochemical
101 determination, free acidity by titration and salt concentration by Mohr method. After 180 days,
102 brined olives (BO) were dried at the same thermal conditions illustrated above ("DBO 50" and
103 "DBO 70", depending on the drying temperature).

## 2.3 Methods

### *2.3.1 Physicochemical analyses*

For pH and free acidity determinations, olives were submitted to the following extraction procedure: 10 g of each sample were mixed with 30 mL of distilled water three times with an Ultraturrax at 11000 rpm and the filtrated solution was then 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.

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 homogenized pulp samples. Dry matter content was determined by oven drying at 105 °C up to constant weight. These analyses were carried out on ten homogenized olives.

### *2.3.2 Colour determination*

The colour of the olives was measured using a reflection colorimeter (Minolta CR 300, Osaka, Japan). The CIE L*a*b* coordinates were measured using a D65 illuminant. L* represents the lightness, a* and b* the amount of red-green and blue-yellow tones, respectively.

This analysis was assessed on two points of each olive and for ten olives randomly chosen for each sample. Chroma (C*), which represents the degree of saturation or fullness of colour, was calculated as $(a^2 + b^2)^{1/2}$.

### *2.3.3 Total polyphenol content*

Polyphenols of the olives were extracted according to the method reported by Amiot, Fleuriet and Macheix (1986). About 10 g of olives were homogenized with 75 mL of methanol/water (80:20) solution containing 20 mg/L sodium dietyldithiocarbamate trihydrate (DIECA). The extract was centrifuged and the extraction was repeated twice. Samples were filled up to a volume of 250 mL

and analysed spectrophotometrically at 725 nm after reaction with the Folin-Ciocalteu reagent. Results were expressed as mg gallic acid equivalent (GAE) /100 g of dry weight (d.w.).

### *2.3.4 HPLC analyses*

Preparation of olive extract, phenolic standards and HPLC analysis of phenols were carried out according to McDonald, Prenzler, Antolovich, and Robards (2001). Pure standards were purchased from Fluka (vanillic acid, *o*-cumaric acid, caffeic acid, cinnamic acid, ferulic acid, syringic acid, quercetin dihydrate), Sigma Aldrich (gallic acid as internal standard, tyrosol, chlorogenic acid) and Extrasynthèse (hydroxytyrosol and oleuropein). HPLC analysis was conducted using a Knauer HPLC Smartline Pump 1000, equipped with Knauer Smartline UV detector 2600 set at 280 nm. A C18 Knauer Eurospher 100-5 (4.6 x150 mm, 5 μm) column fitted with a guard column were used. The mobile phase utilised consisted of water acidified with acetic acid (98:2, v/v, solvent A) and acetonitrile (solvent B). After 33 min of isocratic conditions in 95% A, the elution gradient was 70% (A) in 10 min, 65% (A) in 10 min, 50% (A) in 10 min, followed by 5% (A) – 95% (B) in 10 min. This condition was maintained for 10 min and then the gradient returned to 95% (A) – 5% (B) in 3 min and was maintained for other 9 min.

The solvent flow rate was 1.0 mL/min and the analysis was performed at 37 °C with an injection volume of 20 μL. Results were expressed as mg/100 g d.w.

### *2.3.5 Melanoidin extraction*

For this procedure, the of Lindermeier, Faist, and Hoffmann method (2002) was followed in which 100 g of pitted and grinded olives (diameter less than 2 mm) were defatted with $CHCl_3$ by stirring. After solvent evaporation in a rotary evaporator, 200 mL of bidistilled water were added to the residual solid and the mixture sonicated at 40 °C for 30 min. The water fraction was collected and the operation was repeated on the solid phase. The two water fractions were combined and centrifuged at 8,500 x g for 15 min at 15 °C and the supernatant was then evaporated under vacuum

(Fraction I). The residual solids were dissolved in 200 mL of ethanol/water (60:40 v/v) solution, and then sonicated for 30 min at room temperature. This operation was repeated twice. The two ethanols: water extracts were combined and centrifuged at 8,500 x g for 15 min at 15 °C, and the supernatant was then evaporated under vacuum (Fraction II). The residual solids were dissolved in 200 mL of 2-propanol/water (50:50 v/v), and then sonicated for 60 min at room temperature. This operation was repeated twice. The two propanol/water extracts were combined and centrifuged at 8,500 x g for 15 min at 15 °C, and the supernatant was then evaporated under vacuum (Fraction III). The remaining solid fraction, consisting of pieces of drupe, represented fraction IV. The yield of each fraction (as gram per 100 g of dried weight) was recorded.

For the antioxidant capacity determination by DPPH assay, 1 g of melanoidin fraction was dissolved in 25 mL of distilled water following the procedure reported by Nakatani, Kajano, Kikuzaki, Sumino, Katagiri, and Mitani (2000) and analysed for total antioxidant capacity.

### *2.3.6.Total antioxidant capacity: DPPH· and ABTS assays*

For the determination of antioxidant capacity by DPPH· method, the procedure to prepare the olive extract was carried out according to Nakatani et al. (2000) with some modifications. 25 mL of distilled $H_2O$ were added to 3 g of the sample, placed in the vortex for 1 min and centrifuged at 6,000 g at room temperature for 5 min. The supernatant was filtered through a Whatman n. 4 filter and then, before spectrophotometrical reading, through a 0.45 µm filter. The total antioxidant activity determination was performed using the Brand-Williams, Cuvelier, and Berset method (1995) which is based on the reaction mechanism between the DPPH (2,2-diphenyl-1-picrylhydrazyl, Carlo Erba, MI, Italy) and the antioxidants present in the samples.

25 µL of the sample extract was made to react 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 spectrophotometrical reading was conducted in darkness in an Agilent 8453 UV-Vis spectrometer at 515 nm wavelength and a temperature of 20 °C to eliminate the risk of thermal degradation of the

molecules tested (Bondet, Brand-Williams, and Berset, 1997). A graph of absorbance versus time showed that the decolouration curve of the radical decrease followed a fourth order kinetic ($r^2$ = 0.99). Results were expressed as $-OD^{-3}$ /min* g d.w.* s, by the following formula:

$[(1/A^3)-(1/A_0^3)] = -3kt$

where $A_0$ is the initial optical density, A is the optical density at rising time t and OD is the optical density.

The methods reported by Othman, Roblain, Chammen, Thonart, and Hamdi (2009) and Re, Pellegrini, Proteggente, Pannala, Yang, and Rice-Evans (1999) were used for extraction and antioxidant capacity determination by ABTS assay respectively. This analysis evaluates the capacity of the studied sample to inhibit $ABTS^+$ radical oxidation, compared with a standard antioxidant (0-15 µM of Trolox). After preparation of the $ABTS^+$ radical, spectrophotometrical analysis was performed at 734 nm and results were calculated as inhibition percentage . The applied formula was the following:

**Inhibition % =** $((OD_0-OD)/ OD_0) * 100$

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 concentration of extracts and Trolox for standard reference data.

Antioxidant capacity was calculated in terms of TEAC (µmol Trolox/ g d.w.).

### *2.3.7. Sensorial analysis*

A panel of nine untrained judges carried out a paired difference test between dried after brining and untreated dried olives regarding the following discriminants: bitter, salty, acid, rancid and chewiness. Panelists indicated the differences between the pairs of samples and the eventual presence of undesired tastes.

### *2.3.8. Statistical analysis*

One-way and two ways 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 DPPH·assay and total phenol content. SPSS software (Version 11.0, SPSS Inc., Chicago, IL, USA) was used for data processing.

## 3. Results and discussions

Carpological measurements revealed an optimal flesh/pit ratio (4.75±1.39) confirming the aptitude of the Carolea cultivar for table olive, due to a good commodity value. With regard to dehydration, the time necessary to reach the estimated value of dry matter varied, as expected, according to fresh sample humidity. The drying process was stopped when 70 % of the estimated dry matter was reached, but the measured value did not always correspond to this value. Processing times were 58 h and 48 h for fresh and brined olives respectively at 50 °C and 22 h and 20 h at 70 °C. The observed differences were due to the different values of olive humidity before the thermal process ($P < 0.05$): in particular brined olives had a lower amount of $H_2O$ on dry matter than fresh olives (Table 1). This is also correlated to the $a_w$ values which diminished after dehydration in a range suitable for a proper preservation. Only DO 50 possessed a value above the safety limit because the lower applied temperature resulted in a smaller reduction of the high original water content as reported in Table 2. A decrease in humidity was instead observed after olive brining and therefore the drying temperatures applied contributed to a further diminution in water content.

The total acidity content generally decreased after heat application with the exception of DO 50. In particular, the BO sample obtained values of total acidity and pH revealed an unsatisfactory fermentation due to the natural method which did not produce the correct progress that can be obtained by an induced acidification. Indeed, about pH value on samples two-way ANOVA revealed the highest influence of the brining pretreatment and no influence due to the temperature of the drying process. Two-ways ANOVA revealed high influence of the brining pretreatment and drying temperature on total acidity, water activity and total polyphenols of olives. The combined effect of both treatments (brining and drying temperatures) did not only influence the dry matter

content of the olives (Table 2). After the brining period, a slight diminution of pH was observed as previously mentioned by other authors (Gambella et al., 2000), whereas pH generally increased after thermal treatments.

Browning was observed in both processed olives: a decrease of the L* parameter developed initially after fermentation and continued during dehydration and lightness was better preserved in DO 50. The application of high temperatures in vegetable processing involved a number of chemical-physical modifications which affected the colour pigments, for example the green to brown toning change and the breakdown of chlorophyll or other pigments, as also previously observed by Palou Lopez-Malo, Barbosa-Cánovas, Welti-Chanes, and Swanson (1999).

The significant decrease in L*, b* and Chroma parameters are also associated with a greater degree of browning; indeed, a* (red colour index) tended to increase in all samples compared to the fresh sample (Table 3). The origin of the brown pigment is complex and not fully understood but it is known to involve the oxidation of polyphenols by enzymatic activity, i.e. peroxidase (POD) and polyphenol oxidase (PPO) (Todaro, Cavallaro, Argento, Branca, and Spagna, 2011; Nicoli, Elizalbe, Piotti, and Lerici, 1991) or by chemical reactions which involve the oxidisation of *o*-diphenols of olives in quinones and their subsequent transformation into different dark compounds (Romero Brenes, García, and Garrido, 1998). Also, the formation of high molecular weight compounds upon heating, derived from non-enzymatic reactions, reflected the browning intensity on samples (Rhim, Nunes, Jones, and Swartzel, 1989).

Recent interest has been directed to olive polyphenols which in fact play an important role in human nutrition as preventive agents against several diseases, protecting body tissue against oxidative stress (Boskou, Salta, Chrysostomou, Mylona, Chiou, and Andrikopoulos, 2006). Highly significant differences ($P < 0.01$) were observed among samples for total phenol content. Compared to the amount of the fresh sample (1364 mg GAE/100 g d.w.), a general decrease was observed for these antioxidant compounds after applied processes. For the drying treatment, the greatest loss of total phenolic compounds in unbrined olives was observed at 50 °C (454 mg GAE/100 g dw) due to the

polyphenol oxidase activity which could be active around that thermal regime and in that temporal condition. Moreover, a reduction of total phenols was observed in the BO sample (1031 mg GAE/100 g d.w.) following the brining process because of the osmotic release between olive pulp and the brine. The drying of the brined samples involved a further oxidation, especially in DBO 50 (277 mg GAE/100 g d.w.) Both processes therefore affected total phenol content with significant differences among samples. This general trend was confirmed by individual phenolic compound quantification reported in Table 4. The major phenols in fresh samples were oleuropein and hydroxytyrosol while tyrosol was present in lower quantities according to Vinha et al., 2005. It is well-known that phenols are hydrolyzed during fermentation, in particular oleuropein, whereas hydroxytyrosol increases (Marsilio, Campestre, and Lanza, 2001) due to acid and enzymatic hydrolysis of oleuropein. In the present study, also the concentration of caffeic acid, a phenolic derived by verbascoside hydrolysis, tended to increase after brining, as mentioned by Brenes, García, and Garrido (1992). A similar trend was manifested for quercetin, a flavonoid compound responsible of some scavenging properties ascribed to olive fruit. Hydroxymethylfurfural (HMF) is an intermediate of the Maillard reaction and it is an indicator of antioxidant presence: in DO 70 it was quantified as 58.5 mg /100 g of dry weight.

Figure 1 describes the results of two antioxidant capacity assays. The DPPH· method is employed to test several foods as fruits (Lachman, Šulc, and Schilla, 2007), vegetables (Arslan and Özcan, 2011) and oils (Minioti and Georgiou, 2010) and the applied ABTS method (Re et al., 1999) studies both water-soluble and lipid-soluble antioxidants in food extracts. The results of the present work attest the increase of radical scavenging activity by thermal treatments at 70 °C, confirming the assessment of several authors who have studied the effect of thermal processing on vegetable composition (Piga, Del Caro and Corda, 2003b; Cossu et al., 2012). Comparing the two drying temperatures, the same trend was observed in the applied antioxidant assays. A higher activity was specifically obtained in samples dried at 70 °C than those dried at 50 °C. Moreover Pearson's correlation between DPPH· assay and total phenol content ($r= 0.769$; $P<0.05$) validated the not

285 exclusive contribution of polyphenols to the total antioxidant activity of table olives as can be seen
286 by comparing the reducing power of DO 70 and fresh olives.
287 A further detailed study was conducted to investigate the presence of new-formed compounds
288 derived by the Maillard reaction by fractionation and measurement of antioxidant activity of
289 melanoidins. In all samples, Fraction I had the greatest relative amount with several differences due
290 to the applied processes and the sample's response to the treatment. Its perceptual amount varied
291 from 45% in DO 70 and 40% in DO 50 to 15% in FDO 70 and 12% in FDO 50. The less polar
292 extracts represented by Fraction II and Fraction III were present with content above 7% in all
293 samples. Moreover, the water-soluble Fraction I contributed most of all to the overall sample
294 antioxidant property, as confirmed by Madrau et al. (2010) for other fruits of the Drupaceae family.
295 In particular, the highest values were observed in DO 70 and in DO 50 (243.9 and 64.5 $-OD^{-3}/min^{*}$
296 g d.w.* $s^{-}$ respectively). As reported in Table 5, all total sums of relative fraction antioxidant
297 properties showed higher values than those manifested by whole dried samples, probably due to a
298 non-synergic effect of total antioxidants. Most of the reducing power ascribed to dried olives can
299 therefore be attributed to Maillard reaction products formed after thermal treatments: higher thermal
300 treatments resulted in a more prolonged Maillard reaction as proved by results obtained for DO 70.
301 From a sensorial evaluation, brined olives were better accepted than unbrined olives due to a
302 savoury taste that balanced the residual bitter taste. So, for a direct consumption, brined dried olives
303 were preferred to dried ones, which instead are considered healthiest for a qualitative point of view.
304

305 **4. Conclusions**

306 This study evidenced the influence of thermal drying process on table olive quality. The observed
307 depletion of antioxidant compounds was balanced at higher temperatures by an increased
308 antioxidant capacity compared to the untreated sample, due to the Maillard reaction products, as
309 showed by the HMF formation. The applied higher drying temperature (70 °C), promoted the new
310 formation of antioxidants, while the temperature of 50 °C was negative for the depletion of

311 endogenous ones. Brining reduced the total antioxidant composition of olives, in particular for
312 phenol amount. Moreover the Maillard reaction did not develop in brined olives probably as a result
313 of the low sugar availability in these partially fermented olives.
314 In conclusion, dried olives could be rationally considered for "ready-to-eat" use or as ingredients in
315 food formulations with an added value derived by their increased functional property.
316
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Table 1. Compositional characteristics of fresh olives (brined and unbrined)

| Sample[¥] | $a_w$ | Dry matter (%) | Total acidity (% citric acid d.m.$^{-1}$) | pH | Total polyphenols (mg GAE/100 g d.w.) |
|---|---|---|---|---|---|
| **O** | 0.996±0.00 | 31.34±0.27 | 0.57±0.02 | 5.26±0.02 | 1364±64 |
| **BO** | 0.967±0.00 | 35.60±1.45 | 0.51±0.07 | 5.22±0.07 | 1031±6 |
| **Sign.** | ** | ** | n.s. | n.s. | ** |

[¥]Data are mean ±SD. Results followed by different letters are significantly different by Tukey's multiple range test. **Significance at $P<0.01$; * Significance at $P<0.05$; n.s. not significant.

409 Table 2. Influence of brining and drying on chemical analyses of green table olives

| | Drying temperatures | BO | O | | Sig. | |
|---|---|---|---|---|---|---|
| Total acidity (% citric acid d.m.$^{-1}$) | 50 °C | 0.28±0.02 | 0.90±0.02 | 0.59 | Brining | ** |
| | 70 °C | 0.42±0.06 | 0.33±0.03 | 0.38 | Temperature | ** |
| | | 0.35 | 0.61 | | Brin. xTemp. | ** |
| pH | 50 °C | 5.13±0.02 | 5.60±0.01 | 5.36 | Brining | ** |
| | 70 °C | 5.35±0.04 | 5.40±0.02 | 5.37 | Temperature | n.s. |
| | | 5.24 | 5.50 | | Brin. xTemp. | ** |
| $a_w$ | 50 °C | 0.756±0.01 | 0.833±0.01 | 0.795 | Brining | ** |
| | 70 °C | 0.730±0.00 | 0.686±0.00 | 0.708 | Temperature | ** |
| | | 0.743 | 0.760 | | Brin. xTemp. | ** |
| Dry matter (%) | 50 °C | 72.56±2.11 | 70.28±1.56 | 71.42 | Brining | n.s. |
| | 70 °C | 76.55±4.41 | 77.54±0.67 | 77.04 | Temperature | ** |
| | | 74.55 | 73.91 | | Brin. xTemp. | n.s. |
| Total polyphenols (mg GAE 100 g d.w. -1) | 50 °C | 277±10 | 454±10 | 365 | Brining | ** |
| | 70 °C | 454±39 | 1,138±149 | 796 | Temperature | ** |
| | | 365 | 795 | | Brin. xTemp. | ** |

410 BO (Brined Olives), O (Unbrined olives), Brin. * Temp. (Combined effect of both treatments applied on samples)
411 Data are mean ±SD. **Significance at $P<0.01$; * Significance at $P<0.05$; n.s. not significant.
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Table 3. Colour parameters before and after applied processes on green table olives

| Sample | L* | a* | b* | Chroma |
|---|---|---|---|---|
| **O** | 61.70±3.79[a] | -9.56±4.96[c] | 37.54±3.05[a] | 39.05±3.05[a] |
| **DO 50** | 42.83±1.31[c] | 2.89±0.71[b] | 3.91±1.16[c] | 4.87±1.16 |
| **DO 70** | 24.84±1.83[d] | 5.87±1.20[a] | 5.63±[b1.96c] | 8.15±1.96[b] |
| **BO** | 50.86±4.14[b] | 5.44±1.74[a] | 38.81±4.56[a] | 39.24±4.[illegible][a] |
| **BDO 50** | 27.08±1.46[d] | 6.41±1.35[a] | 7.00±1.75[b] | 9.51±1.75[b] |
| **BDO 70** | 25.41±2.23[d] | 5.80±1.35[a] | 5.66±1.65[bc] | 8.11± 1.[illegible][b] |
| **Sign.** | ** | ** | ** | ** |

Results followed by different letters are significantly different by Tukey's multiple range test.
**Significance at $P<0.01$; * Significance at $P<0.05$; n.s. not significant.

Table 4. Phenolic composition of green table olives before and after processes (mg /100 g d.w.)

| | | Samples | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Compound | | O | DO 50 | DO 70 | BO | BDO 50 | BDO 70 | Sign. |
| Hydroxymethylfurfural | Mean | NDc | NDc | 58.5 a | NDc | 0.5 b | 0.3 b | ** |
| | SD | - | - | 7.07 | - | 0.05 | 0.03 | |
| Hydroxytyrosol | Mean | 23.1 bc | 3.1 d | 17.2 cd | 123.8 a | 2.7 d | 35.5 b | ** |
| | SD | 6.56 | 0.33 | 2.38 | 6.57 | 0.05 | 0.17 | |
| Tyrosol | Mean | 4.2 abc | 0.3 c | 4.9 ab | 8.4 a | 1.5 bc | 5.1 ab | ** |
| | SD | 2.07 | 0.00 | 0.42 | 1.45 | 0.09 | 0.58 | |
| Chlorogenic acid | Mean | 6.4 a | 2.2 b | 2.3 b | 4.5 ab | 1.8 b | 0.8 b | * |
| | SD | 2.34 | 0.05 | 0.43 | 0.21 | 0.01 | 0.03 | |
| Vanillic acid | Mean | ND b | ND b | ND b | 1.7 a | 1.9 a | 1.4 a | ** |
| | SD | - | - | - | 0.38 | - | 0.05 | |
| Caffeic acid | Mean | 0.4 bc | ND c | 5.5 bc | 8.8 a | 5.7 abc | 6.4 ab | ** |
| | SD | 0.04 | - | 1.26 | 1.86 | 0.03 | 0.29 | |
| Syringic acid | Mean | 1.9 cd | 3.4 bc | 6.2 a | 6.7 a | 1.2 d | 4.8 ab | ** |
| | SD | 0.90 | 0.05 | 0.99 | 0.17 | 0.14 | 0.11 | |
| Ferulic acid | Mean | 2.6 b | 19.8 bc | 3.1 b | 11.9 a | 0.6 d | 0.8 cd | ** |
| | SD | 0.80 | 0.02 | 0.12 | 0.16 | - | 0.03 | |
| *o*-Coumaric acid | Mean | 31.5 a | 2.1 b | 1.2 b | 4.2 b | 1.2 b | 2.9 b | ** |
| | SD | 14.14 | 1.21 | 0.31 | 0.47 | 0.34 | 0.09 | |
| Oleuropein | Mean | 416.1 a | 0.2 c | 135.1 b | 1.1 c | 0.4 c | 0.5 c | ** |
| | SD | 30.03 | 0.07 | 17.01 | 0.03 | 0.02 | 0.00 | |
| Quercetin | Mean | 8.6 bc | 3.9 c | 6.7 bc | 17.1 a | 11.8 ab | 11.9 ab | ** |
| | SD | 3.80 | 0.41 | 0.82 | 1.71 | 0.04 | 0.02 | |

Table 5. Relative and total contribution to antioxidant activity of melanoidin fractions of whole drupes (-$OD^{-3}$/min* g d.w.* s)·

| Sample | Fraction I | Fraction II | Fraction III | ∑ | Total antioxidant activity |
|---|---|---|---|---|---|
| DO 50 | 64.52 b | 13.52 c | 4.89 b | 82.93 | 23.66 |
| DO 70 | 243.92 a | 29.68 a | 9.59 a | 283.19 | 141.63 |
| BDO 50 | 8.14 d | 6.52 d | 1.83 c | 16.49 | 7.38 |
| BDO 70 | 28.24 c | 23.12 b | 1.12 c | 52.48 | 11.54 |
| **Sign.** | ** | ** | ** | | |

Results followed by different letters are significantly different by Tukey's multiple range test. **Significance at $P<0.01$; * Significance at $P<0.05$; n.s. not significant.

Figure caption

Figure 1 Antioxidant activity of green olives by DPPH and ABTS assay.