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Research Article

Heat treatment effect on tocopherols, total phenolics and fatty acids in table olives (*Olea europaea* L.)

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Abstract

The olive fruits are rich source of oil, vitamins, minerals, organic acids and pigments. The fruits contain high level of bioactive compounds. The aim of this study was to examine the effect of heat treatment on tocopherols, total phenolics and antioxidant activity in green and black olives, as well as their fatty acid composition. The instrumental methods used in this experiment were high performance liquid chromatography (HPLC), gas chromatography with flame ionization detection (GC/FID) and spectrophotometric methods. The results revealed that the β+γ-tocopherols content after the heat treatment had the biggest reduction, which was 68.4% for green and 80.2% for black olives. Also, a significant loss of total phenolic content was observed after heat treatment in green and black olives by 18.6% and 18.4%, respectively, as well as antioxidant activity (decrease up to 28.1%). The most abundant fatty acids in green and black olives were oleic (C18:1), palmitic (C16:0) and linoleic acid (C18:2). The changes in fatty acids composition during the heat treatment occurred mostly at the level of polyunsaturated fatty acids, especially linolenic acid (C18:3) in black olives had the significant reduction (by 57.4%) in relation to the initial quantity.

Keywords: fatty acids; GC/FID; HPLC; olive; tocopherols; total phenolics

Introduction

The olive (*Olea europaea* L.) is one of the oldest and most important fruit in Mediterranean countries, which is grown for its edible fruits (Boskou, 2006). As the fruit ripens it changes colour from green to bluish-purple, and at full maturity it turns black (Boskou, 2006). The green colour of the fruits comes from chlorophyll, while the purple and bluish colour comes from anthocyanins. The black colour is formed by the oxidation of phenolic compounds including oleuropein (Boskou, 2006; Omar, 2010). The chemical

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composition of the olive fruits varies in relation to the variety, agroecological conditions and fruit maturity. The fruits contain approximately 70% water, 1.6% proteins, 22% oil, 19.1% carbohydrates, 5.8% cellulose and 1.5% ash (Maqueda, 2005). The oil accumulates during fruit ripening. The main category of constituents of olive oil are triacylglycerols (about 98%), whereas the rest 2% is comprised of variety of compounds belonging to different chemical categories, i.e hydrocarbons, alcohols, sterols, waxes and other components (Maqueda, 2005; Gomez Herrera, 2009). In the human diet olives are used as table olives or to obtain oil. Table olives are the most widely consumed fermented food in the Mediterranean countries (Campus *et al.*, 2018). Due to the presence of glucosides (e.g., oleuropein), olives cannot be consumed after harvest because of the unpleasant bitter taste and astringent effect (Servili *et al*., 2006; Omar, 2010). While the fruits are green, their flesh has a bitter taste, and as they ripen, the bitterness decreases. In the food industry, after harvesting, olives are treated with sodium hydroxide solution to eliminate the bitterness. In order to prolong the shelf time and maintain quality of table olives, one of the preserving methods that can be applied is pasteurization (Aponte *et al*., 2010; Catania *et al*., 2014). However, the heat treatment can negatively affect the quality of table olives in terms of decreasing the content of polyphenols, lipids, proteins and vitamins, changing the colour and texture (Campus *et al.*, 2018).

The bioactive compounds of olives have shown anti-inflammatory and antioxidant properties (Perez *et al*., 2019). Due to their fatty acid composition, where oleic, palmitic, linoleic and linolenic acids are prevalent, olives in diet regulate cholesterol and triacylglycerols levels, preventing many health problems related to the cardiovascular system (Sakouhi, 2008; Cano-Lamadrid *et al.*, 2017; Flores *et al.*, 2017). Linoleic (ω-6) and linolenic (ω-3), called essential fatty acids, have a great importance in metabolism, and they must be ingested through food because the human body is not able to produce them (Cano-Lamadrid *et al*., 2017). Due to stability of fatty acids, olives have a protective effect on cells, especially in correlation with vitamin E. Their interaction reduces the risk of cell damage and the development of inflammatory processes (Tucker and Townsend, 2005; Niki, 2014). The term vitamin E refers on two classes of compounds, tocopherols and tocotrienols. Each class is composed of four isomers designated as alpha (α), beta (β), gamma (γ) and delta (δ), depending on the number and position of methyl groups bounded with chroman structure of the molecule (Preedy and Watson, 2007). All eight forms have different chemical and biological functions. Alpha tocopherol is the most common form with pronounced biological activity and the highest bioavailability (Jiang *et al.*, 2001; Sen *et al.*, 2007; Perez *et al.*, 2019). Vitamin E is essential for the maintenance and development of nervous and muscular system functions, with effect on cellular immunity because it contributes to the formation of lymphocytes, in the prevention of Alzheimer's disease and cancer (Tucker and Townsend, 2005; Niki, 2014). Processing and storage conditions have an effect on the tocopherol content (Gómez-Alonso, 2007). Vitamin E, especially alpha tocopherol, is sensitive to heat, almost 60% is lost by cooking, and significant amounts are lost by baking and frying, as well as grinding, peeling and chopping during food processing (Henry and Chapman, 2002; Eitenmiller and Lee, 2004; Rickman *et al*., 2007). Phenolic compounds exhibit antioxidant, anti-inflammatory, anti-mutagenic and anti-carcinogenic effects (Khoddami *et al*., 2013). In addition, phenolic compounds may contribute to fruit quality in terms of sensory attributes such as colour and flavour. According to literature data, numerous phenolic compounds have been detected in olive fruits (oleuropein; caffeic, gallic, syringic and vanillic acid; luteolin; hesperidin etc.) (Ryan and Robards, 1998). Generally, the heating process caused a reduction of total phenolic content and antioxidant activity in plant material (Murakami *et al*., 2004; Özcan *et al*., 2018; Ghafoor *et al*., 2019).

Olive fruits are most often consumed fermented and preserved in brine or pasteurized, but they are also often used as an ingredient on pizza, where they are treated with high oven temperatures. Although it is proven that different culinary conditions, especially the temperature and the time of heat processing, can influence composition of olive oil, information regarding heating and its impact on fatty acid composition and tocopherols content of table olive does not exist so far. The aim of this study was to determine the tocopherols content in green and black table olives after heat treatment, by HPLC method, in relation to their initial

content, total phenolic content and antioxidant activity, by spectrophotometric methods, as well as the affect of high temperature on their fatty acid profile, by GC/FID method.

Materials and Methods

Samples of green and black olives in brine (pitted, chemical preserved, not pasteurized) were procured in a retail store in Belgrade, Serbia. Olives were produced in Italy (the same producer for both products). The nutritional value (data from the declaration of the product) is given in Table 1.

Table 1. The nutritional value per 100 g of product

Nutritional value per 100 g	Green olives	Black olives
Energy	665 kJ/159 kcal	592 kJ/142 kcal
Fat	14.0 g	10 g
of which: saturates	2.2 g	1.4 g
Carbohydrate	5.9 g	10.6 g
of which: sugars	2.6 g	7.5 g
Fibre	2.6 g	2.4 g
Protein	1.1 g	1.2 g
Salt	4.4 g	4.2 g

The samples (50 fruits of each; the average weight of the fruits was 5.6 g and 6.3 g for green and black olives, respectively) were treated in the oven at 180 °C for 20 minutes in order to imitate pizza production. All samples (green and black olives before and after heat treatment) were subjected to oil extraction and then analysed.

Oil extraction

About 10 g of mashed olives were extracted with 50 ml of hexane using an ultrasonic bath for two hours, and then the sample was left overnight in hexane. After filtration of the extract, the resulting filtrate was evaporated in a stream of nitrogen. Obtained oil was stored at -20 °C in glass vials until further analysis.

Analysis of tocopherols

Quantification of tocopherols was carried out using high performance liquid chromatography (Waters M600E, Milfold, USA) on a reversed phase column Nucleosil 50-5 C18 (Machery-Nagel, Germany) with fluorescence detection. The samples were prepared according to the procedure described in Rabrenović *et al.* (2021).

Analysis of total phenolic content and antioxidant activity

Samples preparation

The samples were prepared according to the procedure described in Mylonaki *et al*. (2008), with some modifications. Briefly, the samples were extracted using the conventional extraction method by adding 20 ml of the appropriate solvent (ethanol/water (60% v/v)) to 1 g of each homogenized sample in closed glass vessels and the extraction was performed using an orbital shaker at 400 rpm for 5 h at room temperature (25±1 °C) in the dark. Then olive extracts were filtered through six-layer medical gauze and stored in the dark at 4 °C until further analysis (maximum 72 h).

Total dry matter content

Total dry matter content (dry weight - DW) was determined by using standard gravimetric method (AOAC, 2005).

Total phenolic content

Total phenolic content (TPC) was determined according to the Folin-Ciocalteu method (Singleton and Rossi, 1965; Singleton *et al.*, 1999), with some modifications. Briefly, 0.5 mL of diluted samples were mixed with 2.5 mL of 10-fold diluted Folin-Ciocalteu's phenol reagent and allowed to react for 5 minutes in dark place, at room temperature. Two millilitres of sodium carbonate solution (75 g/L) were added to the mixture and then shaken. After 2 h of reaction, in dark place, at room temperature, the absorbance at 760 nm was measured using a UV/Vis spectrophotometer (model HALO DB-20S, Dynamica Scientific Ltd., Livingston, UK). The calibration curve was prepared with gallic acid monohydrate solution, and the results were expressed as milligrams of gallic acid equivalents on 100 g dry weight (mg GAE/100 g dry weight). The measurements were performed in triplicate.

Antioxidant activity

DPPH (2, 2- diphenyl - 1- picrylhydrazyl) radical – scavenging activity of samples (AA1) was evaluated the procedure described by Brand–Williams *et al*. (1995), with some modifications. Briefly, each diluted sample (0.1 mL) was added to the DPPH working solution (1.9 mL) (0.094 mmol/L DPPH in methanol). The absorbance at 517 nm was measured using a UV/Vis spectrophotometer (model HALO DB-20S, Dynamica Scientific Ltd., Livingston, UK) after the solution had been allowed to stand in the dark for 30 min. The Trolox calibration curve was plotted as a function of the percentage of inhibition of DPPH radical. The results were expressed as millimoles of Trolox equivalents on 100 g dry weight (mmol TE/100 g dry weight). The measurements were performed in triplicate.

ABTS method (AA2) was carried out according to the procedure described by Re *et al*. (1999) and Salević *et al*. (2022), with some modifications. In order to prepare the $ABTS^{\bullet+}$ solution, stock ABTS solution (14 mmol/L) and potassium persulphate (4.9 mmol/L) - both in phosphate buffer (5 mmol/L, pH 7.4) - were mixed at a volume ratio of 1:1, and stored in the dark at room temperature for 16 h. Before the analysis, the $ABTS^{\bullet+}$ solution was diluted with the phosphate buffer to reach an absorbance of 0.70 ± 0.02 at 734 nm. An aliquot of this working $ABTS^{\bullet+}$ solution (3 mL) was added to 30 µL of the previously dissolved and appropriately diluted samples. After the reaction mixtures were stored in the dark at room temperature for 6 minutes, the absorbance was measured at 734 nm using a UV/Vis spectrophotometer (model HALO DB-20S, Dynamica Scientific Ltd., Livingston, UK). Calibration curve was prepared with Trolox as a standard, and was used to determine the antioxidant activity of the samples. The results were expressed as mmol Trolox equivalents (TE)/100 g dry weight. The measurements were performed in triplicate.

FRAP assay (AA3) was carried out according to the procedure described by Benzie and Strain (1996), with some modifications. Briefly, 3 ml freshly prepared FRAP reagent (mixture of acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM $FeCl_3 \times 6H_2O$ in dH_2O, in volume ratio 10:1:1, respectively), 0.1 mL diluted sample and 0.3 ml H_2O was vortexed and warmed to 37 °C in dark place. Absorbance was reading after 40 min at 593 nm using a UV/Vis spectrophotometer (model HALO DB-20S, Dynamica Scientific Ltd., Livingston, UK). Calibration curve was prepared with Trolox as a standard. The results were expressed as mmol Trolox equivalents (TE)/100 g dry weight. The measurements were performed in triplicate.

Determination of fatty acid composition

The fatty acid composition of olive fruit oil was determined by capillary gas chromatography according to ISO 12966-2:2017. GC instrument Agilent Technologies 6890 (USA) equipped with capillary column SP-2560 (100 m x 0.25 mm x film thickness 0.20) (Supelco, Bellefonte, USA) was used for separation of prepared

fatty acid methyl esters (ISO 12966-1:2014). Injector and detector temperatures were 250 °C and 260 °C, respectively. As carrier gas, helium was used at a flow rate of 5 mL/min. The injection volume was 1 μL, and the injector was set in splitless mode. The oven temperature was programmed from an initial 120 °C to 240 °C (hold 7 min), with a temperature rate of 4 °C/min. The chromatographic peaks in the samples were identified by comparing the relative retention times of FAME peaks of Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA), and individual fatty acids were expressed in relative quantities as mass % of total fatty acids.

Sensory analysis

Sensory analysis was assessed by a trained panel of seven members using five-point hedonic scale (Karagul-Yuceer and Drake, 2006; Šeregelj *et al*., 2019). Panellists evaluated colour and appearance, odour, flavour, texture, general acceptability and the results were presented in radar diagram.

Statistical analysis

Statistical analysis was performed using statistical software STATISTICA 12. Determination of tocopherols, total phenolic content, antioxidant activity and fatty acids, in all samples, were performed in triplicate. Data reported were expressed as means ± standard deviation. The statistical analysis was performed using one-way analysis of variance (ANOVA) and significant differences among means were determined by Duncan's comparison test. Differences at a confidence level of 95% were considered significant.

Results and Discussion

Table olives are considered as a high nutritional values fruit (Pereira *et al*., 2006). The nutritional benefits are mainly related to α-tocopherol and fatty acid contents (Ribarova *et al*., 2003). According to Sakouhi *et al.* (2008) α-tocopherol and fatty acid are cultivar, ripening and processing dependent.

Tocopherol content

In the analysed olive samples, a significantly higher presence of α-tocopherol was evident compared to the other isomers of tocopherol. The results showed that the highest tocopherol content was determined for α-tocopherol in untreated black olive (383.1 μg/100 g) (Table 2). The obtained data of α-tocopherol content in olive samples were in accordance with the results in Hassapidou *et al*. (1994) study. Since black olives are usually harvested 1-2 months after green ones, the higher content of α-tocopherol in black olives could be related to an increase in tocopherol content during fruit ripening. However, according to the results obtained in study Perez *et al*. (2019) it can be noticed that the level of tocopherol gradually decreased in the final stage of maturation. In our study, these conclusions can be related to the content of β+γ and δ tocopherols, since these compounds had a lower content in black olives compared to green olives.

Table 2. Tocopherols content in olive samples

Tocopherols (μg/100 g)	Green olive		Black olive	
	Before heat treatment	After heat treatment	Before heat treatment	After heat treatment
α-tocopherol	$361.7^{a} \pm 3.4$	$295.3^{b} \pm 2.7$	$383.1^{A} \pm 3.6$	$260.1^{B} \pm 2.4$
β+γ-tocopherol	$62.1^{a} \pm 1.0$	$19.6^{b} \pm 0.3$	$52.5^{A} \pm 0.8$	$10.4^{B} \pm 0.2$
δ-tocopherol	$56.1^{a} \pm 1.1$	$31.0^{b} \pm 0.6$	$51.9^{A} \pm 1.0$	$24.4^{B} \pm 0.5$

All data are means ± standard deviation (n=3). Different small letter superscripts within the same row differ significantly ($p < 0.05$), different capital letter superscripts within the same row indicate significant differences between tocopherols in untreated and heat-treated black olives ($p < 0.05$).

Based on the obtained results, it can be concluded that green and black olives had a significantly lower level of all forms of tocopherols after heat treatment. The loss of α-tocopherol in black and green olives was 32.1% and 18.4%, respectively, in relation to their initial content in samples that were not heat-treated. The largest loss after heat treatment was determined in β+γ-tocopherol content, 80.2% in black and 68.4% in green olives. The obtained results were in accordance with Hui (1991) and Eitenmiller and Lee (2004), who determined that significant content of tocopherols was lost during heat treatment. Maguire *et al*. (2004) analysed the tocopherols content in nuts (almonds, hazelnuts, pistachios and walnuts) before and after heat treatment. Roasting at 170 °C significantly reduced the level of α-tocopherol in almonds and hazelnuts, γ-tocopherol in hazelnuts and walnuts, but did not affect the content of α and γ-tocopherol in pistachios. Based on reported data, it can be concluded that the loss of all forms of tocopherols in our study is consistent with most of the literature data, but we cannot fail to mention that in some studies, increases in the content of these compounds have also been reported. According to the results obtained in study Kodad *et al*. (2016), the content of γ and δ tocopherols was increased in all almond samples after roasting. Our assumption is that the stability of all isomers of vitamin E depends of the chemical categories of compounds for which they are bound (phospholipids, proteins, etc.) and certain chemical reactions occur during heat treatment, as well the temperature and period of heating.

Total phenolic content and antioxidant activity

The obtained results of total phenolic content and antioxidant activity (Table 3) show an expected quantitative decrease which is in line with numerous literature data regarding various plant material (Murakami *et al*., 2004; Özcan *et al*., 2018; Ghafoor *et al*., 2019).

Table 3. Total phenolic content and antioxidant activity of olive samples

Parameters	Green olive		Black olive	
	Before heat treatment	After heat treatment	Before heat treatment	After heat treatment
DW (%)	$21.62^{b} \pm 0.23$	$34.11^{a} \pm 0.12$	$37.74^{B} \pm 0.36$	$40.69^{A} \pm 0.26$
TPC (mg GAE/100 g dw)	$1152.45^{a} \pm 12.60$	$938.35^{b} \pm 15.52$	$1918.56^{A} \pm 19.62$	$1565.49^{B} \pm 40.59$
AA1 (mmol TE/100 g dw)	$3.15^{a} \pm 0.10$	$2.93^{a} \pm 0.06$	$6.68^{A} \pm 0.14$	$5.90^{B} \pm 0.11$
AA2 (mmol TE/100 g dw)	$15.13^{a} \pm 0.14$	$14.99^{a} \pm 0.06$	$36.25^{A} \pm 0.36$	$29.79^{B} \pm 0.14$
AA3 (mmol TE/100 g dw)	$6.22^{a} \pm 0.48$	$5.53^{a} \pm 0.33$	$17.03^{A} \pm 0.80$	$12.25^{B} \pm 0.30$

All data are means ± standard deviation (n=3). Different small letter superscripts within the same row differ significantly ($p < 0.05$), different capital letter superscripts within the same row indicate significant differences between parameters in untreated and heat-treated black olives ($p < 0.05$).

In this study, total phenolic content after heat treatment significantly decreased in green and black table olives by 18.6% and 18.4%, respectively (Table 3). Also, antioxidant activity analysed by three methods showed decrease after heat treatment in relation to initial value in untreated samples (Table 3). In heat-treated black olive sample, antioxidant activity was significantly decreased by 11.7-28.1% (Table 3). On the other hand, in heat-treated green olive sample antioxidant activity decreased by 0.9-11.1% (Table 3) which was not statistically significant. Murakami *et al*. (2004), analysing the antioxidant activity of single and mixed polyphenolic compounds, have reported that the presence of certain polyphenolic compounds, e.g. chlorogenic acid, could reduce a decomposition of other polyphenolic compounds at high temperatures, and thus make the antioxidant capacity more stable. We can only assume that there was a higher content of those components

with protective role in green olives in relation to black olives, but this certainly requires further research on this topic.

Fatty acid composition

The most abundant fatty acids in green and black olives were oleic (C18:1), palmitic (C16:0) and linoleic acid (C18:2) (Table 4). The content of oleic acid was higher in black olives in relation to green fruits, while the content of stearic (C18:0), linoleic (C18:2) and palmitic acid (C16:0) was lower. The table olives used in this experiment were not of the same variety, so we cannot compare these contents. We can only assume that changes in the fatty acid profile could occur due to specific enzymatic activities and climatic environments during the ripening process. Similar conclusions reported Poiana and Mincione (2004) and Beltran *et al*. (2004), comparing the different stages during fruits maturation of same varieties. Cooler environments produce higher oleic acid levels, while hot seasons and environments increase palmitic and/or linoleic acids (Lombardo *et al*., 2008; Rouas *et al*., 2016). Besides temperature, the oleic acid content depends on tree age and rainfall (Beltran *et al*., 2004).

Table 4. Fatty acid composition in olive samples

Fatty acid (%)	Green olive		Black olive	
	Before heat treatment	After heat treatment	Before heat treatment	After heat treatment
Palmitic C16:0	$13.12^{a} \pm 0.22$	$13.33^{a} \pm 0.20$	$11.09^{B} \pm 0.25$	$12.10^{A} \pm 0.32$
Palmitoleic C16:1	$1.26^{b} \pm 0.03$	$1.49^{a} \pm 0.04$	$1.22^{B} \pm 0.04$	$1.38^{A} \pm 0.02$
Stearic C18:0	$3.03^{b} \pm 0.05$	$3.39^{a} \pm 0.05$	$2.66^{B} \pm 0.04$	$2.97^{A} \pm 0.06$
Oleic C18:1	$68.10^{a} \pm 0.04$	$68.03^{a} \pm 0.04$	$72.59^{A} \pm 0.27$	$72.16^{A} \pm 0.18$
Linoleic C18:2	$13.78^{a} \pm 0.40$	$13.10^{a} \pm 0.29$	$11.49^{A} \pm 0.32$	$10.86^{A} \pm 0.23$
Linolenic C18:3	$0.61^{a} \pm 0.02$	$0.49^{b} \pm 0.01$	$0.68^{A} \pm 0.02$	$0.29^{B} \pm 0.01$
Arachidic C20:0	$0.19^{a} \pm 0.01$	$0.17^{a} \pm 0.01$	$0.27^{A} \pm 0.01$	$0.24^{B} \pm 0.01$
ΣSFA	$16.34^{a} \pm 0.28$	$16.89^{a} \pm 0.26$	$14.02^{B} \pm 0.30$	$15.31^{A} \pm 0.39$
ΣMUFA	$69.36^{a} \pm 0.07$	$69.52^{a} \pm 0.08$	$73.81^{A} \pm 0.31$	$73.54^{A} \pm 0.20$
ΣPUFA	$14.39^{a} \pm 0.42$	$13.59^{a} \pm 0.30$	$12.17^{A} \pm 0.34$	$11.15^{B} \pm 0.24$

All data are means ± standard deviation (n=3). Different small letter superscripts within the same row differ significantly ($p < 0.05$), different capital letter superscripts within the same row indicate significant differences between fatty acids in untreated and heat-treated black olives ($p < 0.05$).

The changes in fatty acids composition during the heat treatment occurred mostly at the level of polyunsaturated fatty acids, which was to be expected. Although the linolenic acid (C18:3) content was very low in both untreated samples and thus not statistically significant, this fatty acid, especially in black olives, had the significant reduction (by 57.4%) in relation to the initial quantity (Table 4). However, these changes are significantly lower than in olive oil subjected to high temperatures, although this oil showed the least changes in chemical composition during frying, compared to other edible vegetable oils (Sioen *et al*., 2006; Procida *et al*., 2009). This can be explained by the fact that in our study, the oil in olives was incorporated in the undamaged plant tissue cells and was thus protected from thermooxidative degradation. On the other hand, the numerous chemical reactions of hydrolysis, oxidation, isomerisation and polymerisation during deep frying contributed to the change of fatty acids profile and oil quality (Matthäus, 2007; Choe and Min, 2007).

Monitoring of fatty acid changes during heat treatment is an effective method to assess thermal oxidative changes in the oils (Siragakis and Karamanavi, 2017), and thus assess the quality of oil or products that contain it. As an indicator of the degree of oil degradation, linoleic acid (C18:2) content is frequently used, since this polyunsaturated fatty acid is highly susceptible to oxidation. In our study, the content of linoleic acid was reduced by 4.9% and 5.5% in green and black olives, respectively, after the heat treatment (Table 4). These results could indicate that the degradation changes did not occur in a high degree during the olives heating. Our assumption is that the heat treatment period of time was relatively short in order to significant degradation to occur, as well as the oil in fruits was incorporated in cells, as we already explained. In addition to linoleic (C18:2) and linolenic (C18:3) acid, it can be observed that content of oleic (C18:1) and arachidic (C20:0) acid also had a significant decrease after heat treatment, while an increase was noticed in palmitic (C16:0), palmitoleic (C16:1) and stearic (C18:0) acid content. To our knowledge, there is no available literature data related to the stability of tocopherols and fatty acid composition during the heat treatment of whole olives fruits, so the results of this study could be the basis for further research on this topic.

Sensory analysis

The sensory attributes, colour and appearance, odour, flavour, texture, and general acceptability, as well, were assessed by a trained panel of seven members using the 1 to 5 intensity scale and results are presented in Figure 1.

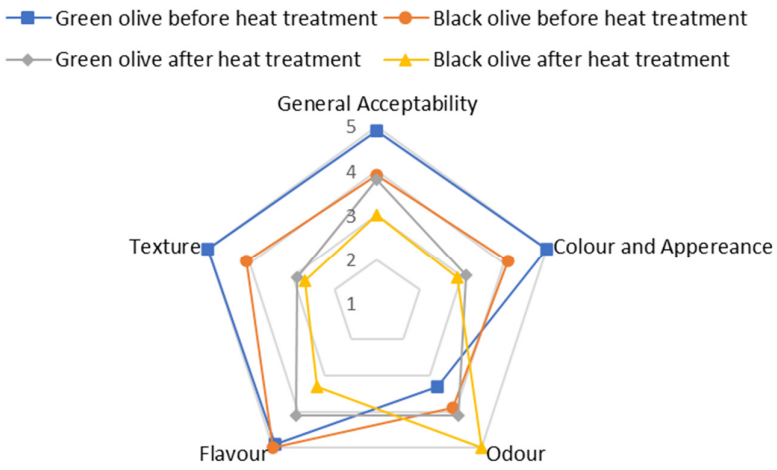

Figure 1. Sensorial attributes of green and black olives before and after heat treatment

As expected, the untreated samples of green and black olives were rated with very high scores in terms of colour and appearance, flavour, texture and general acceptability, while these sensory attributes were evaluated with lower scores in heat-treated olive samples. The only exception is the attribute odour, which was rated with very high scores in both heat-treated samples.

Based on the results obtained in tis study, it can be concluded that heat treatment certainly effects on the bioactive compounds and sensory properties of green and black olives. In further research on this topic, the heating period and temperature range could be varied, which would provide a more detailed insight into the degradation level of table olives compounds.

Conclusions

In this study, the effect of high temperature on tocopherols and total phenolic content in green and black olive, as well as the effect on their fatty acid composition and antioxidant activity, was investigated. The obtained results indicated that all tocopherol isomers content was significantly reduced after oven heating. The loss of α-tocopherol in black and green olives was 32.1% and 18.4%, respectively, in relation to their initial content in samples that were not heat-treated. The largest loss after heat treatment was determined in β+γ-tocopherol content, 80.2% in black and 68.4% in green olives. A significant loss of total phenolic content was observed after heat treatment in green and black olives by 18.6% and 18.4%, respectively, as well as antioxidant activity (decrease up to 28.1%). Also, the heat treatment had an effect on the fatty acids' composition in olives fruits. The results showed that the heat treatment occurred mostly at the level of polyunsaturated fatty acids. The content of linolenic acid, especially in black olives was significantly decreased (by 57.4%), while the content of linoleic acid was reduced by 4.9% and 5.5% in green and black olive, respectively, in relation to the initial amount. These results open the possibility for further research on this topic.

Authors' Contributions

Conceptualization: DM, BR and SČ; Data curation: JM and DP; Formal analysis: SČ and BR; Funding acquisition: JR; Investigation: DM, BR and DP; Methodology: SČ, BR and JM; Software: JM and TP; Supervision: SČ; Writing - original draft: DM and BR; Writing - review and editing: SČ. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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