Effect of irradiation on total biophenol and antioxidant activity quantity during storage of natural black table olives obtained using starter culture

Şahnur Irmak¹, feriste öztürk güngör², erkan susamcı², Didar Sevim³, and Oya Köseoğlu⁴

¹Olive Research Institute ²olive research institute ³olive research institute of turkey ⁴Affiliation not available

April 26, 2023

Abstract

In this study, It was aimed to determine the effects of different production and preservation methods on the shelf life and quality of the Gemlik variety natural black table olives produced with low salt (2%). For this purpose the olives processed with traditional Turkis turning black olive using starter culture The MAP (Modified athmosphere packaging), vacuum and gamma irradiation method (1, 3 and 5 kGy) was applied for the first time in the preservation of table olives. The reducing effect of 5 kGy dose on total phenolic substance and DPPH antioxidant activity was found to be higher than other doses. During storage, pH (4.30-4.83) and titratable acidity (0.68-1.02%) values of black table olives changed in normal course. At the and of the fermentation and storage, total phenolic substance and DPPH antioxidant activity decreased and were found statistically significant (p<0.01). During the storage period, the total amount of phenolic matter in olives in normal production (without starter culture) decreased from 232 to 144 mgCAE/100g and in starter culture added table olives from 200 to 138 mgCAE/100g. As a result of the study, it was ensured that table black olives produced with less salt (2%) could be stored on the shelves for at least 6 months without using any preservatives. In addition, radiation effect on quality was relatively similar to other applications.

INTRODUCTION

Table olives are the traditional fermented product of Mediterranean countries, but today the production and consumption of table olives has spread around the world (Arroyo-Lopez et al., 2008). Turkey is the third biggest producer of table olives in the world, with a production of 399,9 tons in 2022 (IOC, 2022).

The Gemlik cultivar itself has a distinguished place among the olive cultivars grown in Turkey and its cultivation area continues to expand across the country (Ben Ghorbal et al, 2018), and almost constitutes half of the olive tree presence in Turkey (Ozaltas et al., 2016).

Among the 94 local olive variety, due to its high flesh/stone ratio, good shape, texture and excelent organoleptic characteristics "Gemlik" is the most important and economically valuable variety for black table industry in Turkey and mostly processed with naturel fermentation known as Gemlik style traditionally. In this method, Gemlik olives produced with high amount of salt (10-12% salt for winter days and 12-18% salt in summer) and preserved in this way. In recent years, consumers have developed an attitude towards low sodium intake because the sodium-rich diet causes higher blood pressure. The recommended daily intake of salt has been established as 5 g/day (\sim 2000 mg Na⁺/day) by the World Health Organization (WHO) (Bautista Gallego et al., 2011). Good process control is necessary to improve fermentation and produce standard and quality final products. The main drivers of fermentation are the availability of fermentable substrates, salt content, pH, aerobic/anaerobic conditions and temperature control. In order to avoid these disadvantages, the use of starter cultures in the modern table olive industry is recommended (Aponte et al., 2012).

The fermentation process, generally performed by indigenous microorganisms, is one of the best and oldest procedures of treating food products to preserve them and produce other products. Spontaneous fermentations are uncontrolled and not predictable. These spontaneous processes are inefficient since they do not ensure the expected quality and safety characteristics of the final product, the sensorial and structure features, the limitation, or absence of growth of harmful or undesired spoilage organisms. In order to obtain a more controlled process and to improve the quality and safety levels of table olives, the selection and use of starter cultures is diffusing. In fact, several studies demonstrated the usefulness and the benefits of starters in table olives production (Boskou and Clodoveo, 2016).

Besides, the use of starter culture in black olive fermentation was found to be important in terms of LAB dominance on yeast (Montet et al, 2014). Although the starter culture inoculation rate varies depending on the variety and process, it is usually around 1%. The final concentration of inoculated microorganisms in the brine is between 10^{6} - 10^{7} cfu/ml (Erten et al., 2016).

Vacuum and MAP preservation techniques and are widely used in the table olive industry. Gamma irradiation is another preservation method applied to several food products. Food irradiation is a processing technique applied to decontaminate and extend the shelf life of foods by exposing them to ionizing radiation in order to increase the shelf life and safety of the food. Ionizing radiation is highly effective in inactivating microorganisms in various vegetables and it offers a safe alternative as a food decontamination method. There has been a number of research studies directed at examining the effects of irradiation against fresh vegetables and fruit aimed at delaying the ripening, control of the pathogen and pest (Kader, 1986; Niemira et al., 2001).

Black olive production constitutes 80% of the table olive production in our country and consumers prefer black olives for consumption. It was aimed to improve the traditional production method by using starter culture and less salt and to increase the shelf life by irradiation. It is important to find appropriate technologies for table olive preservation. It is seems that irradiation is a potential tool in extending shelf-life of table olives.

To our knowledge, no studies have shown the effect of gamma irradiation on the antioxidant capacity and phenolic compounds of table olives. The aim of this study was to evaluate and compare the effects of different preservation methods (irridation, MAP, vacuum) on some quality paratmeters and antioxidant properties of less salted and starter culture added black table olives during storage. Our secondary aim was to evaluate the effect of starter culter adding on the some quality paraö-meters and total phenol phenolic content of Gemlik olive.

MATERIALS AND METHODS

Olive samples

Gemlik variety olives (240 fruit/kg) were harvested from Bornova Olive Research Institutes' orchard in Izmir, Turkey in two crop seasons. Olives were harvested by hand in the second week of November with 5.3 maturity index as stated by Arslan and Özcan (2011). Maturity index (MI) was calculated on the basis of color changes of peel and pulp and varied between 0 and 7 in eight categories.

Sample names were coded related to processing method; processing style as dry-salted olives with 2% salt (N), dry-salted olives with 2% salt and starter culture (K), packaged olives as modified atmosphere packaged (MP) olives (NMP: dry-salted olives with 2% salt packaged in modified atmosphere, KMP: dry-salted olives with 2% salt and starter culture packaged in modified atmosphere), vacuum packaged (V) olives (NV: dry-salted olives with 2% salt packaged in vacuum, KV: dry-salted olives with 2% salt and starter culture packaged in vacuum, KV: dry-salted olives with 2% salt and starter culture packaged in vacuum, KV: dry-salted olives with 2% salt and starter culture packaged in vacuum) and irradiated olives with different dosages (0, 1, 3, 5 kGy) such as NV0, NV1, NV3, NV5.

A flowchart of experimental procedure and abbreviations were given in Figure 1. The code description of samples were given in Table 1.

Table 1. The code description of samples

Processing Methods of Olives

Dry-Salted Olives

For dry-salting process, harvested olives were placed in plastic containers with the addition of 2% NaCl. The contents of the plastic containers were mixed by shaking every two days. The dry-salting process lasted for about 3 months.

Dry-Salted Olives with Starter Culture

For dry-salting process, harvested olives were placed in plastic containers with the addition of 2% NaCl and starter culture $(1 \times 10^7 \text{ cfu/g})$. The contents of the plastic containers were mixed by shaking every two days. The dry-salting process lasted for about 3 months.

Starter Culture

Lactobacillus plantarum (ATCC*14917) was used as a starter culture. Lactobacillus plantarum was directly inoculated into the plastic containers (1:1 ratio) to reach a final cell density of 7 log colony forming units per ml (cfu/ml).

Packaging

Olives were packaged under conditions of 60% N₂ and 40% CO₂ (MAP) and vacuum (VP) using a packaging instrument (Adona-ADN350, Istanbul, Turkey) using polyamide /polyethylene vacuum bags with low gas permeability and stored at ambient temperature (22-25 degC). The properties of the packages were 90 +-3 μ m thickness, O₂ permeability of 30 cm³/24 h/m²/atm, N₂permeability of 130 cm³/24 h/m²/atm, water vapor permeability of 100 cm³/24 h/m²/atm.

FIGURE 1. Flowchart of experimental procedure

Gamma-Irradiation Treatment of Olives

Gamma-irradiation was implemented by an automatic Tote Box Irradiator (JS 9600, IR-185, Canada) from the Gamma-Pak Sterilization Company in Cerkezkoy, Tekirdag, Turkey. Table olive packages were put in aluminum-steel irradiation boxes and transferred to irradiation rooms by an automatic conveyor. In the irradiation rooms, olives were exposed to gamma rays released from the irradiation source of double capsule Cobalt-60 radioactive welding chisels in metallic form with a loading capacity up to 101 Petabecquerel (PBq) by moving with pneumatic pistons around the source. 1, 3, 5 kGy irradiation dosages were applied to the table olives.

Analytical Parameters of Table Olives

The pH of the olives was directly measured by a pH-meter (WTW 330 Germany) (Anonymous, 2015). Titratable acidity and NaCl % content were tested according to standard AOAC (Anonymous, 1990). Reducing sugar were tested with Mohr method (Irmak,2015). Analitical parameters were measured in raw material and at the and of the fermentation.

Total Phenolic Compounds (TPC)

Total phenolic compound content was determined with a spectrophotometer (UV2450, Shimadzu, Japan) utilizing the sample preparation method that Günç Ergönül (2006) had used. Calibration curve was obtained using the standard solutions in different concentrations prepared from cafeic acid stock solution. 1 g of homogenized olive sample mixed with 5 mL of methanol:water (60:40) solution and shaked for 2 min. Then, it was centrifuged at 3500 rpm for 10 min. The supernatant was taken into a 10 mL tube filtering through a coarse filter paper. The supernatants obtained from the extraction which was performed two timeswere

combined. and was filled with distilled water to the volumeline . Once the sample was well mixed, 0.1 mL was taken into 50 mL volumetric flask and 5 mL of distilled water and 0.5 mL Folin-Ciocalteu reagent were added. After 3 min, 1 mL of 36% Na₂CO₃ solution was added. Then, the sample solution was made up to the volume with distilled water. After the solution was kept in the dark for 2 h, the absorbance were measured at 725 nm wavelength using UV/VIS Spectrophotometer. The concentration values that correspond to the absorbance values were determined in the calibration curve for each sample and the results were calculated as mgCAE/100g olive considering the dilution factor (CAE: cafeic acid equilibrium).

Olive Oil Extraction

Gemlik olive contains 20-34% oil. Different preservation techniques (especially irridation) applied to table olives are likely to affect olive oil properties. Therefore, the oil of the table olives was extracted to perform antioxidant activity analyzes in the oil. Olive oils were obtained using a laboratory scale Abencor system (MC2 Ingenierias y Sistemas Sevilla, Spain) equipped with a hammer crusher, malaxer, and centrifuge. Clean and healthy olives (2 kg) were collected. The malaxation temperature was adjusted to 26-30 °C for 30 min. All olive oil samples were filtered and stored under dark conditions in amber glass bottles (100 mL) at 4 ± 1 °C temperature.

Antioxidant Activity Analysis

The DPPH^{*} (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay (RSA) was performed according to Sevim et al. (2013). The samples were measured with a spectrophotometer at 517 nm (Shimadzu Spectrophotometer UV- 1700 PharmaSpec, Japan). The trolox equivalent (TE) of the DPPH^{*} RSA was calculated against the standard curve prepared with known concentrations of Trolox. The data are expressed as μ mol TE of 100 g of each sample (R² = 0.994) (Carrasco-Pancorbo et al., 2005).

Statistical Analysis

All determinations were obtained from duplicate measurements. Results are expressed as mean \pm standard error. Data analysis was performed by applying an analysis of variance (ANOVA) test. LSD test was applied to determine the differencies between means in the level of p<0.01.

RESULTS AND DISCUSSION

pH, Titratable Acidity and Reducing Sugar Contents of

Raw and Dry Salted Olives

In the table olive processing, salt concentration, temperature and pH adjustments during fermentation and the use of starter culture increase the formation of lactic acid, resulting in more delicious and durable olives (Mcfeeter, 2004; Oliveira et al, 2004; Randazzo et al, 2010; Irmak, 2015).

TABLE 2 Some physikochemical properties of raw and dry salted olives

Although the pH values of raw olives were high, with the development of lactic acid, it decreased at the end of the fermentation. It reached the final values of pH-4.78 in traditional olives and pH-4.23 in culture added olives (Table 2). The final pH value reached by starter culture used indicates a successful lactic fermentation.

The effect of starter culture use on pH and titratable acidity values was found to be statistically significant (p<0.01). Titratable acidity of starter culture added olives was found to be higher than normal production. The pH values of the samples decreased below the safe pH value of 4.6 after 3 months of processing. The pH decrease in both olives during processing is mainly due to the production of lactic acid, the main LAB metabolic product, and the conversion of some phenols on acids such as the glucoside elenolic acid, which is released after hydrolysis of oleuropein (Kiai et al., 2020). It is also stated that other organic acids such as acetic, malic, citric, formic and succinic contribute to the overall pH.

Parallel to the findings of the study, Benincasa et al. (2015) and Ünal & Nergiz (2003) mentioned, similar pH values at the end of fermentation. On the contrary of Benincasa et al. (2015), in particular, since the

starter culture added table olive samples showed higher pH and titratable acidity content, they did not seem to be affected by phenolics.

Differences detected in titratable acidity values according to processing methods showed that processing methods were effective on titratable acidity value. The change in titratable acidity values determined depending on the olive processing methods was found to be statistically significant at the p<0.01 level with the variance analysis applied on the titratable acidity values obtained from the olive samples.

As shown in Table 2, the acidity of raw olives was found to 0.46% (as lactic acid). The dry salting process caused an increase in the acidity of olive fruits. After fermentation, it increased to the value of 0.54% and 0.71% in normal and starter culture added samples respectively. The reason of this increase in the acidity is the lactic asid formed by lactic acid bacteria.

Parallel to our findings the highest titratable acidity, were determined at inoculated samples (Kumral and Sahin, 2009). Ünal and Nergiz (2003) found acidity in black table olives slightly lower than our research findings (0.45%).

The reducing sugar value of the raw olives was 1.92%. dry salting method reduced sugar content of olives and determined 0.45% (N) and 0.36% (K) at the end of fermentation. The change in reducing sugar values depending on the olive processing methods was found to be statistically significant at the p<0.01 level.

The initial sugar concentration in traditional olives was almost 20% higher than in starter culture added olives. This may be because, during the fermentation phase, cultured olives produce higher titratable acidity and therefore more degradation at sugar levels. It is seen in our study that the production of more titratable acidity causes the breakdown of sugars and with it, faster removal of bitterness and a lower pH development.

The total phenolic compound in the raw olive samples is 389 mgCAE/100g, the total phenolic substance values of the olive samplesobtained according to the processing methods are in the normal production and the cultured production methods 238 and 206 mgCAE/100g, respectively.

Our results showed that processing methods affected the phenolic contents and they decreased in both olive processing methods. The phenolic concentration is quite low in olives produced by the culture added method. Due to the rapid pH and titratable acidity changes during fermentation, the faster decomposition and diffusion of water-soluble phenols increases the losses. In traditional production, relatively low titratable acidity causes less degradation of phenols and may be slower to pass to the external environment as it affects the fruit peel less. Besides, we can say that the phenolics in the olive do not adversely affect the culture inoculated for a rapid fermentation.

The processing method of the final products has also a significant effect on the phenolic compound concentration. Olive fruit processing methods affect the taste and can significantly alter the health properties of the olive fruit, mostly because different processing methods can lead to different capacity of hydrolysis products (Marsilio et al., 2005; Zoidou et al., 2010; Charoenprasert & Mitchell, 2012; Salis et al., 2021). Research results showed that table olive processing techniques have a significant effect on the total phenolic content of olives and that the applied processing techniques cause a significant reduction in the total phenol content of olives (Irmak et al., 2010).

As a result, the level of phenolic compounds of olives decreased during fermentation and during storage compared to raw fruit. This is also in line with previous studies (Blekas et al., 2002; Romero et al., 2004; Irmak et al., 2010; Sahan et al., 2013; Kiai et al., 2020). On the other hand, polyphenol contents of end products of all studied treatments were decreased comparing to the traditional one (Abd El-Samie Ibraheem, 2015).

Generally the results of polyphenols were in agreement with those reported by Marsilio et al. (2006), who reported that, processing of olive resulted in a 35-40 % loss of total phenols (during 7 months), where the results indicated that they were lost more during the first 3 months of fermentation. (Abd El-Samie Ibraheem, 2015)

The differences detected in the total phenolic substance values according to the processing methods showed that the processing methods were effective on the total phenolic compound value. The change in the total phenolic matter values determined depending on the olive processing methods was found statistically significant at the p<0.01 level with the analysis of variance applied on the total phenolic matter values obtained from the olive samples (Table 7). Along with the differences detected in the total phenolic compound values of the processing methods, the irradiation and storage process were found statistically significant at the p<0.01 level.

According to the Durante et al. (2018) and Blekas et al. (2002) total phenol of black olives vary from 10 mg/100 g of flesh to 83.3 mg/100 g of fresh weight. These results are lower than the results obtained from our study. This may be due to the different olive cultivars and processing methods they used. Kiai and Hafidi (2014) stated a significant loss in the total amount of phenolic substances in olives during fermentation due to the diffusion of water-soluble phenolic compounds into the brine, and similar results have been reported by other researchers (Alvarez et al., 2014; Othman et al., 2009; Romero et al., 2009).

Changes of pH values during storage

The pH values of the table olive is crucial parameter from technological and sanitary point of view, so pH values measured during storage period (Table 3). pH values were determined between 4.64-4.83 in normal produced olives and between 4.30-4.55 in starter culture added olives.

TABLE 3 pH values of dry salted olives during storage

Irradiation applications together with processing methods were also statistically effected pH values of samples (p<0.01). There was no statistical difference between irradiation doses (1, 3, 5 kGy) on pH values. The applied irradiation doses were found to be different only from the non-irradiated group.

pH values did not change significantly but slightly decreased during storage and at the end of storage, all samples were found to be appropriate according to the Turkish Food Codex (Anon., 2014). Parallel with our research findings, Sanchez et al. (1997) reported that pH increased during storage.

Değirmencioğlu (2011) states that the pH changes of olives stored at 4°C and 20°C after fermentation vary between 5.05-5.25 and 4.99-5.36. Değirmencioğlu (2011) determined that the pH values of olives stored at 4°C and 20°C ranged between 5.05-5.25 and 4.99-5.36. Panagou, (2006) and Ramírez et al., (2013) reported pH as 5.0–5.2 for black dry-salted olives These values are quite higher than the pH values obtained in our study. This may be due to the differences in olive processing and preservation methods.

Fluctuations observed in pH values durung storage. It might be due to the the organic acids formed by the microflora

Titratable acidity changes of dry salted olives during storage

Titratable acidity of the table olive is also crucial parameter from technological and sanitary point of view when black olives are processed according to the naturally dry-salted black olives, Titratable acidity values during storage are shown in Table 4. Olive titratable acidity change was similar to pH change in both methods during fermentation. However, this increase was more rapid in olives using starter culture Table 4.

TABLE 4 Titratable acidity values of samples during storage (%)

The processing method, irridation and storage time effect on titratable acidity were found statistically significant (p <0.01).

Preservation methods applied increased the acidity of olives. Starter culture added and vaccum applied group were found to be the highest (1.02%) and the group without culture were the lowest (0.68%). This may be due to the high number of lactic acid bacteria because of the addition of starter culture.

The highest acidity values obtained in the storage period were 0.79% (NV0) in the 1st month of storage and as 1.02% (KV0 and KV1) in the 1st month of storage. The lowest values were found as 0.68% and 0.83% in

the NV3 and KV5 in 8th month of storage, respectively (Table 4).

As noted by Rodriguez-Gomez et al. (2014), there were significant decreases in titratable acidity during the storage. The highest acidity drop was observed in KV5.

According to the LSD test applied, in terms of acidity values, 1 and 3 kGy doses of irradiation were in the same group, while the sample with 5 kGy irradiation was in a separate group from the other samples.

Reducing sugar changes during storage

Soluble sugars extracted from olive fruits into the brine are the substrates for microbial fermentation, leading to the production of acids responsible for the low pH, but also to the production of secondary metabolites responsible for the organoleptic characteristics of the final product (Kailis and Harris, 2007).

The highest values obtained at the end of the storage period were 0.46% (NMP0) in the 1st month of storage and as 0.36% (KV0 and KV1) in the 1st month of storage. The lowest values were found as 0.37% in the NV1, NV3, NV5 and as 0.29% KMP5 in 8th month of storage, respectively.

TABLE 5 Reducing sugar values of dry salted olives during storage (%)

Effect of processing methods, irradiation and storage on reducing sugar were found to be statistically significant (p<0.01). According to the LSD test applied for the difference between irradiation applications, the sample with non- irradiated samples was in a separate group from the other samples but there was no difference between the 1, 3, 5 kGy irradiation doses. Data obtained by other authors also showed that gamma irradiation, using a doses up to 10 kGy, did not induce significant loss in water soluble components such as, sugars and

the sugar content of with and without added culture processed olives followed a similar trend and slightly decreased during storage (Table 5). This may be due to continued microbial activation during storage. Our results, seems to be compatible with the decrease in the amount of sugar that stated by López-López et al. (2007) and Kiai et al. (2020)

Total phenolic compound (TPC) changes during storage

Phenolics are a major category of components with important biological properties present in olive drupes. Apart from their contribution to sensory and aromatic characteristics of olives, they are also regarded as natural antioxidants due to their reducing properties as hydrogen- or electron-donating agents (Grounta et al., 2017).

The highest values obtained at the end of the storage period were 232 mgCAE/100g in the first month of storage in NV0 and 200 mgCAE/100g in the second month of storage in KV0. The lowest values obtained at the end of the storage period were 144 mgCAE/100g in the eight month of storage in NMP5 and 138 mgCAE/100g in the eight month of storage in KV5. The total phenolic compound values of olives are shown in Table 6.

Total phenolic content of the olive samples (wet basis) range between 229.12 and 415.34 mgCAE/100g (Yıldız and Uylaşer, 2015). Total phenol content obtained in this study were lower. Such differences may be due to the varieties amd processing methods studied.

TABLE 6 The TPC values of dry salted olives during storage

Processing method, irridation and srorage were significantly effect the TCP of olives (p<0.01)

TABLE 7 Applications with significant statistical differences in the TPC values

Gamma irradiation provoked significant changes in TPC of olives. According to the LSD test applied to determine the differences between applications, the unirradiated group differed from the 3 and 5 kGy irradiated groups. A seen in the Table 6, irradiation slightly decreased the total amount of phenol of table olives and continued to decrease during the storage period. Similarly De Toledo et al., (2007) observed that

gamma irradiation decreased phenolics. Contrary to our findings Stajner et al. (2007) and Harrison and Were, (2007) found significant increase in total phenolic by applied doses of γ -irradiation in soybeans and almond skin, respectively. This increase in phenolics may be partly due to the higher extractability of these materials after irradiation

Antioxidant activity changes during storage

The DPPH* RSA amount was determined as $67.28 \,\mu$ molTE/100g oil in oils obtained from raw olive samples. The amount of DPPH* RSA at the end of the fermentation period was 54.47 μ molTE/100g oil in the normal production group of the turned olives obtained according to different processing methods, and the DPPH* RSA amount was 51.54 μ molTE/100g oil in the group produced by adding culture (Table 8).

During the post-packaging storage period, DPPH* RSA values were determined in olive samples in the 4th and 8th months of storage. In the normal production N group, the highest DPPH* RSA value was determined as 57.35 μ mol TE/100g oil in the irradiated NV3 coded sample, and the lowest was 47.14 μ molTE/100g oil in the 5 kGy irradiated vacuum sample in the NV group. In the K group produced with culture addition, the highest value was 60.94 μ mol TE/100g oil in the KMP3 coded sample at the 4th month, and the lowest was 49.97 μ molTE/100g oil in the KV0 coded sample. The differences detected in the DPPH* RSA values according to the processing methods showed that the processing methods were effective on the antioxidant activity values. The change in the antioxidant activity values determined depending on the olive processing methods was found statistically significant at the p<0.01 level with the analysis of variance applied on the antioxidant activity values obtained from the olive samples. Along with the differences detected in DPPH* RSA values according to processing methods, packaging, irradiation and storage were statistically significant (p <0.01 level).

TABLE 8 The DPPH* RSA values of olive oils obtained from dry salted olives

In the DPPH* RSA values determined in the olive oil samples that obtained from dry salted olives during the research, the applications that are found to be statistically significant and their significance levels are presented in Table 9.

TABLE 9 Applications with significant statistical differences in the TPC values

It was observed that at higher dose of irridation decreased the free radical activity of oils as compared to raw fruit oils. However 3 and 5 kGy doses significantly increased the antioxidant activity of oils obrained from table olive after processing. The enhanced antioxidant capacity/activity of a plant after irradiation is mainly attributed either to increased enzyme activity (e.g., phenylalanine ammonialyase and peroxidase activity) or to the increased extractability from the tissues (Althoman et al. 2009). Similar to the findings of the study, Latreche et al., (2018) reported that the irradiation at 10 kGy increases antioxidant activity.

According to several authors, there is a linear correlation between the total polyphenol content in table olives and their antioxidant activity (Othman et al., 2009; Romero et al., 2004; Sousa et al., 2014). The study of Irmak et al. (2017) showed that processing style of olives had significant effects on quality parameters of olive oils obtained from raw and fermented olives. Kiai et al. (2020) states that during the fermentation, there is a decrease of approximately 43% in total phenolic components. For this reason, it is stated that phenol losses during fermentation reduce the antioxidant activity of olives. They also report that changes in processing methods change the profile and level of phenolic compounds and thus affect the antioxidant activity of the final product.

Contrary to our study findings some studies showed that gamma irradiation did not change the radical scavenging activities of some medicinal plants (Jeong et al., 2009, Brandstetter et al., 2009), dried spices (Nagy et al., 2011) and olive leaves (Aouidi et al., 2011). Others reported that low or mild gamma irradiation could increase or slightly increase the antioxidant activities of seeds of soybean and cumin (Dixit et al., 2010; Kim et al., 2009) and peach fruit (Hussain et al., 2010).

Aşık & Özkan (2011) stated that tocopherol, carotene, chlorophyll and especially phenolic compounds cha-

racterize the antioxidant activity in extra virgin olive oils, and that these natural antioxidants protect the activity by decomposing peroxides and preventing the formation of free radicals. In our research, it is observed that storage affects the antioxidant activity values. It has been determined that the decrease in the total amount of phenolic substances during storage also determines the decrease in antioxidant activity. Besides, it is one of the results obtained in our study that MAP packaging has a preventive effect on the decrease of antioxidant activity.

According to several authors, there is a linear correlation between the total polyphenol content in table olives and their antioxidant activity (Othman et al., 2009; Romero et al., 2004; Sousa et al., 2014). They found a positive corelation between the TPC and DPPH. The study of Irmak et al. (2017) showed that harvest year, process type and salt content of olives had significant effects on quality parameters of olive oils of raw olives and fermented olives.

CONCLUSION

This work proposed a modification for the traditional way that black-table olives have been processed by a great part of the Turkey. The traditional dry salted method was modified by lowering salt and adding starter culture. At the same time, a new preservation method (irradiation) has been proposed to the black table olive sector. Starter culture use in dry salted olives produced a final product with lower pH value and higher free acidity. Fermentation of olives with starters lower salt concentration compared to those traditionally used by the producers. It is important because it allows the production of Gemlik table olives with low salt content.

In this study, the effect of irradiation on black table olive storage was investigated for the first time. Gammairradiation at the doses tested (1, 3, 5 kGy) seems to have little effect on total phenolic amount and antioxidany capacity of the black table olive. Generally no significant differences between the 1 kGy and 3, 5kGy has been found. 1 kGy irridation seemed to be a suitable method for black olive preservation and lower doses may be studied in future studies. This study suggested that suitable doses of irradiation might be carefully selected and used to minimise the loss of antioxidant phenolic compounds in black table olive during storage.

Due to market demand for new preservaion methods, the results of this study will be beneficial for the consumers and the table olive sector. The resulting product could be of higher quality with extended shelf-life while being at the same time safer for the consumer.

Dry salted table olives must be valued in the future taking into consideration their high content in bioactive compounds.

ACKNOWLEDGMENTS

This project was supported by the Republic of Türkiye Ministry of Agriculture And Forestry General Directorate of Agricultural Research And Policies (TAGEM).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Şahnur IRMAK: Methodology, Investigation, Analysis, Writing, Editing, Supervision, Ferişte ÖZTÜRK GÜNGÖR: Olive Harvesting, Processing, Packaging, Analysis Erkan SUSAMCI : Olive Harvesting, Processing, Packaging, Analysis Didar SEVİM: Oil Extraction, Analysis, Editing, Oya KÖSEOĞLU: Oil Extraction, Analysis,

ETHICS STATEMENT

This research did not involve any human or animal study and institutional ethical approval was not required.

ORCID

Sahnur Irmak https://orcid.org/0000-0003-0488-9647

Ferişte Öztürk Güngör https://orcid.org/0000-0002-0678-7305

Erkan Susamci https://orcid.org/0000-0003-1956-4634

Didar Sevim https://orcid.org/0000-0003-0236-2294

Oya Köseoğlu https://orcid.org/0000-0002-3297-3355

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

REFERENCES

Anonymous 1990. AOAC Official methods of Analysis. 15th ed. Association of Official Analytical Chemists. 1990. Washington, DC. USA

Anonymous, 2014, Turkish Food Codex Table Olive Communiqué (No:2014/33), Ankara.

Anonymous, 2015, Table Olive Standard, TS/774, Turkish Standards Institute. 2015. Ankara.

Abd El-Samie Ibraheem AEH., Fouad Abou-Zaid FO. Removing Bitter Compounds from Green Olives using Different Concentrations of Citric Acid. Middle East J. Appl. Sci. 2015; 5: 39-46.

Althoman M, Rajeev B, Karim AA (2009) Effects of radiation processing on phytochemicals and antioxidants in plant produce. Trends in Food Science and Technology 20(5):201–212.

Alvarez, D.M.E., López, A. and Lamarque, A.L., 2014, Industrial improvement for naturally black olives production of Manzanilla and Arauco cultivars, Journal of Food Processing and Preservation, 38; 106.

Aouidi, F., Ayari, S., Ferhi, H., Roussos, S., 2011. Gamma irradiation of air-dried olive leaves: Effective decontamination and impact on the antioxidative properties and on phenolic compounds. Food Chemistry 127(3):1105-1113

Aponte M, Blaiotta G, La Croce F, Mazzaglia A, Farina V, Settanni L, Moschetti G. Use of selected autochthonous lactic acid bacteria for Spanish-style table olive fermentation. . Food Microbiol. 2012;30 : 8-16.

Arroyo-Lopez FN, Querol A, Bautista-Gallego J., Garrido-Fernandez A. Role of yeasts in table olive production. Int. J. Food Microbiol. 2008;128 :189- 196.

Arslan, D., Özcan, M.M., 2011. Influence of growing area and harvest date on the organic acid composition of olive fruits from Gemlik variety. Sci. Hortic. 130 (3), 633–641.

Aşık H, Özkan G. Olgunlaşma derecesinin Memecik zeytin çeşidinin fiziksel özellikleri üzerine etkisi (In Englis: The effect of ripening degree on physical properties of Memecik olive variety). Hasad Gıda. 2011; 27 :36-41.

Bautista Gallego, J., Arroyo Lopez, F. N., Romero Gil, V., Rodriguez Gomez, F. and Garcia Garcia, P., 2011, Chloride salt mixtures affect Gordal cv. green spanish-style table olive fermentation, Food Microbiology, 28: 1316-1325

Ben Ghorbal, A., Leventdurur, S., Agirman, B., Boyaci Gunduz, C. P., Kelebek, H., Carsanba, E., Darici, M., Erten H. 2018. Influence of geographic origin on agronomic traits and phenolic content of cv. Gemlik olive fruits. Journal of Food Composition and Analysis 74

Benincasa C., Muccilli S., Amenta M., Perri E., Romeo FV. Phenolic trend and hygienic quality of green table olives fermented with *Lactobacillus plantarum* starter culture. Food Chem. **2015** ; 186: 271–276.

Blekas G., Vassilakis C., Harizanis C., Tsimidou M., Boskou D. Biophenols in table olives. J. Agric. Food Chem. 2002; **50** : 3688–3692.

Borçaklı M., Özay G., Alperden I., Fermentation of Turkish Black Olives with Traditional and Aerated Systems. In "Food Flavours, Ingredients and Composition", Ed. G. Charalambous, Elsevier Science Publisher. 1993; 265-277.

Boskou D., Clodoveo M. Products From Olive Tree Book, Intech, 2016.

https://doi.org/10.5772/61902.

Brandstetter, S., Berthold, C., Isnardy, B., Solar, S., Elmadfa, I., 2009. Impact of gamma-irradiation on the antioxidative properties of sage, thyme, and oregano, Food and Chemical Toxicology, Vol. 47, 2230-2235.

Byun, M.; Kang, I.; Mori, T. Effect of γ -irradiation on the water soluble components of soybeans. Radiat. Phys. Chem 1996, 47, 155–160.

Carrasco-Pancorbo, A., Cerratani, L., Bendini, A., Sequra Carretero, A., Del Carlo, M., Gallina-Toschi, T., Lercker, G., Compagnone, D., and Fernandez-Gutierrez, A., 2005, Evaluation of the antioxidant capacity of individual phenolic compounds in virgin olive oil, Journal of Agricultural and Food Chemistry, 53:8918-892

Charoenprasert S., Mitchell A. Factors influencing phenolic compounds in table olives (*Olea europaea*). J. Agric. Food Chem. 2012.60: (29):7081-7095.

De Bruno A., Zappia A., Piscopo A., Poiana M. Qualitative evaluation of fermented olives grown in Southern Italy (cvs. Carolea, Grossa of Gerace and Nocellara Messinese), Emir J Food Agric. 2019. **31** (8): 587-596.

Değirmencioğlu, N. Gemlik tipi sele zeytininin mikrobiyolojik kalitesi üzerine sıcaklık ve modifiye atmosferde paketlemenin etkisi (In English: The effect of temperature and modified atmosphere packaging on the microbiological quality of Gemlik type saddle olives). Ulusal zeytin Kongresi. 2011.

Dixit, A.K., Bhatnagar, D., Kumar, V., Rani, A., Manjaya, J.G., Bhatnagar, D., 2010. Gamma Irradiation Induced Enhancement in Isoflavones, Total Phenol, Anthocyanin and Antioxidant Properties of Varying Seed Coat Colored Soybean. Journal of Agricultural and Food Chemistry 58(7):4298-302.

Durante M, Tufariello M, Tommasi L, et al. (2018) Evaluation of bioactive compounds in black table olives fermented with selected microbial starters. J Sci Food Agric 98, 96–103

Erten H., Boyacı-Gündüz CP., Ağırman B., Cabaroğlu T. Fermentation, Pickling and Turkish Table Olives. Handbook of Vegetable Preservation and Processing. 2016; 209-230.

Grounta, A., Tassou, C. C., Panagou, E. Z. 2017. Greek-Style Table Olives and their Functional Value. Book Editor(s):Fereidoon Shahidi, Apostolos Kiritsakis. https://doi.org/10.1002/9781119135340.ch16.

Günç Ergönül P.. Zeytin meyvesinin olgunlaflması sırasında, bileflimindeki organik asit miktarındaki değiflimler ve bu değiflimlerin yağ birikimiyle olan iliflkisinin arafltırılması. CBÜ FBE Yüksek Lisans Tezi. (In English: Investigating the changes in the amount of organic acid in the composition of olive fruit during ripening and the relationship of these changes with fat accumulation, Master Thesis) 2006; 98.

Harrison, K. and Were, L.M., 2007. Effect of gamma irradiation on total phenolic content yield and antioxidant capacity of Almond skin extracts, Food Chemistry, Vol. 102, 932-937.

Hussain, P.R. . Wani, A.M., Meena, R.S., Dar, M.A. 2010. Gamma irradiation induced enhancement of phenylalanine ammonia-lyase (PAL) and antioxidant activity in peach (Prunus persica Bausch, Cv. Elberta), Radiation Physics and Chemistry. Vol. 79, 982-989

IOC, International olive council, 2022. http://www.internationaloliveoil.org/estaticos/view/132-world-tableolive-figures. Date of access: 06.01.2022). Irmak Ş. Gemlik Çeşidi Sofralık Siyah Zeytinlerin Muhafazasında Yararlanılan Farklı Yöntemlerin Raf Ömrü ve Kalite Üzerine Etkileri. Ege Üniversitesi FBE Doktora Tezi. (In English: The Effects of Different Methods Used in the Storage of Black Table Olives of Gemlik Varieties on the Shelf Life and Quality. PhD Thesis) 2015; 451.

Irmak Ş., Kadiroglu P., Ötles S. Evaluation of olive preservation methods on bioactive constituents and antioxidant properties of olive oils. J Am Oil Chem Soc. 2017; 94:595–609. DOI 10.1007/s11746-017-2971-5

Irmak Ş., Öztürk-Güngör F., Susamcı E., Bazı Sofralık Zeytin Çeşitlerimizin Toplam Fenolik Madde Miktarları ve İşleme Tekniklerinin Bu Bileşikler Üzerine Etkileri (In English: Total Phenolic Substance Amounts of Some Table Olive Varieties and Effects of Processing Techniques on These Compounds). Zeytin Bilimi, 2010;1 (2):57-64.

Kader A. A. Biochemical and physiological basis for effects of controlled and modified atmospheres on fruits and vegetables. Food Tech. 1986;40:99–104.

Kailis S. and D. Harris. 2007. The olive tree Olea europaea, pp. 17–66. In: Kailis S. and D. Harris (eds). Producing Table olives. Landlinks Press, Collingwood, USA

Kiai, H. and Hafidi, A., 2014, Chemical composition changes in four green olive cultivars during spontaneous fermentation, LWT - Food Science and Technology, 57:663-670.

Kiai H., Raiti J., El Abbassi A., Hafidi A. Chemical profiles of Moroccan picholine olives and its brines during spontaneous fermentation. Int. J. Fruit Sci. 2020; **20** (3): 1297–1312.

Kim, J.H., Shin, M.H, Hwang, Y.J., Srinivasan, P., Kim, J.K., Park, H. J., Byun, M.W., Lee, J.W, 2009. Role of gamma irradiation on the natural antioxidants in cumin seeds. Radiation Physics and Chemistry. Vol. 78, 153-157.

Köseoğlu O., Sevim D., Kadiroğlu P. Effects of filtration on the quality properties of extra virgin olive oils during storage. J. Am. Oil Chem. Soc. 2019; **96** : 291-301.

Kumral, A., Basoglu, F., Sahin, I2009. Effect Of The Use Of Different Lactic Starters On The Microbiological And Physicochemical Characteristics Of Naturally Black Table Olives Of Gemlik Cultivar, Journal of Food Processing and Preservation, 651-664.

López-López A., Jiménez-Araujo A., García-García P., Garrido-Fernández A. Multivariate analysis for the evaluation of fiber, sugars, and organic acids in commercial presentations of table olives. J. Agric. Food Chem. 2007; **55** : 10803-10811.

Marsilio V., Seghetti L., Iannucci E., Russi F., Lanza B., Felicioni M. Use of a lactic acid bacteria starter culture during green olive (Olea europaea L., cv. Ascolana tenera) processing. J. Sci. Food Agric. 2005;85 : 1084-1090.

Marsilio V., d'Andria R., Lanza B., Russi F., Iannucci E., Lavini A., Morelli G. Effect of Irrigation and Lactic Acid Bacteria Inoculants on the Phenolic Fraction, Fermentation and Sensory Characteristics of Olive (Olea Europaea L. cv. Ascolana Tenera) Fruits. J. Sci. Food Agric. 2006;86 :1005-1013.

Mcfeeter RF. Fermentation microorganisms and flavor changes in fermented foods. J. Food Sci. 2004; 69 (1):35-37.

Montet D., Ray RC., Zakhia-Rozis N. Lactic acid fermentation of vegetables and fruits. Microorganisms and Fermentation of Traditional Foods. (1st ed.). CRC Press. 2014;33.

Nagy, T.O, Solar, S., Sontag, G., Koenig, J., 2011. Identification of phenolic components in dried spices and influence of irradiation. Food Chemistry 128(2):530-534.

Niemira, B. A., Christopher, Sommers, C., Glenn, B., 2001. Irradiation Inactivation of Four Salmonella Serotypes in Orange Juices with Various Turbidities. Journal of Food Protection. Vol. 64, 614-617.

Oliviera, M., Brito D., Catulo, L., Letao, F., Silva, S., Vilas-boas, L., Peito, A., Fernandez, I., Gordo, F., Peres, C., 2004, Biotechnology od olive fermantationof "Galega" Portuguese variety.Grasas Y Aceites 55(3).219-226.

Othman NB., Roblain D., Chammen N., Thonart P., Hamdi M. Antioxidant phenolic compounds loss during the fermentation of Chétoui olives. Food Chem. 2009; **116** : 662-669.

Özışık S., Öztürk F. Türkiye'de zeytin ve zeytinyağı sektörünün mukayeseli analizi (In English: Comparative analysis of olive and olive oil sector in Turkey). Ulusal Zeytin Kongresi, 2011.

Özaltaş, M., Savran, M.K., Ulaş, M., Kaptan, S., Köktürk, H.(2016) Türkiye Zeytincilik Sektör Raporu, ISBN: 978-605-9175-57-9. SS: 302.

Panagou, E. Z. (2006). Greek dry-salted olives: Monitoring the dry-salting process and

subsequent physico-chemical and microbiological profile during storage under different packing conditions at 4 and 20^{*} C. LWT–Food Science and Technology, 39, 322–329

Perricone M., Bevilacqua A., Corbo MR., Sinigaglia M. Use of *Lactobacillus plantarum* and glucose to control the fermentation of "Bella di Cerignola" table olives, a traditional variety of Apulian region (Southern Italy). J Food Sci. 2010; **75** : 430-436.

Ramirez, E., Garcia-Garcia, P., de Castro, A., Romero, C., & Brenes, M. (2013). Debittering of black dry-salted olives. European Journal of Lipid Science and Technology, 115, 1319–1324.

Randazzo CL., Rajendram R., Caggia C. "Lactic acid bacteria in table olive fermentation," in olives and olive oil in health and disease prevention. eds R. Victor, Preedy, and Ronald Ross Watson (Oxford: Academic Press). 2010;369–376.

Rodriguez-Gomez F., Arroyo-Lopez FN., Romero-Gil V., Jimenez-Diaz R., Garrido-Fernandez A., Garcia-Garcia P. Table Olive Fermentation with Multifunctional *Lactobacillus pentosus Strains*. Food Cont. 2013; **34**: 96-105.

Rodriguez-Gomez, F., Lopez-Lopez, A., Gomero Gil, V., Arroyo-Lopez, F. N. (2014). Effect of post-fermentation storage on Spanish-style green Manzanilla olives. Food Science and Technology, 57(2), 789-793pp. doi:10.1016/j.lwt.2014.01.041

Romero C., Brenes M., Yousfi K., Garcia P., Garcia A., Garrido A. Effect of cultivar and processing method on the contents of polyphenols in table olives. J Agric Food Chem. 2004. **52** (3):479-84. doi: 10.1021/jf0305251.

Sahan Y., Cansev A., Gulen H. Effect of processing techniques on antioxidative enzyme activities, antioxidant capacity, phenolic compounds, and fatty acids of table olives. Food Sci. Biotechnol. 2013;22 (3):613-620. https://doi.org/10.1007/s10068-013-0122-9

Salis C., Papadakis IE., Hagidimitriou M. Identification and quantification of phenolic compounds in fresh and processed table olives of cv. 'Kalamata'. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2021; 49 (2), 12394.https://doi.org/10.15835/nbha49212394

Sanchez, A. H., Montano, A. and Rejano, L. (1997). Effect of preservation treatment, light, and storage time on quality parameters of Spanish style green olives, Journal of Agriculture and Food Chemistry, 45, 3881-3886pp

Sevim D., Tuncay O., Koseoğlu O. The effect of olive leaf addition on antioxidant content and antioxidant activity of "Memecik" olive oils at two maturity stages. J. Am. Oil Chem. Soc. 2013; 90 :1359–1369.

Sousa C., Gouvinhas I., Barreira D., Carvalho MT., Vilela A., Lopes J., Martins-Lopes P., Barros AI. 'Cobrançosa' olive oil and drupe: Chemical composition at two ripening stages. J. Amer. Oil Chem. Society. 2014;**91** : 599-611.

Stajner, D., Mişosevic., M., Popovic, B., M., 2007. Irradiation effects on phenolic content, lipid and protein oxidation and scavenger ability of soybean seeds, International Journal of Molecular Sciences, , 618-627

Ünal, K., Nergiz C. The effect of table olive preparing methods and storage on the composition and nutritive value of olives. Grasas Aceites. 2003. 54 :71-76.

Varol N., Erten L., Turanlı T."Zeytin kitabı" (In English: Olive book) T.C. Tarım ve Köyişleri Bakanlığı Teşkilatlandırma ve Destekleme Genel Müdürlüğü, Televizyon Yoluyla Yaygın Çiftçi Eğitimi Projesi (YAYÇEP). 2009; 86-89.

Villavicencio , A.L.C.H, Mancini-Filho, J, Delincée, H., Greiner, R., 2000. Effect of irradiation on antinutrients (total phenolics, tannins and phytate) in Brazilian beans. Radiation Physics and Chemistry , Vol. 57, 289-293.

Yıldız G., Uylaşer V. Profile and total content of phenolics and antioxidant activity of commercial table olives from Turkey. Qual. Assur. Saf. Crops Foods. 2015; 5: 635-642.

Zoidou E., Melliou E., Gikas E., Tsarbopoulos A., Magiatis P., Skaltsounis AL. Identification of Throuba Thassos, a traditional Greek table olive variety, as a nutritional rich source of oleuropein. J. Agric. Food Chem. 2010; **58** (1):46-50.

Figure Captions

FIGURE 1 Flowchart of experimental procedure of olives

Table Captions

TABLE 1 The code description of samples

TABLE 2 Some chemical parameters of raw and dry salted olives

TABLE 3 pH values of samples during storage

TABLE 4 Titratable acidity values of samples during storage (%)

TABLE 5 Reducing sugar values of samples during storage (%)

TABLE 6 The TPC values of dry salted olives

TABLE 7 Applications with significant statistical differences in the TPC values

TABLE 8 The DPPH* RSA values of olive oils that obtained from dry salted olives

TABLE 9 Applications with significant statistical differences in the TPC values

Hosted file

Answer to reviewrs 26.04.2023.docx available at https://authorea.com/users/611609/articles/ 640026-effect-of-irradiation-on-total-biophenol-and-antioxidant-activity-quantityduring-storage-of-natural-black-table-olives-obtained-using-starter-culture

Hosted file

R1 FIGURES 16.03.2023.docx available at https://authorea.com/users/611609/articles/640026effect-of-irradiation-on-total-biophenol-and-antioxidant-activity-quantity-duringstorage-of-natural-black-table-olives-obtained-using-starter-culture

Hosted file

REV. TABLES 11.04.2023.docx available at https://authorea.com/users/611609/articles/640026effect-of-irradiation-on-total-biophenol-and-antioxidant-activity-quantity-duringstorage-of-natural-black-table-olives-obtained-using-starter-culture