**GRASAS Y ACEITES** 71 (1)
January–March 2020, e348
ISSN-L: 0017-3495
https://doi.org/10.3989/gya.0103191

# Effect of ozone treatment on the physical, microbiological and sensorial properties of Spanish-style table olives

F. Öztürk Güngör[a,✉], Ö. Özdestan Ocak[b] and M.K. Ünal[b]

[a]Olive Research Institute, University Avenue No: 43 35100, Bornova, Izmir, Turkey.
[b]Ege University, Faculty of Engineering, Department of Food Engineering, Izmir, Turkey.
✉Corresponding author: feriste@gmail.com

Submitted: 09 January 2019; Accepted: 01 April 2019; Published online: 14 January 2020

**SUMMARY:** Ozone has been widely used in the food industry as an effective antimicrobial agent. In this study the possibilities of using ozone in table olive preservation was investigated for the first time. For this purpose, the Domat variety of table olives was processed according to the Spanish style and treated with aqueous ozone for 5, 10, and 20 minutes at 0.5, 1, 2 and 4 ppm. The effects of ozonation on the microbiological, physical and sensory characteristics of the table olives were evaluated during the storage period (up to 180 days). The pH, color and firmness of the ozone treated table olives showed higher stability. Statistically significant reductions in the total bacteria and yeast/mould counts were obtained ($p < 0.05$). *Enterobacteriaceae* and *Escherichia coli* were not found in the samples. After 60 days of storage the control samples (ozone untreated olives) obtained low values for sensory analysis, and did not meet market requirements. The results indicate that treating green table olive with ozon (1 ppm concentration) for 10 min in aqueous form reduces the microbial population without any negative effects on the firmness, color (L*, a* b*) or sensory attributes of the table olives.

***KEYWORDS***: *CV. Domat; Microbiological safety; Ozone; Pasteurization; Sensory analysis of table olives; Storage; Table olive; Table olive preservation*

**RESUMEN:** ***Efecto del tratamiento con ozono sobre las propiedades físicas, microbiológicas y sensoriales de las aceitunas de mesa de estilo español.*** El ozono se ha utilizado ampliamente en la industria alimentaria como un eficaz agente antimicrobiano. En este estudio se investigaron por primera vez las posibilidades de utilizar ozono en la conservación de la aceituna de mesa. Para este propósito, las aceitunas de mesa de la variedad Domat procesadas según el estilo español se trataron con ozono acuoso durante 5, 10 y 20 minutos a 0,5, 1, 2 y 4 ppm. El efecto de la ozonización sobre la calidad microbiológica, física y sensorial de la aceituna de mesa se evaluó durante el período de almacenamiento (hasta 180 días). El pH, color y la firmeza de las aceitunas de mesa tratadas con ozono mostraron una mayor estabilidad. Se obtuvo una reducción estadísticamente significativa en el recuento total de bacterias y levaduras/mohos ($p < 0,05$). No se encontraron enterobacterias ni *Escherichia coli* en las muestras. Después de 60 días de almacenamiento, las muestras de control (aceitunas sin tratar con ozono) obtuvieron valores bajos para el análisis sensorial, sin cumplir la condición para el mercado. Los resultados indican que el tratamiento de la aceituna de mesa verde con ozono (concentración de 1 ppm) durante 10 min en forma acuosa reduce la población microbiana sin efectos negativos sobre la firmeza, el color (L *, a * b *) y los atributos sensoriales de las aceitunas de mesa.

***PALABRAS CLAVE***: *Aceituna de mesa; Almacenamiento; Análisis sensorial de aceitunas de mesa; Conservación de aceitunas de mesa; CV. Domat; Ozono; Pasteurización; Seguridad microbiológica*

**ORCID ID:** Öztürk Güngör F https://orcid.org/0000-0002-0678-7305, Özdestan Ocak Ö https://orcid.org/0000-0003-0967-8865, Ünal MK https://orcid.org/0000-0001-6283-7517

**Citation/Cómo citar este artículo:** Öztürk Güngör F, Özdestan Ocak Ö, Ünal MK. 2020. Effect of ozone treatment on the physical, microbiological and sensorial properties of Spanish-style table olives. *Grasas Aceites* **71** (1), e348. https://doi.org/10.3989/gya.0103191

## 1. INTRODUCTION

Table olives are among the main fermented vegetables in Mediterranean countries (Arroyo-López *et al.*, 2006). With an annual production rate of 412 thousand tonnes, Turkey is the third largest table olive producer in the world after Egypt (IOC, 2016). 91 olive varieties (*Olea europaea* L.) are cultivated in Turkey. Due to its high flesh/stone ratio, good shape, texture and excelent organoleptic characteristics "Domat" is the most important and economically valuable variety for the green table olive industry in Turkey and is mostly processed with alkali, also known as Spanish-style.

In the food industry, it is important to protect the quality of the product, to extend the shelf-life and to reduce the losses to minimum levels. The shelf-life of foods is closely related to microbial safety and biochemical and enzymatic reactions (Romeo *et al.*, 2009).

Preservation methods such as pasteurization or sterilization are frequently used to guarantee the stability and safety of fermented olives (Medina-Pradas and Arroyo-López, 2015). Heat treatments are particularly important for microbial safety, although they can be the cause of negative effects on the chemical characteristics, consistency, texture and color of the final product (Catania *et al.*, 2014).

One of the methods developed for food preservation is an ozone gas-based system which has the potential to be used successfully in the food sector due to its disinfectant effect and the absence of residue left behind. Ozone as an aqueous disinfectant was declared to be generally recognized as safe (GRAS) for food contact applications in 1997 (Xu, 1999). $O_3$ is a strong oxidizer, and there are numerous applications for gaseous and dissolved $O_3$ (ozonated water) in disinfection and food processing. Because $O_3$ is highly unstable and decomposes rapidly, it does not leave a potentially harmful residue on fresh products (Renumarn *et al.*, 2014). In some studies, it was declared that ozonated water has been shown to reduce the microbial population and extend the shelf-life of fresh-cut fruits and vegetables (Sapers, 2003).

Ozone application has been used as a post-harvest treatment and to regenerate fermentation brines in Spanish-style green olives (Segovia-Bravo *et al.*, 2008) and iron solutions in oxidized black olives (García-García *et al.,* 2014; Romero-Barranco *et al.*, 2016) or as preservative agent in table olive packaging (Arroyo-López *et al.*, 2006). In considering other non-thermal disinfectant approaches, ozone seems to be an effective sanitizer with great potential applications in the food industry. For effective and safe use in food processing, optimum ozone concentration, contact time and other treatment conditions should be defined for all products (Karaca and Velioğlu, 2007).

To the best our knowledge, there has been no research on the application of ozone as a preservation method of table olives. The main purpose of this study was to evaluate the effects of ozone application on some physical, microbiological and sensory characteristics of green table olives during storage.

## 2. MATERIALS AND METHODS

### 2.1. Raw material and sample preparation

Green olive fruits (Domat variety), with an average weight of 160-180 fruit/kg were harvested from the Olive Research Institutes orchard in Kemalpaşa, Izmir, Turkey, in October 2013 at 1 maturity index ánd by hand. The olives were then processed according to Spanish style table olive preparation method. The olives remained in the lye solution (1.9% NaOH) until 2/3 of the fruit flesh had been penetrated. Then the olives were washed with tap water for 18 h, to completely remove NaOH. After this pre-treatment, the olives were placed in brine (7 g/100 mL of NaCl) for three months at ambient temperature for fermentation to take place.

At the end of fermentation, the pH value (3.78), free acidity (0.88%) of the brines and L* (56.19), a* (0.59), b* (37.59), and hardness (535.32 g) of the olive fruits was measured.

### 2.2. Ozonation system design and ozone treatments

An ozonation system was designed to apply ozone effectively to the table olives (Figure 1). This system was formed by a perforated nickel chromium basket where olive samples were placed and an application tank (50 L, KenaTek Machine, Izmir, Turkey). Two small diffusers were installed into the application tank to ensure a homogeneous distribution of the ozone gas. The concentration of ozone in the tank was measured by an ozone measurement sensor (Electrode SZ 283, ITALY) in the range between 0-20 ppm. Ozone gas was generated using a corona discharge ozone generator which generates ozone from the air stream (TEKNOZON the model TKZ-25 g, Izmir, Turkey).

To determine the effect of ozone application on the physical, microbiological, and sensory properties of table olives, the experiment was designed to be one-factor (14 levels by adding control and pasteurization groups for concentration * time combination). Table olives were treated with 0.5, 1, 2, and 4 ppm ozone for 5, 10, and 20 minutes before packaging. 5 kg of olives were treated with ozone each time. The water temperature used during the ozonation applications was 20 °C ± 1 °C. At the end of each treatment, the water was refreshed and about 30 liters of water were added to the tank. To disinfect the water, it was treated with 0.5 ppm ozone for 5 minutes. Each application was repeated twice. The ozonated olives were packed in glass jars which had been sterilized at 121 °C for 15 min, in portions of

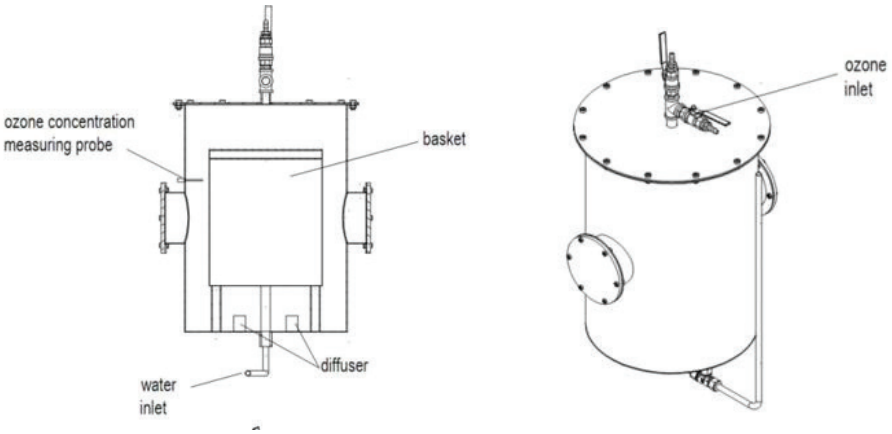


FIGURE 1. Developed ozonation system and prototype.

approximately 540 g and covered with 460 mL brine prepared with ozonated water (4% NaCl and 0.6% citric acid) and stored for 6 months at 20 ± 2 °C. Additional groups of not treated samples (without ozone treatment) and pasteurized samples (80 °C, 15 minutes) were added to the experiment following storage under the same conditions as the ozonated samples. During the storage period (180 day) a pH measurement, microbiological analysis, water activity determination, color determination and sensory analysis were carried out at day 0 (right after the ozone treatment) and at monthly intervals.

### 2.3. Physico-chemical analysis

***pH measurement.*** The pH of the brines was measured by WTW 330 (Germany) (Anon., 2015).

***Water activity ($a_w$) measurement.*** The water activity of the table olives was measured by Pre Aqua Lab (2365 NE, USD).

***Color.*** The color of the table olives was measured by colorimeter (Minolta, model CR-400 chromameter, Japan).

For each sample 20 olives were analyzed to evaluate the skin color. The color of the olive surface was evaluated in terms of lightness (L*, where L=0 is black and L=100 is white), greenness to redness (a*, a+ is red and a- is green) and blueness to yellowness (b*, b+ is yellow and b- is blue) (Panagou, 2004).

***Hardness.*** Hardness and its evolution in olives were determined through a texture analyzer (TA. XT plus, Stable Micro Systems, Godalming, UK) equipped with a 2 mm diameter cylinder probe. By puncturing the olives, the target value of 4 mmthe maximum force (N, g) applied against the time (sec) was measured. The settled parameters of the test were: pre-test speed 2 mm·sec$^{-1}$, test speed 0.5 mm·sec$^{-1}$, post-test speed 4 mm·sec$^{-1}$, and load cell 30 kg (Romeo *et al*., 2012).

### 2.4. Microbiological analysis

The automated system TEMPO (Biomerieux) was used for the enumeration of TAMB (total aerobic mesophilic bacteria), lactic acid bacteria (LAB), yeasts and moulds (YM), *Escherichia coli* (EC) and *Enterobacteriaceae* count (EB) in the olive samples. TEMPO uses a dehydrated culture medium and an enumeration card containing 48 wells across 3 different dilutions for the automatic determination of the most probable number (MPN) (User's Manual, Application Guide of TEMPO®, Biomerieux, Available from:

https://techlib.biomerieux.com/wcm/techlib/techlib/applications/guidedSearch/cleverLink/CleverLink.jsp?productnumber=tempo./Accessed 06.08.12).

Samples of table olive pulp (10 g) and sterile peptone saline diluent (90 mL) were mixed in plastic bags with a filtered compartment. Each of samples was homogenized for 2 min. in a stomacher (easy MIX AES Chemunex 1068, France). After homogenization, for LAB and TAMB, 0.1 mL and for YM, EB and EC, 1 mL of the homogenized sample suspension was taken using a sterile pipette from the filtered bag and transferred into the vial containing culture media previously mixed with 3 mL (for YM, EB, EC) and 3.9 mL (for TAMB, LAB) of sterile distilled water. The vials were mixed with a vortex for 4 seconds. The inoculated media were moved into the TEMPO cards. Cards and reagents were then loaded into the TEMPO Preparation Station according to the manufacturer's instructions, to automatically fill the cards with the appropriate sample dilution. The cards were incubated for 48 h at 30 °C (TAMB and LAB tests), 72 h at 25 °C (YM test) 24 h at 30 °C (EC test) and 24 h at 35 °C (EB test). After incubation, the cards were placed in an automated reader which detects fluorescence. The results were expressed in colony-forming units per 1 g of sample (CFU·g$^{-1}$).

### 2.5. Sensory analysis

During the storage period, the olives were submitted to a sensory analysis by a trained taste panel from the Olive Research Institute (Izmir, Turkey) according to the methodology described in the IOC method (IOC, 2011). The panel consisted of 8 different judges, 5 women and 3 men, between 31-50 years old. The evaluated sensory characteristics were: *a)* negative sensations or defects (abnormal fermentations and other defects such as butyric, putrid and zapatería, winey-vinegary, soapy, metallic, cooking effects, rancid, musty and earthy defects); *b)* gustatory sensations (salty, bitter, acidic) and *c)* kinesthetic sensations (hardness, fibrousness, crunchiness). The samples were coded with random three-digit numbers. Three to four olives were given to the panelists in individual booths in a sensory laboratory. Between samples, water was used to clean out the pallet.

All of these analyses were carried out in duplicate for each sample. To elaborate the sensory data, the method for calculating the means and the confidence intervals was used, as detailed in Annex 1 (COI/OT/MO/No 1/Rev.2 Annex 1 Method for calculating the means and the confidence intervals) (IOC, 2011), taking into account the attributes with a robust coefficient of variation of 20% or less. The computer program for carrying out the calculations was as presented in Annex 3 (COI/OT/MO/No 1/Rev.2 Annex 3 Sensory analysis of table olives computer program) (IOC, 2011). For classification purposes, only the mean of the defect predominantly perceived (DPP) was considered, in other words, perceived with the greatest intensity.

According to the DPP intensity, the samples were classified into four categories:

- Extra or Fancy: DPP ≤ 3
- First, 1st, Choice or Select: 3 < DPP ≤ 4.5
- Second, 2nd or Standard: 4.5 < DPP ≤ 7.0
- Olives that should not be sold as table olives: DPP > 7.0

### 2.6. Statistical Analysis

A statistical analysis of the obtained data was carried out using the SPSS version 20.0 (SPSS, Inc., Chicago, IL, USA). Significant differences among treatments were determined by variance analysis and means were separated by the Duncan test with a 95% significance level.

## 3. RESULTS AND DISCUSSION

### 3.1. Effect of ozonation on pH and water activity (aw)

At the end of fermentation, the pH decreased to 3.78, and thus the acidity 0.88% increased, which ensures the preservation of the product. The initial pH values of ozonated olives were slightly lower than the values of pasteurized and control samples (Figure 2 (a)). The concentration-time combination and storage time effect on the pH value were statistically significant ($p < 0.05$). The values obtained for the pH values of the cover brines obtained at the end of the storage period were in agreement with the IOC limits (IOC, 2004). However, the final pH value for the

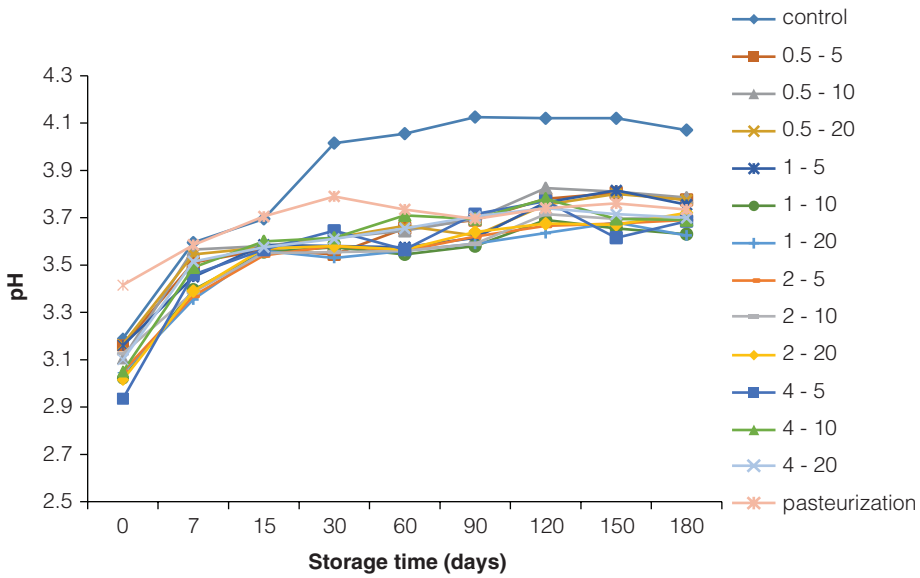


FIGURE 2. Changes in pH values of ozonated, pasteurizated and control group olives (average; n=2 at each sampling point).
NOTE: ozone concentration (ppm): 0.5, 1, 2, 4; application time (min): 5, 10, 20

control group was close to the limit of 4.3. At the end of the storage period, the pH values for 1-10 and 1-20 applications were smaller than the other applications. The pH values of the brines were very similar and did not show significant differences among treatments. The brine pH of the control group was the highest at the end of storage and was in a different group from the other applications. In line with the findings of this study, Alexandre *et al*., (2005) reported that the pH of green basil and bell pepper was significantly affected by ozone application.

Ozone application did not affect the $a_w$ value of the table olives. There was no significant difference between the control group, pasteurized olives or ozonated olives. A slight increase was determined until the 90$^{th}$ day for aw values and after the 90$^{th}$ day, there was a slight decrease in aw values. At the end of storage, the $a_w$ values for the table olives increased compared to the beginning of storage.

### 3.2. Effect of ozonation on hardness

The hardness of olives is one of the key quality parameters for commercial value. As there is no standard for hardness, it is important for the olives to preserve their original firmness as much as possible during storage (Romeo *et al*., 2009). In fact, a non-appropriate fruit texture can be one of the main reasons for rejection by the consumer. In addition, a lack of fruit hardness may cause high economic losses to the processing industry because of difficulties in fruit pitting and stuffing after lactic fermentation (Garrido Fernandez *et al*., 1997). In our work ozone application significantly affected the hardness of the olives (Fig. 3). The hardest olives were detected in pasteurized olives (310.9) and the softest olives in the control group (219.8 g). The hardness of ozonated olives was found to be softer than pasteurized olives and harder than the control group. Compared with the control group, ozone treatment increased the hardness of the olives.

Koyuncu *et al*., (2008) reported that the hardness of cherries treated with ozone increased slightly during storage. The hardness did not reveal significant differences among ozonated groups, but showed a decrease in the values during the storage period. The greatest loss of hardness in the olives occurred on the 60$^{th}$ day. The decrease in hardness during the storage is in accordance with data reported by Sánchez-Gómez *et al*., (2013).

### 3.3. Effect of ozonation on color

The role of color is a highly significant attribute in the quality evaluation and choice made by consumers (Romeo *et al*., 2012).

While the effect of ozone application was not significant for the a* values of the olives (Table 1 (b)), it was found to be significant for L* (Table 1 (a)) and b* (Table 1 (c)) values. In our study, the L* values of the ozonized olives were found to be lower than the control group and higher than the pasteurized olives (except for 2-20 applications). As the lowest L* value was measured in pasteurized olives with 54.72, it can be said that the pasteurization process caused some darkening of the olives. The L* values for the ozonated olives were lower than the control group. This can be attributed to the fact that ozone is relatively blackish due to its oxidizing properties. At the end of storage, the L* value for the olives had increased compared to the beginning of storage. In other words, the olives had been tinted.

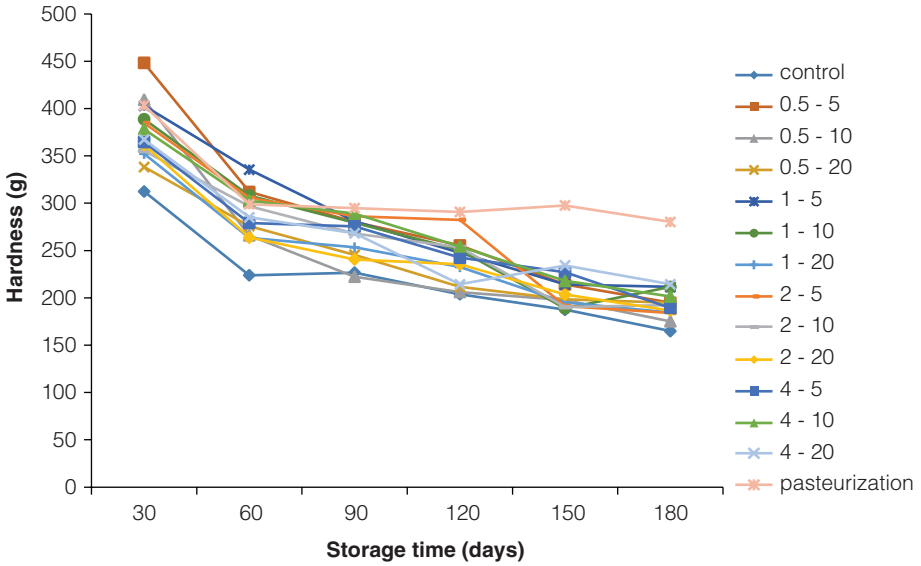


FIGURE 3. Changes in hardness values of ozonated, pasteurizated and control group olives (average; n=2 at each sampling point).
NOTE: ozone concentration (ppm): 0.5, 1, 2, 4; application time (min): 5, 10, 20

Table 1. L* (lightness) (a), a* (red-green) (b) and b* (yellow-blue) (c) values for ozonated, pasteurizated and control group olives

Table 1 (a)

| Applications (conc.-time/ ppm-min.) | Storage time (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **Conc.-time mean.*** |
| 0.5 - 5 | 55.34 | 56.72 | 56.60 | 57.02 | 55.84 | 57.05 | 55.20 | $56.25^{f}$ |
| 0.5 - 10 | 55.32 | 55.60 | 55.82 | 57.25 | 56.10 | 56.23 | 54.99 | $55.90^{ef}$ |
| 0.5 - 20 | 54.12 | 54.22 | 55.02 | 56.57 | 55.31 | 53.38 | 55.54 | $54.88^{bc}$ |
| 1 - 5 | 55.14 | 56.29 | 56.41 | 54.93 | 56.76 | 55.34 | 55.67 | $55.79^{def}$ |
| 1 - 10 | 54.64 | 55.78 | 55.21 | 56.09 | 56.00 | 54.46 | 55.78 | $55.42^{bcdef}$ |
| 1 - 20 | 54.94 | 54.86 | 54.64 | 54.77 | 56.11 | 55.60 | 54.39 | $55.04^{bcd}$ |
| 2 - 5 | 54.75 | 54.96 | 56.64 | 54.65 | 55.48 | 55.56 | 55.08 | $55.30^{bcde}$ |
| 2 - 10 | 53.71 | 54.70 | 54.11 | 54.17 | 56.03 | 54.98 | 54.85 | $54.65^{b}$ |
| 2 - 20 | 51.27 | 53.46 | 53.60 | 52.30 | 52.98 | 52.33 | 54.30 | $52.89^{a}$ |
| 4 - 5 | 54.52 | 56.47 | 55.38 | 56.40 | 56.95 | 56.72 | 54.67 | $55.87^{ef}$ |
| 4 - 10 | 55.66 | 55.34 | 54.26 | 55.78 | 57.52 | 56.79 | 54.45 | $55.68^{cdef}$ |
| 4 - 20 | 53.54 | 56.45 | 56.93 | 56.85 | 56.04 | 56.25 | 56.18 | $56.03^{ef}$ |
| Pasteurization | 54.59 | 54.22 | 56.18 | 57.43 | 52.99 | 54.48 | 53.14 | $54.72^{c}$ |
| Control | 55.84 | 57.40 | 57.50 | 57.51 | 57.59 | 56.50 | 57.18 | $57.07^{g}$ |
| Storage time mean.* | $54.53^{a}$ | $55.46^{bc}$ | $55.59^{bc}$ | $55.84^{c}$ | $55.83^{c}$ | $55.40^{bc}$ | $55.10^{b}$ | |

*Lowercase letters in the same row and column are evaluated independently and different letters indicate different groups according to Duncan's test ($p < 0.05$). The data are reported as the average of duplicate (n=2). conc: ozone concentration (ppm): 0.5, 1, 2, 4; time:application time (min): 5, 10, 20

Table 1 (b)

| Applications (conc.-time/ ppm-min.) | Storage time (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **Conc.-time mean.*** |
| 0.5 - 5 | 0.02 | -0.68 | -0.59 | -0.63 | -0.81 | -1.00 | -0.90 | -0.65 |
| 0.5 - 10 | 0.37 | -0.56 | -0.23 | -0.45 | -0.63 | -0.45 | -0.39 | -0.33 |
| 0.5 - 20 | 0.12 | -0.22 | 0.23 | -0.56 | -0.32 | -0.30 | 0.22 | -0.12 |
| 1 - 5 | 0.12 | -0.70 | -0.12 | -0.24 | -0.62 | -0.20 | -22.80 | -3.51 |
| 1 - 10 | 0.28 | -0.54 | -0.13 | 0.02 | -0.66 | 0.05 | -0.44 | -0.20 |
| 1 - 20 | 0.44 | -0.54 | -0.29 | -0.28 | -0.45 | -0.30 | -0.75 | -0.31 |
| 2 - 5 | 0.90 | -0.31 | -0.22 | -0.14 | -0.66 | -0.19 | -0.66 | -0.18 |
| 2 - 10 | 0.73 | -0.10 | 0.39 | -0.05 | -0.75 | -0.25 | -0.63 | -0.09 |
| 2 - 20 | 1.49 | 0.11 | 0.32 | 0.10 | -0.07 | 0.06 | -0.58 | 0.20 |
| 4 - 5 | 0.66 | -0.10 | -0.11 | 0.00 | -0.45 | -0.17 | -0.64 | -0.12 |
| 4 - 10 | 0.69 | 0.41 | 0.06 | 0.35 | -0.46 | -0.57 | -0.07 | 0.06 |
| 4 - 20 | 1.04 | -0.23 | -0.39 | -0.05 | -0.45 | -0.38 | -0.30 | -0.11 |
| Pasteurization | 1.64 | 0.26 | 0.72 | 0.46 | 1.26 | 1.15 | 0.65 | 0.87 |
| Control | 0.54 | -0.13 | -0.16 | -0.18 | 0.00 | -0.32 | -0.56 | -0.11 |
| Storage time mean.* | 0.64 | -0.24 | -0.04 | -0.12 | -0.36 | -0.20 | -1.99 | |

*Lowercase letters in the same row and column are evaluated independently and different letters indicate different groups according to Duncan's test ($p < 0.05$). The data are reported as the average of duplicate (n=2), conc: ozone concentration (ppm): 0.5, 1, 2, 4; time: application time (min): 5, 10, 20

Table 1. L* (lightness) (a), a* (red-green) (b) and b* (yellow-blue) (c) values for ozonated, pasteurizated and control group olives (*Continued*)
Table 1 (c)

| Applications (conc.-time/ ppm-min.) | Storage time (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 30 | 60 | 90 | 120 | 150 | 180 | Conc.-time mean.* |
| 0.5 - 5 | 37.23 | 38.18 | 37.46 | 37.98 | 36.72 | 37.65 | 37.29 | $37.50^{g}$ |
| 0.5 - 10 | 35.56 | 35.72 | 35.65 | 36.77 | 36.64 | 35.57 | 34.95 | $35.83^{bcd}$ |
| 0.5 - 20 | 35.79 | 36.05 | 34.78 | 36.97 | 35.47 | 33.74 | 33.39 | $35.17^{b}$ |
| 1 - 5 | 37.15 | 37.85 | 37.08 | 36.90 | 38.28 | 35.77 | 35.79 | $36.97^{efg}$ |
| 1 - 10 | 36.41 | 37.90 | 36.08 | 36.98 | 38.02 | 35.26 | 35.94 | $36.65^{defg}$ |
| 1 - 20 | 35.83 | 37.01 | 36.75 | 36.88 | 36.69 | 35.66 | 35.67 | $36.35^{cdef}$ |
| 2 - 5 | 36.36 | 36.30 | 37.28 | 35.64 | 35.85 | 37.19 | 35.02 | $36.23^{cde}$ |
| 2 - 10 | 35.15 | 35.00 | 34.47 | 34.49 | 36.59 | 34.90 | 35.61 | $35.17^{b}$ |
| 2 - 20 | 32.73 | 33.76 | 34.48 | 33.16 | 33.26 | 31.66 | 33.52 | $33.22^{a}$ |
| 4 - 5 | 38.15 | 37.64 | 38.05 | 38.46 | 38.23 | 37.19 | 33.57 | $37.32^{fg}$ |
| 4 - 10 | 37.39 | 35.50 | 35.44 | 37.00 | 38.81 | 37.42 | 35.21 | $36.68^{defg}$ |
| 4 - 20 | 36.09 | 38.66 | 38.15 | 38.62 | 37.37 | 38.60 | 34.72 | $37.46^{g}$ |
| Pasteurization | 35.90 | 35.80 | 37.65 | 38.73 | 32.15 | 34.87 | 33.89 | $35.57^{bc}$ |
| Control | 38.22 | 40.15 | 38.83 | 39.04 | 38.46 | 37.21 | 37.33 | $38.46^{h}$ |
| Storage time mean.* | $36.28^{bc}$ | $36.82^{bc}$ | $36.58^{c}$ | $36.97^{c}$ | $36.61^{c}$ | $35.90^{b}$ | $35.13^{a}$ | |

*Lowercase letters in the same row and column are evaluated independently and different letters indicate different groups according to Duncan's test ($p < 0.05$). The data are reported as the average of duplicate (n=2), conc: ozone concentration (ppm): 0.5, 1, 2, 4; time: application time (min): 5, 10, 20

Regarding the b* parameter, for the index of blue/yellow coloring, the highest value was measured in the control group (38.46). As the b* values of ozonated olives were lower than the control group, it was concluded that the application of ozone led to a decrease in the yellowness of the olives.

As in the case of texture, it could be indicated that the values for the parameters L*, a*, b * were of the same order as those found by Sánchez-Gómez *et al*., (2013) for the Spanish style green olives of the cultivars used in Spain.

### 3.4. Effect of ozonation on microbiological population

The results from the microbiological analyses of the olive samples are shown in Table 2. The EB and EC loads in the table olives after fermentation were below the detection limits (< 10). Undesired microorganisms, such as *Enterobacteriaceae* and *Clostridium* spp. are present at the beginning of olive fermentation but at the end of the process, when the pH decreases, they are not generally detected (Alves *et al*., 2012; Randazzo *et al*., 2012). According to the results of the statistical analysis, the concentration-time combination, storage time and interaction effect on total aerobic bacteria, lactic acid bacteria and moulds and yeasts of the olives were found statistically significant ($p < 0.05$).

The initial counts of total aerobic mesophilic bacteria, lactic acid bacteria and yeasts and molds in the untreated table olives were 4.96, 2.49 and 3.46 log CFU $g^{-1,}$ respectively. After ozonation, total aerobic mesophilic bacteria ranged from < 100 to 3.10 log CFU $g^{-1}$. At the end of 180 days storage, the total aerobic mesophilic bacteria of the pasteurized olives was below the detection limits (< 100), and the highest total aerobic mesophilic bacteria number was found in 2-20 applications with 6.14 log CFU $g^{-1}$. Unexpectedly, at 120 and 150 days of storage, the microbial load was observed in the pasteurized jars. It is thought that it can be caused by contamination from the other jars during analysis.

The differences among the responses observed for ozone concentration due to ozone treatments can be explained by the diversity of native flora enumerated in each case, and consequent different sensitivity to the ozone oxidizing effect. The nature and composition of food surface, the degree of attachment to or association of microorganisms with food and biofilm formation are other possible justifications (Alexandre *et al.*, 2011.The lower microbial inactivation achieved in our study can be explained by the fact that bacterial spores are more resistant to environmental abuses and toxic chemicals than vegetative cells. According to Alexopoulos *et al.,* (2017) no statistical differences in the viable counts of the lactic acid bacteria were observed between the control and the samples treated with ozone. This indicates that ozone did not alter the population of beneficial microflora (Alexopoulos *et al.*, 2017).

Table 2. TAMB (total aerobic mesophilic bacteria) (a), LAB (lactic acid bacteria) (b) and YM (yeast and moulds) (c) values for ozonated, pasteurizated and control group olives (CFU.g-1)

Table 2 (a)

| Applications (conc.-time/ ppm-min.) | Storage time (days) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **0** | **7** | **15** | **30** | **60** | **90** | **120** | **150** | **180** | **Conc.-time mean*** |
| 0.5 - 5 | 1.33 | 2.68 | 2.83 | 4.39 | 5.22 | 5.22 | 5.10 | 5.78 | 5.10 | $4.18^{de}$ |
| 0.5 - 10 | <100 | 4.49 | 2.96 | 4.94 | 5.44 | 5.44 | 5.07 | 5.79 | 5.00 | $4.35^{e}$ |
| 0.5 - 20 | 1.00 | 3.56 | 3.14 | 4.91 | 5.30 | 5.30 | 5.24 | 4.92 | 4.50 | $4.21^{de}$ |
| 1 - 5 | <100 | <100 | 1.74 | 3.42 | 5.69 | 5.69 | 4.51 | 4.51 | 5.04 | $3.40^{b}$ |
| 1 - 10 | <100 | <100 | 2.48 | 3.92 | 4.77 | 4.77 | 5.39 | 5.29 | 4.50 | $3.46^{b}$ |
| 1 - 20 | 1.00 | 1.16 | 2.83 | 3.24 | 5.15 | 5.15 | 5.55 | 5.66 | 4.50 | $3.81^{bcd}$ |
| 2 - 5 | <100 | <100 | 3.12 | 4.34 | 5.18 | 5.18 | 5.41 | 4.05 | 5.68 | $3.66^{bc}$ |
| 2 - 10 | <100 | <100 | <100 | 4.42 | 5.11 | 5.11 | 5.30 | 4.78 | 5.54 | $3.36^{b}$ |
| 2 - 20 | 1.82 | <100 | 2.55 | 4.69 | 5.63 | 5.63 | 5.64 | 6.05 | 6.14 | $4.24^{de}$ |
| 4 - 5 | <100 | <100 | 2.83 | 5.36 | 5.87 | 5.87 | 5.95 | 5.54 | 5.91 | $4.15^{de}$ |
| 4 - 10 | <100 | <100 | 2.93 | 5.32 | 6.03 | 6.03 | 5.73 | 5.25 | 5.66 | $4.10^{cde}$ |
| 4 - 20 | <100 | <100 | 3.17 | 5.37 | 5.30 | 5.30 | 6.12 | 5.68 | 5.56 | $4.06^{cde}$ |
| Pasteurization | <100 | <100 | <100 | <100 | <100 | <100 | <100 | 5.69 | <100 | $0.63^{a}$ |
| Control | 3.10 | 5.38 | 5.67 | 5.83 | 6.18 | 6.18 | 6.09 | 8.43 | 6.00 | $5.87^{f}$ |
| Storage time mean.* | $0.59^{a}$ | $1.23^{b}$ | $2.59^{c}$ | $4.30^{d}$ | $5.06^{e}$ | $5.06^{e}$ | $5.08^{e}$ | $5.53^{f}$ | $4.94^{e}$ | |

*Lowercase letters in the same row and column are evaluated independently and different letters indicate different groups according to Duncan's test ($p < 0.05$).
The data are reported as the average of duplicate (n=2)
conc: ozone concentration (ppm): 0.5, 1, 2, 4; time: application time (min): 5, 10, 20

Table 2 (b)

| Applications (conc.-time/ ppm min.) | Storage time (days) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **0** | **7** | **15** | **30** | **60** | **90** | **120** | **150** | **180** | **Conc.-time mean.*** |
| 0.5 - 5 | <100 | 1.39 | 1.33 | 4.46 | 5.75 | 5.90 | 6.38 | 7.68 | 5.71 | $4.29^{de}$ |
| 0.5 - 10 | <100 | <100 | 2.52 | 5.11 | 5.76 | 5.53 | 6.22 | 7.38 | 5.70 | $4.25^{de}$ |
| 0.5 - 20 | <100 | <100 | 2.24 | 5.05 | 5.54 | 5.75 | 5.99 | 7.99 | 5.60 | $4.24^{cde}$ |
| 1 - 5 | <100 | <100 | 2.47 | 3.61 | 5.81 | 5.73 | 5.67 | 7.83 | 5.26 | $4.04^{bcde}$ |
| 1 - 10 | <100 | <100 | 1.16 | 4.37 | 5.58 | 5.65 | 5.58 | 6.46 | 4.50 | $3.70^{b}$ |
| 1 - 20 | <100 | <100 | 2.26 | 5.51 | 5.69 | 5.24 | 5.64 | 7.46 | 4.76 | $4.06^{bcde}$ |
| 2 - 5 | <100 | <100 | 1.00 | 5.69 | 5.69 | 5.64 | 4.76 | 5.54 | 5.84 | $3.80^{bc}$ |
| 2 - 10 | <100 | 2.00 | 2.00 | 5.69 | 5.69 | 5.51 | 5.69 | 5.60 | 5.54 | $4.19^{cde}$ |
| 2 - 20 | <100 | 1.00 | 0.00 | 5.58 | 5.77 | 6.02 | 5.98 | 5.59 | 6.14 | $4.01^{bcd}$ |
| 4 - 5 | <100 | 1.56 | 2.99 | 5.73 | 5.93 | 6.40 | 5.85 | 5.26 | 5.91 | $4.40^{de}$ |
| 4 - 10 | <100 | 0.00 | 2.82 | 5.69 | 5.76 | 6.07 | 6.07 | 5.56 | 5.66 | $4.18^{cde}$ |
| 4 - 20 | <100 | 1.00 | 3.46 | 5.73 | 5.63 | 6.35 | 6.28 | 6.49 | 5.56 | $4.50^{e}$ |
| Pasteurization | 1.00 | <100 | <100 | <100 | <100 | <100 | 5.04 | <100 | <100 | $0.67^{a}$ |
| Control | 2.80 | 4.86 | 5.65 | 6.69 | 6.60 | 6.45 | 7.19 | 7.26 | 5.97 | $5.94^{f}$ |
| Storage time mean.* | $0.27^{a}$ | $0.84^{b}$ | $2.14^{c}$ | $4.92^{d}$ | $5.37^{e}$ | $5.44^{e}$ | $5.88^{f}$ | $6.15^{f}$ | $5.15^{de}$ | |

Lowercase letters in the same row and column are evaluated independently and different letters indicate different groups according to Duncan's test ($p < 0.05$). The data are reported as the average of duplicate (n=2), conc: ozone concentration (ppm): 0.5, 1, 2, 4; time: application time (min): 5, 10, 20

At the end of storage, the lowest and the highest lactic acid bacteria were determined in pasteurized olives and in the control group, respectively. Ozonation produced nearly 1.5 log and 2 log decreases in the number of lactic acid bacteria and total bacteria, respectively, due to its strong oxidative power against a wide spectrum of microorganisms (Guzel-Seydim *et al.*, 2004). However, no apparent

TABLE 2. TAMB (total aerobic mesophilic bacteria) (a), LAB (lactic acid bacteria) (b) and YM (yeast and moulds) (c) values for ozonated, pasteurizated and control group olives (CFU.g-1) (*Continued*)
TABLE 2 (c)

| Applications (conc.-time/ ppm-min.) | Storage time (days) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **0** | **7** | **15** | **30** | **60** | **90** | **120** | **150** | **180** | **Conc.-time mean.*** |
| 0.5 - 5 | 2.01 | 2.98 | 2.98 | 1.99 | 2.24 | 2.20 | 1.04 | 1.95 | 0.67 | $2.01^{bcde}$ |
| 0.5 - 10 | 1.34 | 4.32 | 4.32 | 2.80 | 3.07 | 3.18 | 1.90 | 1.56 | 1.19 | $2.63^{e}$ |
| 0.5 - 20 | 1.06 | 2.30 | 2.30 | 2.69 | 1.59 | 2.90 | 2.72 | 2.35 | 2.29 | $2.24^{de}$ |
| 1 - 5 | <10 | 1.77 | 1.77 | 3.47 | 2.10 | 1.13 | 1.97 | 0.00 | 0.52 | $1.41^{bcd}$ |
| 1 - 10 | <10 | 2.83 | 2.83 | 1.95 | 2.42 | 1.00 | <10 | 0.52 | 0.00 | $1.28^{b}$ |
| 1 - 20 | <10 | 2.33 | 2.33 | 2.41 | 3.08 | 1.07 | 1.27 | 2.13 | <10 | $1.48^{bcd}$ |
| 2 - 5 | <10 | 3.66 | 3.66 | 2.64 | 2.57 | 1.98 | 2.65 | 2.44 | <10 | $2.18^{cde}$ |
| 2 - 10 | <10 | 3.63 | 3.63 | 1.27 | 2.68 | <10 | 1.39 | 0.67 | 0.86 | $1.57^{bcd}$ |
| 2 - 20 | <10 | 2.45 | 2.45 | 3.18 | 3.39 | 1.82 | 2.17 | 2.74 | 0.52 | $2.08^{cde}$ |
| 4 - 5 | <10 | 2.98 | 2.98 | 2.94 | 2.74 | <10 | <10 | 1.89 | <10 | $1.50^{bcd}$ |
| 4 - 10 | <10 | 3.57 | 3.57 | 3.88 | 2.67 | 1.57 | 1.61 | <10 | 2.16 | $2.11^{cde}$ |
| 4 - 20 | <10 | 3.09 | 3.09 | 3.00 | 2.94 | 1.02 | 0.52 | <10 | <10 | $1.52^{bcd}$ |
| Pasteurization | 1.78 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | $0.20^{a}$ |
| Control | 2.31 | 3.04 | 3.04 | 3.47 | 2.97 | 3.45 | 2.02 | <10 | <10 | 2.25d$^{e}$ |
| Storage time mean.* | 0.61a | $2.78^{c}$ | $2.78^{c}$ | $2.55^{c}$ | $2.46^{c}$ | $1.52^{b}$ | $1.28^{b}$ | $1.16^{b}$ | $0.59^{a}$ | |

*Lowercase letters in the same row and column are evaluated independently and different letters indicate different groups according to Duncan's test ($p < 0.05$). The data are reported as the average of duplicate (n=2), conc: ozone concentration (ppm): 0.5, 1, 2, 4; time: application time (min): 5, 10, 20

effect of ozone on the lactic acid bacteria population was observed by Arroyo-Lopez *et al.,* (2006). In line with our research findings, Venturini *et al*., (2002) reported that ozone treatment prevented microbiological developments which occurred on the fruit during storage and was effective in reducing the yeast-mold load.

It is observed that the application of ozone is particularly effective in reducing the total bacterial load in olives at the beginning of storage. However, apart from pasteurized olives, total aerobic mesophilic bacteria development was observed during storage in both control group and olives subjected to ozone application. The total number of aerobic mesophilic bacteria of the ozonated olives was about 2 log units lower than the control group, and about 4 log units higher than the pasteurized olives. In this case, it can be said that the ozone application significantly reduced the total aerobic mesophilic bacteria growth that occurred during the storage period. Although the $O_3$ treatments reduced the microbial population in green table olives, the population gradually increased with storage time.

### 3.5. Effect of ozonation on sensory characteristics

The presence and intensity of organoleptic negative attributes affect the quality of table olives (Lanza., 2018). Although not legally required, table olives may be classified according to the evaluation of negative sensory attributes, performed by a trained sensory panel (IOC, 2011).

As the pH value exceeded 4.0 and the yeast-mould layer developed on the surface after the 30th day in the control group, the sensory analysis was not performed after the 30th day for this group.

The results of the organoleptic assessment of the green Domat table olives after ozonation and during storage period are shown in Table 3.

The saltiness of the ozonated olives was determined to be lower and statistically significant than the control group (Table 3 (a)). As reported in Table 3 (c), the bitterness of ozonated olives showed similarity with the control group, but lower than pasteurized olives and statistically found in different groups (except 1-20 application).

Fruit hardness is an important organoleptic characteristic for table olives (IOC, 2014) and also one of the sensory attributes of greatest importance to consumer acceptance (Lanza, 2018). The application of ozone results in a lower sensation of salinity and hardness and a higher perception of fibrousness and crispness than the control group (Table 3 (a) and (d)). The acidity and bitterness of the olives were similar to the control group (Table 3 (b) an (c)).

All the samples show a mean value of DPP ≤ 3.0 and therefore they were classified as "Extra or

TABLE 3. Changes in the sensory attribute means of ozonated, pasteurizated and control group olives (a): saltiness. (b) acidity (c) biterness. (d) hardness (e) fibrousness (f) crispness values for ozonated, pasteurizated and control group olives

TABLE 3 (a)

| Applications (conc.-time/ ppm-min.) | Storage time (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **Conc.-time mean.*** |
| 0.5 - 5 | 5.28 | 4.98 | 4.25 | 4.75 | 5.00 | 4.95 | 4.95 | 4.88ab |
| 0.5 - 10 | 5.08 | 4.18 | 4.20 | 4.95 | 4.93 | 5.05 | 4.95 | 4.76a |
| 0.5 - 20 | 5.18 | 4.25 | 4.18 | 4.93 | 4.83 | 5.05 | 5.08 | 4.78a |
| 1 - 5 | 5.35 | 4.60 | 5.20 | 5.35 | 5.05 | 5.05 | 4.58 | 5.03bcd |
| 1 - 10 | 5.68 | 4.63 | 4.83 | 5.35 | 5.18 | 4.83 | 4.33 | 4.97bcd |
| 1 - 20 | 5.08 | 4.43 | 4.95 | 5.00 | 4.95 | 4.63 | 4.45 | 4.78a |
| 2 - 5 | 5.33 | 4.30 | 5.05 | 5.00 | 5.18 | 5.00 | 5.08 | 4.99bcd |
| 2 - 10 | 5.18 | 4.03 | 5.05 | 4.73 | 4.98 | 5.13 | 5.10 | 4.88ab |
| 2 - 20 | 5.38 | 4.43 | 4.90 | 4.70 | 5.08 | 5.13 | 5.48 | 5.01bcd |
| 4 - 5 | 5.60 | 4.93 | 5.15 | 4.90 | 4.93 | 4.50 | 5.35 | 5.05bcd |
| 4 - 10 | 5.55 | 4.98 | 5.30 | 5.00 | 5.03 | 4.63 | 5.13 | 5.09cd |
| 4 - 20 | 5.28 | 4.43 | 4.95 | 4.88 | 5.13 | 4.85 | 4.98 | 4.93abc |
| Pasteurization | 5.73 | 4.93 | 5.40 | 5.15 | 4.90 | 5.15 | 4.68 | 5.13d |
| Control | 5.60 | 5.23 | 5.15 | | | | | 5.33e |
| Storage time mean.* | 5.38$^{c}$ | 4.59$^{a}$ | 4.90$^{b}$ | 4.98$^{b}$ | 5.01$^{b}$ | 4.92$^{b}$ | 4.93$^{b}$ | |

*Lowercase letters in the same row and column are evaluated independently and different letters indicate different groups according to Duncan's test ($p < 0.05$). The data are reported as the average of duplicate (n=2), conc: ozone concentration (ppm): 0.5, 1, 2, 4; time: application time (min): 5, 10, 20

TABLE 3 (b)

| Applications (conc-time/ ppm-min.) | Storage time (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **Conc.-time mean.*** |
| 0.5 - 5 | 4.90 | 4.50 | 4.25 | 4.25 | 3.43 | 4.10 | 3.63 | 4.15$^{ab}$ |
| 0.5 - 10 | 4.78 | 4.50 | 4.20 | 4.20 | 4.08 | 4.10 | 3.78 | 4.23$^{abc}$ |
| 0.5 - 20 | 4.85 | 4.25 | 4.18 | 4.18 | 3.25 | 3.95 | 3.75 | 4.06$^{a}$ |
| 1 - 5 | 4.75 | 3.75 | 5.20 | 5.20 | 3.15 | 4.05 | 3.45 | 4.22$^{abc}$ |
| 1 - 10 | 5.25 | 4.13 | 4.83 | 4.83 | 3.18 | 4.05 | 3.63 | 4.27$^{abc}$ |
| 1 - 20 | 4.95 | 3.75 | 4.95 | 4.95 | 3.63 | 3.13 | 3.75 | 4.16$^{ab}$ |
| 2 - 5 | 5.38 | 4.38 | 5.05 | 5.05 | 3.75 | 3.69 | 3.68 | 4.42$^{cd}$ |
| 2 - 10 | 5.25 | 4.45 | 5.05 | 5.05 | 3.05 | 3.68 | 3.80 | 4.33$^{bc}$ |
| 2 - 20 | 5.15 | 4.33 | 4.90 | 4.90 | 3.48 | 3.50 | 4.20 | 4.35$^{bc}$ |
| 4 - 5 | 4.95 | 4.13 | 5.15 | 5.15 | 3.20 | 3.50 | 4.05 | 4.30$^{abc}$ |
| 4 - 10 | 4.65 | 3.35 | 4.80 | 4.80 | 3.83 | 3.80 | 3.93 | 4.16$^{ab}$ |
| 4 - 20 | 4.55 | 3.53 | 5.15 | 5.15 | 3.25 | 4.13 | 3.95 | 4.24$^{abc}$ |
| Pasteurization | 5.30 | 4.80 | 5.15 | 4.65 | 4.13 | 4.10 | 4.05 | 4.60$^{d}$ |
| Control | 4.65 | 3.65 | 4.65 | | | | | 4.32b$^{c}$ |
| Storage time mean.* | 4.95e | 4.11$^{c}$ | 4.82$^{de}$ | 4.80$^{d}$ | 3.49$^{a}$ | 3.83$^{b}$ | 3.82$^{b}$ | |

*Lowercase letters in the same row and column are evaluated independently and different letters indicate different groups according to Duncan's test ($p < 0.05$). The data are reported as the average of duplicate (n=2), conc: ozone concentration (ppm): 0.5, 1, 2, 4; time: application time (min): 5, 10, 20

TABLE 3. Changes in the sensory attribute means of ozonated, pasteurizated and control group olives (a): saltiness. (b) acidity (c) biterness. (d) hardness (e) fibrousness (f) crispness values for ozonated, pasteurizated and control group olives (*Continued*)

TABLE 3 (c)

| Applications (conc.-time/ ppm-min.) | Storage time (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 30 | 60 | 90 | 120 | 150 | 180 | Conc.-time mean.* |
| 0.5 - 5 | 5.18 | 4.95 | 4.93 | 5.18 | 5.28 | 5.00 | 4.88 | $5.05^{bcd}$ |
| 0.5 - 10 | 4.88 | 5.15 | 4.98 | 5.10 | 5.48 | 5.03 | 4.48 | $5.01^{bc}$ |
| 0.5 - 20 | 5.13 | 5.08 | 4.55 | 5.18 | 5.15 | 5.33 | 4.35 | $4.96^{ab}$ |
| 1 - 5 | 5.13 | 5.58 | 5.35 | 5.58 | 5.25 | 5.30 | 4.35 | $5.22^{cd}$ |
| 1 - 10 | 5.35 | 4.88 | 5.03 | 5.25 | 5.58 | 5.05 | 4.83 | $5.14^{bcd}$ |
| 1 - 20 | 5.35 | 5.25 | 5.43 | 5.50 | 5.48 | 5.03 | 5.00 | $5.29^{d}$ |
| 2 - 5 | 5.03 | 5.18 | 5.50 | 5.23 | 5.68 | 5.13 | 4.20 | $5.13^{bcd}$ |
| 2 - 10 | 5.73 | 5.28 | 5.25 | 5.05 | 5.53 | 5.48 | 4.60 | $5.27^{d}$ |
| 2 - 20 | 5.23 | 5.55 | 5.03 | 5.05 | 5.30 | 5.45 | 4.25 | $5.12^{bcd}$ |
| 4 - 5 | 5.80 | 5.23 | 5.28 | 5.38 | 5.13 | 5.00 | 4.58 | $5.20^{bcd}$ |
| 4 - 10 | 5.40 | 5.38 | 5.33 | 5.40 | 5.35 | 5.08 | 4.73 | $5.24^{cd}$ |
| 4 - 20 | 5.25 | 5.28 | 5.33 | 5.30 | 5.33 | 5.03 | 4.85 | $5.19^{bcd}$ |
| Pasteurization | 5.13 | 4.23 | 5.00 | 4.93 | 4.70 | 4.83 | 4.65 | $4.78^{a}$ |
| Control | 5.33 | 4.60 | 5.28 | | | | | $5.07^{bcd}$ |
| Storage time mean.* | $5.28^{cd}$ | $5.11^{b}$ | $5.16^{bc}$ | $5.24^{bcd}$ | $5.32^{d}$ | $5.13^{bc}$ | $4.59^{a}$ | |

*Lowercase letters in the same row and column are evaluated independently and different letters indicate different groups according to Duncan's test ($p < 0.05$). The data are reported as the average of duplicate (n=2), conc: ozone concentration (ppm): 0.5, 1, 2, 4; time: application time (min): 5, 10, 20

TABLE 3 (d)

| Applications (conc.-time/ ppm-min.) | Storage time (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 30 | 60 | 90 | 120 | 150 | 180 | Conc.-time mean.* |
| 0.5 - 5 | 5.05 | 4.00 | 3.48 | 3.75 | 3.63 | 3.93 | 3.95 | $3.97^{ab}$ |
| 0.5 - 10 | 4.95 | 3.68 | 3.53 | 3.95 | 4.25 | 3.13 | 4.10 | $3.94^{ab}$ |
| 0.5 - 20 | 4.83 | 3.60 | 3.90 | 3.88 | 4.33 | 4.18 | 4.00 | $4.10^{abcd}$ |
| 1 - 5 | 4.68 | 4.00 | 3.70 | 3.90 | 4.08 | 4.23 | 3.68 | $4.04^{abc}$ |
| 1 - 10 | 4.63 | 4.20 | 4.45 | 4.58 | 4.28 | 4.23 | 3.90 | $4.32^{de}$ |
| 1 - 20 | 4.80 | 3.78 | 3.83 | 4.23 | 3.50 | 3.70 | 3.38 | $3.89^{a}$ |
| 2 - 5 | 4.73 | 4.10 | 4.25 | 4.13 | 4.20 | 3.88 | 3.95 | $4.18^{bcd}$ |
| 2 - 10 | 4.60 | 3.48 | 3.88 | 4.00 | 4.30 | 3.68 | 3.93 | $3.98^{ab}$ |
| 2 - 20 | 4.75 | 4.10 | 4.00 | 4.00 | 4.08 | 3.95 | 3.55 | $4.06^{abc}$ |
| 4 - 5 | 4.90 | 3.90 | 3.68 | 3.98 | 3.65 | 4.10 | 3.95 | $4.02^{abc}$ |
| 4 - 10 | 4.70 | 4.53 | 4.13 | 4.13 | 4.23 | 4.13 | 3.80 | $4.23^{cd}$ |
| 4 - 20 | 4.80 | 4.55 | 4.00 | 4.13 | 4.43 | 4.20 | 3.63 | $4.25^{cd}$ |
| Pasteurization | 5.28 | 3.63 | 4.08 | 4.38 | 4.01 | 3.83 | 3.65 | $4.12^{abcd}$ |
| Control | 5.00 | 4.35 | 4.25 | | | | | $4.53^{e}$ |
| Storage time mean.* | $4.83^{c}$ | $3.99^{b}$ | $3.94^{ab}$ | $4.08^{b}$ | $4.07^{b}$ | $3.93^{ab}$ | $3.80^{a}$ | |

*Lowercase letters in the same row and column are evaluated independently and different letters indicate different groups according to Duncan's test ($p < 0.05$). The data are reported as the average of duplicate (n=2), conc: ozone concentration (ppm): 0.5, 1, 2, 4; time: application time (min): 5, 10, 20

TABLE 3. Changes in the sensory attribute means of ozonated, pasteurizated and control group olives (a): saltiness. (b) acidity (c) biterness. (d) hardness (e) fibrousness (f) crispness values for ozonated, pasteurizated and control group olives (*Continued*)
TABLE 3 (e)

| Applications (conc.-time/ ppm-min.) | Storage time (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **Conc.-time mean.*** |
| 0.5 - 5 | 4.35 | 4.13 | 3.93 | 3.75 | 3.85 | 3.63 | 3.75 | 3.91$^{a}$ |
| 0.5 - 10 | 4.33 | 3.55 | 3.93 | 3.63 | 3.98 | 3.55 | 3.78 | 3.82$^{a}$ |
| 0.5 - 20 | 4.43 | 3.63 | 4.00 | 3.55 | 4.10 | 4.03 | 3.50 | 3.89$^{a}$ |
| 1 - 5 | 4.73 | 3.50 | 4.00 | 4.10 | 4.25 | 3.95 | 3.43 | 3.99$^{ab}$ |
| 1 - 10 | 4.43 | 3.68 | 3.85 | 4.10 | 4.15 | 3.60 | 3.60 | 3.91$^{a}$ |
| 1 - 20 | 4.60 | 3.88 | 4.13 | 4.00 | 4.05 | 3.55 | 3.40 | 3.94$^{a}$ |
| 2 - 5 | 4.58 | 3.93 | 3.88 | 4.03 | 3.75 | 3.73 | 3.65 | 3.93$^{a}$ |
| 2 - 10 | 4.43 | 3.48 | 3.80 | 4.05 | 3.58 | 3.78 | 3.65 | 3.82$^{a}$ |
| 2 - 20 | 4.25 | 3.85 | 3.88 | 4.03 | 3.98 | 3.75 | 3.48 | 3.89$^{a}$ |
| 4 - 5 | 4.43 | 4.13 | 4.30 | 4.13 | 4.23 | 4.03 | 3.85 | 4.15$^{b}$ |
| 4 - 10 | 4.60 | 4.25 | 4.23 | 4.33 | 4.05 | 3.90 | 3.73 | 4.15$^{b}$ |
| 4 - 20 | 4.63 | 4.20 | 3.88 | 4.50 | 4.30 | 4.00 | 3.73 | 4.18$^{b}$ |
| Pasteurization | 4.70 | 4.00 | 4.68 | 4.00 | 4.20 | 3.93 | 3.85 | 4.19$^{b}$ |
| Control | 4.70 | 4.45 | 4.35 | | | | | 4.50$^{c}$ |
| Storage time mean.* | 4.51$^{e}$ | 3.90$^{bc}$ | 4.06$^{d}$ | 4.01$^{cd}$ | 4.03$^{cd}$ | 3.80$^{b}$ | 3.64$^{a}$ | |

*Lowercase letters in the same row and column are evaluated independently and different letters indicate different groups according to Duncan's test ($p < 0.05$).
Note: ozone concentration (ppm): 0.5, 1, 2, 4; application time (min): 5, 10, 20

TABLE 3 (f)

| Applications (conc.-time/ ppm-min.) | Storage time (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **0** | **30** | **60** | **90** | **120** | **150** | **180** | **Conc.-time mean.*** |
| 0.5 - 5 | 4.20 | 3.58 | 3.53 | 3.50 | 3.10 | 3.28 | 3.05 | 3.46$^{a}$ |
| 0.5 - 10 | 4.13 | 3.45 | 3.55 | 3.38 | 3.33 | 3.45 | 3.10 | 3.48$^{a}$ |
| 0.5 - 20 | 3.90 | 3.70 | 3.68 | 3.38 | 3.43 | 3.80 | 3.00 | 3.55$^{a}$ |
| 1 - 5 | 4.08 | 3.78 | 3.75 | 3.70 | 3.00 | 3.53 | 3.08 | 3.56$^{a}$ |
| 1 - 10 | 3.90 | 3.70 | 3.55 | 3.43 | 3.38 | 3.15 | 3.25 | 3.48$^{a}$ |
| 1 - 20 | 3.93 | 3.63 | 3.38 | 3.68 | 3.35 | 3.20 | 2.95 | 3.44$^{a}$ |
| 2 - 5 | 4.08 | 3.80 | 3.70 | 3.65 | 3.23 | 3.40 | 3.15 | 3.57$^{a}$ |
| 2 - 10 | 3.80 | 3.43 | 3.55 | 3.50 | 3.13 | 3.28 | 3.30 | 3.43$^{a}$ |
| 2 - 20 | 3.75 | 3.55 | 3.43 | 3.33 | 3.13 | 3.25 | 3.10 | 3.36$^{a}$ |
| 4 - 5 | 3.93 | 3.68 | 3.65 | 3.75 | 3.43 | 3.38 | 3.25 | 3.58$^{a}$ |
| 4 - 10 | 4.00 | 3.83 | 3.83 | 3.53 | 3.30 | 3.18 | 3.20 | 3.55$^{a}$ |
| 4 - 20 | 3.75 | 3.55 | 3.58 | 3.40 | 3.50 | 3.25 | 3.13 | 3.45$^{a}$ |
| Pasteurization | 4.43 | 4.18 | 3.88 | 3.90 | 3.75 | 3.75 | 3.35 | 3.89$^{b}$ |
| Control | 4.30 | 4.20 | 3.80 | | | | | 4.10$^{c}$ |
| Storage time mean.* | 4.01$^{e}$ | 3.72$^{d}$ | 3.63$^{cd}$ | 3.55$^{c}$ | 3.31$^{b}$ | 3.38$^{b}$ | 3.15$^{a}$ | |

*Lowercase letters in the same row and column are evaluated independently and different letters indicate different groups according to Duncan's test ($p < 0.05$). The data are reported as the average of duplicate (n=2), conc: ozone concentration (ppm): 0.5, 1, 2, 4; time: application time (min): 5, 10, 20

Fancy". It can be said that the application of ozone can improve green table olives with minimal changes in organoleptic properties.

## 4. CONCLUSIONS

The microbiological analyses showed that treatment with ozone reduced the total aerobic mesophilic bacteria, lactic acid bacteria and yeast/mould counts which were statistically lower than those of the untreated control samples at the 95% confidence interval. The applied treatment achieved a significant reduction in microbial load. 1-10 application was markedly different from the other ozonated groups and had the lowest value for total aerobic mesophilic microorganisms. No significant difference among the application groups was found except for the 1-10 application for total aerobic mesophilic microorganisms. It was concluded that an ozone treatment at 1 ppm for at least 10 minutes could be successfully applied for reducing the microbial count of green table olives.

In conclusion, the ozone treatment was applied to extend the microbiological shelf-life and the quality of green table olives during storage time. All stored olives were classified as "extra" commercial category, but consumer perception of food quality depends not only on microbial quality, but also on other food factors such as structural changes. As shown by the results, an advisable storage period for this product should not exceed 5 months; although the hygiene is preserved, after this period, the firmness might compromise its acceptability by consumers.

The treatment at 1pmm for 10min can significantly extend the shelf-life of these products, since it was found efficient at reducing the indigenous microbiota. Thus, ozone may introduce a reliable non-thermal method to extend the shelf-life of fermented green table olives. However, additional research about the effects of ozonation on functional, nutritional, and chemical properties is needed in order to establish ozone as a useful tool for the table olive industry.

## ACKNOWLEDGMENTS

This work was supported by TAGEM (General Directorate of Agricultural Research and Policies) under project number TAGEM/HSGYAD/13/A057P01/21. The authors would like to thank TAGEM for the financial support of the research project, to the panelists of the Olive Research Institute's Tasting Panel for the voluntary contribution and Teknozon Ozone Systems Environmental Technologies Electric Electronic Machinery Industry and Trade Limited Company for supplying the ozone generator.

## REFERENCES

Alexandre EMC, Fundo J, Santos DM, Brandao TRS. 2005. Effect of ozone on the quality of green beans (*Phaseolus vulgaris* L.) and bell peppers (*Capsicum annuum* L). 7° Encontro de Quimica dos Alimentos, Viseu, *Escola Superior de Biotecnologia*, Universidade Catolica Portuguesa.

Alexandre EMC, Brandão TRS, Cristina LM, Silva CLM. 2011. A modelling microbial load reduction in foods due to ozone impact. *Proc. Food. Sci.* **1**, 836–841. https://doi.org/10.1016/j.profoo.2011.09.126

Alexopoulos A, Plessas S, Kourkoutas Y, Stefanis C, Vavias S, Voidarou C, Mantzourani I, Bezirtzoglou E. 2017. Experimental effect of ozone upon the microbial flora of commercially produced dairy fermented products. *Int. J. Food Microbiol*. **246**, 5–11. https://doi.org/10.1016/j.ijfoodmicro.2017.01.018

Alves M, Gonçalves T, Quintas T. 2012. Microbial quality and yeast population dy-namics in cracked green table olives' fermentations. *Food Control* **23**, 363–368. https://doi.org/10.1016/j.foodcont.2011.07.033

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

Arroyo-Lopez FN, Duran Quintana MC, Garrido Fernandez A. 2006. Microbial evolution during storage of seasoned olives prepared with organic acids with potassium sorbate, sodium benzoate, and ozone used as preservatives. *J. Food Protect*. **69,** 1354–1364.

Catania P, Alleri M, Martorana A, Settanni L, Moschetti G, Vallone M. 2014. Investigation of a tunnel pasteurizer for "Nocellara del Belice" table olives processed according to the "Castelvetrano method". *Grasas Aceites* **65**, e049. https://doi.org/10.3989/gya.0578141

Garrido-Fernandez A, Fernandez Diez MJ, Adams MR. 1997. *Table Olives: Production and Processing*. Chapman & Hall, London, United Kingdom, 481.

García-García P, Arroyo-López NF, Rodríguez-Gómez F. 2014. Partial purification of iron solutions from ripe table olive processing using ozone and electro-coagulation. *Sep. Purif. Technol.* **133**, 227–235. https://doi.org/10.1016/j.seppur.2014.06.011

Graham DM. 1997.Use of ozone for food processing. *Food Technol.-Chicago*. **51**, 72–75.

Guzel-Seydim ZB, Greene AK, Seydim AC. 2004. Use of ozone in the food industry. *LWT - Food Sci. Technol.* **37**, 453–460. https://doi.org/10.1016/j.lwt.2003.10.014

International Olive Council (IOC). 2004. Trade standard applying to table olive. COI/OT/NC No. 1, December 2004. Madrid (Spain).

International Olive Council (IOC). 2011. Method for the sensory analysis of table olives, COI/OT/MO No 1/Rev.2, November 2011. Madrid (Spain).

International Olive Council (IOC). 2016. http://www.internationaloliveoil.org/estaticos/view/132-world-table-olive-figures. (Date of access: 10.11.2017).

Karaca H, Velioglu S. 2007. Ozone application in fruit and vegetable processing. *Food Rev. Int.* **23**, 91–106. https://doi.org/10.1080/87559120600998221

Koyuncu MA, Seydim AC, Dilmaçünal T, Savran E, Tas T. 2008. Effects of different precooling treatments with ozonated water on the quality of the sweet cherry fruit cv. '0900 ziraat'. *Acta Hortic.* **795**, 831–836. https://doi.org/10.17660/ActaHortic.2008.795.134

Lanza B. 2018. Measurement of kinesthetic properties of in-brine table olives by microstructure of fracture surface, sensory evaluation and Texture Profile Analysis (TPA). *J. Sci. Food Agric.* **98**, 4142–4150. https://doi.org/10.1002/jsfa.8932

Medina-Pradas E, Arroyo-López FN. 2015. Presence of toxic microbial metabolites in table olives. *Front Microbiol*. **6**, 873. https://doi.org/10.3389/fmicb.2015.00873

Panagou EZ. 2004. Effect of different packing treatments on the microbiological and physicochemical characteristics of untreated green olives of the Conservolea Cultivar. *J. Sci. Food Agric.* **84**, 757–764. https://doi.org/10.1002/jsfa.1732

Randazzo CL, Ribbera A, Pitino I, Romeo FV, Caggia C. 2012. Diversity of bacterial population of table olives assessed by PCR-DGGE analysis. *Food Microbiol.* **32**, 87–96. https://doi.org/10.1016/j.fm.2012.04.013

Renumarn P, Srilaong V, Uthairatanakij A, Kanlayanarat S, Jitareerat P. 2014. The effects of immersion methods and concentration of ozonated water on the microbial counts and the quality and sensory attributes of fresh-cut broccoli. *I. F. R. J*. **21**, 533–539.

Romero-Barranco C, García-Serrano P, García-García P, Brenes-Balbuena M. 2016. Re-use of partially purified iron color fixation solutions using electro-coagulation and ozonation in ripe table olive processing and packaging. *J. Food Eng.* **181**, 28–34. https://doi.org/10.1016/j.jfoodeng.2016.02.020

Romeo FV, De Luca S, Piscopo A, Perri E, Poiana M. 2009. Effects of post-fermentation processing on the stabilisation of naturally fermented green table olives (cv Nocellara etnea). *Food Chem.* **116**, 873–878. https://doi.org/10.1016/j.foodchem.2009.03.037

Romeo FV, Piscopo A, Poiana M. 2012. Effect of drying, chemical and natural processing methods on black Biancolilla olives. *Grasas Aceites* **63**, 223–230. https://doi.org/10.3989/gya.095811

Sánchez-Gómez AH, García-García P, Garrido-Fernández A. 2013. Spanish-style green table olive shelf-life. *Int. J. Food Sci. Tech*. **48**, 1559–1568. https://doi.org/10.1111/ijfs.12124

Sapers GM. 2003. Washing and sanitizing raw materials for minimally processed fruit and vegetable products. *In Microbial safety of minimally processed foods*; Novak JS, Sapers GM, Juneja VK., Eds.; CRC Press: Boca Raton, FL, London, New York, Washington, DC; pp 221–253.

Segovia-Bravo KA, García-García P, Arroyo-López FN, López-López A, Garrido-Fernández A. 2008. Ozonation process for the regeneration and recycling of Spanish green table olive fermentation brines.*Eur. Food Res. Technol.* **227**, 463–472. https://doi.org/10.1007/s00217-007-0742-5

Tzortzakis N, Chrysargyris A. 2017. Postharvest ozone application for the preservation of fruits and vegetables. *Food Rev. Int.* **33**, 270–315. https://doi.org/10.1080/87559129.2016.1175015

Xu L. 1999. Use of ozone to improve the safety of fresh fruits and vegetables. *Food Technol. **53***, 58–61. Proceedings book.