LWT - Food Science and Technology 89 (2018) 572–580

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Effect of *Lactobacillus plantarum* on olive and olive oil quality during fermentation process

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ARTICLE INFO

Keywords:
Antioxidant activity
Fatty acid profile
FT-IR analysis
Lactobacillus plantarum.

ABSTRACT

The olive fruits were fermented with *Lactobacillus plantarum* ATCC 8014 and then some physicochemical properties of the extracted oil including chemical indices, fatty acid composition, functional groups, total phenol content, antioxidant activity and thermal properties were investigated. Some fruit quality indices such as color and texture also were determined. Results showed the concentration of unsaturated free fatty acids in treated samples increased after the fermentation process. The results of 2,2-diphenyl-1-picrylhydrozyl (DPPH) test demonstrated that *L. plantarum* can improve antiradicalactivity of olive oil. According to Pearson's test, phenolic compounds had a strong involvement in the antioxidant activity. FT-IR analysis demonstrated that the oxidation occurred during the brining process of the control sample. Peroxide value and ^{1}H NMR analysis also showed extracted oil from olives treated with *L. plantarum* had more oxidation stability compared to untreated samples. In control sample, DSC analysis showed that the oxidation of oil sample increased during the storage period. Results also indicated that fermentation had positive effects on texture and color of olives. Therefore, fermentation can preserve quality of olive and olive oil during storage.

1. Introduction

Lactic Acid Bacteria can play a significant role in improving the physicochemical properties of raw agricultural products due to the ability to produce various antimicrobial compounds such as bacteriocins, organic acids, fatty acids, hydrogen peroxide and diacetyl as well as antioxidant activity (Gould, 1991, pp. 461–482). Consequently, in recent years, ever-increasing interest to study the influence of starter cultures on qualitative properties of most important industrial varieties of table olive has been observed (Argyri, Nisiotou, Mallouchos, Panagou, & Tassou, 2014; Kiai & Hafidi, 2014; Rodríguez-Gómez et al., 2014; Romeo, De Luca, Piscopo, Perri, & Poiana, 2009).

Natural table olives are currently one of the most important fermented vegetables in western countries. Various factors affect the fermentation process such as indigenous microbiota present on the olive surface, olive cultivar, as well as technological factors such as brine concentration, hygienic practices, and the processing temperature (Tassou, Panagou, & Katsaboxakis, 2002). Organoleptic and preservation features are notably enhanced by the fermentation process (Campus et al., 2015).

Oils are an essential part of human diet due to their nutrimental and technological properties. Additionally, some of them contain polyunsaturated fatty acids which are essential for human health (Moreno, Olivares, Lopez, Adelantado, & Reig, 1999). Oxidation is one of the most important factors in the deterioration of oils and fats due to further decrease in nutritional quality and safety (Muik, Lendl, Molina-Díaz, & Ayora-Cañada, 2005).

Up to date, no research has been conducted in order to investigate the effect of *Lactobacillus plantarum* fermentation on oxidative stability properties of olive oil. Therefore, the current investigation was undertaken to characterize and compare the chemical composition, thermal properties and oxidation stability of extracted oil from fermented olive with that extracted once from control samples. Additionally, texture and color of olives were studied during fermentation.

2. Materials and methods

2.1. Olive samples

Olive fruits (cv. Phishomi) were collected from Darab, Shiraz, Iran (October 2016). Olives were hand-selected to remove fruits with blemishes, defects, and insect damage washed thoroughly with water

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https://doi.org/10.1016/j.lwt.2017.10.025
Received 31 May 2017; Received in revised form 11 October 2017; Accepted 13 October 2017
Available online 15 October 2017
0023-6438/

under pressure to remove all impure matters and then immersed in a solution containing 300 ppm chlorine (Montano, Sánchez, & Castro, 1993). Prior to immersion of olives, they were heated at 80 °C for 10 min to decrease most of the competitive microbiota and improve fermentability (Balatsouras, Tsibri, Dalles, & Doutsias, 1983; Etchells, Borg, Kittel, Bell, & Fleming, 1966).

2.2. Olive treatment

The fruit samples were subjected to the traditional Spanish-style processing. A total of 30 kg olives were dipped in a 1.5% (w/v) NaOH solution for 10 h at room temperature until the alkali reached 2/3 of the flesh as determined from the epidermis to the pit. Then, NaOH solution was replaced with tap water. The process included a quick washing instantly after treatment followed by two water changes at 4 and 10 h. The initial salt concentration of 1.5% was applied.

2.3. Preparation of bacterial inocula for olives

The fermentation was carried out using *L. plantarum* ATCC 8014, a strain isolated from natural Spanish-style green olives. For propagation of inocula, MRS broth (Oxoid, Basingstoke) was applied. In order to harvest *L. plantarum*, cultures were centrifuged at 4000 g for 10 min after 48 h of inoculation at 37 °C and then washed twice with sterile normal saline. Finally, cells were resuspended in saline and used as inoculum.

2.4. Inoculation of olives

Olive samples (30 kg) were divided into two sets: one set was inoculated with *L. plantarum* ATCC 8014. In another one, uninoculated samples were considered as control. All treated olives were ground and malaxated slowly (30 min). Oil extraction was carried out by centrifugation at 3000*g* for 20 min. The collected oil was filtered, filled into dark glass bottles and stored at −18 °C until the experiments.

2.5. Color measurement

In order to determine color attributes of olive samples (L* (lightness), a* (redness) and b* (yellowness)) during storage time a Chromameter (Chroma Meter model CR-410, Minolta Co. Ltd., Osaka, Japan) was used. It was standardized using black and white ceramic plate. CIE (Commission International de l'Eclairage) L*, a* and b* were determined from five surfaces of the samples. The instrument was set at illuminant D-65 and a 2 observer angle.

2.6. Texture analysis

Texture analysis was carried out using a texture analyzer equipped with a 2 mm diameter cylinder probe. Parameters of this test were as follow: pre-test speed 2 mm/s, test speed 0.5 mm/s, post-test speed 4 mm/s, maximum force of 1500 g and the penetration depth was limited to 3 mm.

2.7. Quality indices of the extracted oils

2.7.1. Peroxide value

The peroxide value (PV) determination was performed according to the spectrophotometric method of described by Shantha and Decker (1994). Considering the extent of peroxidation, 0.60–0.30 g of oil samples was mixed in a disposable glass tube with 9.8-mL chloroform–methanol (7:3 v/v) for 2–4 s followed by addition of Ammonium thiocyanate solution (50 mL, 30% w/v). Then, 50 mL of iron (II) chloride solution ([0.4 g barium chloride dihydrate dissolved in 50-mL H_2O] + [0.5 g $FeSO_4$. $7H_2O$ dissolved in 50-mL H_2O] + [2 mL 10 M HCl, with the precipitate, barium sulphate, filtered off to obtain a clear solution]) were added, and the sample was mixed on a vortex mixer for 2–4 s. The mixture was incubated at room temperature for 5 min and the absorbance of the sample was measured at 500 nm against a blank that contained all the reagents without the sample using a spectrophotometer. Results were reported as meq/kg oil.

2.7.2. Acid value

Acid value (AV) was quantify based on Firestone (1989) method. For the AV measurement, the oil samples (10 g) were dissolved in 50 mL of previously neutralized chloroform–ethanol medium (50:50 v/v) and then titrated using an ethanolic solution of 0.1 N potassium hydroxide (KOH) (as the standard reagent). The AV index was expressed as milligrams of KOH needed for neutralization of the free fatty acids of 1 g of the oil sample (g/kg).

2.8. Radical scavenging activity using DPPH assay

The DPPH activity of the oil samples was carried out according to Tuck and Hayball (2002)method. Various concentrations of oil extracts were prepared. The volume was made up to 100 μL with methanol. After addition of 0.1 mmol/L methanolic solution of 2,2–diphenyl-1-picrylhydrazyl radical (DPPH) to the tubes, they were shaken thoroughly and stayed at 27 °C for 20 min. A similar method was used for the preparation of control samples, without any extract, and methanol was utilized for the baseline correction. Changes in the absorbance of the resulting solutions were measured at 517 nm using UV-vis spectrophotometer. The percentage inhibition was calculated as follow:

$$\%\ \text{Radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100 \quad (1)$$

2.9. Measurement of total phenol content

The total phenol content determination was carried out based on the method described by Capannesi, Palchetti, Mascini, and Parenti (2000). Briefly, total phenol was obtained from a solution of oil in hexane by triple-extraction with water–methanol with a respective ratio of 80: 20 v/v. The absorbance measurement was done at 765 nm via UV-spectrophotometer. Results were reported as g of gallic acid/kg oil.

2.10. Fatty acid composition

Gas chromatography (GC) analysis was done based on recommended procedure by Farhoosh, Niazmand, Rezaei, and Sarabi (2008). In order to trans esterification of fatty acids into their corresponding FAMEs, a solution of oil in hexane (0.3 g in 7 mL) with 2 mL of 7 mol/L methanolic potassium hydroxide was vigorously shaken at 50 °C for 10 min. The FAMEs were evaluated using an Agilent gas chromatograph fitted with a CP-FIL 88 (Supelco., Inc., Bellefonte, PA) capillary column of fused silica (60 min length 0.22 mm I.D., 0.2 mm film thickness) and a flame ionization detector (FID). Helium was utilized as the carrier gas with a flow rate of 0.75 mL/min. The oven temperature was held at 198 °C, and that of the injector and the detector at 250 °C.

2.11. Fourier transforms infrared spectra acquisition

A Fourier transform infrared (FTIR) spectrophotometer (AVATAR 370 FT-IR, Thermo Nicolet) was utilized to record the percent transmittance in the absorption mode 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1}. A small quantity (~3 μL) of the extracted oil samples was deposited with the use of a Pasteur pipette between two well-polished KBr disks, creating a thin film (Rohman & Man, 2010).

2.12. Differential scanning calorimetry

Oil sample (8–10 mg) was weighted into aluminum pan and covers were sealed into place. The thermal features of the oil samples were evaluated by differential scanning calorimetry (DSC) (Mettler Toledo, DSC 822). The calibration of the instrument was done using indium and zinc. The nitrogen was used as a purge gas at a flow rate of 100 mL/min and a pressure of 20 psi. An empty sealed pan applied as the reference. Each sample was cooled to 20 °C (held for 3 min), and it was then heated from 20 to 60 °C at the rate of 10 °C/min.

2.13. NMR analysis

Oil sample (5 mg) was dissolved in chloroform with continuous stirring for 2 h. ^{1}H NMR spectra were recorded with an Avance DRX-500 Bruker Spectrometers, equipped with a process controller.

2.14. Statistical analysis

The data obtained in this study were analyzed by one-way analysis of variance (ANOVA) using SPSS 16 (SPSS Inc., Chicago, IL). The comparison of treatment means was carried out using Duncan's multiple range test with 95% of confidence intervals.

3. Results and discussions

3.1. Qualitative properties

The color attribute of food products is main factor in acceptance of a food product. The color parameters of olive surface such as *L*-value, *a*-value, *b*-value during storage time were listed in Table 1. According to the obtained results, there was no significant difference between b* parameter for control and inoculated samples. The value of *L** parameter decreased after fermentation process. Higher value of *L**, which is an indicator for degree of lightness, revealed that the degree of lightness decreased during fermentation process of olive which may be attributed to browning reaction. On the other hand, a significant decrease in *a** parameter was observed after fermentation process, demonstrating a distinct toning from green to red. This effect could be attributed to the presence of chlorophyllase in the first days of fermentation which leads to hydrolysis of phytol (Minguez-Mosquera, Garrido-Fernandez, & Gandul-Rojas, 1990). Furthermore, this effect may be due to reactions of browning or chemical oxidation reactions.

The texture has a considerable influence on consumers' acceptance of foodstuffs. Additionally, in certain products, it may be even more important than sensory properties (Szczesniak & Kleyn, 1963). The results of textural analysis on original and fermented olive are presented in Table 1. It can be seen that the values of stiffness decreased at the end of the process for both inoculated and control samples. The exogenous enzymes derived from microorganisms may lead to a decrease in stiffness due to disruption of the cell wall of the olive (Fernández-Bolaños, Rodriguez, Guillén, Jiménez, & Heredia, 1995; Fernández-Bolaños et al., 2002). Similarly, Fadda, Del Caro, Sanguinetti, and Piga (2014) investigated the effect of brining time on the texture and antioxidant activity of naturally fermented green olives. The authors reported a decrease in the hardness of olive after 30 days of brining.

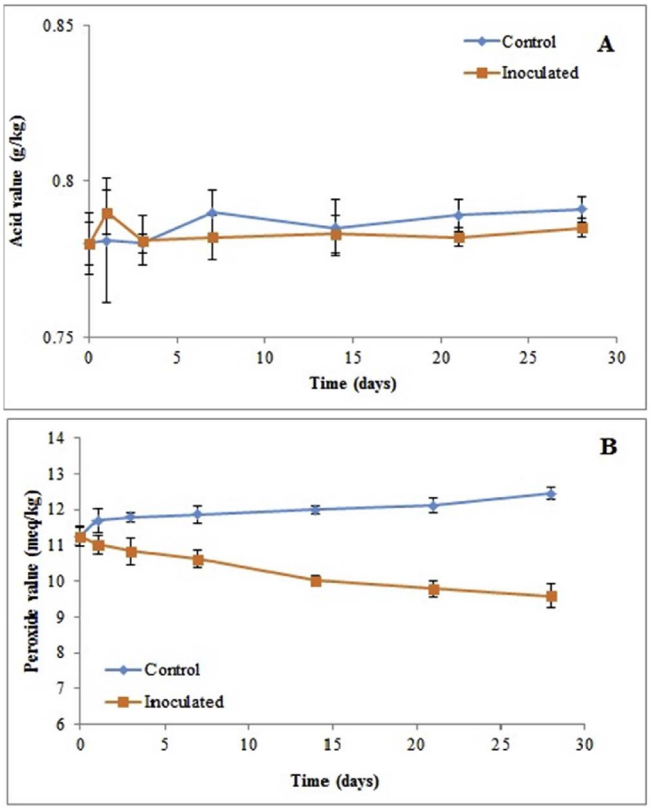

Fig. 1. Effect of storage time on the acid (A) and peroxide values (B) of the extracted oil from control and inoculated olives. Error bars indicate the standard deviation for each sample.

3.2. Quality indices

From the technological and sanitary point of view, acidity is an important factor and should be monitored during the fermentation process (Kiai & Hafidi, 2014). The acid values (AV) of control and inoculated samples during storage are shown in Fig. 1. No significant difference ($p > 0.05$) was observed between the measured AV for fermented and non-fermented samples during storage. The activity of enzymes (esterases and lipases) present in olive tissues or produced by microorganisms can increase free fatty acids of olive oil.

The peroxide values of samples during storage are shown in Fig. 1B. There is a slight increase in PV during the processing of control samples, but by increasing the time of brining, peroxide values of the oil extracted from the inoculated samples decreased ($p < 0.05$). Based on Knauf, Vogel, and Hammes (1992), some species of lactobacilli produce a heme-dependent catalase, which can degrade hydrogen peroxide at a very high rate, preventing the formation of peroxyl radicals. Therefore, the inhibitory influence of LAB is due to the ability of lactic acid bacteria to create low oxidation-reduction potential. This result is in agreement with those reported by Saide and Gilliland (2005) and Lin and Yen (1999).

Table 1
Colorimetric and textural properties of olive samples.

Stiffness (N/m)	b*	a*	L*	Sample
5934.21 ± 311^{a}	3.26 ± 0.17^{a}	0.33 ± 0.03^{a}	29.19 ± 0.88^{a}	Original sample (0 day)
5721.02 ± 366^{a}	3.18 ± 0.08^{a}	−0.41 ± 0.11^{b}	26.14 ± 0.91^{b}	Control sample (28 days)
4129.97 ± 198^{b}	3.16 ± 0.11^{a}	−0.78 ± 0.08^{c}	21.33 ± 1.12^{c}	Inoculated sample (28 days)

Values represent means ± standard deviations. Means within a row with the same superscript lowercase letters are not significantly different at $P < 0.05$.

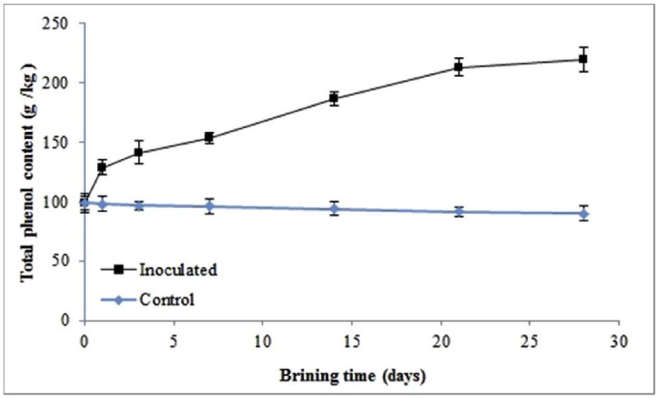

Fig. 2. Effect of storage time on total phenol content of the extracted oil from control and inoculated olives. Error bars indicate the standard deviation for each sample.

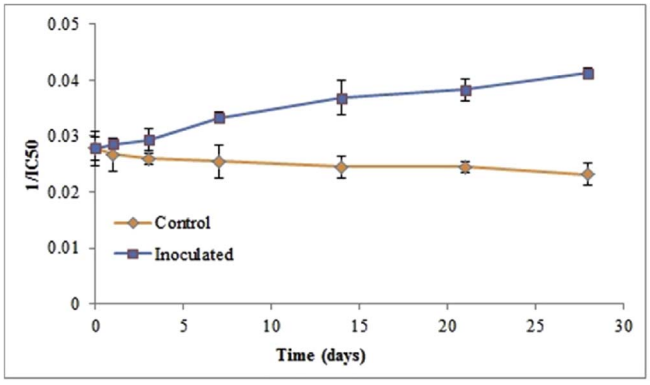

Fig. 3. Effect of storage time on antiradical activity of the extracted oil from control and inoculated olives. Error bars indicate the standard deviation for each sample.

3.3. Antioxidant properties

Phenolic compounds have antioxidant activity due to their ability to scavenge free-radical and active oxygen species. Changes in concentration of total phenolic compounds in control and inoculated samples during brining are depicted in Fig. 2. Total phenolic of the extracted oil from control sample slightly decreased during storage which may be due to the diffusion of these compounds to the brine. Therefore, the diffusion of phenolic compounds from the olive flesh leads to enrichment of brines (Kiai & Hafidi, 2014). Similarly, Kiai and Hafidi (2014) indicated that the total phenol content of olives decreased during boring process. On the other hand, it can be observed that the utilization of *L. plantarum* led to a significant increase ($p < 0.05$) in the total phenolic content in extracted olive oils, which may be related to the ability of *L. plantarum* to trap the oxygen present in the solution that was responsible for the auto-oxidation of phenolic compounds (Kachouri & Hamdi, 2006). Furthermore, it has also illustrated the elevated antioxidant activity of fermented samples is associated with enzymes liberated by bacteria during fermentation. These enzymes are the main reason for the degradation of complex polyphenols to small molecules which in turn leads to the elevation of total phenolic content (Jayabalan, Subathradevi, Marimuthu, Sathishkumar, & Swaminathan, 2008). Considering the notable influence of fermentation on total phenol content, it is reasonable to assume that fermented samples have a different phenolic composition in comparison to control samples. Further analysis should be carried out to evaluate the influence of fermentation process on the composition of the phenolic fraction of olive oil.

DPPH radical scavenging method is one of the most common techniques used for evaluation of antioxidant activities, due to its sensitivity, simplicity, rapidity and reproducibility in comparison to other techniques (Milardović, Iveković, & Grabarić, 2006). The antiradical activity and its evolution during 28 days of brining are given in Fig. 3. It can be seen that the antioxidant capacity of control sample had a slight decrease during fermentation process. On the other hand, with increasing the fermentation time, antiradical activity of inoculated samples increased. The radical scavenging activity of reactive chemical species is attributed to the antioxidant components present in the extracted oil (Campus et al., 2015). Visioli, Bellomo, and Galli (1998) demonstrated that phenolic compounds have strong antioxidant and free radical scavenging activities. These components are known as chain-breaking antioxidants which react with lipid radicals to form nonreactive radicals, interrupting the propagation chain. In another word, these compounds are able to donate an electron or hydrogen atom to the lipid radical formed over the propagation phase of lipid oxidation and stabilize the corresponding phenoxyl radical by delocalizing the unpaired electron (Bendini et al., 2007). As reported in the previous section, *L. plantarum* can trap the oxygen present in the solution that was responsible for the auto-oxidation of phenol components (Kachouri & Hamdi, 2006). Therefore, elevated free-radical scavenging

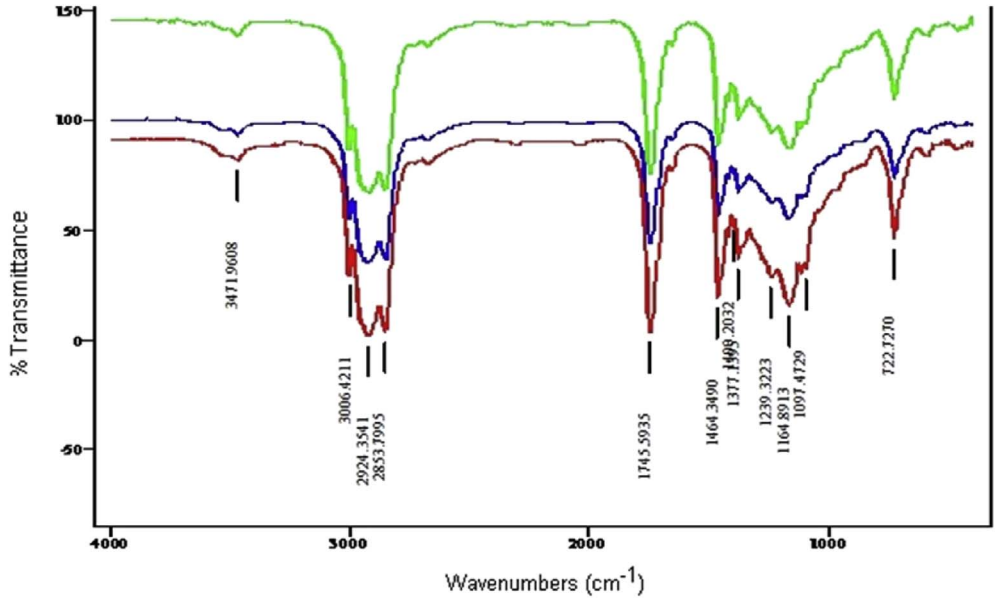

Fig. 4. FT-IR analysis of the extracted oil from control and inoculated olives.

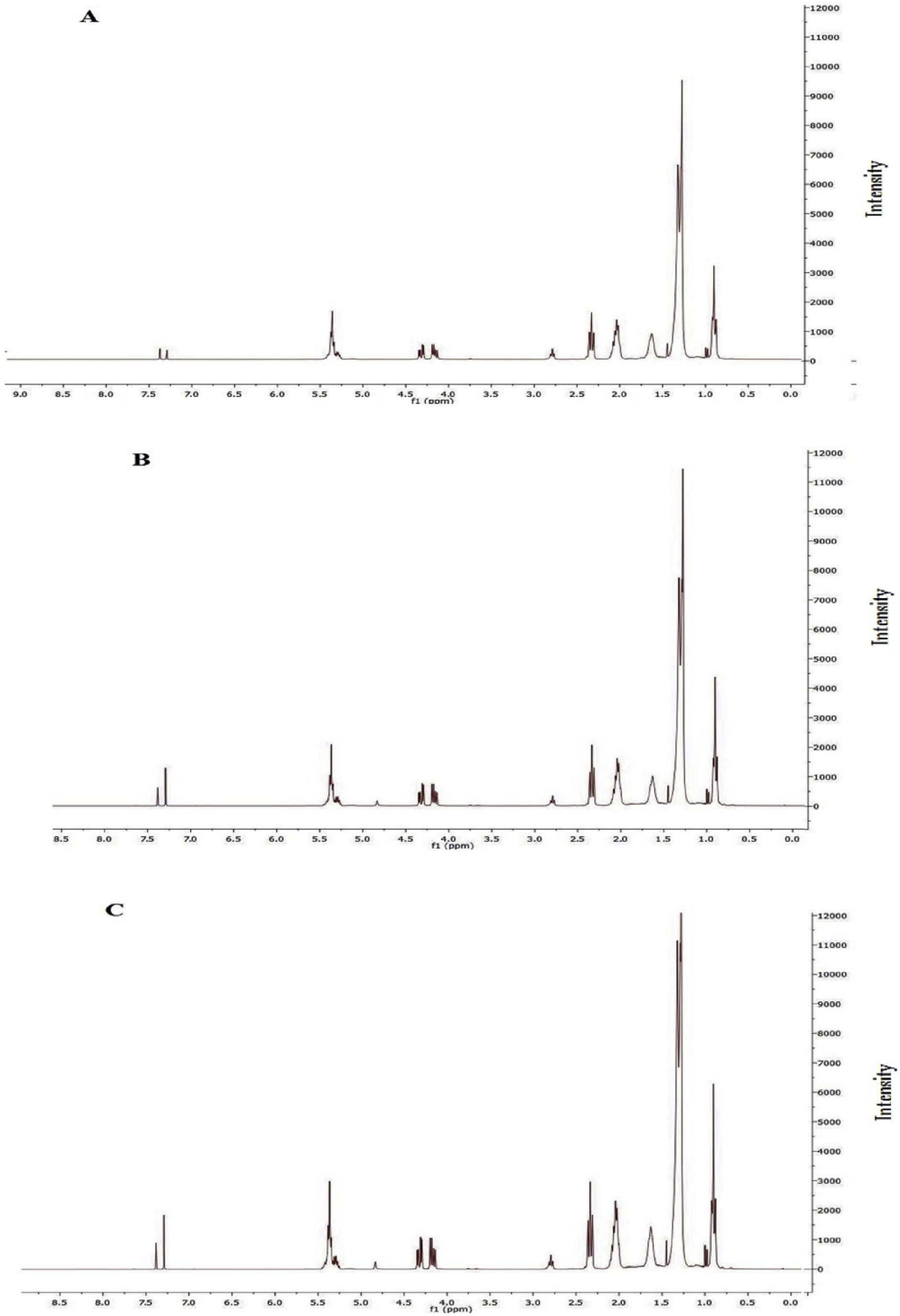

Fig. 5. The 1H NMR spectrum of the extracted oils from control (1day) (A), treated (28 days) (B) and control samples (28 days) (C).

Table 2
Fatty acid profile (g/kg) of original and fermented olive oil.

Inoculated sample (28 days)	Control sample (28 days)	Original sample (0 day)	Fatty acid
603.6 ± 0.6[a]	590.4 ± 1.7[b]	605.8 ± 1.7[a]	Oleic
196.6 ± 1.2[a]	189.8 ± 1.3[b]	197.3 ± 0.7[a]	Linoleic
6.8 ± 0.3[a]	6.1 ± 0.2[b]	6.9 ± 0.0[a]	Linolenic
7.3 ± 0.1[a]	7.2 ± 0.3[a]	7.4 ± 0.7[a]	Arachidic
24.1 ± 0.9[a]	23.9 ± 1.0[a]	24.0 ± 0.9[a]	Stearic
142.3 ± 0.6[a]	142.9 ± 1.1[a]	142.8 ± 1.5[a]	Palmitic
0.8 ± 0.2[a]	0.8 ± 0.0[a]	0.8 ± 0.1[a]	Myristic
14.4 ± 0.8[a]	14.3 ± 0.3[a]	14.6 ± 1.1[a]	Palmitoleic

Values represent means ± standard deviations. Means within a row with the same superscript lowercase letters are not significantly different at $P < 0.05$.

activity during brining could be associated with more presence of phenolic compounds which was confirmed by total phenol analysis. The results obtained in the present study is in agreement with those reported in previous studies (Jain, Yadav, & Sinha, 2009; Kudoh, Matsuda, Igoshi, & Oki, 2001; S.; Li et al., 2012; Osuntoki & Korie, 2010) where indicated that *Lactobacillus* strains had the potential to scavenge DPPH radicals.

Based on literature review, there is a positive relation between total phenol content and antioxidant potential of different foodstuffs such as vegetables and fruits (Baiano, Terracone, Viggiani, & Del Nobile, 2014; Del Carlo et al., 2004; Jayaprakasha, Girennavar, & Patil, 2008; Kornsteiner, Wagner, & Elmadfa, 2006; H.; Li, Wang, Li, Li, & Wang, 2009; Martínez et al., 2012; Sánchez et al., 2007; Ubando-Rivera, Navarro-Ocaña, & Valdivia-López, 2005). In this research, the antioxidant potential of olive samples was evaluated by DPPH radical scavenging. The result of Pearson's test demonstrated that there was a high and significant positive correlation between antiradical power ($1/IC_{50}$) and phenol content of control ($R = 0.957$, $p = 0.001$) and treated ($R = 0.933$, $p = 0.003$) samples. Therefore, it can be concluded that phenolic compounds had a strong involvement in antioxidant activity.

3.4. FT-IR analysis

FTIR analysis was carried out to investigate the structural changes in oil occurred during a storage period of the samples. FTIR spectra of the extracted oil samples from control and inoculated samples are illustrated in Fig. 4. It is evident that there are considerable differences in their bands. Trace compounds present in a mixture have a weak peak which is not detectable in the FTIR spectrum. For example, the band of hydroperoxide functional group (near to 3444 cm^{-1}) is not detected in the FTIR spectrum of the samples. The peak observed around 3470 cm^{-1} is attributed to the overtone of the glyceride ester carbonyl absorption (Guillén & Cabo, 2002). With progressing the oxidation process, the concentration of hydroperoxide groups in the sample increases and as a result, its absorption in the infrared spectrum intensifies (Guillén & Cabo, 2002). Therefore, the intensity of this absorbance can be used as an indicator for hydroperoxide generation throughout the oxidation process. As it can be observed from Fig. 5, this band in control (28 days) sample has more intensity than fresh and inoculated sample oil which indicates that the oxidation occurs during the brining process of the control sample. Comparatively, the absorption intensity at this region for treated sample (28 days) was less than control (28 days) and fresh oil samples. Based on Knauf et al. (1992), some species of lactobacilli produced a heme-dependent catalase, which can degrade H_2O_2 at a very high rate, blocking the formation of peroxyl radicals. Thus, the inhibitory effect of LAB is due to the ability of lactic acid bacteria to create low oxidation-reduction potential.

The wave number at 3006 cm^{-1} is attributed to the C–H stretching vibration of the *cis*-double bond. The frequency of this peak remains constant during the first stage of oxidation and then starts to decrease (Guillén & Cabo, 2002). As it can be seen, there are no considerable differences between the frequency of this band in different samples and thus, it can be concluded that at the end of the storage period, oxidation is yet in the first stage. The diagnostics bands near to 1238, 1163 and 1118 cm^{-1} arise from the stretching vibration of C-O groups of esters.

The original samples indicated a peak around 1743 cm^{-1}, which is associated with ester carbonyl functional group of the triacylglycerols

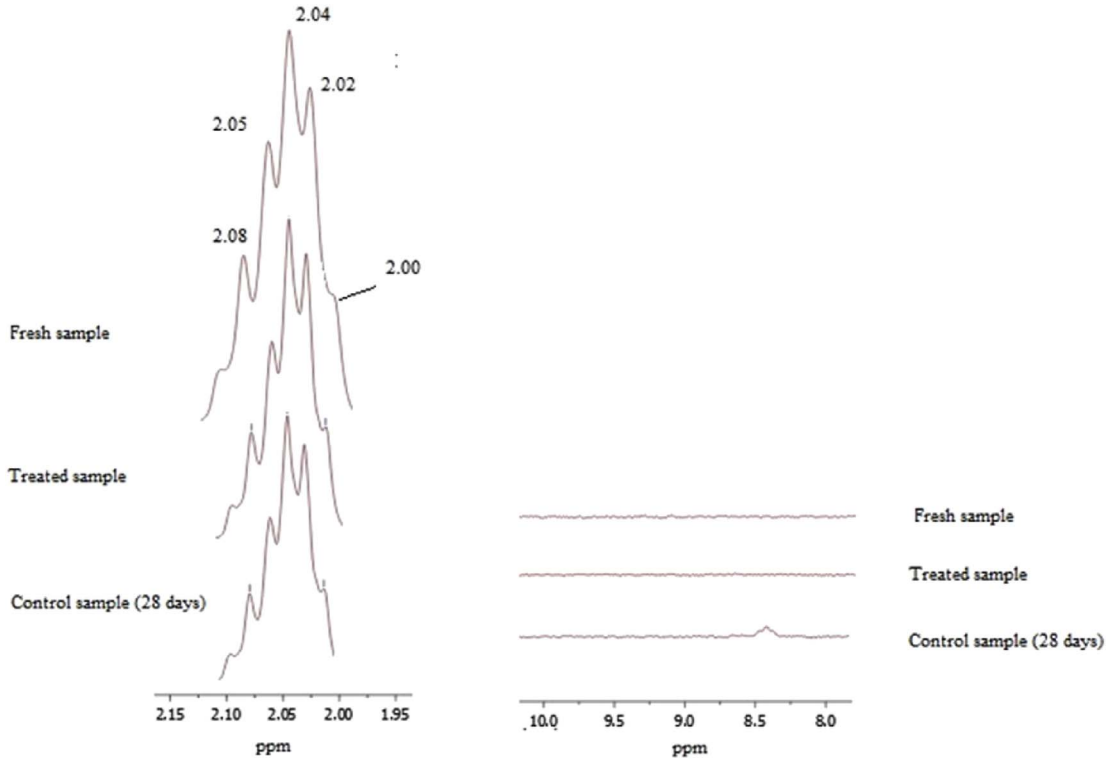

Fig. 6. Enlargement of the allylic hydrogen atoms region (A) and expanded region between 8 and 10 ppm.

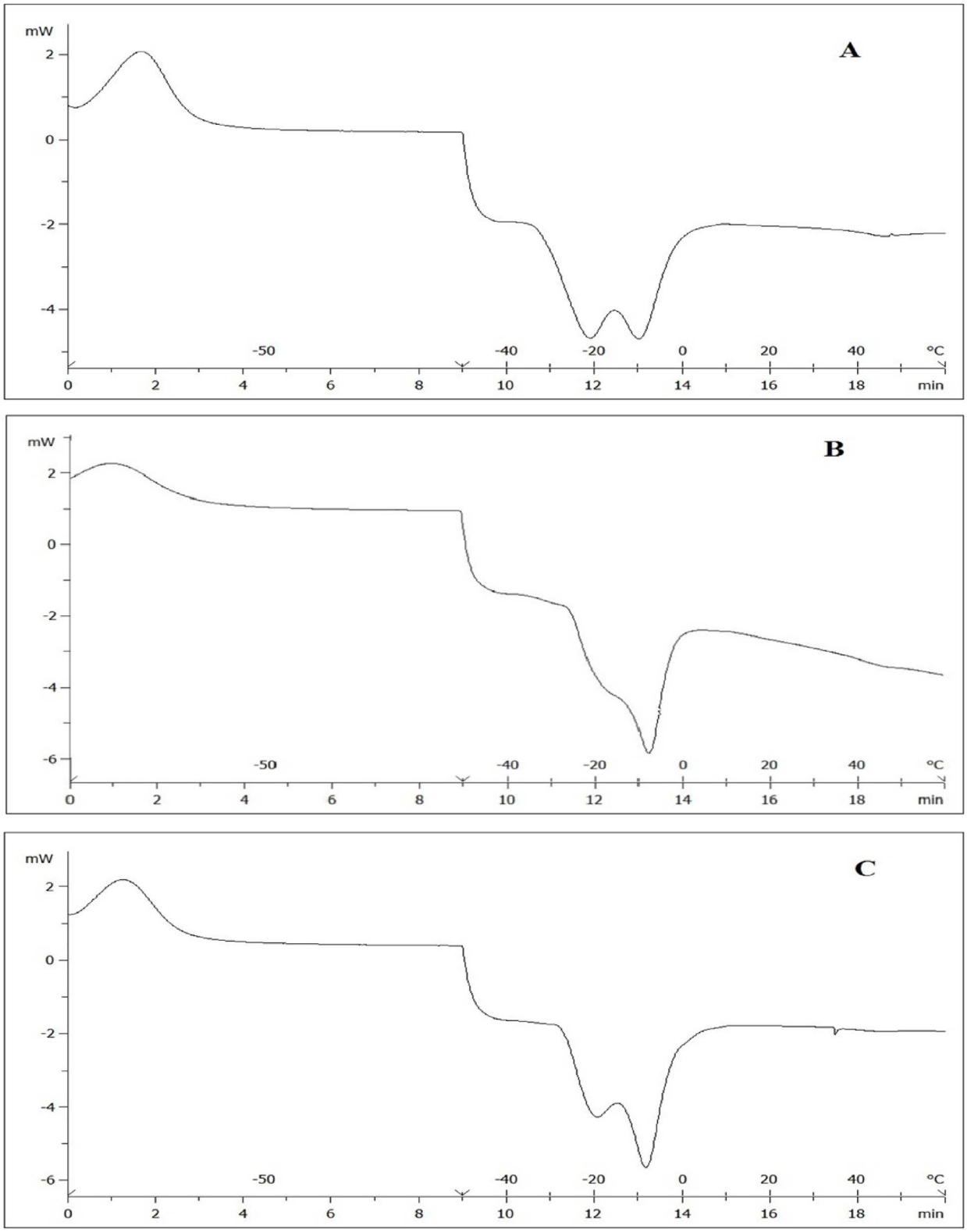

Fig. 7. DSC thermogram of the extracted oils from control (1day) (A), control (28 days) (B) and treated samples (28 days) (C).

(Hashemi, Shahidi, Mortazavi, Milani, & Eshaghi, 2016). During the oxidation process, secondary oxidation products such as aldehydes and ketones produce, which results in the appearance of bands around 1728 cm^{-1}. These bands overlap with that of ester group at 1746 cm^{-1} leading to a broadening of the peak and a decrease in its frequency. It can be seen that these wave numbers for all the samples are approximately similar in intensity and frequency, confirming that the oxidation process is in the initial step.

3.5. Fatty acids profile

The fatty acid profiles of the oils from original and inoculated

samples in initial and the end of period bringing were presented in Table 2. The concentrations of long-chain fatty acids were affected by the fortification of oils with *L. plantarum* cells. Concentrations of long-chain unsaturated fatty acids (oleic, linoleic and linolenic acid) in control sample decreased after bringing which may be due to oxidation of these fatty acids to form hydroperoxides (Hashemi et al., 2016). On the other hand, the concentration of these fatty acids in oils from inculcated samples had no significant difference ($p < 0.05$), indicating that the oil extracted from inoculated sample could prevent the oxidation of these unsaturated fatty acids. This effect may be related to the capacity of *L. plantarum* to prevent the linoleic acid oxidation (Kachouri & Hamdi, 2006). The same results have been reported for the effect of some bacteria strains such as *L. acidophilus, L. plantarum* and *Bifidobacterium longum* on oxidation of linoleic acid (Hashemi et al., 2016; Kachouri & Hamdi, 2006; Kullisaar et al., 2002; Lin & Chang, 2000; Lin & Yen, 1999). In conclusion, *L. plantarum* showed positive influences on fatty acid profiles of olive oil.

3.6. ^1H NMR analysis

The ^{1}H NMR spectra of the extracted oil from fresh and fermented olive are presented in Fig. 5. The region between 0.5 and 5.5 ppm in fresh oil sample contains all the typical ^{1}H NMR signals of vegetable oils. From the nutritional and technological point of view, determination of acyle groups in various types of oils and fats is important (Guillén & Ruiz, 2001). The signals identified at 0.83–0.93 ppm are related to the overlapping of the triplet signals of methyl group hydrogen atoms of saturated, oleic (n-9) and linoleic (n-6) acyl groups. The diagnostic signals at 0.93–1.03 ppm refer to $–CH_3$ (linolenic acyl groups). The resonances for $–(CH2)_n$ –(acyl groups) were identified at 1.22–1.42 ppm. The signals observed at 1.52–2.70 and 2.24–2.36 ppm are related to methylene hydrogen atoms in β and α positions, in relation to the carbonyl group, respectively. The signals for α methylene hydrogen atoms in relation to a single double bond were found at 1.94–2.14 ppm. The diagnostic signals at 2.70–2.88 ppm correspond to the overlapping of the signals of α methylene hydrogen atoms in relation to 2 double bonds which is known as bis-allylic hydrogen atoms. The peaks at 4.05–4.35 ppm are related to the hydrogen atoms on 1 and 3 carbon atoms of the glyceryl group. The peaks identified at 5.20–5.26 have attributed the hydrogen atom on the carbon atom 2 of the glyceryl group, whereas the main ones at 5.26–5.40 are associated with olefinic hydrogen atoms of the different acyl groups.

The intensity of the signals in ^{1}H NMR spectra is directly related to their concentration in the sample. Therefore, the proportion of the acyl groups can be determined from the ^{1}H NMR signal area. As it can be observed from Fig. 5, the intensity of the signal related to linoleic acyl groups in the original sample (signal 5) was less than that obtained for the control sample (28 days). Furthermore, the intensity of this signal for treated sample was more than that of a control sample, indicating the extracted oil from treated sample had more oxidation stability. This result is consistent with those observed in FTIR and GC analysis. The signal of allylic protons of the samples was enlarged to exhibit more clearly the evolution of the degradation of the linoleic acyl group. It can be seen that the linoleic group is slightly degraded in control sample.

The ^{1}H NMR signal related to the hydroperoxide proton appears between 8.0 and 9.0 ppm. It can be seen that in a fresh sample, this region is free of signals; however, in control sample (28 days), the signal of hydroperoxide is detectable. Similarly, the spectrum of the fresh oil sample and treated sample had no signals in this region (Fig. 6).

3.7. Thermal analysis

DSC is an acceptable method for evaluating the thermal properties of oils and fats. DSC thermogram of the oil extracted from treated and control samples are shown in Fig. 7. All the samples exhibited, at first, a minor exothermic event and then several major endothermic peaks in the range of temperature between −40 and 0 °C. The exothermic peak is probably related to crystallization of the unsolidified oil fraction. Based on previous studies, a shift of the crystallization peak toward lower temperatures is due to lipid oxidation (Vittadini, Lee, Frega, Min, & Vodovotz, 2003). It can be seen there is a shift in the crystallization peak toward lower temperatures at the end of period bringing in control sample which indicated that the oxidation of oil samples increased during the storage period. Control samples exhibited a more evident shift of the crystallization peak, demonstrating the oxidation of treated samples was less than controls. Likewise, the crystallization enthalpy of control samples decreased after storage period which is an evidence for the occurrence of the oxidation reaction.

The endothermic event obtained at temperatures above −18 °C is associated with the melting of crystallized lipids (Chiavaro, Vittadini, Rodriguez-Estrada, Cerretani, & Bendini, 2008). Two peaks are observable: first, a major endothermic event around −20 °C and the second event is distinguishable near to −5 °C. The melting point of the control sample was decreased during storage, whereas there was no considerable difference between the melting point of inoculated and fresh samples. The melting point of fats depends on fatty acid composition. In another word, more number of double bonds in a fatty acid is accompanied with the fewer melting points (Yılmaz, Karakaya, & Aktaş, 2010). Consequently, a decrease of unsaturated fatty acids control samples during brining process led to an elevation in melting and transition points.

4. Conclusion

In this study, the effect of fermentation process on quality of olive fruit and physicochemical properties of its oil was investigated. *L. plantarum* indicated considerable free-radical scavenging activities during the fermentation process. Furthermore, it was found that *L. plantarum* decreased the oxidation of unsaturated fatty acids and also led to a decrease in peroxide value. The result of this study confirmed that the fermentation of olive induced a considerable increase in phenolic compounds of olive oil. Fermentation process was relatively effective in retaining firmness and color of olives.^{1}HNMR spectrum showed that the intensity of the signal related to linoleic acyl groups for the treated sample was more than control one, revealing the extracted oil from the treated samples had more oxidation stability. Thermal analysis indicated that the crystallization enthalpy of control samples decreased after storage period which is an evidence for the occurrence of the oxidation reaction. Overall, fermentation can be suggested as an appropriate method for preservation of olive quality and olive oil stability during storage.

References

Argyri, A. A., Nisiotou, A. A., Mallouchos, A., Panagou, E. Z., & Tassou, C. C. (2014). Performance of two potential probiotic *Lactobacillus* strains from the olive microbiota as starters in the fermentation of heat shocked green olives. *International Journal of Food Microbiology, 171*, 68–76.

Baiano, A., Terracone, C., Viggiani, I., & Del Nobile, M. A. (2014). Changes produced in extra-virgin olive oils from cv. Coratina during a prolonged storage treatment. *Czech Journal of Food Science, 32*, 1–9.

Balatsouras, G., Tsibri, A., Dalles, T., & Doutsias, G. (1983). Effects of fermentation and its control on the sensory characteristics of Conservolea variety green olives. *Applied and Environmental Microbiology, 46*, 68–74.

Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, A. M., Segura-Carretero, A., Fernández-Gutiérrez, A., et al. (2007). Phenolic molecules in virgin olive oils: A survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade Alessandra. *Molecules, 12*, 1679–1719.

Campus, M., Cauli, E., Scano, E., Piras, F., Comunian, R., Paba, A., et al. (2015). Evaluation of a single strain starter culture, a selected inoculum enrichment, and natural microflora in the processing of Tonda di Cagliari natural table olives: Impact on chemical, microbiological, sensory and texture quality. *LWT-food Science and Technology, 64*, 671–677.

Capannesi, C., Palchetti, I., Mascini, M., & Parenti, A. (2000). Electrochemical sensor and biosensor for polyphenols detection in olive oils. *Food Chemistry, 71*, 553–562.

Chiavaro, E., Vittadini, E., Rodriguez-Estrada, M. T., Cerretani, L., & Bendini, A. (2008). Monovarietal extra virgin olive oils. Correlation between thermal properties and chemical composition: Heating thermograms. *Journal of Agricultural and Food Chemistry, 56*, 496–501.

Del Carlo, M., Sacchetti, G., Di Mattia, C., Compagnone, D., Mastrocola, D., Liberatore, L., et al. (2004). Contribution of the phenolic fraction to the antioxidant activity and oxidative stability of olive oil. *Journal of Agricultural and Food Chemistry, 52*, 4072–4079.

Etchells, J., Borg, A., Kittel, I., Bell, T., & Fleming, H. (1966). Pure culture fermentation of green olives. *Applied Microbiology, 14*, 1027–1041.

Fadda, C., Del Caro, A., Sanguinetti, A. M., & Piga, A. (2014). Texture and antioxidant evolution of naturally green table olives as affected by different sodium chloride brine concentrations. *Grasasy Aceites, 65*, 002.

Farhoosh, R., Niazmand, R., Rezaei, M., & Sarabi, M. (2008). Kinetic parameter determination of vegetable oil oxidation under Rancimat test conditions. *European Journal of Lipid Science and Technology, 110*, 587–592.

Fernández-Bolaños, J., Rodriguez, R., Guillén, R., Jiménez, A., & Heredia, A. (1995). Activity of cell wall-associated enzymes in ripening olive fruit. *Physiologia Plantarum, 93*, 651–658.

Fernández-Bolaños, J., Rodríguez, R., Saldaña, C., Heredia, A., Guilén, R., & Jiménez, A. (2002). Factors affecting the changes in texture of dressed (" aliñadas") olives. *European Food Research and Technology, 214*, 237–241.

Firestone, D. (1989). *Official methods and recommended practices of the American oil chemists' society*, *Vol. 5*. IL, USA: AOCS Champaign.

Gould, G. W. (1991). *Antimicrobial compounds Biotechnology and food ingredients.* New York, NY: Van Nostrand Reinhold.

Guillén, M. a. D., & Cabo, N. (2002). Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils. *Food Chemistry, 77*, 503–510.

Guillén, M. a. D., & Ruiz, A. (2001). High resolution 1 H nuclear magnetic resonance in the study of edible oils and fats. *Trends in Food Science & Technology, 12*, 328–338.

Hashemi, S. M. B., Shahidi, F., Mortazavi, S. A., Milani, E., & Eshaghi, Z. (2016). Effect of Lactobacillus plantarum LS5 on oxidative stability and lipid modifications of Doogh. *International Journal of Dairy Technology, 69*, 550–558.

Jain, S., Yadav, H., & Sinha, P. R. (2009). Antioxidant and cholesterol assimilation activities of selected lactobacilli and lactococci cultures. *Journal of Dairy Research, 76*, 385–391.

Jayabalan, R., Subathradevi, P., Marimuthu, S., Sathishkumar, M., & Swaminathan, K. (2008). Changes in free-radical scavenging ability of kombucha tea during fermentation. *Food Chemistry, 109*, 227–234.

Jayaprakasha, G., Girennavar, B., & Patil, B. S. (2008). Radical scavenging activities of Rio Red grapefruits and Sour orange fruit extracts in different in vitro model systems. *Bioresource Technology, 99*, 4484–4494.

Kachouri, F., & Hamdi, M. (2006). Use *Lactobacillus plantarum* in olive oil process and improvement of phenolic compounds content. *Journal of Food Engineering, 77*, 746–752.

Kiai, H., & Hafidi, A. (2014). Chemical composition changes in four green olive cultivars during spontaneous fermentation. *LWT-food Science and Technology, 57*, 663–670.

Knauf, H. J., Vogel, R. F., & Hammes, W. P. (1992). Cloning, sequence, and phenotypic expression of katA, which encodes the catalase of *Lactobacillus sake* LTH677. *Applied and Environmental Microbiology, 58*, 832–839.

Kornsteiner, M., Wagner, K. H., & Elmadfa, I. (2006). Tocopherols and total phenolics in 10 different nut types. *Food Chemistry, 98*, 381–387.

Kudoh, Y., Matsuda, S., Igoshi, K., & Oki, T. (2001). Antioxidative peptide from milk fermented with *Lactobacillus delbrueckii* subsp. bulgaricus IFO13953. *Journal-Japanese Society Of Food Science and Technology, 48*(1), 44–50.

Kullisaar, T., Zilmer, M., Mikelsaar, M., Vihalemm, T., Annuk, H., Kairane, C., et al. (2002). Two antioxidative *lactobacilli* strains as promising probiotics. *International Journal of Food Microbiology, 72*, 215–224.

Lin, M. Y., & Chang, F. J. (2000). Antioxidative effect of intestinal bacteria *Bifidobacterium longum* ATCC 15708 and *Lactobacillus acidophilus* ATCC 4356. *Digestive Diseases and Sciences, 45*, 1617–1622.

Lin, M. Y., & Yen, C. L. (1999). Inhibition of lipid peroxidation by *Lactobacillus acidophilus* and *Bifidobacterium longum*. *Journal of Agricultural and Food Chemistry, 47*, 3661–3664.

Li, H., Wang, X., Li, Y., Li, P., & Wang, H. (2009). Polyphenolic compounds and antioxidant properties of selected China wines. *Food Chemistry, 112*, 454–460.

Li, S., Zhao, Y., Zhang, L., Zhang, X., Huang, L., Li, D., et al. (2012). Antioxidant activity of *Lactobacillus plantarum* strains isolated from traditional Chinese fermented foods. *Food Chemistry, 135*, 1914–1919.

Martínez, R., Torres, P., Meneses, M. A., Figueroa, J. G., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2012). Chemical, technological and in vitro antioxidant properties of mango, guava, pineapple and passion fruit dietary fibre concentrate. *Food Chemistry, 135*, 1520–1526.

Milardović, S., Iveković, D., & Grabarić, B. S. (2006). A novel amperometric method for antioxidant activity determination using DPPH free radical. *Bioelectrochemistry, 68*, 175–180.

Minguez-Mosquera, M. I., Garrido-Fernandez, J., & Gandul-Rojas, B. (1990). Quantification of pigments in fermented Manzanilla and Hojiblanca olives. *Journal of Agricultural and Food Chemistry, 38*(8), 1662–1666.

Montano, A., Sánchez, A., & Castro, A. D. (1993). Controlled fermentation of Spanish-type green olives. *Journal of Food Science, 58*, 842–844.

Moreno, M. M., Olivares, D. M., Lopez, F. A., Adelantado, J. G., & Reig, F. B. (1999). Determination of unsaturation grade and trans isomers generated during thermal oxidation of edible oils and fats by FTIR. *Journal of Molecular Structure, 482*, 551–556.

Muik, B., Lendl, B., Molina-Díaz, A., & Ayora-Cañada, M. J. (2005). Direct monitoring of lipid oxidation in edible oils by Fourier transform Raman spectroscopy. *Chemistry and Physics of Lipids, 134*, 173–182.

Osuntoki, A., & Korie, I. (2010). Antioxidant activity of whey from milk fermented with *Lactobacillus* species isolated from Nigerian fermented foods. *Food Technology and Biotechnology, 48*, 505–511.

Rodríguez-Gómez, F., Romero-Gil, V., Bautista-Gallego, J., García-García, P., Garrido-Fernández, A., & Arroyo-López, F. N. (2014). Production of potential probiotic Spanish-style green table olives at pilot plant scale using multifunctional starters. *Food Microbiology, 44*, 278–287.

Rohman, A., & Man, Y. C. (2010). Fourier transform infrared (FTIR) spectroscopy for analysis of extra virgin olive oil adulterated with palm oil. *Food Research International, 43*, 886–892.

Romeo, F. V., 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 Chemistry, 116*, 873–878.

Saide, J., & Gilliland, S. (2005). Antioxidative activity of *lactobacilli* measured by oxygen radical absorbance capacity. *Journal of Dairy Science, 88*, 1352–1357.

Sánchez, C. S., González, A. T., García-Parrilla, M., Granados, J. Q., De La Serrana, H. L. G., & Martínez, M. L. (2007). Different radical scavenging tests in virgin olive oil and their relation to the total phenol content. *Analytica Chimica Acta, 593*, 103–107.

Shantha, N. C., & Decker, E. A. (1994). Rapid, sensitive, iron-based spectrophotometric methods for determination of perorlride values of food lipids. *Journal of AOAC International, 77*, 421–424.

Szczesniak, A., & Kleyn, D. (1963). Consumer awareness of texture and other food. *Food Technology, 27*, 74–75.

Tassou, C., Panagou, E., & Katsaboxakis, K. (2002). Microbiological and physicochemical changes of naturally black olives fermented at different temperatures and NaCl levels in the brines. *Food Microbiology, 19*, 605–615.

Tuck, K. L., & Hayball, P. J. (2002). Major phenolic compounds in olive oil: Metabolism and health effects. *The Journal of Nutritional Biochemistry, 13*, 636–644.

Ubando-Rivera, J., Navarro-Ocaña, A., & Valdivia-López, M. (2005). Mexican lime peel: Comparative study on contents of dietary fibre and associated antioxidant activity. *Food Chemistry, 89*, 57–61.

Visioli, F., Bellomo, G., & Galli, C. (1998). Free radical-scavenging properties of olive oil polyphenols. *Biochemical and Biophysical Research Communications, 247*, 60–64.

Vittadini, E., Lee, J., Frega, N., Min, D., & Vodovotz, Y. (2003). DSC determination of thermally oxidized olive oil. *Journal of the American Oil Chemists' Society, 80*, 533–537.

Yılmaz, M. T., Karakaya, M., & Aktaş, N. (2010). Composition and thermal properties of cattle fats. *European Journal of Lipid Science and Technology, 112*, 410–416.