ISSN 0100-2945 DOI: http://dx.doi.org /10.1590/0100-29452020409

Revista Brasileira de Fruticultura

Processing

Effect of different fermentation processes on the phytochemical properties of green table Olives

Taha M. Rababah[1], Muhammad Al- u'datt[2], Majdi Al-Mahasneh[3], Sana Gammoh[4], Hana'a Mahili[4], Tha'er Ajouly[4]

Abstract - Most olive varieties are grown in Jordan using Baladi, Nabali, Crosodi and k_{18} table olives. This study evaluated the effect of de-bittering fermentation methods on the total phenolic, flavonoid contents, and antioxidants. The phytochemical results of fresh and fermented table olives showed significant decrease after fermentation process and during three months of storage. Baladi fresh table olive presented the highest phenol content (350.9mg GAE/100g) followed by Nabali, Crosodi and k_{18} varieties, respectively. The highest antioxidant value was found in Baladi variety (42.2%) followed by k_{18}, Crosodi and Nabali varieties, respectively. Crosodi fresh table olive presented the highest flavonoid content (107.4 mg CE/100G), followed by k_{18}, Nabali and Baladi varieties, respectively. The storage results showed that total phenol content decreased and significantly varied among de-bittering fermentation methods in all storage periods. Higher content in fresh olives was found, followed by those treated with NaOH, ticked and scratchy, respectively. The antioxidant activity and flavonoid content decreased during fermentation periods. The results of de-bittering fermentation methods varied among storage periods and showed that higher antioxidant activities and flavonoid contents were found in olives treated with NaOH followed by ticked, scratchy, and fresh olives, respectively.

Index terms: Table olives, Phytochemicals, Fermentation, De-bittering.

Efeito de diferentes processos de fermentação nas propriedades fitoquímicas de azeitonas verdes de mesa

Resumo-A maioria das variedades de azeitona é cultivada na Jordânia, usando azeitonas de mesa Baladi, Nabali, Crosodi e k_{18}. Este estudo avaliou o efeito dos métodos de fermentação de remoção do amargor no teor total de componentes fenólicos, flavonoides e antioxidantes. Os resultados fitoquímicos das azeitonas de mesa frescas e fermentadas apresentaram diminuição significativa após o processo de fermentação, durante três meses de armazenamento. A azeitona de mesa fresca Baladi apresentou o maior teor de fenólicos (350,9mg GAE / 100g), seguida pelas variedades Nabali, Crosodi e k18, respectivamente. O maior valor antioxidante foi encontrado na variedade Baladi (42,2%), seguida pelas variedades k_{18}, Crosodi e Nabali, respectivamente. A azeitona de mesa fresca Crosodi apresentou o maior teor de flavonóides (107,4 mg CE / 100G), seguida pelas variedades k18, Nabali e Baladi, respectivamente. Os resultados do armazenamento mostraram que o teor total de fenólicos diminuiu e variou significativamente entre os métodos de fermentação de remoção do amargor em todos os períodos de armazenamento. Foi encontrado maior conteúdo em azeitonas frescas, seguido pelas tratadas com NaOH, marcadas e arranhadas, respectivamente. A atividade antioxidante e o teor de flavonoides diminuíram durante os períodos de fermentação. Os resultados dos métodos fermentativos de remoção do amargor variaram entre os períodos de armazenamento e mostraram que atividades antioxidantes e teores de flavonoides mais elevados foram encontrados nas azeitonas tratadas com NaOH, seguidas pelas azeitonas marcadas, arranhadas e frescas, respectivamente.

Termos para indexação: Azeitonas de mesa, Fitoquímicos, Fermentação, Remoção do amargor.

Corresponding author:

Email: trababah@just.edu.jo

Received: March 05, 2019
Accepted: June 03, 2019

[1]Department of Nutrition and Food Technology, Jordan University of Science and Technology. Irbid, Jordan. E-mail: trababah@just.edu.jo (ORCID 0000-0002-3215-6419)

[2]Department of Nutrition and Food Technology, Jordan University of Science and Technology. Irbid, Jordan. E-mail: malodat@just.edu.jo (ORCID 0000-0002-8976-5004)

[3]Department of chemical Engineering, Jordan University of Science and Technology. Irbid, Jordan. E-mail: mmajdi@just.edu.jo (ORCID 0000-0002-7477-7512)

[4]Department of Nutrition and Food Technology, Jordan University of Science and Technology. Irbid, Jordan. E-mails: sana_gammoh@hotmail.com; hanaa_mahely@hotmail.com; thaer.ajouly@yahoo.com

Introduction

The olive trees (*Olea europaea L.*) are a main crop that grows in the Mediterranean regions Al-Ismail et al. (2011). Therefore, olives from economical point of view are considered one of the significant goods that provide oil with high nutritional value and possible therapy treatments Ribarova et al. (2003). Jordan is considered one of the most important countries in olive trees cultivation, it contributes about 72% of the total planted area which are 36% of Jordanian lands MAJ (2008). Olives are seldom used without processing, because of its bitterness Fernández et al. (1997). Fermentation process of table olives is dominant between other vegetable in the Mediterranean countries Lanza (2003). Californian style, Greek style and the Spanish style are the most de-bittering methods applied to make table olives edible to consumption Boskou et al. (2006). They demonstrated that Californian style for green table olives includes lye treatment process before canning and sterilization. Greek style is directly brining and almost these de-bittering methods for black table olives preparation Ünal and Nergiz (2003).Spanish style preparation de-bittering by NaOH before washing then fermentation and packaging Alygizakis (1982). Table olive components are affected by the processing de-bittering methods of table olives fermentation Nergiz (2003). Table olives are considered of high biological properties Pereira et al. (2006). Phenols content are the most complex constituents that found in table olive fruits Solinas et al. (1975). Consequently, plants proved to be rich in natural phytochemicals such as phenolic compounds, flavonoids and antioxidants such as vitamins C and E, and carotenoids Silva et al. (2006). Flavonoids are considered as the major group of phenolics, they have several biological effects that related to health Harborne, and Williams (2000). It's proved that total phenolic, antioxidant activity, and flavonoids compounds are affected by fermentation de-bittering methods and during storage Asami et al. (2003). Also, the bioactive compounds are susceptible to oxidation reactions during the processing and storage of food because some of these compounds are unstable Robards et al. (1999).

This study was thus undertaken to (i) determine the phenolic compounds, flavonoids and antioxidant activity of fresh table olive varieties, (ii) to determine the effect of debittering method of treatment of table olive varieties on the content of phenolic compounds, flavonoids and antioxidant activity, (iii) to determine the effect of storage time of table olive varieties on the content of phenolic compounds, flavonoids and antioxidant activity

Materials and Methods

Chemicals

Sodium carbonate Na_2CO_3, Aluminum Chloride $AlCl_3$, Sodium Hydroxide NaOH, and Sodium Nitrate $NaNO_2$ were purchased from (Scharlau, Barcelona, Spain). Methanol and Folin-Ciolatea reagents, 2, 2-diphenylydrazyl (DPPH), gallic acid were purchased from (Sigma-Aldreich, Switzerland).

Sample Collection

Four different varieties of green table olives; Nabali, Baladi, Crosodi and k_{18} (their abbreviations included N, B, C and K_{18}, respectively) were collected from Irbid farms in October, 2016.

De-bittering Methods

Olives were hand-picked when they developed a green-yellow surface color and normal large-size. The collected olives were subjected to sorting with regard to their size and washed in tab water to be ready for fermentation process. Four fermentation treatments were applied; whole, ticked scratches and treated with NaOH (their abbreviation H, D, T and M, respectively).The de-bittering methods of table olive treated with NaOH were conducted as described by Ünal and Nergiz (2003) as the following. A 2% NaOH solution was added into the four glasses jars containers and the olives (7.5 kg from each variety of table olive) were kept for 8 hours in that solution. During this de-bittering process, penetration of sodium hydroxide solution into the olive flesh was detected by cutting the fruit halfway down its length to see how far the solution has penetrated the flesh from time to time. After penetration of NaOH in a depth corresponding to 2/3of flesh thickness, the solution was poured with the aid of tap container and the fruits were subjected to water washing several times; 3 time at least to eliminate the excess of alkali solution remaining on the fruits. To ensure all alkaline are removed from brine solution 2 drop of phenol naphthalene was added to brine solution. At the end of this period the water was removed. The olives then placed into glass jars bottles (500 grams capacity) and 10% NaCl brine solution were added to cover the olives. For whole table olives and for de-bittering process olive placed into glass jars container contain tap water and kept for 24 hour, water was changed each 8 hour, and the rest of fermentation process was similar to treated with NaOH table olives. For ticked table olives fermentation process, the olives were beat by hand stone and de-bittering olives process was done as mentioned for the whole table olives. Scratchy table olives processed by knife then completed similarly as mentioned before. Olives filled into glass jars bottles of the four verities stored at room temperature for evaluation during 0, 1, 2 and 3 months for phytochemical (total phenol, antioxidant and flavonoids) properties.

Phytochemical Determinations

Extraction of Total Phenolic and Flavonoids Contents and Antioxidant Activity

The extracts were prepared as described below. Table olives pitted by removing the seeds, then grinding for 10 seconds. About 5 g of each sample was weighed out, and mixed with 100 mL of methanol. Extraction was carried out under shaking water bath for 60 min at 60 ◦C. Each extract was filtered out using Whitman No. 3 filter paper, filled accordingly in a 100 ml volumetric flask, and allowed to set in the dark until analysis.

Determination of the Total Phenolic Contents

Total phenolic contents in extracts were determined according to the Folin–Ciocalteu procedure Singleton and Rossi (1965), as follows: 100 µL of the extracts (triplicate) were transferred into a test tube, and then mixed with 0.4 mL of 10% Folin–Ciocalteu reagent. After 3 min for allowing the reaction to take place, 0.8mL of a 10% Na_2CO_3 was added, then 8.7 ml of distilled water were add. The tubes were allowed to stand for 1 h at ambient temperature, and the absorption was measured at 725 nm using spectrophotometer (CELL, model CE 1020; Cecil Instruments Ltd, Cambridge, England) against a blank, which contained 100 µL of methanol in place of sample. Gallic acid was used as calibration standard, and the results were calculated as gallic acid equivalent (GAE) (mg / 100 g dry weight basis) (Figure 3.5).

Determination of Radical DPPH-Scavenging Activity

DPPH-radical scavenging effect was determined according to the de-bittering method of Brand-Williams et al. (1995).The DPPH were soluble in methanol. Fresh DPPH stock solution (5ml) was prepared daily. The solution was prepared by weighing 2.4 mg of the (6×10^{-5} mol/L) DPPH and dissolved it in 100 ml methanol which resulted in purple color solution. 500 µL of methanol extracts were reacted with 0.2 mL of methanol DPPH solution. The mixture was brought to a total volume of 4.0 mL with the extracting solvent. The mixture was mixed thoroughly and allowed to stand in the dark for 30 min. Absorbance (*A*) was then was determined at 515 nm, against the blank. The radical scavenging activity was expressed as % of inhibition according to the formula below

Inhibition (%) = *Abs* of the control − *Abs* sample × 100; Abs of the control, where, *Abs* control and *Abs* sample are the absorbance of the control and sample, respectively.

Determination of Flavonoid Contents

The flavonoid contents were determined with aluminum chloride colorimetric assay according to Marinova et al. (2005). Aliquot of 0.1 ml of extracts added to 5 ml volumetric flask and mixed with 0.3 ml of $NaNO_2$ (15%) was mixed with them, after 5 minutes a 0.3 ml $AlCl_3$ (10%) was added, followed by addition of 2 ml of NaOH (4%) was also added, the total of volume was brought to 5 ml with 2.3 ml distilled water. The tubes were allowed to stand in the dark for 60 minutes at ambient temperature. The absorption was measured at 510 nm using Spectrophotometer (UV-1800, UK) against a blank. The total flavonoids content was expressed of milligram of (+) catching equivalent (CE/100g). Three replicate were used to calculate the mean value.

Statistical analysis

Data were analyzed using the general linear model (GLM) procedure with JMP statistical package (JMP Institute Inc., Cary, NC, USA). The model included the effect of replicates (three) and five storage times fresh pretreatments, zero (at the beginning of fermentation), one, two and three month of storage of the all de-bittering methods of green table olive varieties. Also, includes the effect of storage time on each treatment of table olive varieties. Measured variables included total phenolic, antioxidant activity, flavonoids contents, color measurements, texture (hardness), chemical composition and sensory evaluation. Means were separated by LSD analysis at a least significant difference of $P \leq 0.05$ values. Mean were separated by LSD (Least significant Difference) analysis at a least significant difference of 0.05 p-values.

Results and Discussion

Total Phenolic Content of Fresh Table Olive Types

Total phenol content values showed variation among the samples as shown in (Table 1). The result of total phenolic content showed that there is a significant difference exists between fresh table olives verities. The higher total phenol value was found in Baladi (350.9mg GAE/100g) followed significantly by Nabali (328.6 mg GAE/100g), Crosodi (310.6 mg GAE/100g) and k_{18} (295.1 mg GAE/100g), respectively. Our results were within Mettouchi et al. (2016) results who found that the total phenolic compound of different kinds of fresh green table olives ranged from162 mg GA/100g to 471 mg GA/100g. These variations in the total phenolic contents in fresh table olives could be due to vary in the table olive varieties Rababah et al. (2004), or to their degree of maturation stage and ripening Talhaoui et al. (2015).

Antioxidant Activity of Fresh Table Olive Types

Antioxidant activities values showed variation among the samples as shown in (Table 1). The result of antioxidant activities showed that there is a significant difference exists between fresh table olives varieties. The higher antioxidant value was found in Baladi (42.2%) followed by k_{18} (41.2%), Crosodi (29.3%) and Nabali (28.8%), respectively. Our results were agreed with Othman et al. (2009) results who reported that the antioxidant activity in different types of fresh table olives were varied, but their values were higher than our results and their range was from 50% to 72%. The explanation of the antioxidants differences between the varieties could be as described by Malheiro et al. (2014) who demonstrated that the antioxidant activity of the table olives is partially related with the phenolic composition of the extracts.

Flavonoid Contents of Fresh Table Olives Types

Flavonoid content values are presented in (Table 1). The result of flavonoids content showed that there is a significant difference exists between fresh table olives varieties. The higher total flavonoids value was found in Crosodi (98.8 mg CE/100G), followed by k_{18} (80.1 mg CE/100g), Nabali (75.6 mg CE/100G) and Baladi (64.7 mg CE/100G), respectively. Our findings were lower than (27, 19) results, they found that the flavonoid contents were 190 mg GAE/100g to 1500 mg GAE/100g. Mettouchi (2016) investigated that the unfermented olives have significantly ($p > 0.05$) lower total flavonoid contents than the fermented olives. There are no studies similar to our investigation were found.

Total Phenolic Contents of Fermented Table Olive Varieties during Storage

Total phenolic contents of different varieties of table olives and four de-bittering methods of fermentation after one day of fermentation (zero time) and during three months of storage are presented in (Table 2). As shown in the results, total phenols are significantly varied between the de-bittering methods of fermentation in all of the periods of storage, which are represented in the columns and take a significantly letters from a to k as shown in (Table 2). In general, the results of de-bittering methods of fermentation (Table 2) showed that the higher total phenols were found in whole followed by treated with NaOH, ticked and scratchy, respectively. The results of storage at zero time ranged from 294.4 to 350.8 mg/100 g in the all of table olive varieties and de-bittering methods of fermentation. The values of total phenols of the rest of storage periods (1, 2, and 3 months) are similar to what were found at zero time that where 281 to 307 , 284.7 to 300 , and 165.4 to 299.8 mg/100g, respectively. The results of total phenols between the four investigated varieties were complying with what are found in fresh samples as explained before. Comparison with our results regarding the differences between varieties, the only studies of de-bittering methods of fermentation were found by treated with NaOH, while no studies were found regarding the ticked and scratchy de-bittering methods. Our observation are agreed with Olivera et al. (2012) who found that during Spanish-style green olive fermentation of different varieties of olives treated with NaOH, the hydrolysis of glycosides occurs due to the NaOH treatment, caused increased in total phenolic compound then decreased due to diffusion into brine solution leading to differentiation on total phenolic content between varieties. Brenes et al. (1995) found that the phenolic content is variety dependent, some variety show rapid decline and other show increased then decreased in total phenolic compound. Malheiro et al. (2011) reports that the phenolic content of olive cultivars ranges from the very high levels found in some cultivar to the very low levels found in other varieties. Vossen (2007) found that the amount of total phenolic compounds was lower for pitted and stuffed green olives and oxidized olives than whole table olives.

Table 1. The Total Phenols, Antioxidant, and Flavonoids Contents of Fresh Table Olives

Trt*	Total phenols (mg GAE/100g)	Antioxidant (%)	Flavonoids (mg CE/100g)
CF*	310.6 ± 10.2 ^{d}	29.3 ± 1. 7 ^{b}	98.8 ± 7.2 ^{a}
kF	295.1 ± 6.1 ^{c}	41.2 ± 2.7 ^{a}	80.1 ± 5.1 ^{b}
NF	328.6 ± 3.1 ^{b}	28.8 ± 1.9 ^{b}	75.6 ± 5.7 ^{c}
BF	350.9 ± 5.1 ^{a**}	42.2 ± 3.1 ^{a}	64.7 ± 3.7 ^{d}

All values are means of three replicates and calculated on wet basis *Trt= Debittering method of different varieties, N= Nabali, C= Crosodi, B= Baladi, K= k_{18}, F= Fresh table olive **Means ± SD in the same column with the same letter are not significantly different ($P \leq 0.05$).

The effect of storage during fermentation on total phenols is shown in (Table 2) with a significantly letter from A to D. In general, the total phenols in all de-bittering methods of fermentation and in table olive verities are significantly reduced. Comparison with other researchers, our results are agreed with Romero et al. (2004). they reported that the total phenolic contents are reduced during fermentation and after storage in brine. The explanation of the total phenol reduction could be due as reported by Romero et al. (2004) who demonstrated that phenol were transferred from table olive and diffused into fermentation solution. In fact, the diffusion of phenolic compounds from olive flesh to the brine depends on several parameters such as cultivar characteristics, fruit

skin permeability, type of polyphenols present in olive flesh and their ability to diffuse outside the fruits, which is agreed with other results. Sayin and alkan (2015) found that phenolic compounds diffused from flesh to olive brine due to the high NaCl concentration. Also, Perpetuini et al. (2013) agreed with that polyphenols, mainly oleuropein, diffuse from olive flesh into the surrounding solution during preservation, and their acid hydrolysis occurs. The reduction of total phenolic compound during storage as explained by Brenes et al. (1993) could be due to that phenolic compounds are subject to autoxidation in the presence of trace amounts of oxygen, therefore the reactivity varies according to the position of the OH group in the molecule. Moussavi (1979) explained that the autoxidation of organic electron rich phenols proceed at high oxygen pressures and elevated temperatures to yield the corresponding hydro-peroxide at slow rates. Also, they found that the origin of this chemistry appears to involve an unfavorable electron transfer from the substrates to oxygen. Although demonstrated that after the oxidation process, the oxidized olives retained a considerable amount of phenolic compounds Romero et al. (2004).

Table 2. Total Phenolic Contents in Fermented Table Olives at Zero Time and During Three Months of Storage

Trt*	Phenolic Compounds			
	Storage period (Months)			
	0	1	2	3
NH*	330.4 ± 19.07 ^{fA}	307.0 ± 11.1 ^{aA}	300.0 ± 12.6 ^{aB}	299.8 ± 19.7 ^{aB}
ND	328.8 ± 15.1 ^{gA}	281.0 ± 12.3 ^{kB}	288.9 ± 11.6 ^{hB}	165.4 ± 10.2 ^{jkC}
NT	325.9 ± 11.3 ^{hA}	300.1± 10.2^{cA}	298.1 ± 10.2 ^{bB}	294.0 ± 11.6 ^{cC}
NM	333.5 ± 13.07 ^{eA}	304.2 ± 21.5 ^{bB}	296.4 ± 13.2 ^{cB}	296.1± 10.6 ^{bC}
BH	350.8 ± 20.1 ^{bA}	299.7 ± 10.5 ^{cdB}	295.0 ± 21.6 ^{dC}	293.8 ± 14.2 ^{cdD}
BD	349.9 ± 22.07 ^{cA}	297.4 ± 13.2 ^{eB}	292.2 ± 10.6 ^{fghC}	266.9 ± 10.6 ^{fD}
BT	348.7 ± 25.07 ^{dA}	298.0 ± 12.5 ^{deB}	293.2 ± 10.2 ^{fC}	267.8 ± 10.6 ^{eD}
BM	351.2 ± 19.3 ^{aA***}	298.6 ± 10.9 ^{dB}	292.8 ± 12.5 ^{fgC}	292.8 ± 31.2 ^{cdeC}
CH	314.9 ± 21.0 ^{ijA}	296.7 ± 14.2 ^{fB}	294.1 ± 14.2 ^{eC}	265.9 ± 13.2 ^{fghD}
CD	303.2 ± 22.07 ^{kA}	299.8 ± 11.6 ^{hijB}	284.7 ± 31.2 ^{ijC}	196.2 ± 10.6 ^{ghiD}
CT	309.8 ± 13.2 ^{JkdA}	294.1 ± 9.6 ^{gB}	285.1 ± 10.06 ^{iC}	198.9 ± 10.2 ^{ghD}
CM	310.2 ± 14.08 ^{JdA}	296.0 ± 8.07 ^{fjB}	292.3 ± 11.6 ^{fghC}	211.5 ± 10.6 ^{gD}
KH	300.6 ± 12.2^{iA}	292.2 ± 10.6 ^{hB}	290.3 ± 10.2 ^{gC}	177.8 ± 14.2 ^{hiD}
KD	295.6 ± 1.5 ^{mnA}	285.0 ± 12.5 ^{jB}	290.3 ± 31.2 ^{gC}	166.9 ± 14.2^{jD}
KT	294.4 ± 3.2 ^{mA}	289.5 ± 12.5 ^{ijkA}	290.3 ± 16.2 ^{gB}	178.4 ± 8.2 ^{hC}
KM	297.7 ± 1.5 ^{lA}	290.0 ± 21.5 ^{iB}	288.0 ± 10.2 ^{hiC}	178.5 ± 7.0 ^{iD}

All values are means of three replicates and calculated on wet basis *Trt= Debittering method of different varieties, N= Nabali, C= Crosodi, B= Baladi, K= k_{18}, H= whole table olive, D= ticked table olive, T=scratchy table olive, M= treated with NaOH table olive. **Means ± SD in the same column with the same letter are not significantly different ($P \leq 0.05$).

Antioxidant Content of Fermented Table Olives Varieties during Storage

Antioxidant activities (DPPH inhibition %) of different varieties of table olives and four de-bittering methods of fermentation after one day of fermentation (zero time) and during three months of storage are presented in (Table 3). As shown in the results, antioxidant activities are significantly varied between the de-bittering methods of fermentation in all of the periods of storage, which are represented in the columns and take a significantly letters from a to l as shown in (Table 3). In general, the results of de-bittering methods of fermentation (Table 3) showed that the higher antioxidant activities were found in treated with NaOH followed by ticked, scratchy and whole, respectively. The results of storage at zero time ranged from 29.3 to 45.2 % in the all of table olive varieties and de-bittering methods of fermentation. The values of total phenols of the rest of storage periods (1, 2, and 3 months) are similar to what were found at zero time that where 26.3 to 42.1, 24.2 to 38.8, 14.4 to 38.5%, respectively. Comparison with other researchers of antioxidant results of table olives, there are no studies of de-bittering methods of fermentation were found regarding the treated with NaOH, ticked and scratchy de-bittering methods. Adetuyi and Ibrahim (2014) demonstrated that the reducing power of antioxidants is an important indicator of potential antioxidant activity that decreased during fermentation time. The explanation to decrease antioxidant as (30,) observed that antioxidant reduction with reduce phenol content with ensure that phenols like; hydroxyl-tyrosol, tyrosol, and verbascoside exhibited important antioxidant capacity. The effect of storage during fermentation on total phenols is shown in (Table 3) with a significantly letter from A to D. In general, the antioxidants in all de-bittering methods of fermentation and in table olive verities are significantly reduced. Our

results were agreed with Rietjens et al. (2007) results; they reported that the antioxidant potential was decrease in the percentage of radical scavenging activity during the fermentation period. The explanation as Othman et al. (2009) found that the antioxidant activity in table olive varieties is related to its phenolic content which confirms that antioxidant activity decrease depends on the rate of phenolic loss during fermentation.

Flavonoids of Fermented Table Olive Varieties during Three Month of Storage

Total flavonoid contents of different varieties of table olives and four de-bittering methods of fermentation after one day of fermentation (zero time) and during three months of storage are presented in (Table 4). As shown in the results, flavonoid contents are significantly varied between the de-bittering methods of fermentation in all of the periods of storage, which are represented in the columns and take a significantly letters from a to k as shown in (Table 4). In general, the results of de-bittering methods of fermentation (Table 4) showed that the higher total flavonoid content were found in treated with NaOH followed by ticked, scratchy and whole, respectively. The results of storage at zero time ranged from 60.2 to 101.9 mg/100g in the all of table olive varieties and de-bittering methods of fermentation. The values of total phenols of the rest of storage periods (1, 2, and 3 months) are similar to what were found at zero time that where 42.9 to 81.7, 33.3 to 67.6 and 2.1to 9.6 mg/100g, respectively. Comparison with our results, there is no studies of de-bittering methods of fermentation were found regarding the treated with NaOH, ticked and scratchy de-bittering methods.

The effect of storage during fermentation on total phenols is shown in (Table 4) with a significantly letter from A to D. In general, the total phenols in all de-bittering methods of fermentation and in table olive verities are significantly reduced. Adetuyi and Ibrahim (2014) results was in agreement with our observation of that fermentation process caused a decrease in the total flavonoid and total non-flavonoid content with increased fermentation time. The explanation of the reduction in the total flavonoid contents could be due to as result of harsh condition during table olive process which could be affect the phytochemicals breakdown therefore cell wall integrity which cause a migration of component and cause a migration of some flavonoids Hunaefi et al. (2013).

Table 3. Antioxidant Activity in Fermented Table Olives at Zero Time and During Three Months of Storage

Trt*	Antioxidant Activity			
	Storage period (Months)			
	0	1	2	3
NH*	28.6 ± 3.2 ^{jA}	27.7 ± 1.6^{iB}	25.2 ± 1.9 ^{hC}	14.3 ± 3.2^{iD}
ND	27.6 ± 2.1 ^{kA}	27.0 ± 0.9 ^{ijkA}	26.7 ± 2.1 ^{ghiB}	14.4 ± 1.9 ^{iC}
NT	26.5 ± 1.9 ^{lA}	26.3 ± 1.6 ^{jA}	26.9 ± 1.9 ^{ghA}	16.6± 0.6 ^{kB}
NM	30.1± 2.1 ^{hA}	27.6 ± 2.1 ^{ijB}	26.6 ± 0.9^{gC}	17.5 ± 2.1 ^{gD}
BH	42.2 ± 3.2 ^{cA}	30.4 ± 1.6^{hB}	27.3 ± 1.9 ^{fghC}	18.9 ± 3.2 ^{ID}
BD	41.6 ± 1.9 ^{dA}	30.6 ± 0.9 ^{fgB}	27.5 ± 2.1^{fgC}	20.2 ± 0.9^{hD}
BT	40.8 ± 1.9 ^{fA}	30.8 ± 1.6 ^{fB}	28.2 ± 3.2 ^{fC}	20.7 ± 2.1 ^{gD}
BM	43.1± 2.1 ^{aA**}	31.8 ± 2.9 ^{eB}	30.2 ± 0.6^{efgC}	25.6 ± 2.1 ^{fghiD}
CH	29.3 ± 3.2 ^{iB}	32.2 ± 2.1 ^{defA}	31.9 ± 2.1 ^{eA}	25.7± 0.6 ^{fghC}
CD	28.7 ± 0.9 ^{jB}	33.2 ± 0.9 ^{defA}	32.5± 0.6 ^{deA}	26.7 ± 1.9 ^{fgC}
CT	27.7 ± 1.9 ^{kD}	33.9 ± 1.6 ^{deA}	32.7 ± 1.6^{dB}	27.3± 2.1 ^{fC}
CM	30.2 ± 2.1 ^{hB}	35.1 ± 3.2 ^{dA}	29.7 ± 2.1 ^{efgC}	24.2 ± 3.2 ^{fghiD}
KH	41.2 ± 0.9 ^{eA}	36.3 ± 0.9 ^{cB}	33.9 ± 1.6^{bcdC}	33.0 ± 0.6 ^{dC}
KD	40.7 ± 1.9 ^{fA}	38.0 ± 2.1 ^{bcB}	36.0 ± 3.2 ^{bC}	35.8 ± 0.9 ^{bcC}
KT	39.7 ± 2.9 ^{gA}	39.3 ± 0.9 ^{bA}	38.8 ± 1.9 ^{abB}	36.0 ± 2.1 ^{bC}
KM	42.8 ± 3.2 ^{bA}	42.1± 0.9 ^{aA}	38.9 ± 2.6^{aB}	38.5 ± 3.2 ^{aB}

All values are means of three replicates and calculated on wet basis.*Trt= Treatments, N= Nabali, C= Crosodi, B= Baladi, K= k_{18}, H= whole table olive, D= ticked table olive, T=scratchy table olive, M= treated with NaOH table olive** Means ± SD in the same column and the same raw with the same letter are not significantly different ($P \leq 0.05$).

Table 4. Flavonoids in Fermented Table Olives at Zero Time and During Three Months of Storage

Trt*	Flavonoid Contents			
	Storage period (Months)			
	0	1	2	3
NH*	75.5 ± 5.6^{jA}	70.4 ± 6.2 ^{defB}	67.6 ± 5.1 ^{aC}	4.4 ± 1.0 ^{deD}
ND	74.8 ± 2.2^{kA}	42.9 ± 2.1 ^{kB}	33.3± 4.1 ^{gC}	2.1 ± 1.6^{fghD}
NT	73.8 ± 6.2^{lA}	44.2 ± 2.6 ^{jB}	38.8 ± 2.1 ^{fghiC}	2.2 ± 1.1 ^{fgD}
NM	76.1 ± 2.07 ^{iA}	50.6 ± 2.4 ^{ghijB}	39.2 ± 1.6^{fghC}	2.6 ± 0.9 ^{fD}
BH	64.7 ± 5.6 ^{nA}	57.4 ± 3.1 ^{gB}	45.8 ± 2.0 ^{efC}	4.6 ± 2.4 ^{dD}
BD	63.7 ± 3.1 ^{oA}	50.7 ± 2.2^{ghijB}	40.5 ± 2.1 ^{fgC}	3.4 ± 1.6^{efgD}
BT	62.8 ± 2.1 ^{pA}	52.7 ± 1.6^{ghiB}	41.9 ± 3.2 ^{fC}	3.7 ± 2.0 ^{efD}
BM	65.3 ± 1.2^{mA}	54.8± 3.1 ^{ghB}	44.5 ± 3.6^{efgC}	3.8 ± 2.1 ^{eD}
CH	94.2 ± 2.1 ^{bA}	81.73 ± 7.7 ^{aB}	52.5 ± 2.1 ^{deC}	7.7± 6.2 ^{bD}
CD	90.5 ± 7.6^{cA}	64.4 ± 2.1 ^{fghB}	46.8 ± 0.1 ^{eC}	6.2 ± 5.0 ^{cdD}
CT	87.6 ± 6.3 ^{dA}	67.4 ± 5.6^{fB}	50.1± 4.1 ^{defC}	6.9 ± 2.6^{cD}
CM	96.9 ± 8.2^{aA**}	82.5 ± 7.1 ^{aB}	52.1 ± 4.6^{defgC}	7.6 ± 5.4 ^{bcdD}
KH	80.1 ± 7.1 ^{fA}	69.3 ± 5.2^{eB}	61.8 ± 4.1 ^{abcC}	9.6± 8.0 ^{aD}
KD	79.8± 6.6 ^{gA}	71.6± 6.1 ^{deB}	50.9 ± 2.1 ^{bC}	8.4 ± 6.2 ^{abcdD}
KT	78.6 ± 2.7 ^{hA}	72.5 ± 02.6^{dB}	58.4 ± 3.2 ^{cC}	8.8 ± 5.6 ^{abcD}
KM	80.9 ± 7.1 ^{eA}	74.1 ± 3.9 ^{cB}	61.7± 1.4 ^{abcdC}	9.0 ± 7.1 ^{abD}

All values are means of three replicates and calculated on wet basis
*Trt= Debittering method of different varieties, N= Nabali, C= Crosodi, B= Baladi, K= k_{18}, H= whole table olive, D= ticked table olive, T=scratchy table olive, M= treated with NaOH table olive
**Means ± SD in the same column with the same letter are not significantly different ($P \leq 0.05$).

Conclusions

Fresh table olive varieties and de-bittering methods of fermentation were investigated for the effect of fermentation on the total phenol, antioxidant activity, total flavonoid, chemical composition, color, texture, pH, acidity and sensory attributes for a storage period of 0, 1, 2, 3 months. Fresh table olives were found to have higher contents of total phenols, antioxidant activity and flavonoids than fermented samples. Total phenols, antioxidant activity and flavonoids contents of fermented table olive decrease significantly during 0, 1, 2 and 3 months of storage, due to the diffusion into brine solution. Despite the reduction in the total phenols, antioxidant activity and flavonoids contents, fermentation process keeps good amounts of investigated phytochemicals during storage.

Acknowledgement

The support that was provided by Deanship of Research at Jordan University of Science and Technology is appreciated.

References

ADETUYI, F.O; IBRAHIM, T. A. Effect of Fermentation Time on the Phenolic, Flavonoid and Vitamin C Contents and Antioxidant Activities of Okra (Abelmoschus esculentus) Seeds. **Nigerian Food Journal**, v. 32, n. 2, , p 128-137, 2014.

AL-ISMAIL, K.M.; AHMAD, R.; AL-DABBAS, M.; AJO, R.Y.; RABABAH, T. Some physiochemical properties of olive and olive oil of three Jordanian olive varieties. **Lakrivista Italiana Delle Sostange Grasse**, Milano, v.88, p.191-198, 2011.

ALYGIZAKIS, M. **The elaboration and canning of the table olit'es**. Athens: Mavrommatis, 1982.

ASAMI, D. K.; HONG, Y. J.; BARRETT, D. M.; MITCHELL, A. E. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. **Journal of Agricultural and Food Chemistry**, Washington, v.51, n.5, p.1237-1241, 2003.

BOSKOU, G.; SALTA, F.N.; CHRYSOSTOMOU, S.; MYLONA, A.; CHIOU, A.; ANDRIKOPOULOS, N.K. Antioxidant capacity and phenolic profile of table olives from the Greek market. **Food Chemistry**, London, v.94, n.4, p.558-564, 2006.

BRAND-WILLIAMS, W.; CUVELIER, M.E.; BERSET, C.L.W.T. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology, 28, n.1, p.25-30, 1995.

BRENES, M.; GARCIA, P.; DURAN, M.C.; GARRIDO, A. Concentration of phenolic compounds change in storage brines of ripe olives**. Journal of Food Science**, Chicago, v.58, n.2, p.347-350, 1993.

BRENES, M.; REJANO, L.; GARCIA, P.; SANCHEZ, A. H.; GARRIDO, A. Biochemical changes in phenolic compounds during Spanish-style green olive processing. **Journal of Agricultural and Food Chemistry**, Washington, v.43, n.10, 2702-2706, 1995.

FERNÁNDEZ, A.G.; ADAMS, M.R.; FERNÁNDEZ-DÍEZ, M.J. **Table olives**: production and processing. Berlin: Springer Science Business Media, 1997.

HARBORNE, J.B.; WILLIAMS, C.A. Advances in flavonoid research since 1992. **Phytochemistry**, New York, v.55, n.6, p.481-504, 2000.

HUNAEFI, D.; AKUMO, D.N.; SMETANSKA, I. Effect of fermentation on antioxidant properties of red cabbages. **Food Biotechnology**, London, v27, n.1, p.66-85, 2013.

LANZA, B. Abnormal fermentations in table-olive processing: microbial origin and sensory evaluation. **Frontiers in Microbiology**, Lausanme, v.4, p.91, 2013.
Lanza , (2013).

MAJ - Ministry of Agriculture of Jordan. **Annual report**. 2008 .

MALHEIRO, R.; MENDES, P.; FERNANDES, F.; RODRIGUES, N.; BENTO, A.; PEREIRA, J.A. Bioactivity and phenolic composition from natural fermented table olives. **Food Function**, Cambridge, v.5, n.12, p.3132-3142, 2014.

MALHEIRO, R.; SOUSA, A.; CASAL, S.; BENTO, A.; PEREIRA, J.A. Cultivar effect on the phenolic composition and antioxidant potential of stoned table olives. **Food and Chemical Toxicology**, Oxford, v.49, n.2, p.450-457, 2011.

MARINOVA, D.; RIBAROVA, F.; ATANASSOVA, M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. **Journal of The University of Chemical Technology and Metallurgy**, Sofia, v.40, n.3, p.255-260, 2005.

METTOUCHI, S.; BEY, M. B.; TAMENDJARI, A.; LOUAILECHE, H. Antioxidant activity of table olives as influenced by processing debittering method. **International Journal of Chemical and Biomolecular Science**, Boston, v. 2, n.1, p.8-14. 2016.

MOUSSAVI, M. Effect of polar substituents on autoxidation of phenols. **Water Research**, New York, v.13, n.12, p.1125-1128, 1979.

OLIVERA, D.F.; MUGRIDGE, A.; CHAVES, A.R.; MASCHERONI, R. H.; VIÑA, S.Z. Quality attributes of Okra , (Abelmoschus esculentus L. Moench) pods as affected by cultivar and fruit size. **Journal of Food Research**, 1, n.4, 224.

OTHMAN, N.B.; ROBLAIN, D.; CHAMMEN, N.; THONART, P.; HAMDI, M. Antioxidant phenolic compounds loss during the fermentation of Chétoui olives. **Food Chemistry**, London, v.116, n.3, p.662-669, 2009.

PEREIRA, J.A.; PEREIRA, A.P.; FERREIRA, I.C.; VALENTÃO, P.; ANDRADE, P.B.; SEABRA, R.; BENTO, A. Table olives from Portugal: phenolic compounds, antioxidant potential, and antimicrobial activity**. Journal of Agricultural and Food Chemistry**, Washington, v.54, n.22, p. 8425-8431, 2006.

PERPETUINI, G.; SCORNEC, H.; TOFALO, R.; SERROR, P.; SCHIRONE, M.; SUZZI, G.; LICANDRO-SERAUT, H. Identification of critical genes for growth in olive brine by transposon mutagenesis of Lactobacillus pentosus C11. **Applied and Environmental Microbiology**, Washington, v.79, n.15, p.4568-4575, 2013.

RABABAH, T.M.; HETTIARACHCHY, N.S.; HORAX, R. Total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and tert-butylhydroquinone. **Journal of Agricultural and Food Chemistry**, Washington, v.52, n.16, p.5183-5186, 2004.

RIBAROVA, F.; ZANEV, R.; SHISHKOV, S.; RIZOV, N. α-Tocopherol, fatty acids and their correlations in Bulgarian foodstuffs. **Journal of Food Composition and Analysis**, New Yok, v.16, p.659–667, 2003.

RIETJENS, S.J.; BAST, A.; HAENEN, G.R. New insights into controversies on the antioxidant potential of the olive oil antioxidant hydroxytyrosol. **Journal of Agricultural and Food Chemistry**, Washington, v.55, n.18, p.7609-7614, 2007.

ROBARDS, K.; PRENZLER, P.D.; TUCKER, G.; SWATSITANG, P.; GLOVER, W. Phenolic compounds and their role in oxidative processes in fruits. **Food Chemistry**, London, v.66, n.4, p.401-436, 1999.

ROMERO, C.; BRENES, M.; YOUSFI, K.; GARCÍA, P.; GARCÍA, A.; GARRIDO, A. Effect of cultivar and processing debittering method on the contents of polyphenols in table olives. **Journal of Agricultural and Food Chemistry**, Washington, v.52, n.3, p.479-484, 2004.

SAYIN, F.K.; ALKAN, S.B. The effect of pickling on total phenolic contents and antioxidant activity of 10 vegetables. **Journal of Food and Health Science**, Istanbul, v.1, n.3, p.135-141, 2015.

SILVA, S.; GOMES, L.; LEITAO, F.; COELHO, A.N.; BOAS, L.N. Phenolic compounds and antioxidant activity of Olea europaea L. fruits and leaves. **Food Science and Technology International**, Ibaratá, v.12, n.5, p.385-395, 2006.

SINGLETON, N.L.; ROSSI, J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. **American Journal of Enology and Viticulture**, Davis, v.16, n.3, p.144-158, 1965.

SOLINAS, M.; DI GIOVACCHINO, L.; CUCURACHI, A. I polifenoli delle olive e dell'olio di oliva. **Ann. Ist. Sperimen. Elaiotec**, v.5, p.105-126, 1975.

TALHAOUI, N.; GÓMEZ-CARAVACA, A.M.; LEÓN, L.; DE LA ROSA, R.; FERNÁNDEZ-GUTIÉRREZ, A.; SEGURA-CARRETERO, A. Pattern of variation of fruit traits and phenol content in olive fruits from six different cultivars. **Journal of Agricultural and Food Chemistry**, Washington, v.63, n.48, p. 10466-10476, 2015.

ÜNAL, K.; NERGIZ, C. The effect of table olive preparing debittering methods and storage on the composıtıon and nutrıtıve value of olives. **Grasas y Aceites**, Sevilla, v.54, n.1, p.71-76, 2003.

VOSSEN, P. Olive oil: history, production, and characteristics of the world's classic oils. **HortScience**, Alexandria, v.42, p.1093–1110, 2007.