VOL. 12, NO. 6, JUNE 2017 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

www.arpnjournals.com

ANTIOXIDANTS CONTENT OF OLIVE OIL PRODUCED FROM TWO OLIVE VARIETIES AT DIFFERENT HARVESTING STAGES

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ABSTRACT

The objective of this study was theinvestigation of the antioxidant content of olive oil from two varieties Nabali and Rumiat different harvesting stages in order to achieve the optimum harvesting date. The proper harvesting dates were determined using maturation index according to International Olive Council method. Our results showed that the proper harvesting stage for Rumi olives was during the periods October 24thtoOctober 28th, whereas, the proper harvesting stage for Nabali olives was between November 4thandNovember 7th. The acidity and peroxide value of olive oil from two varieties was within the range of extra virgin olive oil according to IOC. The results showed a significant decrease in squalene contents in Rumi and Nabali olive oil parallelly with the progress of the harvesting stages. In general, most of antioxidants contents of olive oil of the two selected olive varieties were gradually increase up to the proper harvesting dates then declined.

Keywords: antioxidant, maturation index, harvesting stage, olivevarieties, oliveoil.

INTRODUCTION

Olive tree *Olea europaea*, a native tree of the Mediterranean area and parts of Asia,is now widely cultivated in many other world countries for production of olive oil and table olives (Saunders, 2014). The vast majority of olives (over 90%) are grown for oil (Carr, 2014). olive oil is the most common component in the Mediterranean diet due to its nutritional value and benefits for human health. Consumption have becoming maximized in the last few years (Escrich, *et al*., 2011), Mediterranean countries are considered more producing and exporting olive and its products. Together they manufacture approximately 90% of the total olive oil produced (Ramos and Santos, 2010; Girelli *et al*. 2016).

Olive oil is composed primarily by triacylglycerols (~99%) and secondarily contains small quantities of free fatty acids (FFA), glycerol, phosphatides, pigments, flavor compounds, sterols, unidentified resinous substances and other constituents (Boskou *et al*., 2006). Olive oil constituents can be divided into two categories: the saponifiable fraction (triacylglycerols, FFA, phosphatides, etc.), and the unsaponifiable fraction (hydrocarbon, fatty alcohols, etc.). The unsaponifiable constituents of virgin olive oil account for 0.5 to 1.5% of the oil. Some of the non- glyceride constituents (unsaponifiable matter) contribute to the flavor quality of olive oil (Kiritsakis *et al*., 2000).

Ben Témime *et al*. (2006) showed that the climatic conditions, in particular the rainfall during the growing and the ripening of the olive fruits, all influence biosynthesis of the oil of composition, quality and concentration of phenolic compounds within the fruit. During maturing, important chemical changes occur inside the fruits, which are related to the synthesis of chemical compounds that may affect virgin olive oil quality (Salvador *et al*., 2001). Unless the mainly effects of the maturation degree over yield, color, fruit deterioration, etc., ripening degree should be considered a priority criterion in order to define the physicochemical and organoleptical quality of oil (Negre *et al*. 2011).

The total antioxidant activity of commercial oils is mainly due to their polyphenol and alpha-tocopherol content (Tawalbeh *et al*., 2006). Polyphenols from olives and olive oil are believed to be effective antioxidants owing to their free radical-scavenging and metal-chelating properties (Keceli and Gordon, 2001). Squalene exhibited a weak antioxidant activity at the early stages of autoxidation whereas tocopherols showed their antioxidant effect after 3 weeks of storage (Naziri, *et al*. 2016), also efficients as singlet oxygen scavengers, thereby inhibiting photooxidation (Dessi, 2002).

The optimam harvesting time is the first and most crucial decision to make in the production process of virgin olive oil. It is determined by several factors: fruit retention force, oil content in the olive fruit, chemical composition, sensory attributes of olive oil and industrial yield Guzmán *et al* (2015). Because these factors evolve as the fruit matures, it is necessary to use methods to monitor the maturity process. The most common method for evaluating maturity in olives is based on the visual determination of a maturation (harvest) index (Kiritsakis *et al*., 2000). Other methods to determine the stage of maturity in olives include determining fruit firmness.In addition, Salvador *et al*., (2001), have proposed the changes in total polyphenols content of the oil.

The main objective of this study was to determine the optimum harvesting periodes and the influence on antioxidant contents of the most popular olive varieties in Jordan (Rumi and Nabali).

MATERIAL AND METHODS

Olive samples

Olive samples were obtained from homogeneous olive fruits of two varieties: Nabali and Rumi. Varieties were collected at three harvesting stages at Maturation Index) 4, 5 and 6) from different trees of the same varieties at the same farm (containing Nabali and Rumi olive trees) located in Bani Kanana district (North of Jordan). The harvesting stages of olive fruit were determined by calculating the Maturation Index mentioned below.

Determination of the olive harvesting stages

The harvesting stages were determined by the maturation index (MI) according to the IOC method (2011). One hundred olive fruits of each variety (one sample) were randomly taken from different sites of each olive variety trees, the process was repeated eight times and the average was calculated, the number of olive samples at each harvesting stage (Maturation Index 4, 5 and 6) was eight samples from each variety. The determination of the maturation index was done as follows:

Maturity index category

0: skin colour deep green category
1: skin colour yellow-green category
2: skin colour green with reddish spots on < half the fruit surface. Start of colour change category
3: skin colour with > half the fruit surface turning reddish or purple. end of colour change category
4: skin colour black with white flesh category
5: skin colour black with < half the flesh turning purple category
6: skin colour black with not all the flesh purple to the stone category
7: skin colour black with all the flesh purple to the stone the maturity index is obtained by applying the following formula where a, b, c, d, e, f, g, and h are the number of fruits in each of the colour categories 0, 1, 2, 3, 4, 5, 6, and 7 respectively:

$$\text{Maturation Index} = \frac{a0 + b1 + c2 + d3 + e4 + f5 + g6 + h7}{100}$$

Olive oil extraction

Olive's samples collected at each harvesting stage for each variety were sending directly to the olive press. The olive oil extraction of the collected olives was carried out in Al-Sadoon press (Alpha Laval, type) located in Bani Kanana districtin Jordan. All samples were cold pressed (28-32^{o}C) in the end of the olive collection day. An olive oil sample (1 liter) of each harvesting stage for each variety was withdrawn at the middle of its extraction process period. The samples were kept at freezing temperature -20 Cfor further experimentation in the dark.

Chemical analysis

Acidity avalue and peroxide value was determined according to AOCS (1998) method

Total phenols analyses

The Phenol content of olive oil was determined following the method of Gutifinger (1981). Ten grams of olive oil were weighed into a 250ml Erlenmeyer flask and dissolved with 50ml hexane. Twenty ml of aqueous methanol (60%) were added and mixed vigorously for 2 minutes. The methanolic phase was removed and placed in a beaker (at each two phases separated). The combined extracts were layout to dryness in a vacuum rotary evaporator (Heidolph, Germany) at 70ºC. The residue was dissolved in 100ml methanol and stored at low temperature. One ml from the methanolic extract was placed into a 10ml volumetric flask. Five ml distilled water and 0.25ml (2N) Folin Ciocalteau was added and mixed well for 3min. Two ml of Na_2CO3 (17%) were added and the flask was filled with distilled water up to the mark. The absorbance of the blue color formed was measured, after 1 hr, at 725nm. The phenolic compounds were extracted by aqueous alcohol solution (40:60) and the extraction process was repeated 2 times.

Analysis of squalene

Squalene was determined in olive oil samples according to the method of (Bianchia *et al*., 2001) as follows;

Cold saponificatiom

Accurately, about 200mg of filtered olive oil was weighed into a 15ml screw capped test tube, 10ml of 1N KOH (85%GCC laboratories, UK) dissolved in methanol, 1ml of a squalan solution (1mg/ml) as an internal standard (IS) were added to the sample. The mixture was shaken until diminishing of dispersed oil particles; the sample was then placed in a dark place at room temperature for 20-24 hours (Park and Addis, 1986). The saponified oil was transferred into a 100ml separatory funnel and 10ml of distilled water was added. Unsaponifiables were extracted 3 times with 10, 5 and 5ml of diethyl ether (99% HPLC grade, GCC laboratories, England). Pooled diethyl ether extract was washed once with 0.5N KOH (85%GCC laboratories, UK) and 5 times with 5ml distilled water. The ether was filtered using Whatman No.1 filter paper and dried over anhydrous sodium sulfite (Na_2SO4) (GCC laboratories, UK). The filter paper and the anhydrous sodium sulfite were washed twice with 5ml diethyl ether (99% HPLC grade, GCC laboratories, England), the solvent was concentrated using rotary evaporator (Heidolph, Germany) at 25ºC and under vacuum to about 1ml, then dried under nitrogen stream (ultra pure) after being transferred to a 5ml vial.

Derivatization of the unsaponifiables

The trimethylsilyl derivatives (TMS) of the components of the unsaponifiables were obtained according to Giacometti (2001) as follows: a portion of 500*M*l of silylation reagent, consisting of a mixture of

pyridine (CBH, Nottingham, UK) / hexamethyldisilazane (Janssen, Belgium) / trimethylchlorosilane (Fluka, Switzerland), 9:3:1 (V/V/V) (prepared previously and stored at refrigerator) was added to the unsaponifiable matter, the vial was left for 40 minutes at ambient temperature. The pyridine was evaporated under a stream of nitrogen (extra pure), then 4ml of hexane (GC grade, Lab scan, Dublin) was added and shaked vigorously, the solution was transferred into a 5ml screw capped test tube and centrifuged at 4000 round/minute for 5 minutes (Hettich EBA-20, Germany). The hexane layer was analyzed immediately by gas chromatography

Gas chromatographic analysis

The TMS derivatives of the unsaponifiables were analyzed on Shimadzu gas chromatograph (model GC-2010, Shimadzu Inc., Koyoto, Japan) supplied with split-splitless injector port and flame ionization detector, an RTX-65TG (Restek, USA) capillary column (30m x 0.25mm internal diameter; film thickness was 0.01*m*m; and the active ingredients were 35% diphenyl-65%dimethyl polysiloxan) was used. Oven analysis temperature started at 180ºC and was raised at rate of 3ºC/minute to 280ºC then left at 280ºC for 5 minutes. The injector temperature was 290ºC, and the detector temperature was 310ºC, flow rate of nitrogen (carrier gas) was 1.2ml/minute and split ratio was 1:30. Squalene peaks were identified by comparison with the retention times of reference standards of squalane (sigma, 98%, GC grade, Japan).The concentration of the analyte was calculated using the internal standard technique (IS) of squalane. The chromatogram and the peak area were recorded by a digital integrator (Shimadzu C-R8A).

Statistical analysis of the experimental data

Three variables were statistically analyzed atthree harvesting stages of virgin olive oils of two olive varieties; each sample was analyzed three times. The available data for the two varieties were analyzed by the complete random design (CRD) in factorial arrangement according to the general linear model procedure of SAS (SAS, 1994). Data were analyzed using the analysis of variance (ANOVA) procedure. Duncan's multiple range test ($P < 0.05$) was applied to determine significance between different treatments.

RESULTS AND DISCUSSIONS

Determination of the proper harvesting stages

Table-1 showes the MIfor the two varieties (Rumi and Nabali); the proper maturation stage for Rumi olives is earlier than that in Nabali olives. This finding agreed with that founded by (Tawalbeh *et al*., 2006) with differences in dates corresponding to the MI which may be explaind by differences of several factors such as quantities and date of rainfull, temperatures, weathers from year to year. Qutub *et al.* (2010) they reported that all parameters regarding fruit ripening and qualitative attributes showed that a good harvest period seemed to be mid-late October for Nabali Baladi while the good harvest period for Nabali Mohassan seemed to be late October-early November.

Al-Maaitah *et al.* (2009) reported that the majority of olive oil produced (94%) is not of the top marketable quality, as the fruit has not been picked at the ideal harvest time. Time of harvesting may have a significant effect on oil quality as well as, yield, oil stability and sensory characteristics.

Table-1. Maturation index (MI) for two varieties of olive fruit at different harvesting dates.

Maturation index	**Date day/ month**
Rumi olive variety	
4.0± 0.1*	1st October – 3rd October
5.0± 0.1	24th October-28th October
6.0± 0.1	12th November– 17th November
Nabali olive variety	
4.0± 0.1	11th October – 15th October
5.0± 0.1	4th November – 7th November
6.0± 0.1	22nd November - 27th November
*Each value is a mean of eight replications, ±standard deviation	

Traditionally, olives are harvested at the green-yellow or black-purple stage. Since not all of the fruits mature simultaneously at the same tree, harvesting should take place when the majority of the fruits are at optimum maturity. This is not always possible because other factors may also affect harvesting time, such as availability of enough farm labor, availability of olive oil mills and weather conditions, etc. (Kiritsakis *et al.,* 2000). According to (Tawalbeh *et al*., 2006), the proper ripening stage of olives in Jordan corresponding to MI range between 4.5 and 5.5, which deviates from the universally accepted index (3.5-4.5) set up by Aparicio and Luna (2002). It is believed that this range is more appropriate for our local conditions and varieties (Tawalbeh *et al*., 2006). Maturation index values lesser or higher than this range corresponds to unripe and over ripe olives respectively.

The maturity level of fruit for olive oil production with the best quality characteristics coincides with a maturation index of 5 (Kiritsakis *et al*., 2000). TheMI values of Italian olive cultivars for olive oil production were 2.48, 2.74, 3.25, 3.57 and 6.2 at November.14, Nov. 27, December.10, Dec.20 and January 1 respectively (Kiritsakis *et al*., 2000). This indicates that the proper harvesting dates for Italian cultivars were between December 20 and January 1, which is later than the Jordainian olive cultivars. ″These differences indicate that the MI should be evaluated for each case separately, since it depends on olive cultivar, growing area, climate conditions, etc.″

Olive oil acidity and peroxide value

Table-2 shows that the acidity of Rumi olive oils was slightly but significantly increased ($p < 0.05$) and remained in the range of extra virgin oil during the harvesting dates whereas the Nabali oil samples increased only at the last date with less than Rumi olive. These results agree with those obtained by Al-Rousan (2004) for Nabali Mohassan cultivar, as well as "Cornicabra" a Spain variety (Salvador *et al.*, 2001). On the other hand, these results did not agree with those reported by Humeid *et al*., (1992) who reported that there was no significant effect of the time of harvesting of olives on the acidity of the extracted Nabali olive oil in Jordan. Additionally, Salvador *et al*. (2001) reported that olives at a later stage of ripening give oils with higher levels of free acidity since they undergo an increase in enzymatic activity, especially by lipolytic enzymes, and are more sensitive to pathogenic infections and mechanical damage. However, the relatively low levels of acidity in both Rumi and Nablai olive oil reflects the high quality of the olive fruits before pressing and indicates that the found differences between the two varieties are probably only due to endogenous enzymes activity.

Table-2. Acidity and peroxide value of olive oils produced at differrnt harvesting stages of Nabali and Rumi olives.

Maturation index	Rumi olive variety		Nabali olive variety	
	Acidity (Oleic acid)%	**Peroxide value** (mEq O_2/kg oil)	**Acidity** (Oleic acid)**%**	**Peroxide value** (mEq O_2/kg oil)
4.0± 0.1	0.48 ±0.03^{c}	4.6± 0.4^{c}	0.46±0.01^{b}	4.8±0.6^{b}
5.0± 0.1	0.59 ± 0.02^{b}	5.5±0.7^{b}	0.53±0.03^{ab}	5.4±0.5^{ab}
6.0± 0.1	0.64 ±0.05^{a}	6.6±0.8^{a}	0.57±0.02^{a}	7.4±0.9^{a}

*Each value is a mean of three replications, ±standard deviation.
Means with different matching letters within a column and variety are significantly different ($p < 0.05$) according to Duncan's Multiple Range test

The peroxide value test was the most effective in evaluating the rancidity of virgin olive oil during all stages of rancidity except the late declining stage (Amr and Abu-Al-Rub, 1995). The results in table 3 showed gradual and significant ($p < 0.05$) increase in PV of the two oilvariates. PV values of olive oil for both varieties were about 4.3 meq O_2/kg at the N4 MI to be about 6.4 meq O_2/kg at the N6 MI for Rumi oil, while for Nabali oil was 4.5 meq O_2/kg at N4 MI to 7.1 meq O_2/kg at N6 MI.

These results agree with those reported by Abu-Al-Rub (1992) who found that the peroxide value of Nabali virgin olive oil was 4.2 mEq O_2/kg oil, and with those found by Al-Rousan (2004) who found that they ranged between (2.5-11.2) at five harvesting periods of Nabali Mohassan. On the other hand, (Humeid *et al*., 1992) reported that the peroxide values did not exceed 2.35 mEq O_2/kg in Nabali olive oil extracted at laboratory scale and various levels of ripening.

Antioxidants content in olive oil at different harvesting stage

Table-3 Showed that the total phenol contents in olive oil were significantly ($p < 0.05$) increased until the proper harvesting stage then significantly decreased for both olive varieties.

This finding can be explained because some phenolic acids (Gallic, benzoic, caffeic, ferulic, vanillic, p-coumaric and shikimic acids are destroyed during olive fruit maturation, while some acids are synthesized (Kiritsakis *et al.*, 2000). This also might be attributed to the fact that the phenolic substances in olives progressively increase to a maximum level at the half pigmentation stage (proper harvesting date) and decreasing sharply as ripening progresses (Rotondi *et al,* 2004; and Salvador *et al,* 2001). Additionally, it was found that the concentration of oleuropein (the principal phenolic compound) in olive changed significantly during fruit development (Ryan *et al.*, 1999). Also it was reported that the virgin olive oil obtained from overripe olives is characterized by a higher yield but it contains smaller amounts of total phenols. The decrease in total phenols content "as ripening progresses" may be due to the presence of protocatechuic (3, 4-dihydroxybenzoic acid) and Gallic acids, which could also be used as a good marker for the stages of ripeness. The two compounds are easily degraded during the ripeness process (Aparicio and Luna, 2002).

Table-3. Total phenols, Tocopherol and Squalene contents of olive oils produced at differnt harvesting stages of Nabali and Rumi olives.

Maturation index / Rumi olive variety	Date day/ month	Totalphenols mg/kg	Tocopherols mg/kg	Squalene mg/kg
4.0± 0.1*	1st October–3ed October	244c± 6	271b± 12.6	6118a± 140.1**
5.0± 0.1	24th October-28th October	362a ± 9	348a ± 22.6	4847b± 130.6
6.0± 0.1	12th November– 17th November	301b ± 11	252b± 18.2	3788c± 68.9
Nabali olive variety				
4.0± 0.1	11th October – 15th / October	210c± 8	252b± 12.5	6167a± 177.4
5.0± 0.1	4th November – 7th November	358a± 11	332a± 16.7	4917b ± 161.4
6.0± 0.1	22nd November - 27th November	298b± 10	240b± 10.7	3814c ± 88.4

*Each value is a mean of eight replications, followed by the standard deviation.
** Means with different matching letters within a same column and variety are significantly different ($p < 0.05$) according to Duncan's Multiple Range test.

Several factors may explain the differences in the total phenols content in olive oil such as cultivation procedures, environmental factors, harvesting stage, storage conditions and processing of the fruit (Mousa *et al.*, 1996; Kiritsakis and Min, 1989). Therefore, it can be suggested that the main factors that may affect on the quantities of total phenol contents of the main Jordanian olive oil varieties (Rumi and Nabali) are the olive varieties and the olive harvesting stages.

The data in Table-3 indicates that harvesting stages had significant ($p < 0.05$) effect on the total phenols content of olive oil of both studied varieties. The total phenols content in Rumi olive oil was 244, 362, and 301 mg/kg, while their content in Nabali olive oil was 210, 358, and 298 mg/kg before, at and after the proper harvesting stages respectively. These findings agreed to some limits with those found by similar studies investigated the contents of total phenols of olive oil in Jordanian Rumi and Nabali olive varieties (Tawalbeh *et al*., 2006).

In Table-3, The Tocopherol content in olive oil was significantly ($p < 0.05$) increased until the proper harvesting stage then decreased for both olive varieties. Kiritsakis *et al.*, (2000) theyreported that the fluctuation in Tochopherol content of olive oil is due to their gradualdestruction during processing and storage. Furthermore, (Salvador *et al*., 2001) observed that alpha-tocopherol content in olive oil varied slightly during "Cornicabra" olives ripening (Spain variety) and a clear trend with maturity was not clear. Variety of olives had significant ($p < 0.05$) effect on the tocopherols content in olive oil. Tocopherols content were found to be significantly higher ($p < 0.05$) in Rumi olive oil than those of Nabali olive oil throughout the harvesting stages. However, the tocopherols content of both studied varieties also was relatively high. Almost a close result (240mg/kg) for tocopherols content in olive oil was reported earlier by (Gutifinger and letan, 1974). Olive harvesting stages had no significant ($p< 0.05$) effect on the tocopherols content in both Rumi and Nabali olive oil.

In this study, Squalene was determined also before, during and after the proper harvesting stages in Nabali and Rumi olive oils. The results in Table-3 show a significant decrease in squalene ($p < 0.05$) contents in Rumi and Nabali olive oil parallel with the progresse of the harvesting stages. These results may be explained on the basis that squalene is a biochemical precursor of sterols; therefore, the biosynthesis of sterols (especially *B*-sitosterol) may be on the expense of the squalene content. These results agree with those reported by (Rodriguez-Estrada, 2000) who found that in oil samples extracted from olives harvested at different ripening levels, the level of epoxy-squalenes varies. Since they were practically absent in the oils obtained from mature olives, it can be stated that epoxy-squalenes are precursors to the steroid biosynthesis, i.e. lanosterol in the animal and cycloarthenol in the vegetal ones. Data in Table-3 indicate that the squalene contents in olive oil obtained from both olive varieties and at all harvesting stages) are nearly agreed with that found by (Tawalbeh *et al*., 2006), but relatively high level in comparison with data of virgin olive oil in other countries, where other researchers informed that the concentration of sequalene in olive oil ranged 170-4600 mg/kg (Grigoriadou *et al*., 2007) and Popa *et al* (2015) reported that the concentration was 5640mg/kg. Wihle, Psomiadou and Tsimidou, (2002) reported a squalene content ranging between (1360-7080mg/kg).

Table-3 indicates that variety of olives had no significant ($p < 0.05$) effect on the content of squalene in olive oil, The squalene content in Rumi olive oil was slightly lower than those in Nabali olive oil.

CONCLUSIONS

According to the obtained results, the acidity and peroxide value of olive oil from two varieties was within the range of extra virgin olive oil according to IOOC. However, oil samples can be considered as a good source of total phenols, tocopherols, and squalene. The total phenols and tochopherols of olive oil of the two selected olive varieties were gradually increased up to the most

suitable harvesting stages then declined. The proper harvesting atage, where the maturation index is around 5, depends clearly on olive variety. The proper harvesting stage for Rumi olives in the studied farm /2009 seasons was during the periods 24/10-28/10.While, the proper harvesting stage for Nabali olives was between 4/11-7/11. The results showed a significant decrease in squalene contents in Rumi and Nabali olive oil parallelly with the progress of the harvesting stages. Harvest olives during the proper harvesting stages using harvesting index proposed by International Olive Council in order to produce olive oil with high quality.

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