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ORIGINAL RESEARCH PAPER

CHARACTERIZATION OF OLIVE OIL OBTAINED FROM WHOLE FRUIT AND FRUIT FLESH OF CULTIVAR: KAISSY GROWN IN SYRIA

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The quality of extra virgin olive oils (EVOO) from whole fruits and fruit flesh of Kaissy olive (*Olea europaea*) cultivar was investigated in this study. Acid value (AV), peroxide value (PV), iodine value (IV), specification number (SV), Thiobarbituric acid (TBA) value, phenol content, refractive index (RI) and viscosity were measured after 0, 6 and 12 months of storage. The physicochemical properties of oil extracted from whole fruit and fruit flesh samples of olive were: AV (0.32 and 0.40%), PV (4.79 and 6.13%), TBA (0.056 and 0.052 mg MDA kg^{-1} oil), IV (84.41 and 83.87 g^{-1} oil), SV (195.48 and 187.56 mg KOH g^{-1} oil), total phenolic (339.52 and 226.68 mg gallic acid kg^{-1} oil), RI (1.4669 and 1.4668) and viscosity (129.33 and 130.00 mPa s^{-1}) respectively. The results demonstrated that the AV, PV, RI and viscosity values significantly ($p<0.05$) increased, while TBA value and total phenolic content significantly ($p<0.05$) decreased during storage.

Keywords: Olive oil, Storage time, Phenolic contents, Acidity, Peroxide, Syria

Introduction

Vegetable oils have become significant ingredients in food preparation or processing in homes, restaurants, food manufactures or for medicinal purpose (Dawodu *et al.*, 2015; Swaminathan and Jicha, 2014).

Olive oil is unique among other oils in that it is a product of a fruit, rather than a seed. Currently, olive oil is very popular for its nutritive and health-promoting potential (Sarolic *et al.*, 2014). Table olives and olive oil are traditional components of the Mediterranean culture and diet. About 90% of the olive fruit world production is used for oil extraction; according to the International Olive

Council (IOC), the annual world production of olive oil is 2,425,000 tones, making the olive tree the sixth most relevant oil crop in the world (Bajoub *et al.*, 2015). On commercial product value basis, olive oil has an important place in the world's production, having a 15% share of the world oil trade (Luchetti, 2000). The price of olive oil is usually 2-5 times higher than that of other vegetable oils depending on many factors such as the origin and the harvesting period of the olive fruits (Luchetti, 2000). There are several ways of defining quality which have been proposed over the last few years to evaluate extra-virgin olive oil (EVOO) and virgin olive oil (VOO) quality, and to ensure its authenticity (Ben-Ayed *et al.*, 2013).

Several studies investigate the effect of olive storage, processing systems, and oil storage conditions on the flavor and overall quality of olive oil (Angerosa, 2002). One of the most severe quality problems of oil is its oxidative rancidity, due to both autoxidation and photo-oxidation (Cecchi *et al.*, 2010). Much work has been done on the oxidative evolution of virgin olive oils to investigate the effects of specified variables on the oxidative stability of oil, or to identify the oxidized products (Bajoub *et al.*, 2015; Ben-Ayed *et al.*, 2013; Cecchi *et al.*, 2010; Gomez-Alonso *et al.*, 2007; Luchetti, 2000; Mendez and Falque, 2007). To our knowledge, no studies have evaluated the physicochemical characteristics on the composition of produced or marketed local cultivated olive oil in Syria. Therefore, the objective of this study was to evaluate the quality characteristics of olive oil extracted from different parts of olive fruits (whole fruits or fruit flesh) of Kaissy cultivar olives produced in Syria.

Materials and methods

The studied olive cultivar was Kaissy, the most widespread in Syria. The olive fruits of good quality and in the mature firm condition were harvested in the crop year 2009/2010, from the trees grown in grove located in the countryside near Damascus (Deer Al Hajar, Syria), under conventional agriculture practices. Then olive fruits were weighed as in the sampling plan and transferred into polyethylene pouches for storage. Each pouch of olive fruits (1 kg) was considered as a replicate. Then olive fruits were divided into two groups: group 1 used for extraction of the oil from the whole fruits, and the second group used for extraction of the oil from fruit flesh (pulp).

Oil extraction

The oils from whole fruit and fruit flesh were extracted at the shortest time possible using mechanical and physical processes (Blatchly *et al.*, 2014). Olive fruits with or without stone were crushed with hummer crusher and slowly mixed for about 30 min at 27 ^{o}C, then the past mixed was centrifuged at 3000 rpm for 3 min without addition of water to extract the oil. Finally, the oils were decanted and immediately transferred into dark glass bottles and stored at room temperature (20 – 25 ^{o}C) for the irradiation treatment and physicochemical properties. Physical and chemical

analysis of oils extracted from the whole fruits and from fruit flesh (pulp) were performed immediately after harvest, and after 6 and 12 months of storage.

Physical and chemical analysis of oils

Free fatty acids (FFA), peroxide value (PV), iodine value (IV), saponification (specification) value (SV) and the refractive index (RI) at 25 ^{o}C were determined according to standard methods (AOAC, 2010). The results for FFA, PV, IV and SV were expressed as percent oleic acid (Oleic acid %), milliequivalents of active oxygen per kilogram of olive oil (mEq O_2 kg^{-1} oil), gram iodine per 100 g oil (g I_2 100 g^{-1}) and (mg KOH g^{-1} oil) respectively. TBA value (Thiobarbituric acid) in terms of malonaldehyde (mg MDA kg^{-1} sample) was determined using direct method (IUPAC, 1992). The viscosity of the oils was measured with HAAKE viscometer 6 R plus Model (RTM) using a R2 column at 200 rpm, and the results of viscosity were expressed as mPa s^{-1}. The refractive index (nD) of olive oil samples was determined with an Abbe refractometer (VED Carl Zeiss JENA, German) calibrated against pure water at 25 ^{o}C. These determinations were carried out in triplicate.

Determination of total phenol content of olive oil extracts

Phenolic compounds were isolated from olive oil by a 3-time extraction of solution of oil in hexane with water mixture (60:40. v/v). The Folin-Ciocalteau reagent (Merck Schuchardt OHG. Hohenbrunn, Germany) was added to a suitable aliquot of the combined extracts, and the absorption of the solution was measured at 725 nm using UV-VIS spectrophotometer. Results were expressed in milligrams of gallic acid per kilogram of oil (Gutfinger, 1981).

Statistical analysis

The experiment was designed to determine the effect of the oil source and storage period on Syrian olive oil composition. Data were subjected to statistical analysis and were successively studied by analysis of variance test (ANOVA) using the SUPERANOVA computer package (Abacus Concepts Inc, Berkeley, CA, USA; 1998). The differences were considered significant at $p<0.05$. (Snedecor and Cochran, 1988).

Results and discussion

The effect of the oil source and storage period on the acid value of olive oil

Acid value (AV) or titratable acidity of oils is usually expressed as percent of free fatty acid content on the basis of oleic acid. Acid value is not only an important quality factor, but also has been extensively used as a traditional criterion for classifying olive oil in various commercial grades (Salvador *et al*., 2000). The AV of the extracted virgin olive oils in the present study indicated the total acidity as contributed by the fatty acids in the sample. Table 1 details averaged results obtained for AV indices and their respective standard deviations of oil extracted from whole fruits and fruit flesh during the storage time. AV of oil extracted from whole fruits (0.32%) was significantly ($p <0.05$) lower than the AV of oil extracted from fruit flesh (pulp) (0.40%) and it was always below its threshold value (0.8%)

that could be classified as extra- virgin olive (IOC, 2015). The results obtained for the free fatty acid content of the oils of Kaissy cultivar were in good agreement with those obtained by Abu-Reidah *et al.* (2013) who indicated that the values of the acidity of Palestinian olive cv. Nabali cultivated in different locations varied from 0.33% to 0.51%. In fact, the low values of acidity recorded in the current study were due to the use of fresh and healthy olive fruits. Data present in Table 1 showed that storage time has significant ($p<0.01$) effect on the AV of olive oil. It is clear that FFA increased during storage time, and a maximum increase in AV (1.35%) was observed in olive oil extracted from whole fruits after 12 months of storage. The high AV of olive oil may be attributed to the hydrolysis of glycerides to yield fatty acids during storage. Fatty acids are normally found in the form of triglycerides, but they may break down into free fatty acids during processing (Atinafu and Bedemo, 2011).

The effect of oil source and storage period on the peroxide value of olive oil

The effect of the source of the extracted oil from whole fruits or from flesh fruits, and storage time for 0, 6 and 12 months, on the peroxide value (PV) of virgin olive oil is presented in Table 1. The results indicated that the source of the oil and storage time have significant ($p<0.01$) effect on the peroxide values of virgin olive oil. The PV of oil extracted from whole olive fruit (4.79 meq O_2 kg^{-1} oil) was significantly ($p<0.01$) lower than the PV extracted from olive fruit flesh (6.13 meq O_2 kg^{-1} oil). The lipid oxidation is attributed to the combination of free radicals with O_2 to form hydro peroxides. In this study, PVs were increased significantly ($p<0.01$) in both stored olive oils. It is worth mentioning that during storage, PV in all cases did not exceed the value of 20 meq O_2 kg^{-1} of olive oil (Table 1), which is the maximum established by the Council for International Olive Oil in order for some oil to be considered as virgin oil. After 12 months of storage, the PVs of oil extracted from whole fruits and from fruit flesh were 11.19 and 19.43 mEq O_2 kg^{-1} oil respectively. We explain these results by the fact that the virgin olive oil has high content of unsaturated fatty acids, which are responsible for oxidative rancidity (Ramadan and Morsel, 2002). Peroxide values of all oil samples were under the maximum Codex and International Olive Oil standard peroxide value (20 meq O_2 kg^{-1} oil) for virgin olive oil deterioration (IOC, 2015). Virgin olive oil in the present study has relatively high degree of un-saturation, which could suggest that virgin olive oil has high content of unsaturated fatty acids. The PV is a measure of the content of hydroperoxides, which are primary oxidation products, and the freshness of lipid matrix. Also, the peroxide concentration, usually expressed as peroxide value, is a measure of oxidation or rancidity in main oil stages. (Wannahari and Nordin, 2012). Peroxidation of oils leads to by-products that negatively affect nutritive values of oils and polyunsaturated fatty acids are especially susceptible to this type of oxidation (Fernandes *et al.*, 2012). This leads to reduction in oil quality (Babalola and Apata, 2011).

The effect of oil source and storage period on the TBA value of olive oil

Thiobarbituric acid (TBA) values (mg MDA kg^{-1} oil) were calculated as a measure of the degree of oxidation during the storage period of virgin olive oil extracted

from different parts of olives. The effects of the oil source and storage time on the TBA values of olive oil are presented in Table 1. Olive oil samples extracted from whole fruits had significantly ($p<0.01$) higher TBA value (0.056 mg MDA kg^{-1} oil) than those extracted from fruit flesh (0.052 mg MDA kg^{-1} oil). During storage, the TBA value of both oil samples extracted from whole fruits or fruit flesh significantly ($p<0.01$) decreased. After 12 months of storage, the TBA values for oil extracted from whole fruits and fruit flesh were 0.030 and 0.044 mg MDA kg^{-1} oil, respectively. This may be due to the presence of antioxidant compounds presumably coming from the olive fruits. These results are somehow in contrast with those of Al-Bachir (2015) who determines the changes of the TBA value during the storage period of pistachio oil samples. Makhoul *et al*. (2006) reported that the TBA of sunflower oil increased upon storage in accordance with the double bond shifts in fatty acids and caused the formation of hydroperoxides which created alcohols, aldehydes and aldehyde esters in oils. Thiobarbituric acid reactive substances (TBARS) are normally considered as major TBA-reacting compounds that indicate the magnitude of the oxidative stress (Qureshi *et al.*, 2007).

Table 1. Effect of oil type and storage time on acid value (% oleic acid), peroxide value ($mEqO_2$ Kg^{-1} Oil) and TBA value (mg MDA Kg^{-1} Oil) of olive oil

Storage period/Months	0 Month	6 Months	12 Months	p-value
Oil type	**Acid value Free Fatty Acid (%)**			
Total Fruits	0.32±0.01bB	0.28±0.01bB	1.35±0.03aA	**
Pulp	0.40±0.02bA	0.35±0.01cA	0.49±0.01aB	**
P-Value	**	**	**	
	Peroxide value ($mEqO_2$ Kg^{-1} Oil)			
Total Fruits	4.79±0.12cB	8.64±0.56bB	11.19±0.78aB	**
Pulp	6.13±0.22cA	16.42±0.17bA	19.43±0.35aA	**
P-Value	**	**	**	
	TBA value (mg MDA Kg^{-1} Oil)			
Total Fruits	0.056±0.001aA	0.028±0.001bB	0.030±0.006bB	**
Pulp	0.052±0.001aB	0.032±0.001cA	0.044±0.005bA	**
P-Value	**	**	*	

[abc] Means values in the same column not sharing a superscript are significantly different.
[ABC] Means values in the same row not sharing a superscript are significantly different.
* Significant at $p<0.05$.
** Significant at $p<0.01$.

The effect of oil source and storage period on the iodine value of olive oil

The iodine value (IV) is a measure of the degree of un-saturation in oil; it gives valuable information on the drying property of the oil as well as the extent of adulteration of the oil (Yahaya *et al*., 2012). Table 2 shows that the IVs of oil

extracted from whole fruits and from fruit flesh were 84.41 and 83.87 g I_2 100 g^{-1} oil, respectively. There was no significant difference ($p>0.05$) between oil extracted from whole fruit or fruit flesh. Also, the IV of oil obtained from whole fruits did not change significantly ($p>0.05$) during storage. While oil extracted from fruit flesh showed significantly high iodine value (87.42 g I_2 100 g^{-1} oil) at 6 months of storage, and significantly low iodine value (81.78 g I_2 100 g^{-1} oil) at 12 months of storage comparable to those found at 0 month of storage (83.87 g I_2 100 g^{-1} oil) (Table 2). Oil extracted from all fruit samples and stored for 0, 6 and 12 months had a relatively high iodine value ranging between 81.78 and 87.42 g I_2 100 g^{-1} oil. This result indicates that these oils are non-drying, highly unsaturated and suggests that they contain high levels of oleic acids (Elleuch *et al.*, 2007).

The effect of oil source and storage period on the saponification value of olive oil

The saponification value (SV) of virgin olive oil extracted at the beginning of the storage period (at zero month) from whole fruits (195.48 mg KOH g^{-1} oil) was significantly ($p<0.05$) higher than the SV extracted from fruit flesh (187.56 mg KOH g^{-1} oil). The SVs of the studied samples of virgin olive oil (extracted from whole fruits and or flesh fruits) were much higher than the values of 130.53 mg KOH g^{-1} oil for melon seed, 147.04 mg KOH g^{-1} oil for castor seed, and 172.50 mg KOH g^{-1} oil for African oil bean seed and much lower than the value of 358.69 mg KOH g^{-1} oil for Locust bean seed (Talabi and Enujiugha, 2014). The saponification value in oil extracted from total fruit decreased gradually, while the saponification value in oil extracted from fruit flesh increased gradually during storage (Table 2). After 12 months of storage, the saponification values of oil extracted from whole fruits and flesh fruits were 187.66 and 197.71 mg KOH g^{-1} oil respectively.

The effect of oil source and storage period on the phenolic content of olive oil

Table 2 shows the representative analytical data for the total phenolic content of assayed virgin olive oil samples. Oil extracted from whole olive fruit, at the start of storage (month zero) has significantly ($p<0.05$) higher total phenolic content (339.52 ± 13.56 mg gallic acid equivalents (GAE kg^{-1})) and oil extract from fruit flesh has significantly ($p<0.05$) lower phenolic content (226.68 ± 3.92 mg GAE kg^{-1}). The contribution of phenols to the virgin olive oil stability and antioxidant contribution was observed to be higher than that of other compounds since it was quantified to approximately 50% by Gutierrez *et al.* (2001). The earlier study by Pellegrini *et al.* (2001), Gutierrez *et al*. (2002) and Jiang *et al*. (2005) reported that the total phenol contents of olive oil in Italy, Spain, and Japan were 73-265 mg GAE kg^{-1}, 89.0-228.5 mg GAE kg^{-1} and 9-172 mg GAE kg^{-1}, respectively. Moreover, the phenol content in olive oil is affected by several factors (Cicerale *et al.*, 2009). The phenolic content of both oils significantly ($p<0.05$) decreased during storage. After 12 months of storage, the phenolic contents of oil extracted from whole fruits and from fruit flesh were 160.05 and 84.55 mg GAE kg^{-1} oil, respectively (Table 2). There was a sharp decrease in phenolic contents in all samples during storage, as a consequence of the hydrolysis and oxidation of these compounds, which involve a loss of stability and a deterioration of the sensorial properties and therefore, a deterioration of the commercial quality of the olive oil

(Mendez and Falque, 2007). A similar effect was observed on several samples of extra virgin olive oil (Gomez-Alonso *et al.*, 2007), the reduction of total phenolic compounds of olive oil during 21 months of storage ranged from 43% to 73%, being higher in the samples having higher initial phenolic contents.

Table 2. Effect of oil type and storage time on Iodine number (g I_2 100 g^{-1} Oil), Saponification value (mg KOH g^{-1} Oil) and total phenolic (mg gallic acid kg^{-1} oil) of olive oil.

Storage period/Months	0 Month	6 Months	12 Months	p-value
Type	**Iodine number (g I_2 100 g^{-1} Oil)**			
Total Fruits	84.41±1.71aA	84.57±1.66aB	83.05±1.43aA	NS
Pulp	83.87±0.84bA	87.42±0.58aA	81.78±0.34cA	**
P-Value	NS	*	NS	
	Saponification value (mg KOH g^{-1} Oil)			
Total Fruits	195.48±0.75aA	190.81±5.71abA	187.66±1.54bA	*
Pulp	187.56±1.30aB	195.02±0.46bA	187.71±1.19aA	**
P-Value	**	NS	NS	
	Total phenolic (mg gallic acid kg^{-1} oil)			
Total Fruits	339.52±13.56aA	193.80±5.89bA	160.05±0.13cA	**
Pulp	226.68±3.92aB	189.51±4.80bA	84.55±1.14cB	**
P-Value	**	NS	**	

[abc] Means values in the same column not sharing a superscript are significantly different.
[ABC] Means values in the same row not sharing a superscript are significantly different.
NS: not significant.
* Significant at $p<0.05$
** Significant at $p<0.01$

The effect of oil source and storage period on the refractive index of olive oil

The refractive index of virgin olive oil extracted at the beginning of storage period (at zero month) from whole fruits (1.4669) was slightly higher than the one extracted from fruit flesh (1.4668). The refractive index of olive oil in the present study showed that it is not as thick as most drying oils whose refractive indices fell between 1.475 and 1.485 (Ogungbenle and Afolayan, 2015). During storage, the refractive index of both extracted oil slightly significantly ($p<0.05$) increased After 12 months of storage, the refractive indexes of oil extracted from whole fruits and from fruit flesh were 1.4677 and 1.4670, respectively. A significant ($p<0.05$) increase in the refractive index of olive oil samples during storage may be due to the exclusion of some saturated fatty acid and / or compounds which could affect this property.

The effect of oil source and storage period on the viscosity of olive oil

The viscosities of virgin olive oil extracted at the beginning of the storage period (at zero months) from whole fruits, and fruit flesh were 129.33 and 130.00 mPa.s^{-1} at 25 ^{o}C, respectively. The viscosity value in the present study was lower than that reported by Majid *et al.* (2004) for olive oil (466.81 mPa.s^{-1}), sunflower oil (331.12 mPa.s^{-1}), cotton oil (358.43 mPa.s^{-1}), linseed oil (296.08 mPa.s^{-1}), soybean oil (284.98 mPa.s^{-1}), coconut oil (297.90 mPa.s^{-1}), and palm oil (309.24 mPa.s^{-1}). The variation observed for those vegetable oils was probably due to the method of preparation of the oils (Liu *et al*., 2012). In general, the viscosity of the lipids (fats/oil) increases with the increase of intermolecular hydrogen bonding. The low viscosity suggests that there are a few hydroxyl groups in the molecule which is supported by the low refractive index of the oil (Majid *et al*., 2004). With respect to the combined effect of the source of oils and storage time, oil viscosity significantly ($p<0.05$) increased during 12 months of storage. After 12 months of storage, the viscosities of the oil extracted from whole fruits and from fruit flesh were 160.33 and 161.00 mPa s^{-1}, respectively. The oil may thicken and become more viscous as it is stored. This seems to be due to the process of polymerization and also to oxidation, hydrolysis and isomerization.

Table 3. Effect of oil type and storage time on the refractive index (nD 25 ^{o}C) and viscosity (mPa.s^{-1}) of olive oil.

Storage period/Months	0 Month	6 Months	12 Months	p-value
Oil type	**Refractive Index (nD 25 ^{0}C)**			
Total Fruits	1.4669±0.0001aA	1.4668±0.0001aA	1.4677±0.0001aA	**
Pulp	1.4668±0.0001aB	1.4668±0.0001aA	1.4670±0.0001aA	**
P-Value	NS	*	**	
	Viscosity (mPa.s^{-1})			
Total Fruits	129.33±0.58bA	126.33±0.58cA	160.33±0.58aA	**
Pulp	130.00±1.00bA	126.67±0.58cA	161.00±0.00aA	**
P-Value	NS	NS	NS	

^{abc} Means values in the same column not sharing a superscript are significantly different.
^{ABC} Means values in the same row not sharing a superscript are significantly different.
NS: not significant.
* Significant at $p<0.05$.
** Significant at $p<0.01$.

Conclusions

Olive oil is a complex, interesting, and economically important material found in most households in Syria. Using olive oil as the subject of chemical analyses helps to motivate the understanding of basic physical and chemical properties of oil. This study has indicated that virgin olive oil obtained from Kaissy cultivar grown in

grove located in the countryside near Damascus (Deer Al Hajar, Syria) has chemical and physical quality characteristics within the legal and acceptable limits.

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