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Research Article
Effect of Harvesting in Different Ripening Stages on Olive (*Olea europea*) Oil Quality

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Abstract
The aim of the present study is to investigate the chemical and physical characteristics of the olive oil in different ripening stages of Manzanilla and Kalamata varieties to determine the optimum harvesting time of the olive fruits. The oil content was gradually increased due to the accumulation of synthesized oil during maturation process. The oil showed an unstable trend in the relation between the acid value and ripening stages. The peroxide values in all ripening stages were below the standard limit. The K values significantly decreased from S1 to S5 but still within standard limit. The moisture content very slightly decreased in maturity and declared that the maturity stages didn't significantly affect the moisture content. The iodine value was significantly decreased with ripening development. The saponification value was significantly decreased with developing of ripening stages. Refractive index values of Manzanilla and Kalamata oils were below the standard limit. Reddish stage (S4) of ripening showed the best physicochemical characteristics. All stages of ripening showed a high content of unsaturated fatty acid especially oleic acid. The total polyphenols and flavonoids level in early maturation stages higher than late maturation stages. The early ripening stages showed the highest antioxidant capacity while significantly decreased with the developing of ripening stages. Organoleptic scores descended along with advanced ripening stages of olive fruit. Finally, we can conclude that the reddish ripening stage (S4) is the best stage for harvesting of the olive fruits to get the high quality of oil.

Key words: Olive oil, ripening stages, oil quality, physicochemical characteristics, phenolic compounds, antioxidant

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Olive oil is a very common constituent in the Mediterranean diet due to its nutritional value and health benefits for human beings. Consumption of olive oil significantly increased in the last few years as a result of its nutrition value (Martinez-Victoria and Manas, 2004). Olive oil has a unique optimal balance between saturated and unsaturated fatty acids as well as other minor components such as polyphenols, tocopherol and chlorophyll (Lazzez *et al*., 2008). Egypt considered as a place of birth of olive and oil. Egypt cultivated about 110.000 ha. Recently, cultivated area of olive trees was expanded up to 416000 ha. In the same time, the produced olive varieties in Egypt had a unique quality of olive oil, especially the type produced in Siwa Oasis. Mediterranean countries are the biggest producers of olive oil worldwide; they produced about 2.872.088 t virgin olive oil with differences among different states (FAOSTAT., 2011). Factors such as cultivar, weather and soil conditions, fruit ripeness, agronomic practices and oil extraction process modify oil chemical composition and organoleptic characteristics (Uceda *et al*., 2004). During maturation process a significant chemical changes occur in the crops; which related to the synthesis of organic substances, especially triglycerides by enzymatic activities (Boskow, 1996), that play a crucial role in olive oil quality (Montedoro *et al*., 1986). Maturation degree is a limiting factor in the composition of fatty acids and the level of polyphenols, tocopherols and pigments in oil (Gutierrez *et al*., 2000). The oil becomes unstable in mature olives due to the increase of polyunsaturated fatty acids and decrease of the total polyphenol content (Ayton *et al*., 2007). These changes are of great commercial importance as they dramatically influence the sensorial characteristics of the oil and its shelf life. Improvement of oil quality associated with the increase of oil content but the peaks begin to decline when reached a maximum oil content (Tombesi *at al*., 1994). According to Lazzez *et al*. (2008) the choice of the optimal harvesting time is essential for obtaining the highest quantity and quality of olive oil. Olive ripeness is one of the most important factors associated with the quality of virgin olive oil (Youssef *et al*., 2010). The phenolic composition and sensory properties of olive oil vary significantly depending on the olive ripening stage (Rivas *et al*., 2013). Determining the optimal harvest date is particularly challenging because variability in cultivar response between growing seasons and varying crop loads influencing the degree of ripening. In Egypt, there is a lack of information about the impact of the level of maturity on olive oil quality. The aim of the present study is to evaluate physical and chemical characteristics of the olive oil in different ripening stages of Manzanilla and Kalamata varieties to determine the optimum harvesting time of the olive.

MATERIALS AND METHODS

Olive fruit samples: This study was carried out during the 2013-2014 olive season in the farm of City of Scientific Research and Technological Application (SRTA-City), located in Borg El Arab, Alexandria, Egypt; from two varieties (*Olea europaea* L. cv. Manzanilla) and (*Olea europaea* L. cv. Kalamata).

Ripening index: The maturation index of olive fruits was determined according to the method given by Uceda and Frias (1975). Olive fruit (*Olea europaea* L.) samples from two varieties (Manzanilla and Kalamata) were harvested in different ripening stages as follow (stages of maturity-S): 15th August, 2014 (S1) (100% intense green skin), 5th September, 2014 (S2) Yellowish green, 25th September, 2014 (S3) Green with reddish spots, 15th October, 2014 (S4) Reddish and 5th November, 2014 (S5) (100% purple flesh and black skin) were handpicked throughout ripening based on the evaluation of the olive skin and pulp colors and applied directly to oil extraction to determine their oil content.

Oil extraction process and oil yield: Olive fruits were used to cold press machine to avoid heat effect on the extracted oil quality according to the method described by Martinez-Suarez *et al*. (1975). The fruits were applied to oil press machine and the extracted oil was pooled and stood for sedimentation of the solid particles, the clear upper phase was collected and purified by centrifugation for 20 min at 4000 × g. The top oil layer was removed. After filtration; oil stored in a dark glass bottles at 4°C until further analysis. The oil yield was expressed as a percentage of fresh olive paste weight using the following equation:

$$\text{Oil yield} = \frac{\text{VxD}}{\text{W}} \times 100$$

Where:

V = Volume of the obtained oil (mL)
D = Density of oil (0.0915 g mL^{-1})
W = Weight of olive past

Analytical indices: Determination of Free Fatty Acids (FFA) and peroxide value was carried out according the official

methods described in EC Regulation (1991). The FFA was determined by titration of the oil in ethanol with KOH (0.01 N) and expressed as a percentage of oleic acid. Peroxide value was determined by sodium thiosulfate titration of free iodine from the mixture of oil and glacial acetic acid: chloroform (3:2) and expressed as milliequivalents of active oxygen/kilogram oil (meq kg^{-1} oil). Ultraviolet spectrometric absorbence at (K232 and K270) was evaluated according (ISO., 2002). All experiments were carried out in triplicates.

Physicochemical analyses: The moisture content of oil was determined by drying the oil in an oven at 60-80°C until constant weight according to AOAC (1975). Saponification value was determined according to Cooks and Reds (1966). The iodine number of the olive oil was carried out according to Gupta and Bhargava (1992). A refractive index of the examined olive oil was measured as described by Edmiston (2001). Oil was put on the prism of a refractometer and tempering at the determination temperature for 2 min, the refraction of light by the oil can be determined and converted to the refractive index.

Fatty acid analysis: Fatty acid composition was determined as described by Yang *et al*. (2013) using HP 6890 series gas chromatography system with HP 5973 mass selective detector. An oil sample (100 mg) was weighed into a test tube and dissolved in hexane (5 mL). Transesterification agent (11.2 g of KOH dissolved in 100 mL ethanol) was added to the mixture. The tube was stoppered and the content was vigorously mixed with a vortex for 1 min. The mixture was left and for 5 min and 0.5 g solid $NaHSO_4$ was added. The mixture was homogenized and centrifuged for 3 min at 4000×g at room temperature. The aliquots of supernatant were sampled for analysis. The methyl esters obtained were analyzed by Gas Phase Chromatography (GPC) (PERI, Perichorm, France) equipped with glass capillary column 30 mL long and 0.4 mm diameter, impregnated with carbowa×20 M (Applied Science Labs, USA). The analysis was performed at a constant temperature (195°C) with nitrogen flow rate of 3 mL min^{-1} and 0.5 bar of pressure.

Extraction of phenolic compounds: Ten milliliter of methanol/water mixture (80:20 v/v) plus tween 20 was added to 2 g of olive oil sample and mixed with a homogenizer (Heidolph-Silent Crusher M, Germany) at 25000×g for 1 min. the mixture was than centrifuged at 5000×g for 10 min (Nüve NF 615, Ankara, Turkey). After the centrifugation, supernatant (methanolic extract) was collected in a clean tube. The extraction was repeated two times (with an addition of 10 mL methanol/water at each time), the supernatant was collected and recorded as a total volume (El Riachy *et al*., 2012).

Total phenol: Total phenolic content of examined oil samples were determined spectrophotometrically by the Folin-Ciocalteau method at 765 nm, in terms of gallic acid as µg GA kg^{-1} oil (Montedoro *et al*., 1992; El Sohaimy *et al*., 2015). Immediately following the extraction, 1 mL of an aliquot of the aqueous methanol solution was diluted to 6 mL with deionized water. 0.5 mL of Folin-Ciocalteau reagent was added and left for 1 min. Then, 2 mL of Na_2CO_3 solution (15%) was added and diluted with 1.5 mL of deionized water and mixed with a vortex (Velp Scientifika, Europe) for 30 sec. The samples were left in a dark place for 2 h and then the absorbence was measured on a spectrophotometer (T80 UV/VIS, England) at 765 nm, against a gallic acid calibration curve. The phenolic content was expressed as µg GAE/g sample.

Determination of total flavonoid: The total flavonoid content in samples was measured using the colorimetric assay developed by Zhishen *et al*. (1999). Oil extract (1 mL) was measured and 4 mL of ddH_2O were added to the sample and mixed well. After 5 min, 0.3 mL of 5% $NaNO_2$ was added to the mixture and 0.3 mL 10% $AlCl_3$ was added after additional 5 min, followed by 2 mL of 1 M NaOH after additional 6 min and the volume increased to 10 mL with ddH_2O. The mixture was mixed very well and the absorbence was measured at 510 nm. The calibration curve was made using different concentrations of catechol (20, 40, 60, 80 and 100 g mL^{-1}).

Phenolic compounds (HPLC): Determination of biophenols in olive oil was carried out according protocol of International Olive Council (2009), using Agilent 1260 infinity HPLC Series (Agilent, USA), equipped with quaternary pump, a Zorbax Eclips Plus C18 column (150×4.6 mm, 5 µm particle), operated at 25°C. The separation was achieved using (a) Water with 0.2% H_3PO_4 (v/v), (b) Methanol and (c) Acetonitrile. About 20 µL was injected and VWD detector was used at 284 nm.

DPPH free radicals scavenging activity: The antioxidant capacity of olive oil was measured by DPPH method as described by Rakesh and Singh (2010) with minor modification by El Sohaimy *et al*. (2015). Methanolic extract (1 mL) was added to 0.5 (0.15 mM DPPH solution) and mixed vigorously. The mixture was incubated for 30 min at room

temperature (25°C). The absorption was measured at 517 nm and the antioxidant capacity was calculated from the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{A0 - A1}{A0} \times 100$$

Where:
A0 = Absorbence of the control
A1 = Absorbence of the sample

Sensory analyses: Sensory properties of olive oil samples were carried out according to International Olive Council (2015a) by eight of selected and trained panelists from food technology department of Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Application. The necessary attributes color, taste and odor were evaluated for each oil sample on a score of 2.5, 5, 2.5, which is the best for attribute respectively resulting final organoleptic score (10).

Statistical analysis: The data were analyzed using Co-Stat one-way analysis with $p \leq 0.05$ to identify significant differences among all parameters analyzed in extracted olive oils from different harvest stages.

RESULTS

Oil content: In the present study, the oil content of the Manzanilla and Kalamata varieties was determined as shown in Table 1. The oil content of Manzanilla variety in different stages of maturity was ranged from 15.84±0.36% in the intense green stage (S1) to 25.76±0.49% in the black stage (S5) and in Kalamata varieties was ranged from 18.32±0.49% in (S1) to 28.17±0.98 in (S5) ($p<0.05$).

Physicochemical characteristics: The studied oil samples showed an unstable trend in the relation between the acid value and ripening stages. Starting with the fruit ripening stages; no significant differences were observed in acid value between stages; S1 (Intense green), S2 (Yellowish green), S3 (Green with radish spots) and S4 (Reddish) while in S5 (black stage); a significant difference was observed between Manzanilla (0.13±0.005) and Kalamata (1.00±0.0035) where, ($p>0.05$) (Table 2). The peroxide values of Manzanilla and Kalamata at different ripening stages are shown in Table 2. The peroxide values increased significantly with developing in the ripening process for the examined varieties of olive fruits. The lowest peroxide values were observed in S1 ripening stage (Green intense) of the studied varieties (0.10±0.02 and 1.74±0.21) Manzanilla and Kalamata, respectively while, the highest peroxide value was observed in the (S5) ripening stage (black stage) (6.62±0.29 and 11.91±0.43), respectively. The peroxide value in black stage (S5) of Manzanilla was (6.62±0.29), lower than that of Kalamata (11.91±0.43), which still below the standard limit (>20 meq O_2/kg oil) (International Olive Council, 2015b) (Table 2). Table 2 showed the iodine number of Manzanilla and Kalamata oil in different ripening stages. The iodine value of Manzanilla oil was ranged from

Table 1: Oil content of Manzanilla and Kalamata olive at different ripening stages

	Total lipids (%)				
Olive cultivar	S1	S2	S3	S4	S5
Manzanilla	15.84±0.36	16.51±0.36	20.72±0.53	23.29±0.34	25.76±0.49
Kalamata	18.32±0.49	20.89±0.65	22.38±0.25	27.22±0.24	28.17±0.98

All experiments carried out in triplicates and the numbers in tables are the mean of triplicates ±SD, ($p<0.05$)

Table 2: Physicochemical properties of Manzanilla and Kalamata olive oils at different ripening stages

Olive cultivar and ripening stages	Acidity (%) (g oleic acid/100 g oil)	Iodine value mg KI/g oil	Peroxide value (meq O_2/kg)	K 232	K 270
Manzanilla					
S1	0.19±0.003	90.04±1.33	0.86±0.03	2.23±0.021	0.180±0.150
S2	0.23±0.012	84.06±2.34	1.17±0.17	2.41±0.117	0.161±0.042
S3	0.52±0.061	80.47±1.66	3.83±0.16	2.01±0.045	0.140±0.025
S4	0.21±0.008	79.21±0.70	5.73±0.19	1.87±0.082	0.134±0.014
S5	0.13±0.005	74.32±1.40	6.62±0.29	1.65±0.145	0.100±0.024
Kalamata					
S1	0.165±0.007	90.80±2.17	1.74±0.21	2.17±0.095	0.140±0.031
S2	0.18±0.004	87.36±1.81	1.81±0.16	2.11±0.045	0.135±0.019
S3	0.40±0.0053	82.40±1.78	3.18±0.30	1.91±0.089	0.130±0.021
S4	0.32±0.0065	80.34±1.43	6.00±0.20	1.77±0.015	0.123±0.025
S5	1.00±0.0035	75.54±1.76	11.91±0.43	1.50±0.046	0.116±0.016

All experiments carried out in triplicates and the numbers in tables are the mean of triplicates ±SD, ($p<0.05$)

74.32±1.40 to 90.04±1.33 and Kalamata oil was varied from 75.54±1.76 to 90.80±2.17 mg KI/g oil in different ripening stages. The ultraviolet absorbence (K) of the extracted oil was measured at λ = 232 and 270 nm for Manzanilla and Kalamata varieties. The K232 value was ranged between 2.41 and 1.65 while, K270 was between 0.10 and 0.18 for Manzanilla oil ($p<0.005$). K232 was 1.50 -2.17 and K270 was varied from 0.116-0.140 for Kalamata oil ($p<0.005$). In Table 3, Moisture content of examined olive oil of Manzanilla variety ranged from 0.13±0.01 to 0.18% and from 0.14±0.02 to 0.21±0.04% for oil of Kalamata variety. While, the standard limit of moisture in olive oil is (0.2%) according to CODEX (1981). Saponification value of oil of Manzanilla variety was ranged from 182.10±2.94 for S5 to 191.04 for S1 and 185.40±2.49 for S5 to 195.19±0.12 for S1 in oil of Kalamata variety (Table 3). The refractive index for Manzanilla and Kalamata oil in different maturation stages were determined and noted in Table 3. Refractive index values of Manzanilla oil were between 1.4674-1.4677 and 1.4678-1.4683 for Kalamata oil. The obtained results revealed that the refractive index of Manzanilla oil was lower than that of Kalamata oil in all-ripening stages.

Fatty acid composition: The results of fatty acids analysis of olive oil in different maturation stages were presented in Table 4. The obtained results declared that, the level of fatty acids was increased with developing of the maturation process. In Manzanilla olive oil the unsaturated to saturated fatty acid was 77.41: 22.59% for S1 (intense green), 78.82:21.18% for S2 (yellowish green), 77.98: 22.02% for S3 (Green with reddish spots), 86.37:13.63% for S4 (Reddish) and 79.09: 20.91% for S5 (Purple flesh/black skin). While in Kalamata olive oil the unsaturated to saturated fatty acids was 72.64:27.36% in S1, 72.23:27.77% in S2, 73.78:26.22% in S3, 76.45:23.55% in S4 and 76.08:23.92% in S5. The all stage of ripening showed a high content of oleic acid (18:1) (monounsaturated fatty acid) (from 72.55% in S1 to 79.29% in S4), beside other polyunsaturated fatty acid; linolenic acid (18:3) (0.61%), linoleic acid (18:2) (3.25%) and palmitoleic acid (16:1) (1.39%).

Phenolic and flavonoid content: The data of total phenolic content and flavonoids in this study, illustrated in Table 5. The total phenolic content of extracted oil from Manzanilla variety was ranged from 122.25±1.90 to 425.190±1.13 µg GAE/g olive oil and flavonoids ranged from 61.62±1.74 to 139.43±1.63 µg catechol/g. While the phenolic content of Kalamata oil was varied from 93.750±2.21 to 376.213±3.23 µg GAE/g and flavonoids content was ranged from 56.33±1.93 to 134.60±0.94 µg catechol/g. The HPLC analysis of phenolic compounds in olive oil was presented in Table 6. The results showed that, Manzanilla olive oil contained a maximum number of phenolic compounds in S4 ripening stage with high level of caffeine (14.378 µg Ml^{-1}),

Table 3: Physicochemical characteristics of Manzanilla and Kalamata olive oils at different ripening stages

Olive cultivar and ripening stages	Moisture content (%)	Saponification No. (mg KOH/g oil)	Refractive index
Manzanilla			
S1	0.18±0.02	191.04±2.67	1.4674
S2	0.15±0.01	188.50±3.17	1.4661
S3	0.15±0.02	187.14±3.26	1.4660
S4	0.13±0.01	185.70±3.72	1.4673
S5	0.13±0.01	182.10±2.94	1.4677
Kalamata			
S1	0.21±0.04	195.19±0.12	1.4678
S2	0.17±0.01	194.38±0.34	1.4679
S3	0.15±0.03	191.53±0.55	1.4681
S4	0.14±0.02	189.17±1.40	1.4682
S5	0.14±0.04	185.40±2.49	1.4683

Values in table the mean of triplicates ±SD (p-value<0.05)

Table 4: Fatty acids content in olive oil

		Manzaneilla (µg mL^{-1})					Kalamta (µg mL^{-1})				
Fatty acid	Length	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
Palmitic acid	C16:0	2.68	2.85	3.59	2.50	4.43	4.27	4.69	4.80	5.44	6.44
Stearic acid	C18:0	0.59	0.53	0.75	0.54	0.95	0.73	0.68	0.74	0.85	1.06
Oleic acid	C18:1	10.73	11.02	13.68	17.69	17.28	10.08	11.43	11.72	16.04	14.52
Linoleic acid	C18:2	0.48	0.68	1.06	1.18	2.30	1.86	2.47	3.93	4.22	5.23
Linolenic acid	C18:3	0.09	0.087	0.12	0.09	0.15	0.15	0.13	0.15	0.16	0.14
Palmitoleic acid	C16:1	0.22	0.26	0.35	0.31	0.46	0.32	0.40	0.61	0.68	0.76
Arachidic acid	C22:0	0.091	-	-	-	-	-	0.10	0.12	0.116	0.14
Nonadecanoic acid	C20:0	-	-	0.12	-	0.14	-	-	-	0.14	-

Table 5: Total phenolic content and flavonoids of Manzanilla and Kalamata olive oils at different ripening stages

Olive cultivar and ripening stages	Total phenols (μg GAE/g oil)	Total flavonoids (μg catechol/g oil)
Manzanilla		
S1	425.190±1.13	139.430±1.63
S2	307.960±2.64	84.270±1.77
S3	231.860±1.23	81.680±1.63
S4	210.300±4.06	73.920±1.60
S5	122.250±1.90	61.620±1.74
Kalamata		
S1	376.213±3.23	134.600±0.94
S2	242.890±5.83	77.160±1.05
S3	145.680±2.75	73.720±1.78
S4	103.050±2.00	64.690±1.32
S5	93.750±2.21	56.330±1.93
Correlation coefficient	Manzanilla	Kalamata
p-value-TP	0.00**	1.00**
p-value-TF	0.0014**	0.0046**

Values in table the mean of triplicates ±SD (p-value <0.05)

Table 6: Phenolic compounds and flavonoids in olive oil

	Manzanilla (μg mL^{-1})					Kalamata (μg mL^{-1})				
Phenolic compounds	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
Catechol	-	-	-	-	-	-	-	-	-	-
Caffeine	-	-	-	14.378	-	-	-	2.608	4.846	-
Caffeic	-	-	-	4.810	-	-	-	-	8.388	-
Vanillin	8.122	2.951	-	4.597	-	1.033	-	-	9.070	-
Ferulic	1.990	9.253	-	12.657	2.956	5.262	3.297	3.164	2.948	1.970
Rutin	-	-	-	9.287	-	-	-	-	-	-
Elegiac	-	-	-	-	-	-	-	-	-	-
Benzoic	9.190	-	-	6.887	4.958	-	-	2.724	2.479	1.829
Cinnamic	3.441	4.003	-	1.088	-	1.287	7.878	3.769	5.844	-

Values in table the mean of triplicates ±SD (p-value<0.05)

Table 7: IC_{50} values for olive oil in different repining stages

Repining stages	IC_{50} (μg mL^{-1})
Manzanilla	
S1	14.57±0.58
S2	20.03±0.46
S3	28.04±0.72
S4	35.54±0.53
S5	30.92±0.56
Kalamata	
S1	20.26±0.62
S2	25.89±0.35
S3	30.88±0.38
S4	36.44±0.52
S5	40.00±0.27

All values in triplicates ±SD, p<0.05

Ferulic (12.657 μg mL^{-1}), rutin (9.287 μg mL^{-1}) and moderate concentration of benzoic (6.887 μg mL^{-1}), caffeic acid (4.810 μg mL^{-1}) and vanillin (4.597 μg mL^{-1}); while contained a lowest level of cinnamic (1.088 μg mL^{-1}) (Table 6). On contrast, no phenolic compounds were detected in S3. Only two compounds were identified in S5 with low concentration benzoic (4.958 μg mL^{-1}) and ferulic (2.956 μg mL^{-1}). The same trend was appeared with Kalamata oil with lower levels than Manzanilla oil. Maximum number of phenolic compounds in S4 ripening stage with high concentration of vanillin (9.070 μg mL^{-1}), cafeic (8.388 μg mL^{-1}) and cinnamic (5.844 μg mL^{-1}) and contained least concentration of caffeine (4.846 μg mL^{-1}), ferulic (2.948 μg mL^{-1}) and benzoic (2.479 μg mL^{-1}) (Table 7). While only ferulic (1.970 μg mL^{-1}) and benzoic (1.829 μg mL^{-1}) were detected in S5.

Antioxidant capacity: Antioxidant capacity of extracted olive oil in Manzanilla and Kalamata varieties in different ripening stages reported in Fig. 1. The data declared that the early ripening stages showed the highest antioxidant capacity while the values significantly decreased with the developing of ripening stages from S1-S5. Manzanilla variety oil showed IC_{50} ranges from 14.57±0.58 (S1) to 30.92±0.56 (S5) while Kalamata variety oil showed IC_{50} ranged from 20.26±0.62 (S1) to 40.00±0.27 (S5) (Table 7).

Organoleptic properties: Organoleptic properties of Manzanilla and Kalamata olive oils at different ripening stages were evaluated and the obtained data were presented in Table 8. It was clear that Manzanilla olive oil color did not change during ripening stages to have the same score of 2. While Kalamata had some changes; as it had a score of 1.2 at

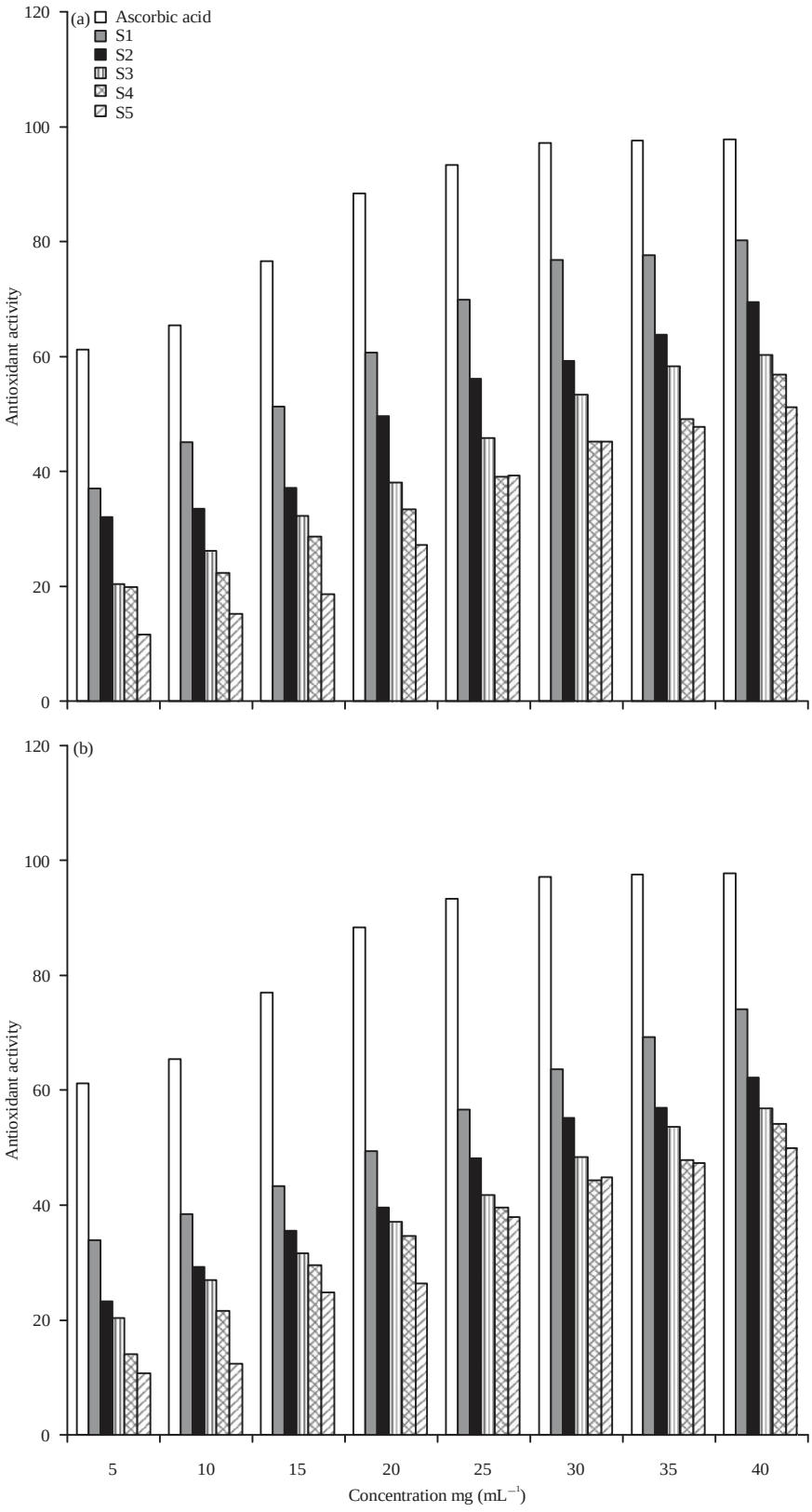

Fig. 1(a-b): Antioxidant capacity, (a) Manzanilla oil and (b) Kalamata oil

Table 8: Organoleptic properties of Manzanilla and Kalamata olive oil

Olive cultivar and ripening stages	Color	Taste	Odor	Total scores
Max. Score	2.5	5.0	2.5	10.0
Manzanilla				
S1	2.0	4.5	2.0	8.5
S2	2.0	4.0	2.0	8.0
S3	2.0	4.1	2.0	8.1
S4	2.0	3.5	2.0	7.5
S5	2.0	3.0	2.0	7.0
Kalamata				
S1	1.5	4.0	2.0	7.5
S2	2.0	4.5	2.0	8.5
S3	2.0	4.0	2.0	8.0
S4	2.0	3.5	2.0	7.5
S5	1.5	3.0	2.5	7.0

Max: Maximum

S1 and score of 2 for each ripening stage of S2, S3 and S4, respectively and in S5 it was 1.5. Concerning taste, both of olive oil varieties recorded slight decrease in the score of taste with developing in ripening stages; as it was four at S1 that dropped to 3 in S5.

DISCUSSION

The level of oil in the fruits is a crucial factor in the suitability of fruits in oil production. The oil content was significantly increased from S1 to S5 in the investigated varieties (Manzanilla and Kalamata) due to the accumulation of synthesized oil during maturation process ($p>0.05$) (Table 1) and the rise in oil content at S5 stage might be due to the decrease of moisture content of the oil during the late maturation stage (Wonder *et al*., 1988), on contrary; disagree with Youssef *et al*. (2010), who observed no significant differences in olive oil yield during maturity process. The acidity, which is an indirect measure of a number of free acids is present in fats and oils. The high the amount of acid value, the high the deterioration or rancidity of the oils and fats are undergone deterioration or rancid. As the rancidity increases, the oil achieves a fuel smell along with a sour taste. The studied oil samples showed an unstable trend in the relation between the acid value and ripening stages. There are no significant differences were observed in acid value from S1 (Intense green) to S5 (black stage). On the other hand, a significant difference was observed between verities Manzanilla (0.13 ± 0.005) and Kalamata (1.00 ± 0.0035) (g oleic acid/100 g oil) (Table 2), where ($p>0.05$). These findings might refer to the essence of olive variety. Nevertheless, the acid value of the oil of two varieties was less than the standard limit (≤ 0.8) (International Olive Council, 2015a). These results proved the low level of free fatty acids in the extracted oil and consequently, very low ability of its deterioration. These results disagreed with Desouky *et al*. (2009), who noticed that, the acidity increased during maturation progress, especially in black stage, which had the highest acidity percentage. Bengana *et al*. (2013), Arslan and Schreiner (2012) and Youssef *et al*. (2010) reported that free acidity increased slightly as fruit ripening progress as during the olive ripening there is progressive activation of lipolytic activity and olives are more sensitive to pathogenic infection and mechanical damage, which result in oils with higher acidity values. Peroxide value is an important test to measure the ability of oil rancidity during storage that produced through reaction with oxygen. The obtained data showed that the peroxide values increased significantly with developing in the ripening process for the examined varieties of olive fruits. The peroxide value in black stage (S5) of Manzanilla was (6.62 ± 0.29 meq O_2/kg oil), lower than that of Kalamata (11.91 ± 0.43 meq O_2/kg oil) which still below the standard limit (>20 meq O_2/kg oil) (International Olive Council, 2015b). The stage Reddish color (S4) shoed the best value for both varieties (5.73 ± 0.19 and 6.00 ± 0.20 meq O_2/kg oil). These findings indicated that the produced oil showed high-quality level and considered as extra virgin oil according the standard limits of International Olive Council (2015a). Desouky *et al*. (2009) remarked that peroxide values in extracted oils from Bouteillan and Koroneiki cultivars in purple as well as in black fruits were significantly higher than those from green fruits. While, Rahmani *et al*. (1997) mentioned that peroxide values did not change significantly during the maturation periods. The ultra violet absorbence on $\lambda = 232$ and 270 nm of the extracted oil showed significant differences in K values in all ripening stages ($p<0.005$). The K values significantly decreased from S1 to S5 for Manzanilla as well as Kalamata varieties ($p<0.05$). The K values in all ripening stages were lower than the standard limit (2.5 for K232 and 0.22 for K270, respectively) (International Olive Council, 2015b). The obtained results confirmed the high purity and freshness of the oil especially in reddish maturation stage. These findings disagreed with the previous study of Rahmani *et al*. (1997), who mentioned that K270 did not change significantly during the maturation stages and Desouky *et al*. (2009), who reported that the K232 or K270 values increased significantly from purple to black fruits. Determination of the moisture content of the oil is imperative for the evaluation of its quality. The higher the moisture content is the higher possibility of deterioration of the oil and might also be a loss of its flavor and reduced levels of antioxidants including polyphenols. The iodine number identified as a number of milligrams iodine used to saturate

the fatty acids present in 100 g of the oil. So, oils rich in unsaturated fatty acid have a high iodine value and vice versa. The significant decrease of iodine value with ripening stages (from S1 to S5) has been remarked ($p<0.05$). Manzanilla oil showed lower iodine values than Kalamata oil. The determination of iodine value in the oil showed the high quality with high level of unsaturated fatty acids especially oleic acid. The moisture content very slightly decreased with the maturity progress and the maturity development did not significantly affect the moisture content, especially when using the same methods of extraction and purification of the oil. On the other hand this study evident the high quality of extracted oil and its low ability for deterioration. These study in agreement with the previous testing olive oil quality by Mailer and Beckingham (2006). Saponification value is another important parameter when oils are considered. It is defined as the number of milligrams of KOH required to combine with fatty acids present in the glyceride form in 1 g of oils or fat. The saponification value was significantly decreased with developing of ripening stages (from S1 to S5) for tow-examined varieties (Table 3) ($p<0.05$). The saponification value of Kalamata variety oil was higher than that of Manzanilla variety oil in all ripening stages but still within the standard limit (184-196 mg KOH/g oil) for virgin olive oil. These findings may due to the glycerides existed in early stages of maturity is higher than that in late stages of maturity and decreased gradually with maturation progress. A refractive index is a considerable tool for detection of the adulteration of oils. The obtained results revealed that the refractive index of Manzanilla oil was lower than that of Kalamata oil in all ripening stages; while both of them were within the standard limit for virgin oil (1.4677-1.44705) (International Olive Council, 2015a). Refractive index parameter evident the freshness of the oil and not stored for a long time after harvesting. These findings revealed that the simple laboratory measurement of refractive index could be used as a quality control technique for finding the adulteration of the oils. Fatty acids are the most crucial components in olive oil. The obtained results in Table 4 declared that the level of fatty acids was increased with developing of the maturation process. The results of fatty acids analysis evident the high level of monounsaturated fatty acids in particular oleic acid (79.29%) compared to polyunsaturated fatty acids (5.25) in olive oil (Manzanilla and Kalamata). This fact guided us to determine the best stage of harvesting (Reddish stage, S4) to get the high quality olive oil with high level oleic acid. The level of oleic acid C18:1 in the olive oil considered a limiting factor to assess the quality of the oil and the optimum harvesting time of the fruits. These results in agreement with International Olive Council (2015b), that mentioned the allowable of oleic acid (C18:1) between 55-83%, linoleic C18:2 from 3.5-21% and linolenic C18:3<1%. The phenolic compounds are not always in agreement in olive oil due to their affecting on sensory properties of the oil. The olive oil has a considerable level of polyphenols and flavonoids, which is good for elongation a shelf life of the oil. The total phenolic content and flavonoids were in a high concentration in the early maturation stages and significantly decreased gradually with the developments of maturation process ($p<0.05$) (Table 6). The high levels of total phenolics and flavonoids in the early maturation stages might refer to the accumulation of these compounds in metabolic processes with the maturation developments and in late stages the phenolase enzyme may cause degradation of phenolic compounds and decreasing their concentrations. A maximum number with highest level of phenolic compounds was noted in S4 ripening stage (caffeine = 14.378 μg mL^{-1}, ferulic = 12.657 μg mL^{-1} and rutin = 9.287 μg mL^{-1} in Manzanilla oil) and (vanillin = 9.070 μg mL^{-1}, cafeic = 8.388 μg mL^{-1} and cinnamic (5.844 μg mL^{-1} in Kalamata oil). The obtained findings of total phenolics and flavonoids are in agreement with early results obtained by Hamidoghli *et al*. (2008) and Katsoyannos *et al*. (2015). They concluded that ripening affected the phenolics and flavonoids content of olive oils as the values of green olive oil were higher than ripe olive oil. The HPLC analysis of phenolic compounds in olive oil showed that, Manzanilla olive oil contained a maximum number of phenolic compounds in S4 ripening stage (Table 6). The synthesis of phenolic compounds was gradually increased and reached to the maximum level of integration in S4. Then in S5 the synthesis process might stop and the phenolase enzymes were activated leading to decrease the level of phenolic compounds. These findings approved the highly beneficial effect of virgin olive oil on lipid oxidation, plasma lipoprotein and DNA damage. These results in agreement with previous studies that demonstrated a reduction in circulating TC and an increase in HDL-C upon virgin olive oil consumption. Furthermore, studies in rats have found that the intake of phenol rich virgin olive oil decreases TC, LDL-C and triglyceride (TG) levels (Gorinstein *et al*., 2002) and substantially increases HDL-C concentrations (Mangas-Cruz *et al*., 2001). Both human and animal *in vivo* studies have shown that the level at which LDL oxidizes decreases linearly with increasing phenolic concentration (Cicerale *et al*., 2010). Olive oil phenolic compounds showed DNA oxidation preventative activity (Fabiani *et al*., 2008). The early ripening stages showed the highest antioxidant capacity while the values significantly decreased with the developing of ripening stages from S1.S5 in Fig. 1. It is clear that the IC_{50}

values are low at the early ripening stages of olive fruits and significantly increased with increasing the ripening stages ($p>0.05$) (Table 7). The linear relationship was remarked between the total phenolic and flavonoids with antioxidant capacity. The results in the present study in agreement with Ninfali *et al.* (2001), who reported that olive oil obtained from mid-period of maturation and stored for two weeks had an antioxidant capacity significantly lower than the top level brand oil. These facts might improve the shelf life of the oil and protect it from oxidation. Organoleptic properties of Manzanilla and Kalamata olive oils cleared that Manzanilla olive oil color did not change during ripening stages to have the same score of 2. Concerning taste; both of olive oil varieties recorded slight decrease in the score of taste with developing in ripening stages. The odor scores were stable. General organoleptic scores descended along with advanced ripening stages of olive fruit. It was observed that scores of organoleptic properties were somehow linked with the total phenolic content of the olive oil that showed the same trend. These results in agreement with Rotondi *et al.* (2004), who presented that the oils produced from olives harvested within the determined time frame showed a superior sensory profile accompanied by the highest possible chemical and nutritional properties.

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