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EVALUATION AND UTILIZATION OF OLIVE EXTRACTS POLYPHENOLS AS NATURAL ANTIOXIDANTS

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ABSTRACT

The objective of this study was to evaluate the polyphenol contents as affected by ripening of Kronaki, Chemlali, Frantoio and Mission olive varieties grown in the New Valley Governorate, Egypt. Besides, the effect of ripe Kronaki olive's total polyphenols addition on sunflower oil stability as natural antioxidants was evaluated. Total polyphenols content of virgin, crude pomace olive oils and vegetation water significantly increased from unripe to the ripe stage in all varieties. Kronaki fruits recorded the highest total polyphenols content followed by Frantoio, Mission and Chemlali in both two ripening stages. Total polyphenol contents were 315 and 335 ppm for virgin oil, 68 and 75 ppm for crude pomace oil and 230 and 250 ppm for vegetation water of Kronaki fruits at unripe and ripe stage, respectively. Polyphenol compounds of the studied virgin olive oils at ripe stage were determined by HPLC. *p*-coumaric acid was the predominant polyphenols compound represented 13.40, 11.53, 9.50 and 7.70 % followed by *o*-coumaric acid which recorded 5.98, 6.54, 8.78 and 7.37 % of total polyphenols in Kronaki, Mission, Frantoio and Chemlali, respectively. Moreover, gallic, catechin, *p*-hydroxybenzoic, salicylic, quercetin, cinnamic acids and phenol present as minor compounds in all studied olive oils. The stability of sunflower oil was increased with increasing the level of olive's polyphenols extract addition. Polyphenols extract addition at 0.15% exhibited remarkable antioxidant activity of sunflower oil and its stability reached to about two-folds compared with the control. Besides, the polyphenols extract from vegetation water was more effective on sunflower oil stability compared with that of virgin or crude pomace oil extracts. That encourages using the vegetation water (a by-product of olive oil industry) polyphenols extract as natural antioxidant source.

Keywords: Virgin olive oil, crude pomace oil, vegetation water, polyphenols, antioxidants.

INTRODUCTION

Olives (*Olea europea* L.) include many cultivars which are used for oil extraction and pickling. Olive oil extracted from olive fruits, which can be consumed in its natural state without begin further treated or refined. The oil obtained from mature fruits by mechanical means, without any chemical treatment, is called "virgin" (Aguilera *et a*l., 2005). Olive pomace and vegetation water are by-products of virgin olive oil processing (Firestone *et al*., 1988). Maturity is one of the most important factors associated with the quality evaluation of olive oil. During ripening, important chemical changes occur inside the drupes which are related to the synthesis of organic substances, especially triglycerides, and other enzymatic activities (Boskou, 1996). The beneficial effects of olive oil are due to not only its high

unsaturated/saturated fatty acid ratio, but also its antioxidants such as vitamin E, carotenoids and phenolic compounds (Visioli andGalli, 1998 and Caruso et al., 1999) and These substances also contribute to the stability of the oil (Papadopoulos and Boskou, 1991; Montedoro *et al*.,1992; and Caruso et al.,1999 .The level of phenolic compounds in virgin olive oil was an important factor in evaluating its quality, as the polyphenols increase the oxidative stability of the oil increase, as well as being responsible for the pungency and bitterness of the oil (Gutiérrez-Rosales *et al*., 1992). Moreover, phytochemicals, like phenolic acid and flavonoids have a very strong antioxidant activity in colon and liver cancer (Liu, 2000). The antioxidant properties of phenolics are mainly because of their rodox properties which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers (Rice-Evans *et al.*, 1997). Polyphenols also, act as chelators of metal ions, preventing metal catalysed formation of initiating radical species (Salah *et al*., 1995). Phenolic fraction of virgin olive oil ranged from 50 to 1000 mg/kg as reported by Montedoro *et al.* (1992) and ranged from 50 to 500 mg/kg as caffeic acid as found by Akasbi *et al.* (1993). Salvador *et al.* (2001) observed that the total polyphenols increased at the last stage of maturity, while Gimeno *et al.* (2002) showed that phenols were decreased during ripening. On the other hand Chimi and Atouati (1994) observed that during ripening, the concentration of phenol compounds progressively increased until it reached a maximum at the "spotted" and "purple" pigmentation stage, after which it decreased. Oxidation of oils not only affect their flavor characteristics, but also influences their nutritive value (Duch *et al.*, 1992). The stability of virgin oil to oxidation has been correlated to the total amount of phenolic components as well as to the *o*-diphenols and to selected simple phenol components (Papadopoulos and Boskou, 1991 and Gennaro *et al*., 1998). The use of synthetic compounds acts as antioxidants not effective in preventing the development of initial off-flavor. Therefore, the use of natural antioxidants is highly desirable to replace the synthetic antioxidants (Papadopoulos and Boskou, 1991 and Satue *et al*., 1995). However, addition of purified polyphenols extracted from olive oil inhibited the oxidative deterioration of soybean oil (Fayed *et al*., 1988) and improved cotton seed oil stability (Khalil, 1994) as well as reduced the oxidation of sunflower oil (Farag *et al.*, 2004). In recent years, the use of some synthetic antioxidant has been restricted because of their possible toxic and carcinogenic effects (Frankel *et al*., 1995; Gazzani *et al*., 1998 and Yen *et al*., 1998). This study aims to evaluate the total polyphenols content of four olive varities as affected by two repining stages, as well as using olive's polyphenol extracts as natural antioxidants for sunflower oil stability.

MATERIALS AND METHODS

Materials:

Olive fruit samples:-

Forty kg fruits of four olive (*Olea europea* L.) varieties namely; Chemlali, Kronaki, Frantoio and Mission were obtained from El-Dakhala Oasis, New Valley Governorate, Egypt, during 2005 season. The olive fruits

were collected at two stages of maturity, the first one was unripe stage (at September, 15) and the second was ripe stage (at November, 15).

Sunflower oil: Sunflower oil was obtained from the local market at Cairo, Egypt.

Methods:

A-Sample preparation:-

Extraction of virgin olive oil, crude pomace oil and vegetation water:

The fruits of the studied olive cultivars were cleaned, crushed by electric crusher, packed in cheesecloth and squeezed with a hydraulic laboratory presser (Craver press.). The resultant extract (oil and vegetation water) was transferred into a separatory funnel and the oil layer was separated, dried by anhydrous sodium sulfate, filterated and kept in brown bottles at -20 ^{0} C until analyses. The vegetation water layer was collected, bottled and stored at - 20^0 C. The crude pomace oil was extracted from the olive pomace by n-hexan, dried and filtered after evaporation of the solvent and kept as above mentioned.

B-Analytical methods:-

Evaluation of sunflower oil: The refractive index (at 20^0 C), iodine value, acid value and peroxide value of sunflower oil were determined according to AOAC (2000) methods.

Determination of total polyphenols:

Extraction of total polyphenols: Total polyphenols were extracted from virgin and crude pomace olive oils according to Gutifinger (1981). Five grams of oils were dissolved in 50 ml n-hexan and the solution was extracted successively with three 20 ml portion of 60% (v/v) aqueous methanol. The mixture was shaken each time for 2 min. the combined extracts were brought to dryness in a vacuum rotary evaporator at 40 ^{o} C. The residue was dissolved in 1 ml methanol and stored at -20^o C until use.

Total polyphenols were extracted from vegetation water according to the method outlined by Capasso *et al.* (1992). A known volume of vegetation water (14 ml) was extracted with ethyl acetate (4×20 ml) and then with n-butanol (4×19 ml). The combined extracts were filtered, dried in precance anhydrous Na_2SO_4 and evaporated to dryness using rotary evaporator at 40^o C. The residue was dissolved in 1 ml methanol and stored at -10^o C until use.

Colorimetric determination of total polyphenols: The concentrated total polyphenols in the methanolic extract was estimated with Folin-Ciocalteau regent (Gutifinger, 1981). 0.1 ml of the extract was diluted by distilled water to 5 ml in a 10 ml voltametric flask, and then Folin-Ciocalteau regent (0.5 ml) was added. After 3 min., one ml of saturated Na_2SO_4 solution was added. The content was measured after 1 h at 725 nm against a reagent blank. Caffeic acid as a standard compound was used to preparation the calibration curve.

HPLC Determination of phenolic compounds:

A Hewlett-Packard HPLC (Model 1100) at Agric. Res., Center, Giza using a hypersil C18 reversed phase column (250 × 4.6 mm) with 5µm particle size-injection by means of Rheodyne injection value (Model 7125) with 50µl fixed loop was used. A constant flow rate of 1 ml/ min. was used

with two mobile phases (A) 0.5% acetic acid in distilled water at pH 2.65 and solvent (B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient was linear starting with (A) and ending with (B) over 35 min, using a UV detector set at wavelength 245 nm. Phenolic compounds of each sample were identified by comparing their relative retention times with those of the standards mixture chromatography. The concentration of an individual compounds was calculated based on peak area measurements, then converted to µ phenolic/ g dry weight. Sixteen standard phenolic compounds were obtained from Sigma (St. Louis, USA) and from Merck-Schuchardt (Munich, Germany) Chemical Companies.

Measurement of sunflower oil stability:

The extracted total polyphenols from virgin, curde pomace olive oils and vegetation water of ripe Kronaki fruits were added to sunflower oil at levels; 0.01, 0.03, 0.05, 0.10 and 0.15%.The oxidative and thermal stability of sunflower oil was measured by Rancimat.

Oxidative stability was evaluated according to Gutiėrrez (1989) by using 679 Rancimat (Metrohm Ltd., CH.9100 Herisau, Switzerland) at Agric. Res., Center, Giza, Egypt. five grams of sunflower oil as well as treated sunflower oil by phenolic extracts were exposed to a stream of atmospheric oxygen (20L/h) at 100±2^0 C. The volatile decomposition products (mainly organic acids) are trapped a measuring detected with distilled water (60 ml) and continuously detected with a conductivity cell (conductivity range 25-200 us/cm).

Statistical analysis: Data were subject to statistical analysis using "F" test according to Snedecor and Cochran (1973) and L.S.D. value for comparisons according to Gomez and Gomez (1984).

RSEULTS AND DISCUSSION

Total polyphenols: Total polyphenols of virgin, crude pomace olive oils and vegetation water of the studied olive varieties were presented in Table (1). Data revealed that there were considerable differences in total polyphenols content between virgin, crude pomace olive oils and vegetation water of all studied varieties. Also, it was clear that total polyphenols content of virgin, crude pomace olive oils and vegetation water significantly increased from unripe to the ripe stage in all varieties. These results are in agreement with those reported by Salvador *et al.* (2001) and Rovellini and Cortesi (2003), they recorded that the content of total polyphenols was increased in olive oils from unripe to ripe stage. While, Gimeno *et al.* (2002) showed that phenols were decreased during ripening. On the other hand, Kronaki fruits recorded the highest total polyphenols content followed by Frantoio, Mission and Chemlali in both two ripening stages. Total polyphenols content was 315 and 335 ppm (virgin oil), 68 and 75 ppm (crude pomace oil) and 230 and 250 ppm (vegetation water) in Kronaki fruits at unripe and ripe stage, respectively. Moreover, the data also indicated that the vegetation water contained considerable amounts of polyphenols in some varieties .The differences in the total phenolic content could be related to the agronomic

characteristics of olive fruits considering the time of their respective ripening (Morelló *et al*., 2005). Besides, the phenolic compound content in oil depends on the place of cultivation, the climate, the variety and the olive's level of maturation at the time of harvesting (Cinquanata *et al.*, 1997 and Visioli and Galli, 1998).

Concerning statistical analysis, it could be noticed that there were no significant differences in all treatments but, there were significant differences in case of variety in vegetation water and in case of interaction between variety and ripening in Virgin olive oil at LSD 5%.

Phenolic compounds composition: Data of polyphenol compounds of the studied virgin olive oils at ripe stage by HPLC apparatus are shown in Table (2). There were twelve identified phenolic compounds and seven unknown fractions. From these results it could be concluded that *p*-coumaric acid was the major polyphenols compound represented 13.40, 11.53, 9.50 and 7.70 % followed by *o*-coumaric acid which recorded 6.54, 7.37, 8.78 and 5.98 % of total polyphenols in Kronaki, Mission, Frantoio and Chemlali, respectively. On the other hand, gallic, catechin, *p*-hydroxybenzoic, salicylic, quercetin, cinnamic acids and phenol present as minor compounds in all studied olive oils with the exception of gallic acid it not found in Mission variety. The concentration of salicylic, catechin, cinnamic acids and phenol in all virgin olive oils varied from 2.99 to 3.56%, 2.50 to 3.45%, 1.82 to 3.75% and 1.50 to 2.76% of total polyphenol, respectively.

Table (1): Total polyphenols content of virgin, crude pomace olive oils and vegetation water (ppm):

Property / Variety	Virgin olive oil			Crude pomace olive oil			Vegetation water		
	Unripe	**Ripe**	**Mean**	**Unripe**	**Ripe**	**Mean**	**Unripe**	**Ripe**	**Mean**
Chemlali	120	130	125	50	63	57	125	136	131
Kronaki	315	335	325	68	75	72	230	250	240
Frantoio	160	170	165	60	70	65	150	240	195
Mission	128	161	146	45	52	49	155	165	160
Mean	181	199	-	56	65	-	165	198	-
L.S.D. 0.05%									
Variety (V)	3			3			6		
Ripening (R)	4			2			3		
V x R	6			1			4		

It was evident from the same data (Table, 2) that the polyphenols; pyrogallic, protocatchuic and coumaric acids recorded the lowest levels. Pyrogallic acid constituted only 0.02, 0.35, 0.20 and 0.30% of total polyphenols for Chemlali, Kronaki, Frantoio and Mission virgin olive oils, respectively. Protocatchuic and coumaric acid percentages ranged from 0.14 and 0.66% for Chemlali to 0.85 and 0.92% for Kronaki, respectively. The relative proportion of phenolic components depends on several factors such as fruit's variety, location, and degree of ripeness (Caponio *et al*., 2001 and Cinquanata *et al*., 1997). Moreoever, polyphenols are present as phenolic acids (caffeic, *p*-coumaric, ferrulic, *p*-hydroxybenzoic and vanillic acids) as well as phenolic alcohols (tyrosol and hydroxytyrosol) in olive oil Koprivnjak and Conte (1998).

Table (2): Phenolic compounds composition (% of total polyphenols):

	Virgin olive oils			
	Chemlali	**Kronaki**	**Frantoio**	**Mission**
Unknown 1	0.06	0.72	ND*	0.09
Unknown 2	0.75	0.34	0.92	0.82
Unknown 3	0.19	0.53	0.45	0.20
Pyrogallic	0.02	0.35	0.20	0.30
Gallic acid	1.98	2.03	3.52	ND
Protocatchuic acid	0.14	0.85	0.34	0.19
Catechin	2.61	3.45	3.20	2.50
P- hydroxybenzoic	2.31	0.70	1.45	1.70
Unknown 4	1.42	1.02	1.95	1.85
P-Coumaric	7.70	13.40	9.50	11.53
Unknown 5	2.23	ND	3.57	2.56
Phenol	2.76	1.75	1.50	2.32
O-Coumaric	5.98	6.54	8.78	7.37
Salicylic acid	2.99	3.56	3.19	3.00
Coumaric acid	0.66	0.92	0.83	0.78
Quercetin	1.53	1.02	1.32	1.28
Unknown 6	0.42	0.30	1.00	0.59
Cinnamic acid	1.82	3.57	2.18	3.04
Unknown 7	66.60	58.97	56.10	62.92

ND* = not detected.

Physico-chemical properties and stability of sunflower oil: The refractive index, acid value, peroxide value and iodine value were determined for the used sunflower oil and the results are shown in Table (3). From these data, it is clear that no hydrolytic and oxidative rancidity has taken place for sunflower oil. Besides, the oxidative stability for sunflower oil was 9.41 h, which be considered initial value before the addition olive's polyphenol extracts to evaluate then effect on the stability of this oil.

Table (3): Physico-chemical indices and stability of sunflower oil:

Indices	**Value**
Refractive index (25 ^{o} C)	1.4643
Acidity (%) as oleic acid	0.01
Peroxide value (meq/ kg)	0.01
Iodine value (g/100g)	134.78
Oxidative stability (hr)	9.41

Effect of olive's total polyphenols addition on sunflower oil stability:
Total polyphenols was extracted from virgin, crude pomace oils and vegetation water of ripe Kronaki olive variety because it has the highest amount of polyphenols among the studied olive varieties. Polyphenols extracts were added at levels of 0.01, 0.03, 0.05, 0.10 and 0.15% to sunflower oil (w/v)and the data of oxidative stability were tabulated in Table 4. Generally, the results revealed that the addition of olive's polyphenols extracts increased the stability of treated sunflower oil compared to the

control. Moreover, increasing the concentration of polyphenols extracts from 0.01 to 0.15% remarkable exhibited the antioxidant activity of sunflower oil. The stability of sunflower oil was nearly duplicated at 0.15% extract addition compared with the control. The data are in the line with those reported by Farag *et al.* (2003 and 2004).

Table (4): Oxidative stability of sunflower oil as affected by Kronaki polyphenol extracts addition:

Polyphenols	Virgin oil		Crude pomace oil		Vegetation water	
	Stability (hr)	Protective factor	Stability (hr)	Protective factor	Stability (hr)	Protective factor
Control	9.41	1.00	9.41	1.00	9.41	1.00
0.01	12.80	1.36	15.00	1.59	12.90	1.37
0.03	14.50	1.54	15.40	1.63	16.00	1.70
0.05	15.20	1.61	15.60	1.65	16.90	1.79
0.10	15.80	1.67	16.30	1.73	19.40	2.06
0.15	17.20	1.82	16.60	1.76	21.50	2.28

On the other hand, it was clear that the polyphenols extract from vegetation water was more effective on sunflower oil stability compared to that of virgin or crude pomace extracts. The high protective effect of vegetation water polyphenols extract may be due to present of some polyphenols compounds have water solubility. Such results are in accordance with those reported by Khalil (1994) and Ismeal et al. (1996).

In conclusion polyphenols extracts of olive oil can be used as natural antioxidants for increasing stability of other oils as well as the importance of using the vegetation water (a by product of olive oil industry) as natural antioxidant source.

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تقييم واستخدام المواد الفينولية لمستخلصات الزيتون كمضادات أكسدة طبيعية

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٣- قسم تكنولوجيا الزيوت والدهون- مركز البحوث الزراعية- الجيزة- مصر

تهدف هذه الدراسة الي تقييم محتوي وتركيب المواد الفينولية خلال مرحلة ماقبل النضج ومرحلة النضج لثمار أصناف الزيتون: الكروناكي ، الشملالي ، الفرانتيو والمشن ، المتحصل عليها من واحة الداخلة بمحافظة الوادي الجديد بمصر. فضلا عن تقييم مدي تأثير اضافة مستخلصات الزيتون الفينولية كمضادات أكسدة طبيعية علي درجة الثبات الأكسيدي لزيت عباد الشمس.

وقد تبين من نتائج هذه الدراسة أن محتوي كلاً من زيت الزيتون البكر ، الزيت المستخلص بالمذيب والماء الخضري من المواد الفينولية يزداد معنوياً بتقدم مرحلة النضج في جميع الأصناف المدروسة. كما سجلت ثمار الصنف كروناكي أعلي محتوي من المواد الفينولية ، يليها ثمار الفرانتيو ، المشن وأخيراً الشملالي. حيث بلغت قيمة المواد الفينولية (مقدرة كجزء في المليون) للصنف كروناكي ٣١٥ ، ٣٣٥ (للزيت البكر) ، ٦٨ ، ٧٥ (للزيت المستخلص بالمذيب) و ٢٣٠ ، ٢٥٠ (للماء الخضري) في مرحلة ماقبل النضج ومرحلة النضج ، علي التوالي.

وبتقدير المركبات الفينولية باستخدام جهاز الـ HPLC تم التعرف علي اثني عشر مركباً فينولياً ، اضافة الي سبعة مركبات أخري غير معروفة. وقد كان حامض *p*- cumaric هو السائد ضمن المركبات الفينولية التي تم التعرف عليها مسجلاً قيماً بلغت ١٣٫٤٠ ، ١١٫٥٣ ، ٩٫٥٠ و ٧٫٧٠% ، يليه حامض *o*- cumaric والذي بلغ ٩٨ ٫٥ ، ٦٫٥٤ ، ٨٫٧٨ و ٧٫٣٧ % من مقدار المركبات الفينولية في الأصناف الكروناكي ، المشن ، الفرانتيو والشملالي علي التوالي.

ومن ناحية أخري سجلت مركبات gallic, catechin, *p*-hydroxybenzoic, salicylic, quercetin, cinnamic acids and phenol القيم الأقل ضمن المركبات الفينولية المتعرف عليها في جميع أصناف الزيتون المدروسة.

ومن الناحية التطبيقية ، أوضحت الدراسة أيضاً أن درجة الثبات الأكسيدي لزيت عباد الشمس (والمقدرة بجهاز الرانسيمات) تزداد بزيادة مستوي اضافة مستخلصات المواد الفينولية ، حيث أظهر اضافة التركيز ٠,١٥ % منها تأثيراً واضحاً علي درجة الثبات الأكسيدي لزيت عباد الشمس لتصل الي حوالي الضعف تقريباً مقارنة بالكنترول.

فضلاً عن ذلك ، فان المستخلص الفينولي للماء الخضري كان أكثر تأثيراً علي درجة ثبات زيت عباد الشمس مقارنةً بتلك المستخلصات لزيت الزيتون البكر أو الزيت المستخلص بالمذيب ، مما يشجع علي استخدام مستخلص المواد الفينولية للماء الخضري (كناتج ثانوي في صناعة زيت الزيتون) كمصدراً لمضادات الأكسدة الطبيعية.