BITTERNESS, NATURAL ANTIOXIDANTS AND QUALITY INDICES OF VIRGIN OLIVE OIL FROM DIFFERENT GROWING AREAS

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ABSTRACT

The purpose of this investigation was to study differences in the bitterness (K_{225}), natural antioxidant (total polyphenol, orthodiphenol, α-tocopherol, chlorophyll and carotenoid) and quality indices (free fatty acids, peroxide value, spectrophotometric K232 and K270, organoleptic testes, color, oxidative stability) of virgin olive oil from koronakii variety cultivated in different olive growing areas in Egypt namely Giza, EL-Sharkia, and EL-Arish were determined. Phenolic compounds were analyzed by HPLC (high performance liquid chromatography).

Results showed that significant differences between olive oil samples extracted from Koronakii variety cultivated in the three different growing areas. Virgin olive oil extracted from EL-Arish region showed higher concentration of natural antioxidants.

Key words: *Virgin olive oil, natural antioxidants, phenolic compound, bitterness, growing areas.*

INTRODUCTION

Virgin olive oil excellent natural food is obtained from olive fruit (*Olea europaea, L.*) by mechanical or physical procedures (such as milling, beating, centrifugation, and decantation) Gandul-Rojas *et al.,* (2000). Olive oil quality is affected by genetic, agronomic, growing region, fruit ripeness, environmental conditions and techniques of processing and storage (Kiritsakis, 1998 and Criado, *et*

al., 2004). The altitude and temperature at which the olive trees are grown affect the olive oil composition (Osman**,** *et al.,* 1994). Seasonal aspects (temperature and rainfall) are part of the agronomic factors and influence by the physiology of the plant. Some studies have shown that the climatic conditions, particularly rainfall during the growing and ripening of the olive fruit, influence the quality of the olive oil (Panelli, *et al*., 1994 and Criado, *et al*., 2004). The stage of ripening of olives and their health mainly affects the sensory quality of oils. Olives harvested relatively early yield oil with a fruity flavor, lower acidity, and greener color than olives harvested late in the season (Romero, *et al*., 2003).

Olive oil is an integral ingredient of the Mediterranean diet and accumulating evidence of its health benefits includes the reduction of risk factors of coronary heart disease, the prevention of several types of cancer, and changes in the immune and inflammatory responses (Keys, 1995, Willett, 1997, Lipworth, *et al*., 1997, and Hannach *et al*., 2008). In this respect, converging evidence indicates that such beneficial effects are related not only to elevated oleic acid content but also to the high level of antioxidants in the nonsaponifiable fraction, including phenolic compounds (Bravo, 1998 and Mateos *et al*., 2005).

Phenolic compounds have been related to the intensity of bitterness, an appreciated organoleptic characteristic of virgin olive oils (Mateos, *et al.,* 2004), although a high intensity of this attribute can be rejected by the consumers even though the oil contains nutritional components of has along shelf live (Basuny and Mostafa., 2004). The intensity of the bitterness of olive oil has linked to the presence of phenolic compounds derived from the hydrolysis of oleuropein, which during oil extraction leads to its aglycon, named secoiridoid derivatives of phenols (Soler-Rivas *et al*., 2000 and Gomez- Rico *et al*., 2008).

Minor compounds are of great importance in the final composition of olive oil because they influence the stability and overall acceptability as well as the nutritional and health related properties of the olive oil (Basuny *et al*., 2008). Compounds such as sterols, squalene, pigments and tocopherols are of great interest as high value added products because of their nutraceutical activities (Kalogeropoulos *et al*., 2007 and Koprivnjak *et al*., 2008).

Tocopherols are well known as components of vitamin E their presences in olive oil are important for their nutritional qualities and

for their antioxidant properties, in that they protect the fat composition from autoxidation (Ibanez *et al*., 2002). The most effective is α-tocopherol, followed by β and δ- tocopherol. Their antioxidant properties in foods have been known for many years (Salvador *et al*., 1998 and Moyano *et al*., 2008). Chlorophyll and carotenoid compounds also play an important role in the oxidative stability of processed food stuffs because they are antioxidant in the dark and pro-oxidation in the light (Gutierrez *et al*., 1992). Sterols are playing a key role in preserving oil from rancidity during storage thus prolonging its shelf- life (Ruiz- lopez *et al*., 1995 and Criado *et al.*, 2008) and squalene is reported to be a quencher of singlet oxygen and a free radical scavenger (Kohno *et al*., 1995). Squalene addition was also proposed to enhance the thermo stability of oils delaying the degradation of unsaturation fatty acids and limiting the extent of polymerization (Manzi *et al*., 1998).

The aim of this work was to study the effect of growing area on the bitter index (K_{225}), minor components (total phenol, orthodiphenol, α-tocopherol, chlorophyll and carotenoid), quality indices (acidity, peroxide value UV absorption K_{232}, K_{270} and sensory analysis) and stability by Rancimat method of virgin olive oil samples extracted from Koronakii variety cultivated in three different growing regions.

MATERIALS AND METHODS

Source of olive fruits:

Olive fruits were collected from different locations in Egypt namely Giza, El-Sharkia, and El-Arish. The fruits were collected in the period which coincided with the time when olives are usually harvested for oil production healthy olive fruit samples were collected at different times between October and November of 2007 season. EL-Arish variety was grown in sandy soil, while Giza and EL-Sharkia variety were grown in clay soil.

Climatogical data were obtained during 2007 from Central Laboratories for Agriculture Climate, Dokki, Giza, Egypt. The climate of the EL-Arish area, the average temperature ranges from a minimum of -2˚C to a maximum of 37 and the annual rainfall varies from 2 to 57 mm / day. Humidity ranges from 33 to 91%. The climate in Giza with temperatures varying from a high 44˚C to a low of 5˚C. Average annual rainfall varies from 1 to 15 mm / day. Humidity varies from 29

– 91 %. The climate in EL-Sharkia with the average temperature ranges from a minimum of 7 to a maximum of 39˚C. Rainfall varies from 1 to 31 mm. Average humidity randes from 32 to 91%.

Solvent, Reagents and Standards:

All solvents were distilled before use, Foline-Ciocaleau reagent was obtained from Gerbsaure Chemical Co. Ltd. Germany and standard phenolic compounds (tyrosol, hydroxytyrosol, gallic, p-hydroxybenzoic, caffic, tannic,vanillic and catechin) and standard α-tocopherol were obtained from Koch-light Laboratories Ltd. Colubrook, Buckingham, Shira, England.

Extraction of olive oil from olive fruit:

The fruit was ground, packed in cheese cloth, then pressed by using hydraulic Laboratory (Carver) press. The separation oil was dried over anhydrous sodium sulphate, then filtered through a whatman filter paper No.1 and kept in brown glass bottle (120 ml) at 5˚C until analysis.

Quality indices of olive oil:

Free fatty acids content (% oleic acid), Peroxide value (meq. O_2 / kg oil), and UV absorption (K_{232} and K_{270}) were carried out following the analytical methods described in International Olive Oil Council (IOOC, 1998).

Total pigment content:

The chlorophyll fraction at 670 nm and the carotenoid fraction at 470 nm were evaluated from the absorption spectrum of each virgin olive oil sample (7.50 g) dissolved in cyclohexane (25 ml). The chlorophyll and carotenoid contents are expressed as mg of major pigment, pheophetin a, and lutein per kg of oil, respectively. (Minguez- Mosquera, et al., 1991).

Oil color:

The color of olive oil sample was estimated using a Lovibond tintometer. The yellow glass filter was fixed at 35 and the intensity of glass color was measured according to the method reported by Nielson (1998).

Total phenol content:

The total phenol content was analyzed using the modified isolation method described by Gutfinger, (1981) with triple extraction

of an oil-in-hexane solution with a 60 % (v/v) water / methanol mixture. The concentration of total polyphenols was estimated with Folin-Ciocalteau reagent at 725 nm. Orthodiphenols were measured calorimetrically at 370 nm after adding 5% (w/v) sodium molybdate in 50 % ethanol to the extract. The results were expressed as mg of Caffeic acid per kg of oil.

Oxidative stability:

Oxidative stability of virgin olive oil is expressed as the oxidation induction time (hrs) measured with a Rancimat 679 apparatus (Metrohm Co., Herisau, Switzerland) using a 5 g oil sample heated to 100˚C ± 2˚C and Lh^{-1} air flow. The time taken to reach a fixed level of conductivity was measured (Laubli and Bruttle 1986).

Bitter index:

The bitter index (K_{225}) was evaluated the extraction of the bitter components of a sample of 1.0 ± 0.01 g oil dissolved in 4 ml hexane passed over a C_{18} column (Waters Sep- Pack Cartridges), previously activated with methanol (6 ml) and washed with hexane (6 ml). After elution, 10 ml of hexane was pressed through to eliminate the fat and the retained compounds were then eluted to 25 ml with methanol / water (1: 1). The absorbency of the extract was measured at 225 nm against methanol / water (1: 1) in a 1- cm (Gutierrez et al., 1992).

Tocopherol content;

α –tocopherol content was evaluated by high performance liquid chromatography (HPLC) with direct injection of an oil-in-hexane solution: 1.5 ± 0.01 g of oil dissolved in hexane to 10 ml. Results are given as milligrams of α –tocopherol per kilogram oil (Ibanez et al., 2000).

Organoleptic test:

The organoleptic test was determined for the extracted oils according to the International Olive Oil Council (IOOC, 1998). The oil samples (15 ml) were presented in covered blue glasses (diameter, 70 mm, capacity, 130 ml) at 28˚C ± 2˚C. The glass warmed and after removing the cover, the sample was smelled and then tested by the panelist to judge its flavor. The different attributes of the oils were assessed and their intensities were evaluated as a mean value of the panelists score.

Phenolic compounds:

The extracted phenolic fraction was dissolved in 1 ml of methanol and analyzed by HPLC. The column was an Inert sil ODS-3(5μm, 15 cm x 4.6 mm). HPLC analysis was performed following the same procedure as Montedoro *et al*., (1992). The eluents were 0.2 % aqueous acetic acid solution and methanol, the flow rate was 1.5 ml/ min, and the injection volume20μL. The total run time was 60 min, the initial composition was 95 % aqueous acetic acid solution (0.2%), and 5% methanol, and the gradient changed as follows. The concentration of methanol was maintained for 2 min, then it was increased to 25 % for 8 min, and finally the methanol percentage was increased to 40, 50, and 100 % for 10 min periods. It was maintained at 100 % for 5 min. Initial conditions were reached in 15 min. Chromatograms were obtained at 280 and 339 nm.

Statistical analysis:

The data was subjected to an ANOVA using SAS. Separation of the means was obtained using the least square means test, and significant difference was defined as $P \geq 0.05$.

RESULTS AND DISCUSSION

Quality indices of virgin olive oil samples:

Table (1) shows the quality indices of koronakii oils from different olive growing areas in Egypt. Results indicated that the free fatty acids (% as oleic acid) content of Koronakii olive oil was below 1 and fell within the accepted value for extra virgin olive oils as the standard free fatty acids limit of extra virgin olive oil is 0.8 maximum (IOOC, 2001). Results, however, revealed that olive oil obtained from Giza region had higher free fatty acid content than those obtained from EL-Arish region. The present study indicated that the geographical region had great influence on the quality indices of olive oil.

Data revealed (Table 1) that in all olive oil samples, the peroxide value and Uv absorption (K_{232} and K_{270}). In none of the oils did it exceed the upper limit (20 meq. O_2 / Kg oil) established by IOOC (2003) from extra virgin olive oil. EL-Arish area showed lower peroxide value and Uv absorption (K_{232} and K_{270}) than those of Giza and EL-Sharkia areas. The present results also supported, again, the influence of the geographical region on olive oil quality.

Also, the results in Table (1) showed the effect of olive oil areas on the organoleptic tests of virgin olive oil obtained from Koronakii variety. The olive oil samples kinds were without defects and therefore qualitative scores were attributed to them only on the basis of their positive organoleptic attributes.

Table (1): Quality indices of virgin olive oil samples obtained from Koronakii variety in three different growing areas in Egypt

Parameters		Growing areas			LSD Values at p ≥ 0.05
		EL-Sharkia	Giza	EL-Arish	
Free fatty acids (% as oleic acid)		0.20b	0.30c	0.08a	0.01
Peroxide value (meq. O_2/kg oil)		2.50b	2.70c	1.50a	0.10
Uv absorption	K_{232}	0.31c	0.25b	0.20a	0.01
	K_{270}	0.07c	0.05b	0.01a	0.01
Organoleptic tests		6.00a	7.10b	7.90c	0.10

Values in each row followed by the same letter are not significantly different at p ≥ 0.05

Chlorophyll and carotenoids pigments and color of virgin olive oil:

Table (2) shows the values corresponding to the concentrations of the chlorophyll and caroteniod pigments and color of koronakii oils from different olive growing area in Egypt. Significant differences (P ≥ 0.05) were observed in the total content of pigments between oils from EL-Sharkia and those coming from two other producing regions. From the results obtained, the behavior of the virgin olive oil from EL-Sharkia is totally different in spite of the geographical proximity to the EL-Arish region. Virgin olive oil from EL-Sharkia showed the least pigmentation. This may be a consequence of the minimum temperatures reached in the former area, implying heavy frosts that could have led to a deterioration of the olive fruit and degradation of the pigment, mainly in the chlorophyll fraction.

The color measured by Lovibond showed significant differences between growing regions and environmental conditions (Table 2). There was a clear relationship between the values of color and the

pigment concentration. The ratio between the chlorophyll and carotenoid fractions was maintained at around one unit in oils from EL-Sharkia showing that the green and yellow fractions were balanced. However, in oils from Giza and EL-Arish this ratio indicated a higher content of chlorophyll than of caroteniods.

Consequently, this result could be due to differences in the photosynthetic apparatus of olive fruit grown in different areas.

Table (2): Chlorophyll and carotenoid pigments and color of virgin olive oil samples obtained from Koronakii variety in three different growing areas in Egypt

Parameters		Growing areas			LSD Values at p ≥ 0.05
		EL-Sharkia	Giza	EL-Arish	
Chlorophyll content (neg / kg oil)		5.50a	10.00b	15.30c	1.50
Carotenoid content (meg / kg oil)		3.90a	7.00b	8.10c	1.00
Color	Yellow	35.00	35.00	35.00	-
	Red	2.10a	3.50b	4.90c	0.20
Chlorophyll / Carotenoids ratios		1.41a	1.43b	1.88c	0.01

Values in each row followed by the same letter are not significantly different at p ≥ 0.05

Total phenol, orthodiphenol, bitter index, α –tocopherol and oxidative stability of virgin olive oils:

Table (3) shows the main components that are related to oil stability. The tocopherol content of virgin olive oil is important to protect lipids against autoxidation and thereby to increase its storage life and value as a wholesome food. The rang of α –tocopherol contents in olive oil from different areas is wide. There were significant differences (P ≥ 0.05) in the α –tocopherol content between growing regions. Virgin olive oil from the EL-Sharkia region showed the α –tocopherol content lower than those and EL-Arish areas.

The amount of phenolic compounds in virgin olive oil is an important factor when evaluating its quality, given that the natural phenols improve its resistance to oxidation Giza and \, to a certain extent, are responsible for its sharp bitter taste. The total phenols in

the oils analyzed in this study varied considerably and a significant effect of the growing area (Table 3). Thus, oils from the EL-Sharkia area had the lowest total phenol concentration, and the highest values were in oils from Giza and EL-Arish areas.

Ortho-diphenol concentration were also studied, exception oils from the different growing areas. Significant differences were found among arthodiphenol content of all the samples from the different growing areas, similar to those observed in the total phenol contents.

The oxidative stability of oils measured as the induction time determined using the Rancimat method, showed the same trend in relation to growing area and total polyphenol and orthodiphenol contents of the oils (Table 3). Significant differences were found between the stability of oils from the different growing areas. The effect of growing areas was very significant in olive oil samples, as it was for phenolic content. It is important to emphasize that the stability of the oils from the EL-Sharkia area, which showed the lowest total phenols concentration. This fact could be explained by a degradation of orthodiphenol compounds, mainly implicated in oil stability, as a consequence of the frequent, frosts in the EL-Arish area.

Bitter taste is one of the characteristic attributes of virgin olive oil. In our study, the bittrer index (K_{225}) was analyzed in oils from the different growing areas. There were significant differences ($p \geq 0.05$) K_{232} between olive oil samples from different areas, similar to that observed in oil phenol content and stability.

Table (3): Total phenol, orthodiphenol, bitter index, α – tocopherol and oxidative stability of virgin olive oil samples obtained from Koronakii variety in three different growing areas in Egypt

Parameters	Growing areas			LSD Values at $p \geq 0.05$
	EL-Sharkia	Giza	EL-Arish	
Phenol content (mg / kg oil)	170.20a	233.40b	390.30c	5.30
Orthodiphenol (meg / kg oil)	15.50a	25.30b	65.40c	3.00
Bitter index (K_{225})	4.12a	5.00b	5.13c	0.50
α – tocopherol	139.30a	185.50b	290.30c	7.30
Oxidative stability (hrs)	18.50a	23.40b	30.50c	2.50

Values in each row followed by the same letter are not significantly different at $p \geq 0.05$

Phenolic compounds of virgin olive oils;

No qualitative differences were observed in HPLC phenolic fraction profile between virgin olive oils from different growing regions. However, significant quantitative differences ($p \geq 0.05$) were observed in a wide number of phenolic compounds (Table 4). As ameans explaining those differences, the phenolic fraction was divided into four main groups (simple phenols, secoiridoid derivatives, flavonoids, and the latter part of the chromatogram).

In relation to simple phenols, higher concentration of hydroxytyrosol and tyrosol were observed in virgin olive oils coming from EL-Arish area. These higher levels could be related to more advanced maturation indexes of olive in this area. Higher concentrations of secoiridiod derivatives were observed in virgin olive oils from Giza region. Low flavonoid levels represented by gallic and vanillin were observed in all the olive oils analyzed. In spite of their low concentrations, flavonoids showed significant differences between olives from the three different growing areas.

The results could be used to characterize virgin olive oils of the Koronakii variety, using the quantitative and qualitative parameters of pigments and phenols as differentiators of the growing areas.

Table (4): Phenolic compounds of of virgin olive oil samples obtained from Koronakii variety in three different growing areas in Egypt

Phenolic compounds	Growing areas			LSD Values at $p \geq 0.05$
	EL-Sharkia	Giza	EL-Arish	
Tyrosol	22.50a	24.10b	25.50c	1.00
Hydroxytyrosol	33.10a	35.20b	37.30c	0.93
P- Hydroxybenzoic	17.20a	18.00b	19.02c	0.70
Caffeic	3.92a	4.11b	5.50c	0.30
Tannic	1.11a	1.19b	1.30c	0.10
Catechin	0.60a	0.80b	1.01c	0.02
Gallic	3.56a	3.88a	4.11a	0.60
Vanillin	3.51a	3.71a	3.80a	0.35

Values in each row followed by the same letter are not significantly different at $p \geq 0.05$

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دليل المرارة ومضادات الاكسدة الطبيعية وخصائص الجودة فى زيت الزيتون البكر من مناطق نمو مختلفة

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يهدف هذا البحث الى دراسة تأثير مناطق النمو المختلفة لثمار الزيتون صنف الكروناكى على خصائص الزيت المستخلص من حيث دليل المرارة على طول موجى 225 نانوميتر ومركبات الاكسدة الطبيعية (الفينولات الكلية – الارثوثنائى الفينول – الفا توكوفيرولات – الكلوروفيل – الكاروتينات) وخصائص الجودة (رقم الحموضة – رقم البيروكسيد – الامتصاص فى منطقة الاشعة الفوق بنفسجية على طول موجى 232، 270 نانوميتير) والخواص الحسية واللون والثبات الاكسيدى . ايضا تم التعرف على تركيب المركبات الفينولية بواسطة جهاز التحليل الكروماتوجرافى السائل فائق الاداء.

اشارت النتائج المتحصل عليها الى وجود اختلافات معنوية بين عينات زيت الزيتون فى المناطق المختلفة. كما وجد ان زيت الزيتون البكر المستخلص من الثمار المزروعة فى منطقة العريش تحتوى على نسب مرتفعة من مضادات الاكسدة الطبيعية مقارنة بالمناطق الاخرى التى تناولتها الدراسة.