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POLYPHENOL CONTENT AND STABILITY OF VIRGIN OLIVE OILS, SEPARATION OF NATURAL ANTIOXIDANT FROM VEGETATION WATER OF OLIVES

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Abstract

Tewenty samples of local olive oils from different location were examined for total polyphenol contents, specific Extinction $E^{1\%}$ at 232 nm and 270nm, peroxide value (Meq/kg oil) and oxidative stability. There was a wide range in the total content (56-460 ppm) , and a polyphenol good correlation was found between polyphenol content and oxidative stability , this suppose the antioxidant role of polyphenols in olive oils. Vegetation water of olive fruits was extracted by ethyl acetate, and the addition of ethyl acetate extract to refined cotton seed oil inhibited the oxidative deterioration when the oil was stored at 100°C.

INTRODUCTION

Virgin olive oil is the oil obtained from the fruit of the olive tree (olea europaea) solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil , and which has not undergone any treatment other than, washing, decantation, centrifugation and filteration. Thus , virgin olive oils contain polyphenols which are usually removed from other edible oils in the various refining stages , Roncero *et al* . (1973).

Several studies concerning the polyphenols in olive oils were published, Roncero et al . (1976) extracted and fractionated polyphenols by thin layer chromatogaphy, several phenolic acids, alcohols, and flavonoids were detected. Gutierrez *et al.* (1977) found a correlation between polyphenol content and odor and flavor , as

determined by organoleptic tests of virgin olive oils.

Gutifinger (1981) noticed that after removal of the polyphenols , the oxidative stability of olive oils decreased considerably and seemed to depend on polyunsaturated fatty acid concentration. It was concluded that polyphenol content can be used as one of the measures for the quality of virgin oil.

On the other hand vegetation water , a major by- product of mechanical extraction , still has little industrial uses and is presumed to contain polyphenols. Roncero *et al* . (1974) and Baice and Cera (1984) determined polyphenols in vegetation water and used paper and gas chromatography for fractionation.

In this study , it was of interest to determine the amounts of total polyphenols in local olive oils of different locations . Also , is was important to determine the quality characteristics and to investigate the effect of polyphenol content on the oxidative stability of olive oils. Also the antioxidative property of total polyphenol of vegetation water of olive was studied.

MATERIALS AND METHODS

Materials:

Fourtteen samples of virgin olive oils were obtained from different locations in the Arab Republic of Egypt, Alexanderia, Vineyards Co. Alexanderia , Gianaclis, (samples 1-6) , Siena (samples No. 7 and 8) , Matroh, (samples 9-11) , General Organization for Agricultural Production, Ministry of Agriculture Giza , (samples 12-14) . The above samples of virgin olive oil, from the 1989- 1990 crop, were produced by mechanical processes, i. e. grinding of the olive fruits , pressing of the pomance and separation of the oil from the vegetation water.

The fruits of three samples of Shemlali, Kronakii and Picual variety olives were obtained from Horticultural Research Institute , Giza, Egypt, and ground , packed in a cheese cloth, then pressed using hydraulic laboratory (Carver) press. After separation of the oil from the vegetation water, the oily layer was dried over anhydrous sodium sulphate, filtered and kept until analysis, (samples 15-17).

Also, the ground fruits of the above olive varieties were dried in an vacuum oven at 60°C for 3 hours, then extracted with a chloroform/methonal mixture, the micella filtered and solvents was evaporated under vacuum at 60°C . The oil produced was dried over anhydrous sodium sulphate and kept until analysis, (samples 18-20).

Refined cotton seed oil was obtained from Cairo Oil and Soap Company , El-Ayat , Giza , Egypt.

Methods:

The total polyphenols were extracted from olive oil using 60% aqueous methanol according to the method described by Roncero *et al*. (1973).

The polyphenols were extracted from the vegetation water by ethyl acetate according to the method described by Baice and Cera (1984).

Specific Extenction $E_{1cm}^{1\%}$ for olive oil, is the U.V. absorbtion of a 1% solution of the oil in Cyclohexane in 1-cm cell was measured according to FAO/WHO (1970). The U. V. absorbtion of the samples were measured at 232 and 270nm using a Shemadzu Spectrophotometer (UV. Vis. 120-02).

Peroxide value (as milliequivalent of peroxide/kg oil) mequvalant of oil samples was determined asd described in A.O.A.C. (1980)

Oxidative stability of the oils was determined at 63°C, oil samples , 100g each were transferred to beakers of 150ml volume and stored at 63°C . Peroxide values of the stored oils were determined asabove method.

The antioxidative activity of polyphenols was concducted by the oven test at 100°C for 120 hr. using refined cotton seed oil. Polyphenols (ethyl acetate extract) from vegetation water at levels 0.100. 200 and 1.000 ppm were added to refined cotton seed oil which were stirred at Ca 60°C for one hr. to ensure the complete dissolution of the antioxidant in the oil . Samples of oil, 50g each were then transferred to open beakers of 100ml volume and stored at 100°C. Peroxide value was determined as above.

RESULTS AND DISCUSSION

The total polyphenols contents of olive oil samples are given in Table1. It can

be noteced that there was a wide range ot total polyphenols contents (56-460 ppm) in olive oil samples of different location in Egypt, also data showed that the method of oil extraction affects the contents of total polyphenols in the oils. Solvent - extracted olive oils were richer in polyphenols than oils obtained by pressing (315-460 VS 56-163 Mg/Kg oil). It can be assumed that the polar polyphenols would dissolve better during solvent extraction of oils than in the polar triglycerides during pressing of the olives. There were a clear differences in total polyphenols contents between olive oil samples which had obtained by solvent extraction and by pressing.

Also from data in Table 1, it can be seen that the olive variety affects the content of total polyphenols. Highest total polyphenols content was noticed for Picual olive oil followed by Shemlali and then Kronakii olive oil. Total polyphenol contents was within the ranges reported by Roncero *et al* . (1973, 1975), Gutfinger (1981) and Sheabar and Neeman (1988).

The specific Extinction $E^{1\%}_{1cm}$ of different virgin olive oils samples were measured at a wave length of 232 nm and 270 nm and the results are tabulated in Table 1. From these results, it can be noticed that the $E^{1\%}_{1cm}$ of virgin olive oil samples at 232 nm, ranged from of 22.7 and 26.9 respectively. The relatively high perioxide values 0.14 to 0.20. It is clear that the absorbances of olive oil samples decreased by increasing the wave length from 232 nm (specific for diene compounds) to 270 nm (specific for triene compounds, Firestone *et al.*, 1985). Mean while , the method of oil extraction affected the U.V. absorbances of olive oils samples at both wave length 232 nm and 270 nm, wher solvent -extracted olive oil samples had $E^{1\%}_{1cm}$ range from 2.25 to 2.75 and from 0.19 to 0.24 at 232 nm and 270 nm respectivley, while olive oil samples obtained by pressing had lower ranges, from 1.90 to 2.20 and from 0.14 to 0.20 at 232 and 270 nm respectively . Such results are in agreement with Codex Alimentarius Commission FAO/ WHO (No. 33-1970), Paganuzzi and Leoni (1979), Khalil (1987), IOOC (1990) Khalil *et al.* (1990) and Khalil and El Agaimy (1991) .

Peroxide value in (Meq/kg oil) which indicate the oxidative rancidity in oils and fats , was determined in all olive oil samples and results showed that peroxide value ranged between 4.20 and 26.9 Meq/kg oil, which was close to the limite reported by IOOC (1990) exept samples No. 7 and No. 12, which had recorded high values which were obtained for some of these samples may be explained by prolonged storage period of the picked olives, before they were pressed.

Table 1. Total polyphenol contents , UV absorbancy $E^{1\%}_{1\,cm}$ at wave lengths 232, 270 nm, peroxide value and oxidative stability of serveral olive oils.

Sample Number	Total Polyphenols (Mg/ Kg oil)	UV absorbancy $E^{1\%}_{1\,cm}$ at wvae lenth		Peroxide value (Mea/kg oil)	Oxidative f stability (days)
		232 nm	270 nm		
1	93	2.15	0.16	12.5	26
2	137	2.10	0.15	6.6	28
3	69	2.20	0.17	16.2	15
4	109	2.08	0.15	13.5	20
5	132	1.95	0.15	10.4	29
6	100	2.08	0.14	11.5	
7	83	2.20	0.18	22.7	11
8	79	2.18	0.16	13.7	16
9	97	2.15	0.16	14.2	15
10	85	2.05	0.15	10.9	13
11	163	1.90	0.14	9.5	31
12	56	2.20	0.20	26.9	9
13	82	2.15	0.16	17.5	18
14	108	2.10	0.15	13.7	19
15 a,d	134	2.10	0.15	6.3	31
16 b,d	125	1.95	0.14	7.5	28
17 c,d	107	2.05	0.14	4.2	25
18 a,e	460	2.75	0.24	13.5	42
19 b,e	412	2.53	0.23	14.5	39
20 c,e	315	2.25	0.19	10.2	36

a : Picual variety olive oils b : Shemlali variety olive oils c: Kronakii vareity olive
d : Laboratory pressed oils e : Solvent - extracted oils

f : Defined as number of storage days at 63°C to obtain peroxide value 70 (Meq/kg oil).

Also, it cauld be observed that olive oils obtained by solvent extraction (samples 18,19 and 20) had peroxide value of 13.5, 14.9 and 10.2 , which were higher than the peroxide value of pressed samples 6.3, 7.5 and 4.2 (samples 15, 16 and 17) respectively this may be due to the oxidation products of phenolic compounds that were extracted by solvent in addition to the lipid peroxides of solvent extracted oil samples. These results are in agreement with the findings reported by Gutfinger (1981) and Kalil *et al.,* (1983).

Oxidative stability evaluations were done by storage of the oil at 63^{o}C and determination of peroxide value. The number of storage days required to obtain peroxide value of 70 Meq/kg oil was taken as a measure of oil's antioxidative stability. From the results of Table 1, it can be seen that the oxidative stability of solvent extracted oils was markedly higher than that of pressed virgin olive oils. The storage time required for the pressed oil samples to reach a peroxide value of 70 Meq/kg was in the range of 9-31 days, wheareas , the solvent extracted oils reached the same peroxide value after 36-42 days. The high resistance to oxidation of the solvent - extracted olive oil samples may be attributed to their total polyphenols contents which considered natural antioxidants. These results are in agreement with those reported by Roncero *et al.* (1973), (1975), Takagi and Iida (1980) , Gutfinger (1981) and Sheabar and Neeman (1988).

The relationship between oxidative stability of virgin olive oils and total polyphenol content, can be shown in Figure 1, A linear relationship is found between total polyphenol content and oxidative stability of olive oils and this supports the antioxidant role of polyphenols in olive oils. This observation is in agreement with the findings of Roncero *et al* . (1973) , Gutfinger (1981) and Sheabar and Neeman (1988).

Oil stability usually is determined at accelerated condition (60^{o}C and more) because ambient conditions demand an excessively long period of time. The antioxidative pot ential of polyphenols extracted from vegetation water was evaluated by their addition to refined cotton seed oil.

The peroxide value serves as an indication of oil quality , genertally it can be stated that the peroxide value is an indication of primary level of oil oxidation. (Augustin and Berry, 1983), the change in peroxide values VS time exhibit both as induction stage, where no secondary oxidative products are formed, and the oxidative stage, where a steep increase in peroxide value occurs.

Addition of total polyphenols (ethyl acetate extract of vegetative water of ol-

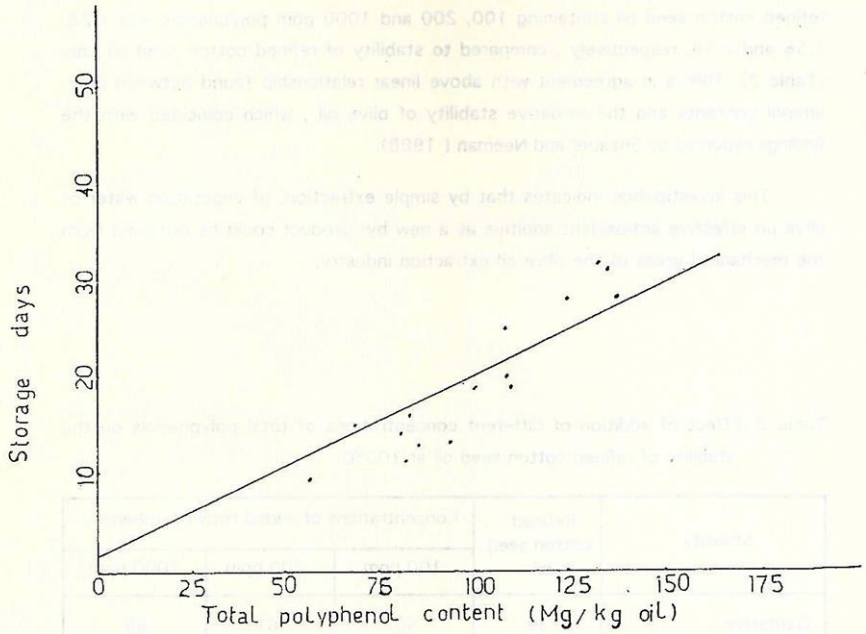

Fig .1. Number of storage days at 63°C required to obtain a peroxide value of 70 Meq/ kg oil vs the total polyphenol content of the oil.

ive) at three different concentrations of 100, 200 and 1000 ppm, enhanced primary oxidative stability compared to control, thus polyphenols added to cotton seed oil improved oil stability (Fig. 2) , the degree of effectiveness was dependent on the concentration used.

After 39 hours at 100°C (the induction stage of refined cotton seed oil), the control showed a continous and rapid increase in peroxides value, whereas the peroxide value of cotton seed oil contain polyphenols, still under 20 Meq/kg. Thus addition of pholyphenols to refined cotton seed oil caused noticeable increase in the induction period of cotton seed oil compared to control. The relative stability of refined cotton seed oil containing 100, 200 and 1000 ppm polyphenols was 1.28, 1.56 and 2.18, respectively , compared to stability of refined cotton seed oil only (Table 2). This is in agreement with above linear relationship found between polyphenol contents and the oxidative stability of olive oil , which coincided with the findings reported by Sheaber and Neeman (1988).

This investigation indicates that by simple extraction, of vegetation water of olive un effective antioxident additive as a new by- product could be obtained from the mechanical press of the olive oil extraction industry.

Table 2. Effect of addition of different concentrations of total polyphenols on the stability of refined cotton seed oil at 100°C.

Stability	Refined cotton seed oil	Concentrations of added total polyphenol		
		100 ppm	200 ppm	1000 ppm
Oxidative Stability at 100°C (time in hours)	39	50	61	85
Relative stability	1	1.28	1.56	2.18

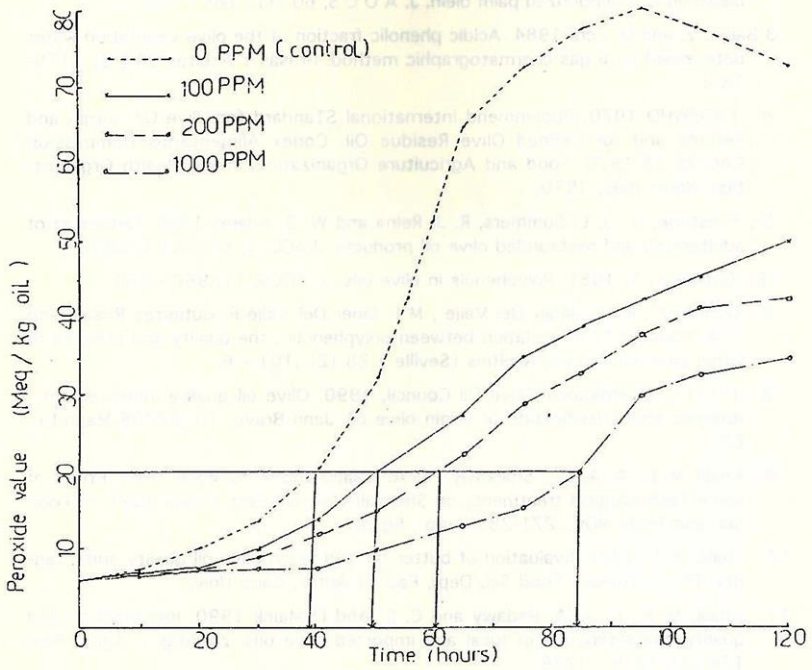

Fig .2. Development of peroxide value in refined cotton seed oil containing ethyl-acetate extract during storage at 100°C.

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محتوي البولي فينولات وثبات زيت الزيتون البكر وفصل مضاد أكسدة طبيعي من المياه النباتيه لعصير الزيتون

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تم دراسة عشرون عينه من زيت الزيتون المحلي من مناطق انتاج مختلفه وذلك لتقدير محتواها من البولي فينولات الكلية - وقياس الامتصاص في منطقة U. V. Specific Extinction $E^{1\%}_{1cm}$ علي طول موجي 270 nm , 232 nm ورقم البيروكسيد (لمكافئ لكل كيلو جرام زيت) وكذلك قياس درجة ثابت هذه العينات.

وقد أظهرت الدرساه أنه يوجد مدي واسع في محتوي العينات من البولي فينولات الكليه (٥٦ - ٤٦٠ جزء في المليون) مع وجود علاقه واضحة بين محتوي عينات زيت الزيتون محل الدراسة من البولي فينولات الكليه ودرجة ثباتها مما يؤكد دور هذه الفينولات كمضادات اكسدة طبيعيه في زتي الزيتون .

وقد استخلصت البولي فينولات الكيه أيضا من المياه النباتية Vegetation water والناتجة من عصير ثمار الزيتون وقد أضيف هذا المستخلص الي زيت بذرة القطن المكرر بتركيزات مختلفه مما ادي الي زيادة درجة ثبات زيت بذرة القطن علي درجة ١٠٠م° مما يوضح أنه بطريه استخلاص بسيطه من المياه النباتيه لعصير الزيتون امكن الحصول علي مضاد اكسده طبيعي من مخلفات معاصر زيت الزيتون .