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Determination of Total Polyphenolic Antioxidants Contents in West-Bank Olive Oil

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Abstract
The purpose of the study was to measure the total concentration of polyphenol in some samples of Palestinian olive oil. The total polyphenol content of the methanol extracts was evaluated colorimetrically using the Folin-Ciocalteau reagent. A diluted extract or phenolic standard was mixed with Folin- Ciocalteau reagent and acqueous Na2CO3 and the total polyphenols were determined colorimetrically at 725 nm. Gallic acid standard solutions were used to calibrate the method. The concentration of polyphenols in olive oil ranges from 150 to 300 mg/kg.
Keywords: Polyphenols, Antioxidant, Olive oils, Gallic acid

1. Introduction

Virgin olive oil is a vegetable oil which can be obtained directly from olive fruit using using various mechanical extraction methods. Its chemical composition consist of major and minor components. The major components, that include glycerol, represent more than 98% of total oil weight. Minor components that amount to about 2% of the total oil weight include more than 230 chemical compounds, e.g. aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds and antioxidants. The main antioxidants of virgin olive oil are carotenes and phenolic compounds including lipophilic and hydrophilic phenols [1]. While the lipophilic phenols, among which are tocopherols, can be found in other vegetable oils, some hydrophilic of VOO are not generally present in other oils and fats. Moreover, the hydrophilic phenols of VOO form a group of secondary plant metabolites that show peculiar sensory and nutritional proprieties [2].

"Phenolic compounds" or "polyphenols" is the term used to define those substance which possess a benzene ring bearing one or more hydroxy groups, including functional derivatives (esters, methyl esters, glycosides, ect.). phenolic compounds are grouped in to the following categories : 1. phenols , phenolic acid , phenyl acetic acids , 2. cinnamic acids , coumarins , isocoumarins and chromones , 3. lignans , 4. ten groups of flavonoids , 5. lignins , 6. tannins , 7. benzophenones , xanthones and stilbenes , 8. quinines , 9. betacyanins . Most phenolic compounds are found in nature in a conjugated form. Phenolic compounds are related to the sensory and nutrititional quality of fresh and processed plant food. The enzymatic browning reaction of phenolic compounds, catalysed by phenoloxidase, is of vital importance in fruit and vegetable processing due to the formation of color and flavour and the loss of nutrients. Oxidative changes of polyphenols are not always undesirable and may contribute directly to enhancing the aroma and flavour of foods. The antioxidant activity of phenolic compounds is of great interest to the food industry .Moreover, their protective role in cardiovascular diseases and cancer as well as the ageing process [3]. The main phenolic compound in olive fruit is oleuropein, a heterosidic ester of elenolic acid with 3,4- dihydroxyphenethylalcohol (hydroxytyrosol) . Other glycosides found in the fruit are ligstroside, aheterosidic ester of elenolic and with 4-hydroxyphenethyalcohol (tyrosol) and verbascoside ,a heterosidic ester of caffeic acid with hydroxytyrosol . Flavones (luteolin -7-gly-coside, apigenin-7-glycoside , luteolin-5-glycoside) and flavonols[3] . Olive oil polar phenol fraction, known for many years as "polyphenols" is in fact a complex mixture of compounds with different chemical structures obtained from the oil by extraction with methanol-water Phenolic compounds are related to the stability of the oil but also to its biological properties. Virgin olive oil phenolic compounds belong to the following classes:

a. tyrosol, hydroxytyrosol, and their derivatives; b. derivatives of 4- hydroxybenzoic, 4-hydroxyphenylacetic, and 4-hydroxycinnamic acids; c. lignans, and d. flavonoids.

Individual phenolic compounds which often appear in lists of olive oil polar phenols are (in alphabetical order): 4-acetoxy-ethyl-1, 2-dihydroxybenzene, 1- acetoxypinoresinol, apigenin, caffeic acid, cinnamic acid (not a phenol), o- and p-coumaric acids, ferulic acid, gallic acid, homovanillic acid, p-hydroxybenzoic acid, hydroxytyrosol, luteolin, oleuropein, pinoresinol, protocatechuic acid, sinapic acid, syringic acid, tyrosol, vanillic acid, and vanillin. The presence of elenolic acid (not a phenol) in the same fraction has also been verified many times. See table 1.

Tyrosol and hydroxytyrosol in their various forms are reported to be the major constituents. The more polar part of the methanol-water extract contains free phenols and phenolic acids. The less polar part contains aglycones of

oleuropein and ligstroside (the hydroxytyrosol and tyrosol esters of elenolic acid), deacetoxy and di- aldehydic forms of these aglycones, the flavones luteolin and apigenin, the lignans 1-acetoxypinoresinol and pinoresinol, and also elenolic acid and cinnamic acid[4],[5]. Polyphenols correlate with key sensory oil properties: bitterness and pungency which are associated with olive oil style. Olive oil classification as mild, medium or robust can be associated to the total phenol content. Robust olive oils tend to have a Total Phenol level above 300 mg/kg, while oils perceived as mild have levels below 180 mg/kg. Thus, oil analysis measuring Total Phenol gives producers guidance for labeling their oil[6].

2. Methodology

2.1 Chemicals and Reagent :

Folin – Ciocalteus phenol reagent solution 1:10 (V/V) in bidistilled water , Sodium Carbonate (Na2CO3) solution 20 % (W/V) , standard solution _ gallic acid 10 mmol/L : 1.8755 g gallic acid in 1000 ml methanol 96 % (V/V) , olive oil sample .

2.2 Procedure:

The researcher collect three sample of olive oil from different location Jerusalem, Tulkarem and Jenin.

The total phenolic levels were determined within a fornight of being judged. 10g of olive oil was dissolved in 50 ml of hexane and extracted three time with 80% aqueous methanol .The extract was then made up to 100 ml with water and left to stand overnight.

Take 1 ml aliquot of the extract and add 5ml folin-ciocalteus phenol reagent. Shake the mixture well and let to stand for 5minutes . 1 ml of saturated sodium carbonate(Na2Co3) was then added and shaker before standing for 1 hour at room temperature . the absorption was read at 725 nm using uv-vis spectrophotometer. For the preparation of calibration curve 1 ml aliquot of 0.05 , 0.2 , 0.4 , 0.5 , 0.6 m mol/l aqueous gallic acid solution were mixed with 5ml folin-ciocalten reagent and 1 ml Na2Co3 (sodium carbonate) solution. And absorption was read after 1 hour at 725 nm in rapport with ablank solution (1 ml bidistilled water ,5ml folin-ciocalten reagent and 1 ml Na2Co3 solution) [8].

2.3 Instrumentation :

Perkin-elmer UV-Vis spectrophotometer with ability to reading absorption at 725 nm. Rectangular glass cuvetts , having an optical length of 1cm were used .The method is most often used in aquantitative way to determine concentrations of an absorbing species in solution , using the beer-Lambert law [9].

3. Results and Discussion

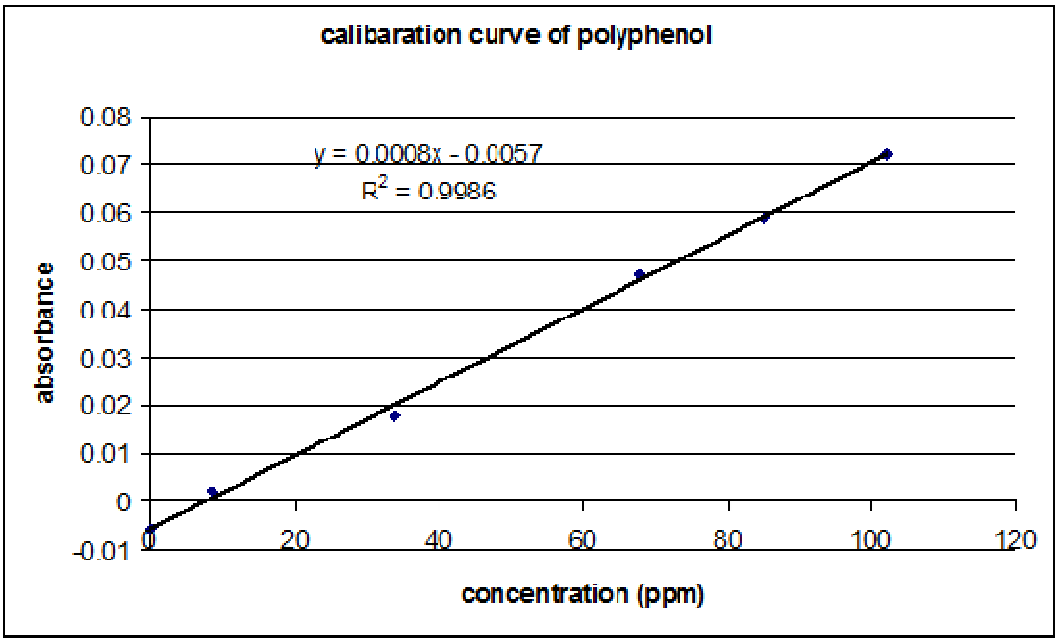
Polyphenols are one type of numerous health-protective antioxidants that are found in extra virgin olive oil. Like other antioxidants, polyphenols fight oxidative stress and may fight against aging-related diseases like heart disease, high blood pressure and cholesterol, and certain types of cancer.

In addition to the anti-aging properties of polyphenols, they also contain strong anti-inflammatory properties. A recent study showed that extra virgin olive oil contains a certain phenol compound called oleocanthal, which acts similar to ibuprofen in the body. This shows the potential for olive oil's ability to help reduce the risk of strokes,. It's thought that two tablespoons of extra virgin olive oil a day is enough to provide these anti-inflammatory benefits [7].

Olives oil samples were analyzed spectrophotometrically for the content of total phenolics, using a modified Folin-Ciocalteu colorimetric method, and calibrated with the absorbance of gallic acid prepared in the same way resulting in concentrations given as mg total phenolics/kg oil expressed as gallic acid equivalents . for plotting the calibration curve of bolyphenol (standard curve of gallic acid).

Table 2: absorbance at different concentration Gallic acid standards at 725 nm

Concentration Of aqueous Gallic acid solution (mM)	concentration Of aqueous Gallic acid solution (ppm)	Absorbance at 725 nm
0.05	8.5	0.002
0.2	34	0.018
0.4	68	0.047
0.5	85	0.059
0.6	102	0.072



The calibration curve equation was : Y= -0.0008 X – 0.0057 and the correlation coefficient (R) for the calibration curve was 0.9986. Then the total concentration of polyphenol in olives oil sample was determined as ppm of Gallic acid.

Table 3: the total concentration of polyphenol in olives oil sample as ppm of Gallic acid

olive oil sample from	absorbance at 725 nm	total concentration of polyphenol (ppm)
Jerusalem	**0.227**	**290.875**
Tulkarem	**0.15**	**194.625**
Jenin	**0.122**	**159.625**

The total concentration of poly phenol for three sample of olive oil from different location Jerusalem, Tulkarem and Jenin was 290.875 , 194.625 , 159.625 ppm of gallic acid respectively .

Polyphenol levels in olives depend on climate, variety, agricultural practices and ripeness at harvest. Polyphenol levels in the fruit are affected by irrigation during the growing season: thrifty watering increases the phenol level. Since polyphenol levels naturally decrease as the olive fruit ripens, harvest time affects their level in the oil: early harvests result in oils with higher polyphenol values.

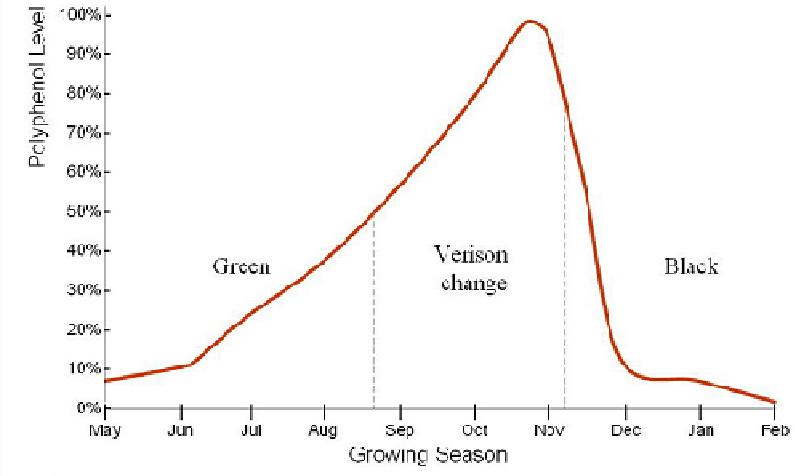


Figure :-4- olive fruit polyphenol level and color during growing season.

Polyphenol levels decrease during milling and storage. Many polyphenols are water soluble and are lost with the vegetation water during processing. In addition, polyphenol levels will slowly decrease during storage, as they dampen oxidation in the oil given these unavoidable losses, an initial high polyphenol level is essential for ensuring longer shelf life and greater health properties [11].

4. Conclusion

A virgin olive oil contains at least 30 phenolic compounds. Phenolic total amount and composition of olive oil varies from 100 to 1000 mg/kg depending on cultivars, place of origin, agronomic techniques, olive ripening, possible infestation by the olives fly Bactrocera oleae , extraction methods, and storage conditions.

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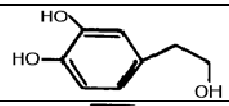
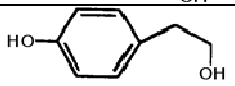
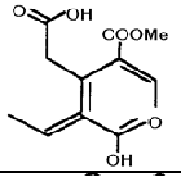
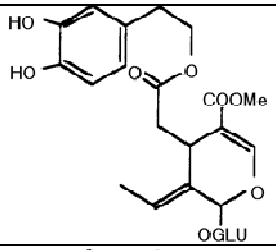
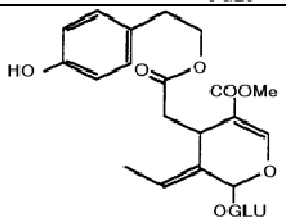
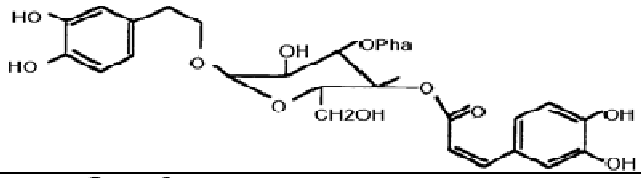
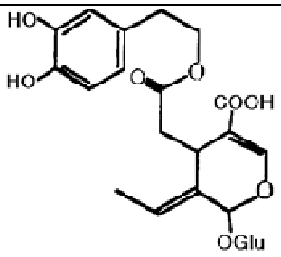
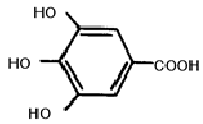
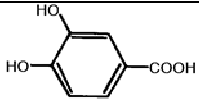
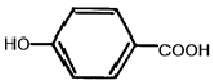
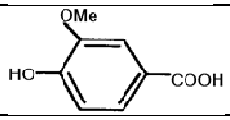
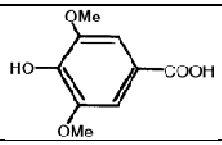
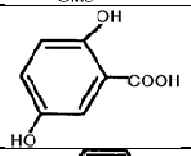
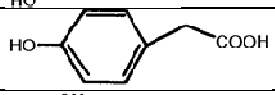
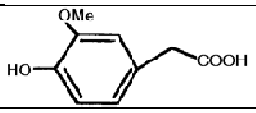
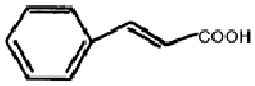
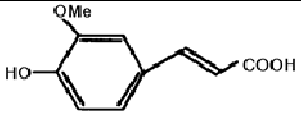
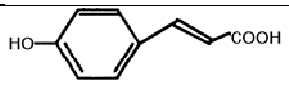
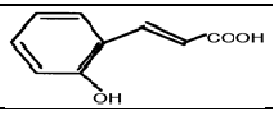
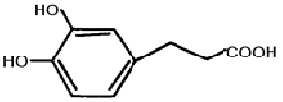
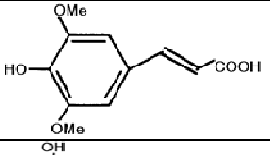
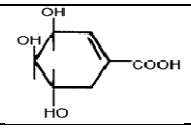
Table 1. Major phenolic compound and related components of olive fruit

Polyphenol	Structure
3,4-dihydroxy-phenethylalcohol (hydroxytyrosol, HYTY)	
4-hydroxyphenethylalcohol (tyrosol , TY).	
Elenolic acid	
Oleuropein GLU=glucose	
Ligstroside GLU=glucose	
Verbascoside	
Demethyloleuropein	
3,4,5-trihydroxybenzoic acid (gallic acid	
3,4-dihydroxybenzoic acid (protocatechuic acid)	
p-hydroxybenzoic acid	
4-hydroxy-3-methoxybenzoic acid (vanillic acid)	

4-hydroxy-3,5-dimethoxybenzoic acid (syringic acid)	
2,5-dihydroxybenzoic acid (gentisic acid)	
p-hydroxyphenyl acetic acid	
4-hydroxy-3-methoxyphenyl acetic acid (homovanillic acid)	
Cinnamic acid	
4-hydroxy-3-methoxy Cinnamic acid (ferulic acid)	
p-hydroxy Cinnamic acid (p-coumaric acid)	
o-hydroxy Cinnamic acid (o-coumaric acid	
3,4-dihydroxy cinnamic acid (caffeic acid)	
4-hydroxy-3,5-dimethoxy cinnamic acid (sinapic acid)	
3,4,5-trihydroxycyclohex-1-enoic acid (shikimic acid)	

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