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Influence of Extraction Method and Production Area on Antioxidant Content (Phenolic Compounds and α-Tocopherol) in Virgin Olive Oil from Iran, Greece and Italy

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In this paper, the effects of the production area and extraction method (traditional and modern process) on virgin olive oil quality were studied . The analysis of α-tocopherol contents in samples appeared that the production area was a critical variable, since this compound of olive oil can vary considerably from area to area. On the other hand, it is also concluded that extraction method affected the presence phenolic compounds.

Key Words: Antioxidant, Extraction method, Phenolic compounds, Production area, α-Tocopherol, Virgin olive oil.

INTRODUCTION

Virgin olive (*Olea europaea* Linn) is one of oldest known vegetable oils and the only one that can be consumed in its crude from (unrefined). People have used olive presses since Greeks first pressing olives over 5000 years ago. It is valued for its fine, balanced, delicious, unique aroma and flavours and long shelf-life. Today its biological, nutritional and healthful effects are universally acknowledged[1,2].

Virgin olive oil quality is affected by several factors, such as a agronomic techniques, seasonal conditions, sanitary state of drupes, ripening stage, harvesting and carriage systems, method and duration of storage and processing technology. However, the environmental and genetic (cultivar) factor are those that basically effect quality and typicality of product. The beneficial effects of olive oil are still due. Not only is its high unsaturated saturated fatty acid ratio, but also to its antioxidants as carotenoids and phenolic compounds[3-6].

Some studies[2,3] have suggested that the antioxidants present in virgin olive protect against cancer and atherosclerosis by impeding the oxidative modification of LDL and its adherence to the arterial wall. These substances also contribute to the stability of oil.

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The levels of antioxidants depend on several factors such as the variety of olive used, the cultivation environment and the method of oil extraction[3,4]. Traditional olive oil extraction is based on applying pressure to olive paste to separate the liquid oil and vegetation water from the solid material. The oil and vegetation water are then separated by standard decantation[2]. This method is still widely used today in Iran (especially in Roodbar and Zanjan) and it is a valid way of producing high quality olive oil. But the modern method of olive extraction uses an industrial decanter to separate all the phases by centrifugation.

The aim of this paper is to investigate the effect of production area and compare the extraction method (traditional and modern process) on several components of the olive oil like quality parameters, phenolic compounds and α-tocopherol content.

EXPERIMENTAL

Virgin olive oils: Four samples of virgin olive oil were obtained from Italy (sample 1), Greece (sample 2), Roodbar (sample 3) and Zanjan (sample 4) and analyzed. Samples were divided in to 2 and 4 groups according to the methods of extraction and production.

Chemicals (α-tocopherol, *n*- hexane, isopropyl, tyrosol, methanol, ethyl nitrile, xylene, potassium hydroxide) were obtained from Merck (HPLC grade). All oil samples were stored in dark brown glass bottles at room temperature until analyzed.

Analytical methods: α-Tocopherol were evaluated by reverse phase high performance liquid chromatography in one run method[2,3,7,8]. Briefly, the method involved a rapid saponification and a subsequent extraction with a mixture of hexane- ethyl acetate (99:1). The chromatographic system consisted *n*-hexane, isopropyl (99:1) at a flow rate 1 mL/min and emission wave length at 254 nm was used.

Phenolic compounds were isolated from a solution of oil in hexane by triple-extraction with water-methanol-ethyl nitrile (60:35:5).

Total phenols, expressed as thyrosol equivalents (mg/kg) were determined with a UV-visible recording RP-HPLC[2,5,7]. The chromatographic system consisted of an C_{18} column.

Statistical analysis: The assays were carried out in 3 replication. The results are shown as labels of mean values and standard deviation. All statistical analysis were performed using statistical manner of company of experimental average.

$$x_1 - x_2 = \pm t\text{spool}\sqrt{N_1 + N_2 \Big/ N_1 N_2}$$

$$S^2 = \sqrt{\frac{N_1 S_1^2 + N_2 S_2^2}{N_1 + N_2}}$$

where x_1 = average of first group; x_2 = average of second group; t = coeffient due to confidence range; Spool = standard deviation; N = number of determination.

RESULTS AND DISCUSSION

α-Tocopherol: Table-1 shows content of α-tocopherol in four different samples. The significant difference is found between Italy and Greece samples when compared the results of their determination. Also, comparing of average for α-tocopherol in Roodbar and Zanjan samples proved significant difference among them.

TABLE-1
CONTENT IN α-TOCOPHEROL (mg/kg)

α-Tocopherol	Sample 1 (Italy) (mg/kg)	Sample 2 (Greece) (mg/kg)	Sample 3 (Zanjan) (mg/kg)	Sample 4 (Roodbar) (mg/kg)
Test 1	12/7	5/4	11/3	6/2
Test 2	13/5	6/6	12/8	6/8
Test 3	14/6	3/8	11/1	6/6

However, the average result of two Iranian samples when compared with Greece and Italy samples separately, showed difference between them but on comparison with Greek sample, no difference is found. The difference is in agreement with Salvador *et al.*[2] production area and extraction technology, which seem to affect the levels of α-tocopherol. But the production area seems more important than other variable.

Phenolic compounds: The contents in phenolic compounds are showed in Table-2. Difference is found between two samples of Greece and Italy. Whereas analysis showed no difference between Iranian samples. In second stage, average results proved difference between the Iranian samples with two other foreign samples separately. This is agreement with the results obtained by Salvador *et al.*[2] and Gimono *et al.*[3] as the production area and extraction technology seem to affects the levels of phenolic compounds.

TABLE-2
CONTENT OF PHENOLIC COMPOUND FROM VIRGIN OLIVE OIL SAMPLES

Phenolic compound	Sample 1 (Italy) (mg/kg)	Sample 2 (Greece) (mg/kg)	Sample 3 (Zanjan) (mg/kg)	Sample 4 (Roodbar) (mg/kg)
Test 1	547/3	326/8	410	419/8
Test 2	513/8	303/6	398/8	412/6
Test 3	524/6	316/8	403/8	405/14

However, the extraction technology seems more effective than other variable because phenols are more hydro soluble than α-tocopherols and are reduced when certain quantity of luke warm water is added during modern extraction for facilitating the extraction process. In addition, better grinding of the olives in traditional method, reduces the releasing of oil oxidation enzymes.

Conclusion

(i) There is a higher content of α-tocopherol and phenolic compounds in Italy virgin olive oil that extracted from modern extraction method. (ii) Regarding the extraction method, the traditional extraction method appears to preserve the phenolic compounds more than modern extraction method. (iii) The results of α-tocopherol determination clearly indicate that the production area is a critical variable.

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