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Phenolic Compounds from Iranian Olive Oil Processing Waste Water

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The purpose of this study was to screen the phenolic compounds extracted from olives grown in the regions of Roudbar and Tarom in Iran. The olive oil residues were tested for their composition in simple phenolic compounds which are usually removed from olive oil at various stages of refining. Total phenolic compounds were extracted from oil samples and oil waste water, and determined spectrophotometrically. The HPLC analysis showed that hydroxytyrosol, vanillic acid and tyrosol were the most abundant phenolic compounds in olive oil, but a substantial proportion of these compounds remains in the water phase generated during olive oil production as waste water.

Key Words: Iranian olive oil residue, Phenolic compounds.

INTRODUCTION

Vegetables, olive oil and fruits are important sources of phenolic type antioxidants. These components are powerful scavengers of free radicals and may therefore protect the human body against free radical initiated diseases[1]. The presence of phenolic compounds in virgin olive oils and their importance as natural antioxidants are known[2–5]. The amount of phenols depends on the variety, climate, location, degree of maturation, type of crushing machine, oil extraction procedures, etc.[6] In the "polar fraction" of olive oil there are more than a hundred different compounds, a significant proportion being the phenolic compounds and polyphenols. The simple phenolic compounds present in olive oil have been identified using commercial standards. However, the identification of complex phenols is a more difficult task given that there are different isomers which co-elute in HPLC and there are no commercial standards or spectroscopic data for most of these compounds. Usually, olive oil is extracted mechanically by pressure and by two or three-phase centrifugation systems, which results in the production of more than 30 million m^3 of black olive mill wastewater [7, 8]. This liquid effluent has a high polluting organic load, due to a high content of organic substances, including sugars, pectins and lipids[9]. Centrifugation, despite its high water consumption, is still the most widely employed method for production of

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virgin olive oil, especially in countries that produce large amounts of olive oil in a short time[10]. Most frequently, olive mill wastewaters are pumped and discharged into evaporation ponds or directly dumped in rivers or spread on soil. This becomes a major environmental problem in the main olive-producing countries of the Mediterranean region, such as Italy, Spain, Greece, Tunisia and Turkey.

The purpose of this study was to screen the major simple phenolic compounds extracted from olives grown in the regions of Roudbar and Tarom in north of Iran and the corresponding olive oil wastewaters were tested for their composition in simple phenolic compounds which are usually removed from olive oil at various stages of refining. Despite the economic importance of the Iranian olive oil there are no complete and reliable data on its chemical composition and phenolic compounds.

EXPERIMENTAL

Acetonitrile, methanol, hexane, acetic acid, ethyl acetate and water were all HPLC grade and were purchased from Merck Company. Methanol, ethyl acetate and hexane for oil extraction were of pro-analysis and were also purchased from Merck.

Cinnamic acid, vanillic acid, 3,4-dihydroxycinnamic acid, 2-(4 hydroxy-phenyl) ethanol, 4-hydroxycinnamic acid, 4-hydroxy-3,5-dimethoxybenzoic acid, 3,4,5-trihydroxybenzoic acid were very pure products obtained from Merk Co. Extra virgin olive oil and olive oil residues were obtained during the 2004–2005 harvest season in the Guilan Province, Roudbar and Tarom area.

Determination of free acidity of olive oil: Free acidity, given as % oleic acid, was determined by titration of a solution of oil dissolved in ethanol/ether (1 : 1) with ethanolic potash.

Peroxide value: Peroxide value, expressed in milliequivalents of active oxygen per kilogram of oil (meq/kg), was determined as follows: a mixture of oil and chloroform/aectic acid was left to react with a solution of potassium iodide in darkness; the free iodide was then titrated with a sodium thiosulfate solution.

Fatty acid composition: Olive oil (0.3 g) dissolved in 4 mL of hexane, shaken vigorously with 0.4 mL of 2 N methanolic potash and analyzed by GC-MS analysis performed on a 30 m capillary column of silicon 5, CB. The temperature programmed was 40°C (held for 2 min), increasing to 250°C. The carrier gas was helium with 10 psi pressure and the amount of sample injection was 1 μL. GC-MS was obtained with a Fisons Instruments GC 8000/Trio 1000. Quantification was achieved using peak area calculation and compound identification was partly carried out using correlation between retention times.

Colorimetric evaluation of total phenol content

(a) Virgin olive oil: 10 mL of a solution of methanol/water (80 : 20 v/v) plus Tween 20 (2% v/w) was added to 10 g of olive oil and mixed with a Vortex at 15000 g for 1 min and centrifuged at 5000g for 10 min; the extraction was repeated two times. To eliminate the oil droplets, the methanolic extract was kept for 24 h at –20°C. The total phenols determined colorimetrically at 765 nm using the folin Ciocalteu reagent and expressed as gallic acid.

(b) Olive oil wastewater: Stabilized and clarified olive oil residues, originating from extraction system, were adjusted to pH 3 with HCl and extracted with ethyl acetate (1 : 1, v/v) two times[11, 12]. The ethyl acetate phase was evaporated to dryness and the residue dissolved in methanol. The total phenols were then determined colorimetrically at 765 nm, using the Folin-Ciocalteu reagent and expressed as gallic acid.

Hydrolysis: The phenolic extracts were alkaline, hydrolysed with 2 M NaOH at 35°C for 30 min[13]. The mixture was then acidified to pH 2 with HCl and extracted twice with ethyl acetate. After evaporation in vacuum, the samples were dissolved in methanol.

HPLC analysis: HPLC system was composed of a 4.6 × 150 mm. LI Bondapak column, coupled with a UV detector (Waters 486): the eluates were detected at 280 nm at 30° C. The flow rate was 1 mL/min; the mobile phase used was 0.01% acetic acid in water (A) *vs.* acetonitrile (B) for a total running time of 50 min. and the gradient changed as follows:

Solvent B started at 5% for 2 min, then increased to 10% in 6 min, to 20% in 17 min, to 70% in 20 min, and to 100% in 1 min, until the end of running. The identity of phenolic compounds was confirmed by standard samples.

Extract purification for HPLC analysis

Virgin olive oil and residues: The methanolic extract was concentrated in vaccum at less than 35°C until it reached a syrupy consistency, was added to 10 mL of acetonitrile and was washed twice with 20 mL of hexane. The acetonitrile fraction evaporated in vacuum until dryness and the residues dissolved in methanol and analyzed by HPLC at 280 nm.

RESULTS AND DISCUSSION

Figs. 3 and 2 depict the representative HPLC chromatogram of phenolic extracts from virgin olive oil of Roudbar and olive oil waste water, respectively. All chromatograms for each sample analyzed were similar, with differences relating only to peak areas. Fig. 1 shows a mixture of hydroxytyrosol and vanillic acid as standard solution when injected separately give these single peaks with different retention times. The median content of total polyphenol compounds in the samples of Roudbar olive oil analyzed was 350 mg/kg (as caffeic acid), as it is shown in Table-1. More than 7% of these compounds transfer into wastewater. Both phenolic extracts from Roudbar olive oil and its wastewater exhibited similar chromatographic profiles figs. 2 and 3 with similar peaks as hydroxytyrosol and vanillic acid by comparing their retention time with standard solution. The main composition characteristics of Roudbar and Tarom oils are a high oleic acid and low linoleic acid content and a large total phenol content (Table-1).

Figs. 4 and 5 depicts the representative HPLC chromatogram of phenolic extracts from Tarom virgin olive oil and the olive oil waste water, respectively. Fig. 6 shows a mixture of hydroxytyrosol and tyrosol as standard solution.

The median content of total polyphenol compounds in the samples of Tarom

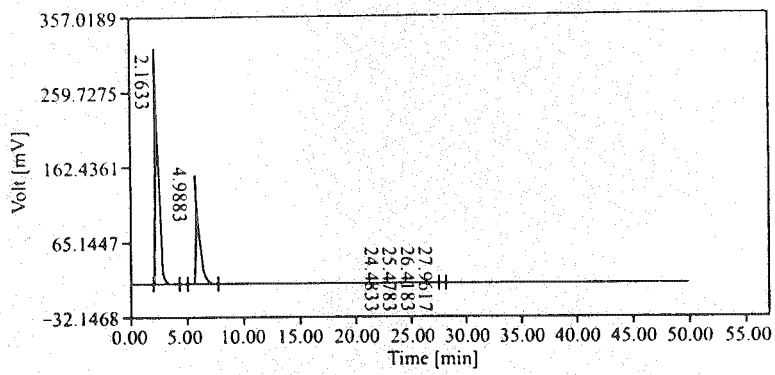
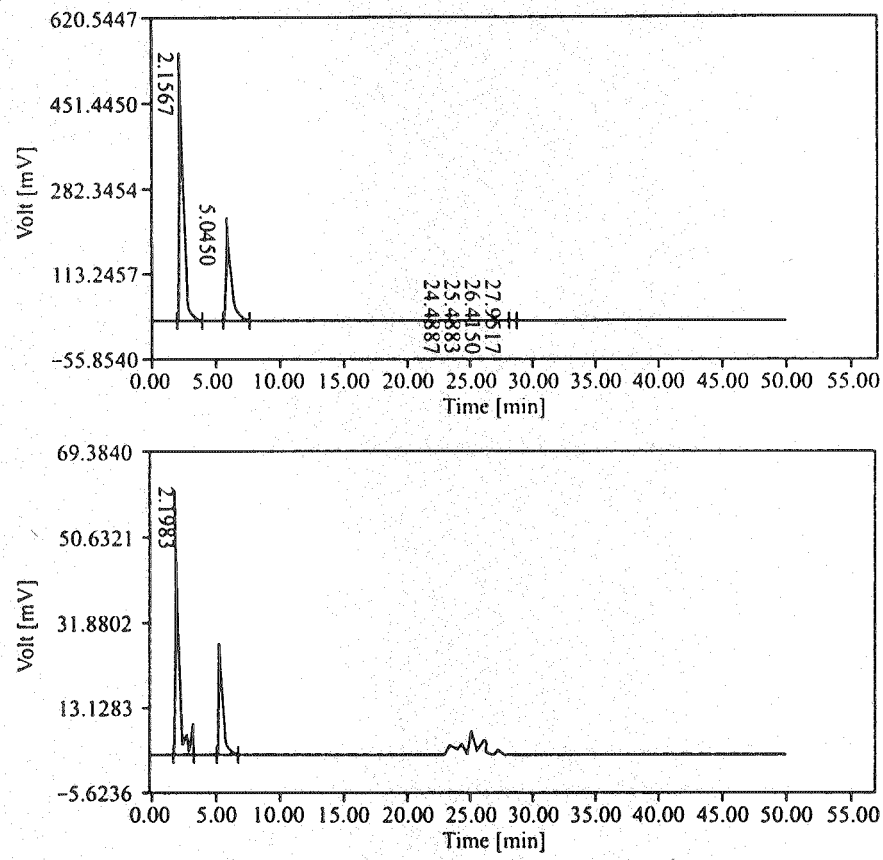


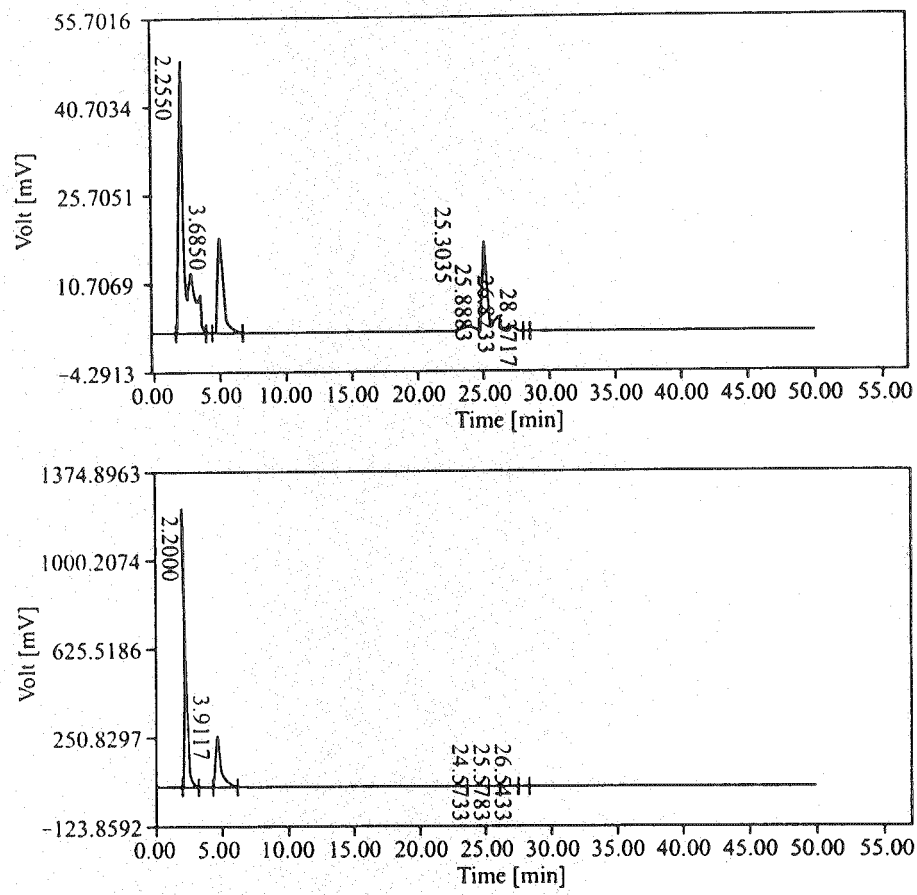
Fig. 1. Standard mixture of hydroxytyrosol RT (2.1633) + vanillic acid RT (4.9883)



Figs. 2 & 3 Separation of phenolic extract of Roudbar olive oil residue and olive oil respectively by HPLC at 280 nm. (hydroxytyrosol + vanillic acid

olive oil analyzed was 85.0 mg/kg (as caffeic acid), as it is shown in Table-2. More than 40% of these compounds transfer into wastewater. Both phenolic extracts from Tarom olive oil and its wastewater exhibited similar chromatographic profiles (Fig. 4 and 5) with similar peaks as hydroxytyrosol and tyrosol by comparing retention times with standard solution. Oleuropein, an ester of elenolic acid and hydroxytyrosol, was found by Visioli[14] as a major compound of olive mill wastewater, did not show absorption at 280 nm. A great number of

simple phenolic compounds have been found in virgin olive oils by several researchers in other countries[15–18], but in our chromatograms only tyrosol (Ty) and hydroxyltyrosol (HTy) and vanillic acid were observed, while all of the other simple phenols reported in the literature are not evidenced.



Figs. 4, 5. Separation of phenolic extract of Tarom olive oil and olive oil residue

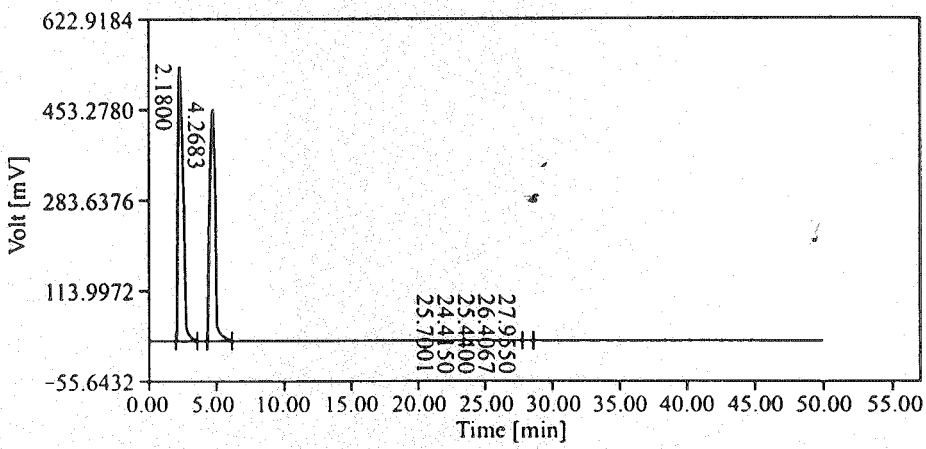


Fig. 6. Standard mixture of hydroxytyrosol + tyrosol

TABLE-1
CHARACTERISTICS OF TAROM & ROUDBAR OLIVE OIL AND WASTEWATER

Parameters	Tarom	Roudbar
	Olive oil	
Free fatty acid (%)	0.3	0.4
Peroxide (meq/kg)	7	6
Oleic acid (%)	74.4	71
Linoleic acid (%)	6.9	8.4
Total phenols (mg/kg)	85.0	350
	Olive oil wastewater	
Dry residue %(w/w)	15.6	17.8
pH	5.9	5.8
Total phenols (mg/kg)	38.0	25.0

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