Physicochemical properties of two varieties of olive oil (Roghani & Zard) in Iran

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Abstract-In this study, the physicochemical properties of the oils of two varieties of olive, named Roghani and Zard, were examined. Selection of varieties was based on the fruit production performance per hectare and the percentage of existing oil in the country. Comparison of fatty acids indicated a significant difference in that the ratio of USFA to SFA of Zard and Roghani oils were 4.62 and 4.15, respectivelyand their oxidizability values were 1.74 and 2.15, respectively. The iodine value of Zard and Roghani oils were determined 77.64 and 80.52, respectively. A significant difference ($p<0.05$) was observed between the saponification number and unsaponifiable matter content and the wax content of the two oils The amount of total tocopherol content (based on α-tocopherol) and polyphenol content (in terms of Gallic acid) in Zard oil were 244.27 and 87.25, and in the oil of Roghani variety were 420.71 and 258.54 ppm, respectively. In addition, sterol compounds in these oils were measured 2.70 and 3.68, respectively. The results revealed that Roghani variety had more appropriate chemical composition than Zard one.

Keywords- olive oil, physicochemical properties, Roghani, Zard

I. INTRODUCTION

Olive oil has a long history and is considered as one of the best edible oils that has amazing effects on human health. This oil is a clear liquid with light yellow, green or brown inclined to green in the mild temperature. According to the Iranian standard, olive oil is divided into four types including virgin, semi-refined, refined and sulfured (Maghsudi, 1999). Regarding the economic importance and status of this valuable vegetable oil, latest researches conducted in the case of its nutritional benefits led to the increased tendency of people toward this product and as a result to the increased production of it throughout the world particularly in Iran. Currently, more than 115 thousand hectares of the orchards of Iran are under olive cultivation and more than $100 million in the country have been invested in the processing and packaging of olive in that 4500 tons of olive oil, including refined and virgin, have been produced and packaged in the current year (Bolandnazar, 2010).

Among the major varieties of olive in Iran, Zard, Roghani and Mari ones can be mentioned. Two varieties of Zard and Roghani have the highest cultivation area and oil yield. Studies have revealed that olive oils resulted from Iranian varieties don't have favorable oxidative stability, comparing with the foreign ones and the researchers maintain that low tocopherol content caused by severe refinement and usage of inappropriate packaging are the main reasons of this shortcoming (Fahimdanesh et al, 2011). Virgin olive oil contains a large number of phenolic compounds including tyrosol, tyrosol hydroxyl, phenolic acids, lutein, apigenin, as well as tocopherols (E vitamins) which in turn include α, β, γ and δ isomers. All these compounds are involved in the category of natural antioxidants which reduce the speed of oxidation and photooxidation reactions. At the same time, they create a favorable flavor in the oil. The total content of tocopherol and phenolic compounds in the virgin olive oil have been accounted for 100-300 and 50-200 ppm, respectively (Maghsudi, 1999). Researchers have shown that the amount of tocopherols depends on the type of variety, and the amount of polyphenol depends on the region of cultivation and that of fatty acids depends on both these factors.

II. MATERIALS AND METHODS

A. Materials

The olive varieties of Roghani and Zard were provided from olive orchard located in Rudbar Olive Research Station. Fresh fruits of them were harvested in the maturation stage based on color index (Uceda & Hemoso, 1989). The reagents were purchased from Merck and Sigma Companies.

B. Oil Extraction

In order to extract the oil, the fruits were crushed by a grinder (FP HP 15 INOX, Pieralisi, Stainless steel, 6 spokes, 2800 rpm, Italy) and the obtained paste was

malaxated at 25°C for 20 min. It was then pressed manually and centrifuged (heraeusSepatechGmbh, Labofuge, Germany) at 7000 rpm for 2 min. Hexane was employed to separate oil from aqueous phase. The obtained oil was kept in amber bottles in 4°C until experiments.

C. Fatty acid composition

The fatty acid composition was determined by gas-liquid chromatography and reported according to relative area percentages. Fatty acids were transesterified into their corresponding fatty acids methylate esters (FAME) by vigorous shaking of a solution of oil in hexane (0.3 g in 7 mL) with 7 mL 2 N methanolic potassium hydroxide at 50 °C for 15 min. The FAME were identified using a HP-5890 chromatograph (Hewlett-Packard, CA, USA) equipped with a BPX 70 (Supelco, Bellefonte, PA, USA) capillary column of fused silica, 60 m × 0.22 mm ID, 0.2 µm of film thickness, and a flame ionization detector (FID). Helium was used as carrier gas with a flow rate of 0.7 mL/min. The oven temperature was kept at 198 °C and that of the injector and the detector at 280 and 250°C, respectively (Farhoosh et al 2008).

D. Calculated oxidizability (Cox) value

The Cox values of the oils based on the percentages of the unsaturated C18 fatty acids were determined by the equation proposed by Fatemi and Hammond:

Cox = [1(18:1%) + 10.3(18:2%) + 21.6(18:3%)]/100

E. Iodine value (IV)

The IV was calculated according to the following equation:

IV = [((%C16:1)0.95)+((%C18:1)0.86)+((%C18:2)1.732) +((%C18:3) 2.616)]

Where C16:1, C18:1, C18:2 and C18:3 represent Palmitoleic, oleic, linoleic and linolenic acid, respectively (firestone 1993).

F. Acid value (AV)

10 g of the sample was weighed in an Erlenmeyer flask and dissolved in 50 ml of ethanol: chloroform (1:1). The resulting solution was titrated against 0.1 N KOH using phenolphthalein as the indicator. The AV was calculated based on the following equation:

$$AV = \frac{N \times V \times 56.1}{W}$$

Where N and V are the Normality and volume of KOH, respectively and W stands for the weight of the sample (AOCS 1993).

E. Oil/oxidative stability index (OSI)

In order to measure the OSI, a Metrohm Rancimat model 743 (Herisau, Switzerland) was applied. The tests were conducted using 3 g of the oil samples at temperatures of 110 and 120 °C and airflow rate of 20 L/h (Farhoosh 2007).

F. Saponification number (SN)

5 g of the oil sample was weighed in an Erlenmeyer flask and mixed with 50 ml of alcoholic KOH. Then, 1 ml of phenolphthalein was added to it and the resulting solution was titrated by 0.5 N HCl till the disappearance of the pink color. Eventually, the entire procedure was performed on the blank which contained all reagents but the oil. The SN was calculated based on the equation below:

$$SN = \frac{(B-S) \times N}{W} \times 56.1$$

Where B and S are the volume of HCl for the blank and the sample, respectively and w is the sample weight (AOCS 1993).

G. Total phenolic (TP) content

A calibration curve of gallic acid in methanol was performed in concentration range of 0.04 - 0.4 mg/ml. The solutions for the spectrophotometric analyses were prepared by the following procedure: in a 50-ml volumetric flask, 1 ml of a standard solution of gallic acid, 6 ml of methanol, 2.5 ml of the Folin-Ciocalteu reagent, 5 ml of %7.5 Na2CO3were added and the final volume was reached with purified water. The solutions were stored overnight and the spectrophotometric analysis was performed at 765 nm. The determination of polyphenols was conducted as follows: 2.5 g of the oil sample was solved with 2.5 ml of n-hexanein a centrifugation tube. The solution was shook on a tube shaker for 1 min and 2.5 ml of methanol: water (80:20, v/v) was added to it and finally the resulting mixture was centrifuged (heraeusSepatechGmbh, Labofuge, Germany) at 5000 rpm for 5 min. The procedure was triplicated. The extract was added to a 50-ml volumetric flask containing 2.5 ml of the Folin-Ciocalteure agent and 5 ml of Na2CO3 (7.5%) and the mark was made with purified water. The samples were stored overnight and their absorbance values were read at 765 nm. The TP content was determined in terms of mg/kg based on the following equation:

$$TP = \frac{Y}{W} \times 1000$$

Where Y is the TP content obtained from the standard curve in terms of mg/ml, and W is the oil weight (Capannesi et al 2000).

H. Total tocopherol (TT) conten

The standard solutions of a-tocopherol in toluene with concentration range of 0-240 µg / ml were prepared. 1 ml of the standard solution or 200±10 mg of the oil sample was weighed in a 10-ml volumetric flask.5 ml of toluene was added and shook well in order to dissolve the oil in toluene. 3.5 ml of 2,2´bipyridine solution (0.07% w/v in 95% aqueous ethanol) along with 0.5 ml FeCl3.6H2O (0.2% w/v in 95% aqueous ethanol) were also added and mixed. Eventually, the resulting solution was made to the volume with 95% aqueous ethanol. After keeping the solution unmoved for 1 min, the absorbance value was read at 520 nm. The calibration curve was depicted and its gradient was calculated. The TT content was determined in terms of mg/kg based on the following equation:

$$TT\ content = \frac{A-B}{M \times W}$$

Where A and B are the absorbance values of the sample and the blank, respectively. M is the calibration curve gradient and W is the sample weight (Wang et al 1998).

I. Unsaponifiable matter (USM) content

0.5 g of the oil sample was weighed in a 15-ml glass tube and saponified with 5 ml of 1 N ethanolic KOH. The tube was sealed and kept at 95°C for 1 hour. After cooling, the tube content was mixed with 10 ml of distilled water. Next, the extraction of USM was triplicated by adding 10 ml of ether into the decanter funnel. The organic phases pertaining to each of etheric extraction replicates were added together and eluted with 10 ml of distilled water in duplicate. After each elution replicate, the organic phases were separated and added together again. In order to ensure the removal of the remaining saponifiable matter, 10 ml of 0.5 N ethanolic KOH was added to the final mixture. Then, 10 ml of distilled water was used to wash out the remaining saponified matter. Finally, the organic phase was separated from the aqueous one and dehydrated sodium sulfate was added to dry the organic phase. After filtering the organic phase through Whatman No. 1 filter paper, the filtrate was transferred to a previously weighed Erlenmeyer flask and dried at 45 °C in a vacuum oven. The percentage of the USM was calculated according to the following equation:

$$\%USM = \frac{(W_u - W_c)}{W_s} \times 100$$

where Wu is the weight of the Erlenmeyer flask as well as the USM. Wc is the weight of the empty Erlenmeyer flask and Ws is the weight of the oil sample (Lozano et al 1993).

J. Wax content

5 g of the oil sample was weighed in an Erlenmeyer flask and five times its volume of acetone was added. The solution (oil/ acetone) was cooled and kept at 4 °C overnight to have the waxes crystallized. The solid fraction was filtered through a previously weighed Whatman No. 1 filter paper, dried at 45 °C in a vacuum oven and then weighed to obtain the acetone in soluble matter (Mezouari et al 2006).

K. Total polar compounds (TPC)

500 mg of the oil sample was weighed in a 5-ml volumetric flask and made to the volume with toluene.1 ml of the solution was poured carefully into the chromatographic column from top. In order to prepare isohexane solvent system, isohexane solvents and diisopropyl ether (85:15 v/v) were mixed. After the sample was macerated on top of the column and vaporization of toluene, 1, 3.5 and 3.5 ml of the separation solvent was injected into the column during three distinct stages. At the end of the process, the bottom of the column was eluted with 500 µl of toluene. After vaporization of the solvent at 40°C in a vacume oven for 30 min, the remaining nonpolar components were weighed. The percentage of the total polar compounds was calculated by the following equation:

$$\%Cp = \frac{W_s - W_n}{W_s} \times 100$$

where Ws and Wn are the weights of the sample and the nonpolar compounds, respectively (Schulte 2004).

L. Sterol compounds

1 g of the oil sample was weighed in a 10-ml volumetric flask and made to the mark with chloroform. 1 ml of the solution was diluted with 10 ml of chloroform. 3 ml of the resulting solution was introduced to a15-ml glass tube and 2 ml of the Lieberman-Butchart reagent was added to it and the solution volume was reached to 7 ml by adding chloroform. After keeping the solution in darkness for 15 min, the spectrophotometric analysis was performed at 640 nm.

The method was calibrated by preparing standards containing 0-2.5 mg of pure cholesterol in 10 ml chloroform and then analyzing as above.

The percentage of the sterol compounds was obtained from the equation below:

$$E = \frac{Y}{W \times X \times 10}$$

where Y is the sterol compounds obtained from the standard curve; W is the sample weight and X is the dilution coefficient which equals to 0.01 in this experiment (Sabir et al 2003).

M. Density

The oil density was determined according to the Pycnometer method. First, the empty 50-ml pycnometer was weighed. Then, the oil sample was weighed in the pycnometer. The density was achieved from the following equation:

$$\rho = \frac{A-B}{V}$$

where A is the weight of the pycnometer and the oil. B is the weight of the empty pycnometer and V is the volume of the oil sample.

N. Viscosity

A rotational viscometer (Visco 88, Bohlin Instruments, Malvern, Worcestershire, UK) equipped with a heating circulator (Model F12-MC, JulaboLabortechnik, Seelbach, Germany) was employed to investigate the rheological behavior of the oils. Appropriate measuring spindle (C30) was used during viscosity measurements according to the viscosity of the oils. Samples were loaded into the cup and allowed to equilibrate at the desired temperature of 25±0.5 °C (Morris, 1983).

O. Refractive Index

The refractive index was determined by a refractometer (Abbe, opticivymen, Spain) equipped with a thermostatic circulator.

P. Statistical analysis

All experiments and measurements were duplicated and the data were subjected to analysis of variance (ANOVA). ANOVA and regression analyses were performed via Minitab (USA) and SlideWrite software. Significant differences between means were determined by Fisher's F-test; ($p<0.05$) was considered statistically significant.

III. RESULTS AND DISCUSSION

The chemical composition of Roghani and Zard olive oils are shown in Table 1. The highest percentage of saturated fatty acids (SFA; primarily palmitic acid, C16:0) was %19.38 in Roghani and %17.78 in Zard. Among the MUFA, the percentage of Palmitoleic acid (C16:1) was 1.58% in Roghani and 1.03% in Zard, where as their percentages of oleic acid (C18:1 ω9) ranged from 65 to 75%; thus, Roghani had a %MUFA (66.1%) lower than that of Zard (72.54). The percentages of polyunsaturated fatty acids (PUFA; mainly linoleic acid, C18:2ω6, and a-linolenic acid, C18:3 ω3) were observed 14.12% and 0.33% in Roghani, and 9.19% and 0.47% in Zard. From the information stated above, Roghani showed a PUFA/SFA ratio of 0.74, Cox value of 2.15 and IV of 80.52 while those of Zard were 0.54, 1.74, and 77.64 revealing the resistance of this oil to oxidation.

The AV of Roghani and Zard were 1.43 and 1.43 mg/g, respectively, indicating that these two oils were unoxidized and of high initial quality (Table 2). Tocopherols along with phenolic compounds are important functional constituents of a small number of vegetable oils. Tocopherols have antioxidant properties and are active as vitamin E, which makes them particularly vital for human health. Interest in phenolic compounds is associated primarily with their antioxidant activity; in addition, they show important biological activity in vivo and may be beneficial in combating diseases related to excessive oxygen radical formation exceeding the antioxidant defense capacity of the human body. There was significant difference between the TT content of Roghani (424.44 mg/kg) and Zard (242.72 mg/ kg). The TP content of Roghani (258.54 mg/kg) was significantly higher than that of Zard (92.26 mg/kg). On the other hand, the TPC of Roghani (3.782%) had no significant difference with that of Zard (3.786%). TPC is regarded as one of the most important tests for evaluating the oil degradation. Polar compounds are the total of non-triglyceride compounds occurring in oils which include alkali contaminations, sterols, tocopherols, mono and diglycerides and etc. It is assumed that the highest amount of the toxic compounds exist within the polar compounds. Sterol compounds are quantitatively the most important portion of the USM in nearly all vegetable oils. The average amount of these compounds ranges from 0.3-2% in edible oils. However it exceeds 10% in some of them. The sterol compounds of Roghani and Zard oils were 3.68% and 2.70%, respectively.

The SN of Roghani (177.88 mg/g) was not significantly different from that of Zard (178.94 mg/g) (Table 2). Since there is an inverse relationship between SN and the weight of fatty acids, and the fatty acid compositions of both oils were similar, the insignificant difference between the SNs was expected. Considering the high SNs of the oils, it could be concluded that both of them contained a large number of low molecular weight fatty acids. Oil molecular weight depends not only on fatty acid composition but extremely on wax and USM contents. The wax content of Zard (5.60%) was higher than that of Roghani (5.11%). Waxes are a group of insoluble high-melting point compounds that occur naturally in crude vegetable oils. These compounds can be disadvantageous as they are primarily responsible for the dark color (turbidity) of refined oil and a high refining loss. The USM content of Roghani (9.78%) was significantly higher than that of Zard (7.86%) (Table 2). Thus, Roghani is more resistant to sensory deterioration than Zard. This can be attributed to the low levels of the PUFA/SFA ratio, the Cox value and the high content of tocopherol compounds in Zard (Tables 1 and 3).

The OSI of Zard at 110 and 120 °C were14.78 and 4.85 h and those of Roghani were 14.33 and 5.53 h which had no significant difference with the corresponding amounts of Roghani. The oxidative stabilities of the oils are relatively high and it could be due to the reasons stated before.

The viscosities of the oils were investigated at various shear rates at 25±0.5°C. The results are shown in tables 3 and 4. It was observed that the Roghani had a higher viscosity than Zard But Newtonian behavior was observed among both. The reason is probably the higher USM content of Roghani.

The refractive indexes of Roghani and Zard were the same and equaled to1.46 and the specific gravities of Roghani and Zard were 0.9147 and 0.9127 (table 2).

IV. CONCLUSIONS

The Roghani and Zard oils are of the most beneficial olive oils. Research about natural antioxidants has been increasing in recent years, since they can protect the human body from free radicals and retard the progress of many chronic diseases. Our results in this research indicated that Roghani and Zard oils can be used as sources of natural antioxidants. More studies concerning separation and identification of their antioxidative components needs are being conducted currently in our research team.

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Table 1.Chemical composition of Zard & Roghani olive oils

Parameter	Zard	Roghani
C16:0	11.82±0.05[b]	13.67±0.33[a]
C16:1	1.03±0.03[b]	1.58±0[a]
C17:0	0.13±0.03[a]	0.09±0.04[a]
C17:1	0.17±0.01[a]	0.11±0.02[a]
C18:0	3.88±0.10[a]	3.83±0.16[a]
C18:1c	69.17±1.13[a]	62.44±0.17[b]
C18:2c	9.19±0.19[b]	14.12±0.11[a]
C18:3	0.47±0.02[a]	0.33±0.04[b]
C20:0	0.71±0.05[a]	0.67±0[a]
C21:0	0.77±0[a]	0.72±0.02[a]
C22:0	0.30±0.06[a]	0.23±0.05[a]
C22:1	2.16±0.60[a]	1.96±0.36[a]
C24:0	0.16±0.02[a]	0.15±0.03[a]
SFA	17.78±0.34[b]	19.38±0.01[a]
MUFA	72.54±0.55[a]	66.10±0.15[b]
PUFA	9.67±0.21[b]	14.45±0.07[a]
USFA	82.21±0.34[a]	80.55±0.08[b]
USFA/SFA	4.62±0.10[a]	4.15±0[b]
COX Value	1.74±0.01[b]	2.15±0[a]
Iodine Value	77.64±0.62[b]	80.52±0.23[a]
PUFA/SFA	0.54±0[b]	0.74±0[a]

Table 2.Physicochemical properties of Zard & Roghani olive oil

Parameter	Zard	Roghani
Acid Value	1.43±0.04[a]	1.43±0.04[a]
OSI (110 °C)	14.78±0.55[a]	14.33±0.16[a]
OSI (120 °C)	4.85±0.49[a]	5.53±0.17[a]
TP	92.26±7.98[b]	258.54±3.90[a]
Specific Gravity	0.9127±0[b]	0.9147±0[a]
Refractive Index	1.46±0[a]	1.46±0[a]
Sterol	2.70±0.22[b]	3.68±0.28[a]
TT	242.72±7.43[b]	424.44±0.53[a]
USM	7.86±0.01[b]	9.78±0.15[a]
SN	178.94±0.24[a]	177.88±0.62[a]
WAX	5.60±0.01[a]	5.11±0[b]
Polar Value	3.786±0.90[a]	3.782±1.99[a]

Table3.Viscosity of Zard olive oil

Time '(s)	Shear Rate '(1/s)	Shear Stress '(Pa)	Viscosity '(Pas)
30	14.44	1.65	0.1141
60.063	14.44	1.61	0.1119
90.125	19.29	1.95	0.1009
120.188	19.28	1.93	0.1001
150.25	23.77	2.28	0.0957
180.313	23.77	2.27	0.0954
210.375	28.47	2.66	0.0935
240.438	28.47	2.66	0.0934
270.5	33.09	3.09	0.0933
300.563	38.01	3.47	0.0913
330.625	42.46	3.82	0.0901
360.688	47.13	4.16	0.0883

390.75	*51.72*	*4.53*	*0.0876*
420.813	*61.09*	*5.26*	*0.0861*
450.875	*65.83*	*5.61*	*0.0853*
480.938	*75.42*	*6.34*	*0.0841*
511	*79.84*	*6.71*	*0.0840*
541.063	*89.08*	*7.42*	*0.0834*
571.125	*103.13*	*8.56*	*0.0830*
601.188	*112.70*	*9.30*	*0.0826*
631.25	*126.45*	*10.43*	*0.0825*
661.313	*140.60*	*11.55*	*0.0821*
691.375	*159.36*	*12.99*	*0.0815*
721.438	*177.95*	*14.43*	*0.0811*
751.5	*196.73*	*15.92*	*0.0809*

Table 4.Viscosity of Roghani olive oil

Time '(s)	*Shear Rate '(1/s)*	*Shear Stress '(Pa)*	*Viscosity '(Pas)*
30	*14.66*	*1.99*	*0.1355*
60.062	*14.57*	*1.88*	*0.1291*
90.125	*19.27*	*2.44*	*0.1266*
120.187	*19.26*	*2.57*	*0.1335*
150.25	*23.76*	*4.02*	*0.1692*
180.312	*23.75*	*3.96*	*0.1668*
210.375	*28.46*	*6.04*	*0.2124*
240.375	*28.46*	*6.01*	*0.2111*
270.437	*33.07*	*7.39*	*0.2236*
300.5	*38.00*	*8.02*	*0.2110*
330.562	*42.45*	*8.49*	*0.1999*
360.625	*47.12*	*8.90*	*0.1890*
390.625	*51.70*	*9.14*	*0.1767*
420.687	*61.07*	*10.12*	*0.1658*
450.781	*65.80*	*10.60*	*0.1611*
480.844	*75.39*	*11.55*	*0.1532*
510.922	*79.82*	*11.93*	*0.1495*
540.984	*89.05*	*12.76*	*0.1433*
571.047	*103.10*	*14.10*	*0.1368*
601.109	*112.65*	*15.00*	*0.1332*
631.109	*126.41*	*16.26*	*0.1286*
661.172	*140.54*	*17.48*	*0.1244*
691.234	*159.31*	*19.24*	*0.1208*
721.297	*177.90*	*20.91*	*0.1176*
751.359	*196.68*	*22.50*	*0.1144*