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Original Research

Physicochemical Traits, Variation in Oil Contents and Comparative Analysis of Selected Varieties of *Olea Europaea* Cultivated under Specific Agro-Ecological Conditions

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Abstract

Fruits of five different varieties of *Olea europea* were taken from the farms cultivated in Pothohar region of Pakistan. Oils were extracted using cold press technique. Different colored oils were obtained in different yields. Physicochemical parameters were carried out by American Oil Chemists Society (AOCS) official methods. Fatty acid profiles were calculated by gas chromatograph equipped with flame ionization detector, after methanolic esterification of olive oils. The most abundant of the fatty acids was oleic acid (62.1-70.15%). The yield percentages (15.3-17.7%), moisture contents (0.4-0.73%), refractive indices (1.4689-1.4691 at 20ºC, 1.4613-1.4615 at 40ºC, 1.4537-1.4540 at 60ºC), saponification value (188-191 mg KOH/g), colors measured by Lovibond tintometer (20Y/2-6 R), acid value (0.17-0.44 mg KOH/g), peroxide value (7.83-17.75 meq/Kg), free fatty acid value (0.08-0.22), iodine value (87-92) were determined. Olive cultivars from Pothohar region indicated very close characteristics to the standard olive oil recommended by International Olive Oil Council (IOOC). Characterized local olive cultivars can contribute as an import substitute of edible oil and a lot of foreign exchange can be saved, as well as lot can be earned by exporting it.

Keywords: olive cultivars, chromatography, functional groups; free fatty acids, yield

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Introduction

Lipids are present in food in various forms and have a high nutritional value. They produce more energy as compared to protein and carbohydrates in animal metabolism. Fatty acids, which are the main component of lipids, oils, fats and esterified waxes, can be categorized on the basis of the length of carbon their chains [1-5]. Those having carbon chains from C1:0 to C10:0 are categorized as short-chain fatty acids (SCFAs). SCFAs are present in milk fat, palm seed oil and palm kernel oil. They are volatile in nature and are thus designated as volatile fatty acids. They have comparatively high calorific value. Long chain unsaturated fatty acids are subdivided into poly unsaturated fatty acids (PUFAs) and mono unsaturated fatty acids (MUFA) [6]. Omega-6 and omega-3 are considered best from a nutritional point of view. The olive tree, *Olea europea,* is is a slow growing, evergreen member of the *Oleaceae* plant family and is known as zaitoon. Its branches are thornless, flowers have characteristic aroma and leaves are elliptical. It is native to Mediterranean basin, Australia and California. It is being cultivated in Baluchistan and Punjab provinces of Pakistan [7-9]. Olive fruits differ in size depending on the cultivar and can weigh from one to ten gram per fruit. They are green when immature, turning to dark blue to violet and ultimately black on ripening, with soft pulp. Oil is extracted from the skin, pulp and seeds. The fruit matures in October to November in the northern hemisphere while its physiological maturity is achieved in January and/ or February. Olives are extremely bitter in taste if unprocessed, because of the presence of oleuropein. Some cultivars, however, have a low level of oleuropein and are known as sweet olives. The fruit, which is classified botanically as a drupe, like the peach and the cherry, is composed of skin, pulp, pit and one or two seeds. The content of the seed is 27% oil, 30% aqueous, 27% carbohydrate and 10% proteins.

Pakistan is the world's fourth largest importer of edible oil. Pakistan import almost 70% of its edible oil to meet the country's requirement and consumes too much foreign exchange as import bills. Domestic production meets just 30% of the total oil requirement . Pothohar is considered the best area for olive cultivation due to the local climate and topography. has therefore been developed as Olive Valley [10].

The current work focuses on the specification of extracted olive oils and detailed studies of fatty acids. The agronomical conditions may affect the nature of oils extracted by specific method. The five locally cultivated varieties in Pothohar region were selected. These trees are cultivated in the Pothohar region of Punjab, Pakistan and the detailed studies have been carried out to evaluate the physicochemical traits of the cold pressed oils.

Materials and Methods

All of the solvents/reagents used were of analytical-grade mostly products of Merck-Darmstadt, Germany. Cultivars named BARI[1] Zaitoon, BARI[2] Zaitoon, Koroneiki, Pendollino, and Arbequina were encoded as A, B, C, D and E respectively. Samples were cleaned and weighed. Olive fruits oils were expressed with cold press as well as with solvent for comparative determinations. Collected olive samples were washed with excess water, dried at 60ºC for 3 h and weighed to obtain yield. *n*-Hexane is used as solvent for extraction of olive oils. Each fruit sample was washed, dried at 60ºC. The cyclic solvent system extracted the oils in about twenty-one cycles. Yield is calculated to assess the productivity of any crop with respect to its oil contents.

$$\text{Oil Yield}(\%) = \frac{\text{weight of extracted oil (g)}}{\text{weight of fruit(g)}} \times 100 \quad (1)$$

The moisture/volatiles are determined by taking weighed amount of sample by sensitive balance at least up to 4th decimal. The sample (15 g) is gently heated on the hot plate up to cessation of bubbles, then cooled in desiccator at room temperature and weighed for calculations.

$$\text{Moisture}\ (\%) = \frac{\text{loss in mass,(g)}\times 100}{\text{mass of sample (g)}} \quad (2)$$

Refractive Index (RI) values are determined by ATAGO Digital Refractometer RX7000 α at 20ºC, 40ºC and 60ºC. The specific amount of KOH required to acidify one gram oil is referred as acid value. 5 g weighed sample is taken in titration flask, added 25 mL ethanol and 3-5 drops phenolphthalein as indicator. Solution was warmed on water bath at 70ºC upto 12 min. Solution was titrated with standard KOH (0.1N) until pink color appeared.

$$\text{Acid value} = \frac{56.1\ \text{xN x V}}{\text{W(g)}} \quad (3)$$

Where, N is normality of KOH, V stands for volume of KOH and W is weight of oil sample.

POV is mill equivalents of peroxide per Kg of oil, it indicates the presence of rancidity or oxidation of sample. The precisely weighed oil sample (5 g) was taken in 250 mL flask. The solvent mixture (50 mL) was added and kept for a minute and then was shaked. Then, titrated $Na_2S_2O_3$ (0.1 M) with vigorous shaking upto disappearance of yellow color of iodine. 0.5 mL of KI (1%) and starch indicator was added and titration was continued up to end point (violet coloration). Parallel procedure followed for blank reading.

$$\text{Peroxide Value(meq /kg)} = \frac{(\text{B}-\text{S})\times \text{V}\times 1000}{\text{W}} \quad (4)$$

Where B is volume of titrant used for blank (mL) and S is vol. of titrant used for sample (mL). SV is mg of KOH to saponify (1 g) of an oil sample. A weighed amount of sample (2 g) was taken in 250 mL titration flask. Alc. KOH (25 mL) was added and reflux heated for 1.5 h on water bath. Three drops of phenolphthalein indicator were added. Mixture was titrated with HCl (0.5N) up to end point (light pink to colorless). The parallel procedure was adopted for blank reading.

$$\text{Saponification value} = \frac{\text{N x } (V_2 - V_1) \text{ x } 56.1}{\text{Weight of oil}} \quad (5)$$

Where N denotes HCl Normality (0.5 N), V_2 shows HCl volume for blank (mL) and V_1 stands for HCl volume for sample (mL). Iodine value expresses the iodine absorbed by 100 g (oil). The oil sample (1 g) was taken in a quick fit iodine flask. 15 mL pure CCl_4 was added to the flask and shaked well. The Wij's solution (20 mL) was added in to the iodine flask. The mixture was placed in dark for 2 h then 15 mL freshly prepared KI was added to the iodine flask followed by 100 cm^3 of distilled water. shake and titrate the excess iodine with 0.1N standard sodium thiosulphate solution till red colour changes to yellow. Then add starch solution as indicator and titrate against 0.1 N standard sodium thiosulphate solution till blue colour disappears.

$$\text{Iodine Value} = \frac{\text{Nx } (V2 - .V1)}{W(g)} \text{x} 12.69 \quad (6)$$

Where W = Weight of oil sample (g), N = Conc. of sodium thiosulphate solution, V_2 = Sodium thiosulphate soln. for blank (mL) and V_1 = Sodium thiosulphate soln. for oil sample (mL). The Lovibond tintometer is used for the measurement of colors and rancidity of all five expressed olive oils. The lovibond cell 5¼ is filled with the sample and the colors are measured interms of red (R) and yellow (Y) scale by increasing color filters on yellow and red scales till colour matching. The same instrument was used for the measurement of rancidity after treating the samples with floroglucinol reagents. The FTIR spectrums of all the samples were taken in the absorption frequency range 600-3900 cm^{-1}. ATR surface was cleaned and washed using methanol, 2-3 drops of sample were poured on the sample slot. FTIR spectrum were recorded for expressed olive oils; BARI[1] Zaitoon, BARI[2] Zaitoon, Koroneiki, Pendollino, and Arbequina.

The methyl esters of extracted oils of all five varieties were esterified prior to GC-FID analysis. The methyl esters of fatty acids were prepared by heating the sample (0.5 mL) under pressure with 1 mL boron trifloride-methanol reagent in Teflon lined screw capped test tube. It was heated in water bath for an hour. The separating funnel was used for the procurement of methyl esters by the use of *n*-hexane and water. The lower water layer was removed and the upper hexane layer containing the methyl esters was filtered through silica gel and anhydrous sodium sulfate to ensure the complete removal of water molecules. The samples were collected in the vials and labeled. Fatty acid analysis was carried out by application of GC-14A, Shimadzu, Japan paired with flame ionization detector (FID). Split injection ratio was adjusted at 1:60. The fatty acids profile of all five varieties were determined. Each of the prepared fatty acid methyl esters (FAME) were run on GC using BPx70 capillary column (0.25 mm id × 60 m) Agilent Inc., USA. High purity nitrogen gas (99.999%) was the carrier gas. High purity hydrogen gas (99.999%) was the detector gas. Detector and injector temperature were set at 280ºC and 250ºC respectively. Column oven temperature was set at 140ºC for 2 min then raised to 170ºC at rate of 6ºC/min and hold for 1 min, then raised to 210ºC at programming rate of 3ºC/min and hold for 10 min and raised to 220ºC having ramping rate 10ºC/min and hold for 5 min. 1 µL sample was injected. Different fatty acids were identified by comparing their retention times with known FAME standards (Supelco® USA) at same operating conditions.

Results and Discussion

The five varieties of olive crops were cultivated in different olive farms in the Pothowar Region, Pakistan. Their physicochemical properties are given in Table 1. The important aspects of cultivation oil yield through cold press expression and oil quality olive cultivation should produce high quantities of very good quality oil (1000 to 2000 kg perhectare) to achieve recognition in the market [11]. The cold press extraction method is considered suitable for the whole fruits of all five varieties (A to E), as it yields a high quality product, while oil yields of 15.3 to 17.7 %. The oil yield per acre is 1.2-2.0 tones if 350 trees are cultivated per acre and they are about 10 feet apart. The Koroneiki (C) fruit had the highest yield, at 17.7 % and the Pendolino (D) the lowest, at 15.3 %. There is thus not significant difference in the oil yields of the selected varieties. In quality control, moisture content is a very important parameter of interest, as it reflects the shelf life, quality and economic importance of edible oils [12]. A higher moisture content increases the probability of rancidity and favors microbial growth. Arbequina (E) had the highest moisture content at 0.72 %, while BARI-1 Zaitoon (A) had the lowest, at 0.43%.

A characteristic parameter of edible oils and fats is the refractive index RI), which decreases with an increase in temperatures. The different pure oils, Vanaspati ghee and milk fats may be characterized by their RI values, while higher values indicating more conjugated unsaturation than non-conjugated oils and fats. The cold press expressed oils have a higher proportion of coloring components as compared to solvent extracted oils and fats. Here, the highest value was for the Pendallino (20 Y/ 6 R), followed by

Table 1. Physicochemical properties and Fatty acids profiles of five olive varieties cultivated in Pothohar region.

S. No.	Parameters	[A]	[B]	[C]	[D]	[E]
1	Oil Yield (%)	17.10	16.10	17.7	15.30	17.50
2	Moisture @105±2ºC (%)	0.43	0.45	0.52	0.71	0.73
3	POV (meq/Kg)	7.83	9.35	17.75	11.98	14.84
4	RI @ 20ºC	1.4691	1.4690	1.4692	1.4689	1.4690
5	RI @ 40ºC	1.4615	1.4613	1.4614	1.4613	1.4614
6	RI @ 60ºC	1.4540	1.4538	1.4539	1.4537	1.4539
7	Color (Lovibond)	20 Y/4 R	20 Y/2 R	20 Y/2 R	20 Y/6 R	20 Y/3 R
8	SV (mg KOH/g)	191	190	189	189	188
9	IV (Wijs' Method)	87	92	89	90	92
10	AV (mg KOH/g Acid)	0.17	0.30	0.34	0.40	0.44
11	FFA (% as Oleic Acid)	0.08	0.15	0.18	0.2	0.22
12	Rancidity (Kries Test)	<1.5 R	<1.5 R	<1.5 R	<1.5 R	<1.5 R
Fatty Acid (%) Profile						
1	Myristic acid ($C_{14:0}$)	0.53	0.44	0.45	0.55	0.61
2	Palmitic acid ($C_{16:0}$)	11.07	14.51	12.07	10.50	15.01
3	Palmitoleic acid ($C_{16:1}$)	0.80	1.54	0.70	0.85	1.25
4	Stearic acid ($C_{18:0}$)	2.40	1.45	2.49	1.50	1.30
5	Oleic acid ($C_{18:1}$)	67.02	63.09	68.02	70.15	62.1
6	Linoleic acid ($C_{18:2}$)	15.03	16.7	13.52	13.7	16.88
7	Linolenic acid ($C_{18:3}$)	1.60	0.96	1.50	1.20	1.36
8	Arachidic acid ($C_{20:0}$)	0.80	0.65	0.5	0.8	0.70
9	Gadoleic acid ($C_{20:1}$)	0.41	0.37	0.42	0.42	0.40
10	Lignoceric acid ($C_{24:0}$)	0.34	0.29	0.33	0.33	0.30

POV: Per oxide value; RI: Refractive index; SV: Saponification value; IV: Iodine value; AV: Acid value; FFA: Free fatty acids

BARI-1 Zaitoon (20 Y/ 4 R). The colors of all varieties are higher, whereas generally the colors of processed cooking oils are lower. The saponification value (188-191 mg KOH/g) of oils indicates that chain length of fatty acids. Butter has higher values than vegetable oils due to higher contents of short chain fatty acids. Arbequina (E) and BAR-1 Zaitoon (A) olives had the same saponification values, showing both has same chain lengths of fatty acids, while Pendallino (D) had the lowest saponification value and thus the smallest proportion of short chain fatty acids.

Iodine values (87-92) indicate the extent of unsaturation in expressed oils. As unsaturation increases, the absorption of iodine increases [13]. According to the literature, olive oil has an iodine value of 80.3, making it fit for human consumption. Arbequina (E) was found to have the highest iodine value, at 92.42, and BARI-1 (A) the showed lowest, at of 86.93. As unsaturated fatty acids are good from a health point of view, Arbequina oil is of higher quality is than BARI-1. The peroxide value (POV; 7.83-17.75 meq/kg) of mechanically expressed oils indicate the extent of rancidity or oxidation of oils. More is the peroxide value; more is the probability of rancidity. Koroneiki indicated highest POV (17.75) BARI-1 Zaitoon the lowest (7.83). BARI-1 Zaitoon was therefore the least likely to become rancid, whereas Koroneiki had the highest probability of rancidity during storage. The olive oil samples had acidity values in the range of 0.17-0.44 mg KoH/g. Higher values occur as a result of the deterioration of fatty acids. Arbequina had the highest acidity, at 0.44, while the BARI-1 variety had the lowest, at 0.17, indicating that BARI-1 Zaitoon is more stable when exposed to air and water, whereas Arbequina should be protected from such exposure. A direct measure of the quality of edible oil is its free fatty acid value (0.08-0.22% as oleic acid) [14]. A low free fatty acid value in edible oils is a good indication of un refined oils, and is useful for refining process. The solvent extraction method produces a higher oil content (15.71-21.52%)

as compared to mechanical expression (12.70-17.50%). However, the conventional single press expressed virgin olive oil is a specialty product. Olive oil contains natural pigments, carotenes, polyphenols, Vitamin-E, and other natural antioxidants which are oils own preservatives. Antioxidants minimize the autoxidation or auto generation of peroxides, delaying the onset of oxidation and rancidity. The values of IV (87-92) and SV (189-192) were found to vary only slightly and to be close to other varieties of different regions and under different agronomical conditions. The relatively high oleic acid content of olive oils gives them a higher IV than palm oil, cotton seed oil and milk fats. FT-IR analysis. Infrared spectroscopy gives information about molecular structure and functional groups by assigning specific absorption bands. In oils, fats and lipids, specific absorption frequencies are attributed to specific functional groups. Absorption peaks were observed in in ranging between 3400 and 3700 cm^{-1}, due to -OH functional group. Peaks at 3003-3005 cm^{-1} indicate $C=CH_2$ alkenyl unsaturation asymmetric vibrations [15-17]. Absorption peaks ranging between 2850 and 3025 cm^{-1} are attributed to the C–H stretching vibration of terminal methyl and methylene groups of fatty acid chains. Vlachos et al. [15] reported that absorption frequencies in the range of 3050-2740 cm^{-1} and 1746 cm^{-1} are due to the production of saturated aldehydes functional groups. Absorption frequencies at 1650to 1500 cm^{-1} indicate the carbonyl absorption of aldehydes and ketones. Absorption frequencies in the range of 1500-900 cm^{-1}, termed the "fingerprint region", are characteristic of the molecular composition of minor component present in oils, fats and lipids. Peaks at 1157-1158 cm^{-1} indicate the stretching vibrations of C-O-C bonds while those at 1104-1105 cm^{-1} correspond to the stretching vibrations of dialkyl C-O groups. FTIR revealed absorption frequencies at 966 cm^{-1} for trans C=C bending [18, 19]. However; trans contents were not identified in all varieties of extracted olive oils. Fatty acids of cold press expressed oils were transesterified with Boron Trifluoride-Methanol Complex (Merck-Schuchardt, Ho-henbrunn, Germany), following the standard method, and the FAME were analyzed on GC-FID. Ten fatty acids were present. Palmitic acid (11.07-15.50%) was found to be the major saturated fatty acid in all varieties, while stearic acid (1.30-2.49%) was the minor saturated fatty acid. Oleic acid (62.10-70.15%) was overall the major component of the whole fatty acids, followed by linoleic acid (12.70-17.0%). The oleic acid (ω_9) in olive oil has nutritional importance, because omega-9 fatty acids have been shown to increase HDL ("good") cholesterol and decrease LDL ("bad") cholesterol, thus helping to eliminate the build-up of arterial plaque, causes heart attack and stroke. Linolenic acids (0.96-1.60%) palmitoleic acids and (0.80-1.54%) were also present in minute quantities. Fatty acid percentages differ from cultivar to cultivar. Those under study exhibited value comparable to the Memecik Ayvalik and Gemlik olive varieties of Turkey [20] close to those of the Greek Koroneiki and Throumbolia cultivars and with approximately the same fatty acid composition as the Mari cultivar from Iran . Saturated fatty acids (SFAs; (12.18-17.43%), unsaturated fatty acids-(82.57-87.82%), MUFAs (62.10-70.15%) and PUFAs (14.9-19.48%) were all present in the cultivars. There were slight differences in each category of fatty acids. The SFA levels of olive oils are lower than those of coconut oil, palm oil, cotton seed oil and soyabean oil, while MUFA levels are higher than in common vegetable oils. The ratio of oleic to linoleic acid, which is an indication of the oxidative stability of olive oil, ranged from 3.67 to 5.12, approximately similar to ratios reported for Throumbolia (3.19) [21] and the Spanish Bodocal (5.07) variety [22].

Conclusion

The results of a detailed study of selected olive fruit oil from the Pothohar region of Punjab, Pakistan was carried out. The results revealed that the agronomical conditions of the region were promising with respect to the qualities of expressed oils. Solvent extraction resulted in higher yield, however first press expressed oils were nutritionally more important. In this study fatty acid compositions, FTIR- spectral analysis and physicochemical analysis were carried out. The results were in comparison to extra virgin olive oils of other regions as recommended by Codex Alimentarius and IOOC. It is concluded here that government should take some viable steps to enhance the olive cultivation at both central provincial levels. It would be possible to meet local demand for consumption of edible oils, increase foreign exchange and boost farmers' income by means of rural development.

Conflict of Interests

The authors declare no conflicts of interest.

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