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Research Article

**PHYSICOCHEMICAL ANALYSIS AND FATTY ACID
COMPOSITION OF OILS EXTRACTED FROM TWO EXOTIC
CULTIVARS OF OLIVE (*OLEA EUROPAEA* L.) CULTIVATED
IN BALOCHISTAN, PAKISTAN****Imran Khan¹, Sabeena Rizwan², Ashif Sajjad^{1*}, Kulsoom Baloch¹, Khalid Mahmood¹,
Irfan Hafeez³, Zahid Mehmood¹ and Mohammad Azam¹.**¹ Institute of Biochemistry, University of Balochistan, Sariab Road, Quetta, Pakistan.² Department of Chemistry, Sardar Bahadur Khan Women's University, Quetta, Pakistan.³ PCSIR Laboratories Complex, Lahore, Pakistan.**Abstract:**

The purpose of this study was to carry out physicochemical analysis and to evaluate the fatty acid composition of imported olive cultivars. Oils were extracted from cultivars Leccino and Coratina which are being cultivated in two districts of Balochistan province. Physicochemical properties of the oils were boiling point, 286-290°C; melting point, -5.3 to -6.8°C; refractive index (25°C), 1.63-1.98; iodine value, 64-72/100 g of oil; acid value, 0.27-0.89 mg of KOH/g of oil; saponification number mg KOH/g of oil, 186.4-198.7; and specific gravity, 0.89-0.93 respectively. Fatty acids which are commonly present in olive oils were analyzed using Gas Chromatograph. The results obtained showed the presence of varied quantities of fatty acids (stearic, arachidic, linoleic, oleic, linolenic, palmitic, palmitoleic). A high percentage of oleic acid (61.56-67.43%) was found, followed by palmitic acid (13.60-17.61%), linoleic acid (12.23-14.45%), and stearic acid (1.40-2.68%) respectively in cultivars from both districts. Physicochemical quality and fatty acid composition of the extracted oils were in conformity with International Olive Oil Council (IOOC). The study concluded that both olive cultivars demonstrated promising physicochemical properties and fatty acid composition. This could be useful as edible oil and would have industrial applications.

Keywords: *olive, olive oil, physicochemical properties, fatty acids.***Corresponding author:****Ashif Sajjad,**

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INTRODUCTION:

The aim of introducing exotic cultivars of olive (*Olea europaea* L.) was to demonstrate that low water demanding crops have more potential, with acceptable economic returns. Olive cultivars; Leccino and Coratina were introduced in Balochistan province a southwestern region of Pakistan for the up gradation of agriculture productions, in order to improve the economic prospects of the province. Both cultivars originated from Italy have distinct set of identification characteristics like medium and large fruits, respectively [1]. Olive oil is used as edible oil in different parts of the world and it has the healthy diet phenomena, which is due to the presence of its monounsaturated fatty acid content. In order to reduce and to prevent the cardiovascular diseases faced by the human health a limited amount of saturated fatty acids are needed. The mono unsaturated fatty acids which are present in olive oil help to lower the large amount of cholesterol contents. An increase consumption of olive oil can decrease the threats from rheumatoid arthritis, an autoimmune disease which leads towards the inflammation and pain especially in joints [2]. Olive cultivars differentiate and vary in their composition because of their maturity of fruits, climate and altitude [3]. These factors contribute in their composition and their slight difference may change the free fatty acid contents of olive oil which plays a pivotal role in digestive disorders created by fat soluble vitamins and facilitates these fats to be absorbed well [2]. It has been reported that 55-66% of the poly phenols are present in olive oil are well absorbed in the small intestine after its consumption [4]. Furthermore, the olive contents like tocopherols and phenolic compounds are used for its antimicrobial and antioxidant properties which have a positive effect on gut health [5]. Nutritionists as well as researchers suggest olive oil for daily consumption due to their antimicrobial activity against diseases. Gastric cancer which can be caused by *Helicobacter pylori*, can be cured by consuming olive oil as its compounds show antimicrobial activity against this disease [6]. The presence of monounsaturated fatty acids in olive oil shows healthy effects on the people's health. The death ratio is less among the people who take un-saturated fats in their diets compared to those people; don't take it in their diet [7]. Olive oil is also used in diet against metabolic syndromes which consists of following risk factors; high blood pressure, high blood sugar and high blood lipids. Eventually, this can lead towards different types of diseases like cardiovascular and type 2 diabetes. Therefore, using regularly olive oil in diet can reduce these factors [8]. Olive oil is processed by

different methods to bring oil to edible grades, these methods including; Greek style naturally black olives in brine, Spanish style green olives in brine and naturally black olives in brine [9]. Among the free fatty acids oleic acid is found to be effective against colon, breast and prostate cancer cell. Researchers discovered that oleic acid has the quality to suppress the oncogene which is critical to etiology [10]. The aim of this study was to investigate physicochemical properties and fatty acid composition of oils extracted from two exotic cultivars of olive (*Olea europaea* L.) 'Leccino' and 'Coratina' grown in two districts of Balochistan, a province of Pakistan.

MATERIALS AND METHODS:**Collection of olive samples**

Olive grown sites were selected for samples collection the north east district Loralai and the central district khuzdar of Balochistan province. Four samples, two olive cultivars Leccino and Coratina were selected from each district. The samples from four trees of each cultivar were handpicked at the stage of maturity and mixed well to give a representative sample.

Olive oil samples

Olives pitting were carried out by olive pitter for oil extraction. Each sample was crushed in grinder and the paste was kept for 30 minutes at room temperature so as to mix properly. The samples were then centrifuged without the addition of water. The oil was separated on the surface and was taken out and stored in the dark bottles. Samples were stored at 4°C for further analysis.

Determination of physicochemical parameters

Extracted olive oils for their boiling point, melting point, refractive index, acid value and specific gravity were measured by following the standard method of American Association of Analytical Chemists AOAC, [11]. The determination of iodine value of the samples was carried out by following standard method of AOCS, [12]. While the saponification value of oils samples were measured by following the standard method of AOAC, [13]. All parameters were determined in triplicate for each oil sample.

Fatty acid profiling

For the fatty acid profile in the olive oil samples was adapted an official method of AOAC, [11] with slight modification and analyses were carried out in triplicate. For fatty acid methyl esters preparation 0.1 g of oil sample was placed in the test tube then 0.2 ml internal standard and 0.5 N methanolic sodium hydroxide 15 ml were added. The tube was placed in

water bath at 80°C for 30 minutes. In another test tube 4 ml of the above solution and 5 ml of BF₃ in methanol was added. The above mentioned heating step was repeated. After that 5 ml of saturated NaCl solution was added and the mixture was vortexed thoroughly. The extraction was carried out thrice with 2 ml of hexane. Water was removed by the addition of small amount of anhydrous sodium sulphate to the hexane extract. For the analyses of prepared fatty acids methyl esters (FAMES), the oven temperature was kept at 84°C for 4 min and was raised to 175°C at a rate of 15°C/min for 15 min, 220°C at a rate of 25°C/min for 25 min and 240°C at a rate of 4°C/min for 10 min. The injector and detector were maintained at 220°C and 240°C respectively, while column temperature was 140°C and the nitrogen was used as a carrier gas with flow rate of 40ml/min. In order to obtain standard and individual peaks of FAMES, a volume of 0.1-0.2 µL hexane solution of methyl ester was injected into GC equipped with flame ionization detector (FID). Separated fatty acid methyl esters were identified by comparing their retention times with those of reference standards. Normalization procedure was used for the quantification of individual fatty acids by calculating the corresponding relative percentage of the total fatty acid methyl esters.

Statistical analysis

The obtained results were analyzed by using IBM Statistical Package Social Sciences (SPSS) v. 20.0. The Kolmogorov–Smirnov (KS) test was applied for the assessment of normality and normal tests were used accordingly. Descriptive analysis was carried out by which frequencies and percentages were used

to depict demographic features. Two sample independent t-test was used to determine the association between study variables with p values of 0.05 taken as significant. The results were presented as mean.

RESULTS AND DISCUSSION:

The oil from two olive cultivars was extracted and their properties were evaluated analytically. Olive samples collected were two exotic cultivars grown at two different districts (Loralai and Khuzdar). Four trees of each cultivar were selected, labeled in field experimental area and about 1 kg of sample was collected from each labeled tree. Physicochemical analyses; boiling point, melting point, refractive index, iodine value, acid value, saponification number and specific gravity were carried out by following standard methods. The obtained results are shown in Table 1 which showed some differences in physicochemical values; boiling point (299°C and 290°C), melting point (-5.7°C and -6.4°C), refractive index (1.68 and 1.63), iodine value (72 and 76), acid value (0.27 and 0.24), saponification number (186.4 and 186.5) and specific gravity (0.93 and 0.89) were found respectively for cultivar Leccino cultivated in Loralai and Khuzdar districts. While Coratina cultivated in Loralai and Khuzdar districts following values: boiling point (286°C and 294°C), melting point (-5.3°C and -6.8°C), refractive index (1.64 and 1.98), iodine value (67 and 64), acid value (0.78 and 0.89), saponification number (195.6 and 198.7) and specific gravity (0.91 and 0.89) were found respectively.

Table 1: Physicochemical properties of oil extracted from Olive cultivars.

Properties	Loralai		Khuzdar	
	Leccino	Coratina	Leccino	Coratina
Boiling point (°C)	299	286	290	294
Melting point (°C)	-5.7	-5.3	-6.4	-6.8
Refractive index (25°C)	1.68	1.64	1.63	1.98
Iodine value (g of iodine/100 g of oil)	72	67	76	64
Acid value (mg of KOH/g of oil)	0.27	0.78	0.24	0.89
Saponification number (mg KOH/g of oil)	186.4	195.6	186.5	198.7
Specific gravity	0.93	0.91	0.89	0.89

Values are means $n = 3$.

The obtained results illustrated that no significant differences were found in physicochemical parameter of oils extracted from two olive cultivars Leccino and Coratina grown in Loralai and Khuzdar districts. Refractive index of oils extracted from cultivar Leccino was (1.68 and 1.63) and from cultivar Coratina it was (1.64 and 1.98). The obtained values were close with other cultivars grown in different parts of the world, which were also ranged between 1.46 to 1.47 reported by Ibrahim *et al*, [14]. Saponification number; Leccino (186.4 and 186.5) and Coratina (195.6 and 198.7) were found respectively, for olive oils from cultivars grown at both locations. The above results were in agreement with its acceptable limits according to International Olive Oil Council IOOC, [15]. The IOOC saponification number of olive oils samples ranged from 187.05 mg KOH/ g to 194.85 mg KOH/ g, were neither below nor above to the range. Furthermore, the results obtained from the studied cultivars from both locations a significant correlation was found in their acid value, iodine value, melting point and boiling point. The above parameters were found to be in accordance to the limits set by the IOOC, [15] standard as in the case of iodine value of the samples ranged from (72 to 76) for Leccino and (64 to 67) for Coratina respectively, from both locations. The obtained results regarding iodine value were different from those mentioned by the Ibrahim *et al*, [14] in

which the amount of iodine value was investigated in more than 10 samples which were found in high percentage from 80 to 90. The observed differences might be due to the maturity of the samples altitude, climate and other reasons which can change and/or influence the percentage of iodine value.

The olive oils quality indicator is its fatty acid composition which should be monitored. Analyzed oil samples and their outcomes were formulated statically in order to calculate their mean value and standard deviation. The results are presented in Table 2 revealed that in the analyzed samples following fatty acids: palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and arachidic (C20:0) respectively. No statistical differences were found in sampling locations and among the studied cultivars. High percentage of oleic acid was found which ranged from (61.56 to 67.34), followed by palmitic acid from (13.60 to 17.61), linoleic acid from (12.23 to 14.45), stearic acid from (1.40 to 2.68), palmitoleic acid from (0.81 to 1.26), linolenic acid from (0.86 to 0.93) and arachidic acid from (0.14 to 0.36) respectively were detected in olive oil samples extracted from both cultivars grown in two districts. Oleic, palmitic and linoleic acids were the main fatty acids in both studied cultivars. No significant differences were found in sample of both districts for oleic, palmitic and linoleic acids.

Table 2: Fatty acid profile of oil extracted from two exotic Olive cultivar.

Fatty acids	Contents (%)			
	Loralai		Khuzdar	
	Leccino	Coratina	Leccino	Coratina
Palmitic (C16:0)	17.43±2.26	17.61±0.08	15.42±0.14	13.60±0.36
Palmitoleic (C16:1)	1.01±0.57	0.81±0.07	1.26±0.07	0.95±0.02
Stearic (C18:0)	2.54±2.19	1.61±0.06	2.68±0.65	1.40±0.06
Oleic (C18:1)	61.56±2.64	65.48±0.72	64.41±0.77	67.34±0.62
Linoleic (C18:2)	14.35±3.82	12.23±0.09	13.62±0.15	14.45±0.29
Linolenic (C18:3)	0.92±0.09	0.86±0.01	0.91±0.04	0.93±0.04
Arachidic (C20:0)	0.36±0.08	0.29±0.01	0.27±0.01	0.14±0.17
Total	98.07	98.49	98.55	99.14

Values are means ± standard deviation for $n = 3$.

Fatty acid composition of the olive in both samples grown at different districts were compared with other already known and analyzed samples from different parts of the world and it is assumed that there is a slight change in their amount and contents. As in the case of Coratina, in which the amount of oleic acid was found less than the cultivars grown in Europe. The obtained results regarding the percentage of oleic acid in olive oils of both cultivars from both locations which was found from 61.56-67.34%, were lower than those reported by Poiana and Mincione, [16] the authors reported a value of about 78% oleic acid in cultivar Coratina cultivated in Europe. Stearic acid in the studied samples was more than 2 percent which was a bit higher than the percentage reported by Poiana and Mincione, [16] as in their study it ranged from 1 to 2 percent. Further by comparing the obtained results of the present study regarding fatty acid contents in studied samples. It was found that in a study conducted in Syria by Al-Bachir and Sahloul, [17] the authors reported oleic acid 68.94% which is close to that of presented study and was more close to that of cultivars grown in Europe. Oleic acid percentage are high in cultivars grown in European countries while, it is less in cultivars cultivated in dried and/or semi-arid places like Balochistan, province of Pakistan and Syria where the weather conditions are dried and the temperature is quite high than the European countries [17]. Therefore, a wide range of variations may occur in its fatty acid contents. The percentage of linoleic acid in Coratina cultivated in both districts was in agreement and/or closed with that of the range 12.22% reported by Al-Bachir and Sahloul, [17]. It is evident that the weather of Syria and Balochistan share some similarities like; climate, altitude and soil pH ICARDA, [3]. The above mentioned arguments can also be strengthened by consulting the work done by Rondanini et al, [18] in which the authors discussed the relation between the cultivars of Argentina and Italy, it was found that Coratina grown in Argentina contain less percentage of oleic acid than the same cultivar grown in Spain and Italy. The decrease in oleic acid content was linked with the increase in temperature. Considering temperature and atmospheric condition effecting olive composition can further be strengthened by comparing the findings of the present work with that of the work done in Khyber Pakhtunkhwa a province of Pakistan in which it was reported that the percentage of oleic acid was quite close to the samples cultivated in both districts of Balochistan. Khyber Pakhtunkhwa has more or less same climate as that of Balochistan. Therefore, the results reported by Muhammad et al, [19] and Ben-Hassine et al, [20] are in agreement with the result found in the present study.

CONCLUSION:

The obtained results from the presented study showed that physicochemical parameters and fatty acid contents in samples collected from both districts of Balochistan were closely related to those areas having a close resemblance in its climate, altitude, temperature and moisture. The variations in studied parameters were found which might be due to the dried condition and temperature difference between the investigated areas. Further research work needed to address these arguments that the temperature plays a pivotal role in fatty acid composition and total phenolic compound which can be reduce during high temperature. However, the result needed to be examined in detail to confirm the sources behind the parallels found in present study. In order to find out whether the studied cultivars have different origin or their connections with the environment, which contributes substantial differences in their fatty acid composition. Further detailed studies are in progress to find out those factors which contributes for the differences in fatty acid contents of olive oil.

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