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Physiochemical and quality parameters of oil extracted from fruit of *Olea ferruginea* from various areas of Pakistan

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Abstract

Purpose of current study was to introduce new edible oil because population needs edible oil which provides them health benefits. Therefore this study focused on extraction and purification of edible oil from fruit of *Olea ferruginea Royle* (Wild olive), as alternative source to overcome shortage of edible oil in country. The fruit samples of *O. ferruginea* were collected from three different locations and various physical and chemical parameters relevant to quality of fruit as well as total oil contents were determined. Oil was further analyzed for fatty acids contents with help of GC-MS and FT-IR techniques. Whereas total phenol, pigments contents as well as oxidative stability parameters of oil were evaluated by using reported methods. According to results physicochemical variations in sizes and weights of fruits as well as yield of oils were observed from three different locations. Results revealed that oil contained higher quantity of essential fatty acids as compared to nonessential fatty acids. The quality parameters like total phenols, chlorophylls contents, oxidative stability (Acidity, peroxide value and UV absorption at K270, K232), iodine value and saponification number were satisfactory. The quantities of these parameters found in this study were equivalent to values suggested by international olive oil council. It is expected that oil of *Olea ferruginea* fruit will be appropriate source of edible oil and will help to reduce burden of foreign exchange of country that currently being used to import oil from abroad.

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Introduction

The major oilseed crops of Pakistan are sunflower, canola, rapeseed/mustard and cotton seed, those has provided only 3.726 Million tons of oil during 2015-2016 that contributed only 14 percent of country s demand (Abdul and Janno, 2018).Demand of edible oil is being increased with the passage of time due to the growing rate of the population and Pakistan became the third largest importer and used 2.6 million tons of oil per annum. It is difficult to make balance between requirement and production of oil,as requirement of edible oil is increasing day by day. Therefore, it is essential to search for new alternative and cheaper resources of edible oil in country (FAO, 2008). *Olea ferruginea* Royle, formerly known as *Olea cuspedata* is naturally growing wild olive broadly available in forest of Murree, Kotli Sattian and Khutta (Rawalpindi) Barakho (Islamabad) Azad Jammu and Kashmir, Hazara Division, Malakand and Swat as well as nearby areas .Fruit of *Olea ferruginea* is edible and good source of oil containing essential non-essential fatty acids (Pervaiz *et al*., 2013).

Virgin olive oil (VOO) is good natural food extracted from Olive fruit from *Olive europaea*. Its composition varies widely depending on variety of fruit, condition of fruit ripeness, environmental and geographical conditions as well as techniques of processing and storage (Barranco *et al*.1996). The nutrition values of olive oil mostly depend on its fatty acid composition as well as its phenolic contents (Caravita *et al*., 2007).The International Olive Council (IOC,2006) as well as European Commission (EEC, 2008) has provided limitation of different quality parameters for a good quality of edible oil. These parameters include acidity, peroxide value, oxidative stabilities and sensory indices. Olive fruit is considered one of the most important crops grown in desert land for their superior ability to cope the deserts conditions like dryness, hard climate and shortage in water supply. Olive trees can be grown in acrimony the nature and climate. The countries around the Mediterranean sea are original zones of olives trees. The total production of these countries is more than 94% of olive fruits and 98% of olive oil from the world production. Therefore, both the olive fruits and olive oil play an important role for supporting the economic situation of many countries. The quantity of free fatty acids is an important factor for classifying oil into commercial grades (Boskou, 1996). The general classification of olive oils into different commercial grades is based on free acidity and sensory characteristic (Taste and aroma). The commercial grade oil of olive fruit merely depend on mechanical or physical methods and organoleptic quality of olive oil depends on several factor including olive cultivar (Kalua *et al*., 2007). Olive oil is very good source of essential fatty acids and by consuming olive oil human body may get good quantity of Omega 3 and Omega 6 fatty acids those may easily absorbed in the body and provides health benefits to consumers. Therefore, keeping in view the importance of facts given above present study was conducted with following aims and objectives (1) To determine physicochemical parameters of fruit of *Olea ferruginea* (2) To assess saturated and unsaturated fatty acids contents of oil (3)To evaluate the levels of total phenols, peroxide and other quality and stability parameters of oil by using various reported methods.

Materials and methods

Collection of fruit samples

A survey was conducted to select fruits bearing trees of *Olea Ferruginea* located in nearby areas of Barakhu (Islamabad), Murree and Kotlisattian areas (District Rawalpindi). This survey was conducted before ripening stage of fruits during August 2015 and 2016. Three kg of olive fruits from each location were collected at ripening stage in fine plastic bags duly labeled with time and name of locations and were transported to Agriculture lab of UIBB PMAS Arid Agriculture University Rawalpindi. Authenticity of fruit samples was carried out by expert from plant sciences department and specimen (voucher no. 131) was deposited for future reference.

Physical characterization of olive fruit

Physical characteristics of fruits were determined by following the procedure of International Olive

Council Standards (IOC, 2015). The weight of pit and pulp was determined by weighing 100 grains of fruits by using electric balance and average weight per fruit was calculated. The length / width (L/W) ratio was calculated on the basis of length and width of grains.

Extraction of oil

The fruits were waterlogged overnight and washed thoroughly to eliminate the pulp. The fruits samples were air dried at room temperature crushed into powder from and used for the estimation of oil contents by using AOAC Official Method (2003). Total 2 grams of fruit sample by addition of appropriate amount of ether in Soxhlet apparatus was used for extraction of oil. The reaction continued for 16 hours and results were expressed as percentage of dry weight of fruit.

Analysis of oil for fatty acids with Gas chromatography- Mass spectroscopy

Fatty acid methyl esters: Olive oil in n-heptane (0.20 g per 2 mL) was transmethylated using a cold solution of KOH (2 mol L-1) 2μL) and methyl esters of (FAME) was analyzed according to European standard method (Dawodu *et al.*, 2015)Gas chromatography and mass spectrometry analysis for the estimation of fatty acids contents of oil was carried out by using shimadzu QP 2010 machine with a 5 columns. The temperature of injector and detector was 275°C, carrier gas (1.0 ml/min N_2), 0.2 µl injection volume and the split ratio was 50:1. The mass spectrum was obtained at 70 ionization voltage, 0.5s scan interval and from 40-950 Dalton, fragments were taken. The obtained significant compounds were identified from the spectral databases of NIST (National institute Standard technology) library (Vazquez *et al.*, 2003; Christopoulaton *et al.*, 2004; Capote *et al.*, 2007).

Analysis with Fourier transform infrared spectroscopy (FT-IR)

FT-IR was used to identify functional groups available on various compounds of oil (Rosenfeld, 2002; Garacia-Gonza *et al.*, 2013). Infrared spectrum FT-IR (Fourier transform Infrared) spectroscopy shimadzu machine, IR affinity 1, Japan and the loaded sample with scan range (400-4000 cm) with 4cm^{-1} resolution.

Determination of the total phenols and o-diphenols contents

Determination of total phenolic contents of olive oil was based on procedure reported by Gutfinger (1981) and using method of Folin- Ciocalteu reagent (Singleton and Rossi, 1965).The optical density was measured at 765 nm using a spectrophotometer and quantity of total phenol was expressed as milligrams of Gallic acid (GA)/kg of oil (Gutfinger, 1981). The concentration level of o-diphenolic in water/methanol extract was determined (Dridi-Gargouri *et al.*, 2013) , optical density was measure at 370 nm using a spectrophotometer and amount of compound was expressed as milligrams of Gallic acid (GA)/ kg of oil .

Quality indices

To characterized newly extracted olive oil, free acidity (FA), peroxide Value (PV) and UV absorption (K270, K232) were determined by using method of (European Community Regulation, 2008). These parameters determine oxidative stability of oil (Ouni *et al.*,2011).

Determination of saponification number

Oil (2 ml) was dissolved in an ethanol solution containing excess of KOH and heated to complete the reaction. The uncombined KOH was assessed by titration with HCl. The saponification number was calculated on the basis of weight / volume of oil and the volume of KOH used. The lesser the saponification number the greater the average molecular weight of the triacylglycerol.

Iodine value

Total 3 g of oil was dissolved in chloroform added 2 ml of pyrimidine dibromide solution in flask having glass stopper and left for 15 minutes at room temperature after which 5 ml of 10 % potassium iodide (KI) was added and mixture was diluted with distilled water followed by titration with 0.02 N

sodium thiosulfate solution as reported by Firestone (1994).

Determination of Pigments contents

Chlorophyll and carotenoids contents were determined following method previously described by (Minguez-Mosquera *et al.*, 1991) with minor modification. Briefly 6.0 g of oil was dissolved in cyclohexane and 25 ml of final volume was prepared. Amount of carotenoids and chlorophylls pigments were quantified on basis of absorbance measured at 470 and 670 nm and expressed by using following formula.

Chlorophyllus (mg/kg)= Abs 670 * 10^6/613* 1000 density……. (1)

Carotenoids (mg/kg) = Abs 470 * 10^6/2000* 1000* density…… (2)

Antioxidants assays

The scavenging ability of oil was assessed by using 1,1diphenyl 1-2 -picryl-hydrazyl (DPPH) assay as reported by (Moon and Shibamoto, 2009).

Statistical analysis

Data obtained was statistically analyzed by ANOVA for mean and standard deviation by using Minitab statistical software version 14.

Results

The physicochemical characteristics of *Olea Ferruginea* fruit observed in current study are given in table 1.Results indicate that fruit of *O. Ferruginae* obtained Barakhu (Islamabad area) have slighter lower size, weight of pit, pulp , grains and other parameter due to probably lower attitude and less rain fall in these areas where similar parameters of fruits were higher for fruit collected from Kotli Sattian and Murree areas might be due to soil condition, higher attitude as well as high ratio of rainfall in those areas.

Table 1. Physical and chemical characteristics of *Olea Ferrugina* fruit from different locations.

Location	Weight of pit (g) 100 grain	Weight of Pulp (g) 100 grain	Length/ Width (L/W)	Weight of 100 grains	Dry matter (g)	Water content (%)	Total oil(%)
Barakhu (Islamabad)	26.54 ± 1.8	125.61 ± 4.2	1.12± 0.8	108.52 ± 2.45	35.7 ±1.2	23.15 ± 2.55	35.1± 1.8
Murree (Rawalpindi)	31.62 ± 2.7	145.65 ±3.6	1.16 ± 0.4	118 .32 ± 2.68	42.5 ±1.6	26.80 ± 5.85	48.3 ± 2.7
Kotlisattian (Rawalpindi)	39.85 ± 3.5	165.28 ±4.35	1.22± 0.6	125.65 ± 3.68	47.3 ± 1.8	28.25 ±3.60	56.4 ± 2.9

Percentage value on dry weight basis.

Results show that oil of *O. Ferruginea* from different locations contained higher quantities of all important fatty acidsm (Fig.1).The fatty acids having higher percentage values are represented in Table 2. 9-Octdecenoic acid (Oleic acid) Hexadecanoic acid (Palmitic acid),Octadeca 9, 12 dienoic acid (Linoleic acid), Stearates (Stearic acid), Cis-9-Hexadecenoic acid (Palmitoleic acid), Eicosanoic acid (Arachidic acid), Alpha linolenicaicd (Linoleic acid) and Cis-9-eicosenoic acid (Gadoleic acid).Higher percentage value of Oleic acid (51.5 – 72.8 %) was analyzed from all oils. Lower percentages of these fatty acids were present in oil obtained from fruit of *O. Ferruginea* from Barakhu areas.

FT-IR spectra for *O. Ferruginea oil* (Fig.2) shows important peaks explaining the stretching, bending and double bond absorptions of the oil. These peaks represent absorption of methylene (CH_2) and methyl (CH_3) groups. Two peaks shows absorption of aldehyde (C = O) and esters (C-O) functional groups.

Results of chlorophyll and carotenoid contents of *O. Ferruginea* oil are provided in Table 3. According to results chlorophyll and carotenoid contents of oil obtained from fruit of *O. Ferruginea* from

Kotlisattina have higher values as compared to those got from Murree and Barakhu. The oil extracted from fruit *O. Ferruginea* from Kotlisattian have higher values of total phenols 139.32 ± 6.47 mg/kg as compared to Murree (132.46 ±8.65 mg/kg) and Barakhu (108.52 ± 5.35 mg/kg) as given in Table 3. Whereas, values of O. diphenols observed for oil of *O. Ferruginea* followed similar pattern. Phenols from olive oil fight various reactive oxygen species and also control the damage caused by free radicals to cells (Lipid peroxidation). Our findings of total phenols and O.diphenols were within the limits.

Table 2. Fatty acid Profile (%) of oil of *O. Ferruginea* from different locations.

Location	Oleic acid C 18:1	Palmitic acid C 16:0	Linoleic acid C18:2	Stearic acid C18:0	Palmitolic acid C16:1	Arachidic acid C20:0	Linolenic acid C18:3	Gadoleic acid C20:1
Barakhu	51.5	6.3	5.4	1.6	1.5	0.3	0.6	0.1
(Islamabad)								
Murree	62.3	8.7	11.7	2.8	2.6	0.5	0.7	0.2
(Rawalpindi)								
Kotlisattian	72.8	11.3	12.8	3.9	2.9	0.6	0.8	0.2
(Rawalpindi								

Value of fatty acids (%) on dry weight basis; ND. Not detected.

Iodine value(IV) represents degree of unsaturation of oil and results of IV of oils are given in Table 3. The values supports that the oil is unsaturated due to higher IV while saturated oils have low iodine values. Variations in saponification value (SV) of *O. Ferruginae* oil from different locations were observed and values are presented in Table 3.The results of DPPH radical-scavenging activities of *O. Ferruginea* oils are shown in Table 3. The significant difference in DPPH radical scavenging activities of oils of *O. Ferruginae* from different locations was observed. The different radical-scavenging activities might be depended on the composition of oil especially phenolic contents and different varieties of fruits particularly it is factual for O-diphenols.

Table 3. Quality indices of oil extracted from *O. Ferruginea* fruits from different locations.

Parameters	Barakhu	Murree	KotliSattian
Free Acidity (% oleic acid)	0.54 ± 0.01	0.35 ±0.01	0.52 ±0.02
Peroxide value (meq O2 kg−1)	7.42 ±0.50	11.36 ±0.35	8.35 ±0.15
K 232	1.65 ±0.04	1.75±0.03	1.86 ±0.05
K 270	0.15 ±0.00	0.17 ±0.00	0.18 ± 0.01
Carotenoids (mg kg−1)	2.36 ±0.08	2.55 ± 0.12	2.63±0.15
Chlorophylls (mg kg−1)	7.25 ±0.28	8.35 ±0.16	11.25± 0.12
Oxidative stability (h)	11.15±0.52	12.82 ±0.25	16.68±0.72
Total phenols (mg kg−1)	108.52 ±5.35	132.46 ±8.65	139.32±6.47
o-Diphenols (mg kg−1)	82. 65 ±6.42	85.34 ±3.65	96.16 ±4.75
DPPH (IC50) µg/ml	41.52 ± 0.2	31.45 ± 0.1	15.38 ±0.2
Iodine value (%)	82.52 ± 1.5	84.56± 2.6	92.67 ±2.5
Saponification number mg/ 1 g of oil	192.26 ±3.6	193.35 ±6.5	196.28 ±3.7

Quality parameters mean value ± Standard deviation(n=3).

Discussion

Physicochemical analysis

Rainfall or supply of water to olive trees increased weight, volume and pulp/pit ratio but not effect on fruit shape .The difference in weight of fruits are mostly due to water contents. Water stress may partially effect and decreased water content / weight of fruit and may delay ripening stage of fruit. Increasing water content results increase of pulp water content but firmness of fruit may be decreased. Reports indicates that moisture content of pulp of fruits and other characteristics of olives (flavor, texture etc.) are not mostly different from wetted and non-wetted olive fruit trees.

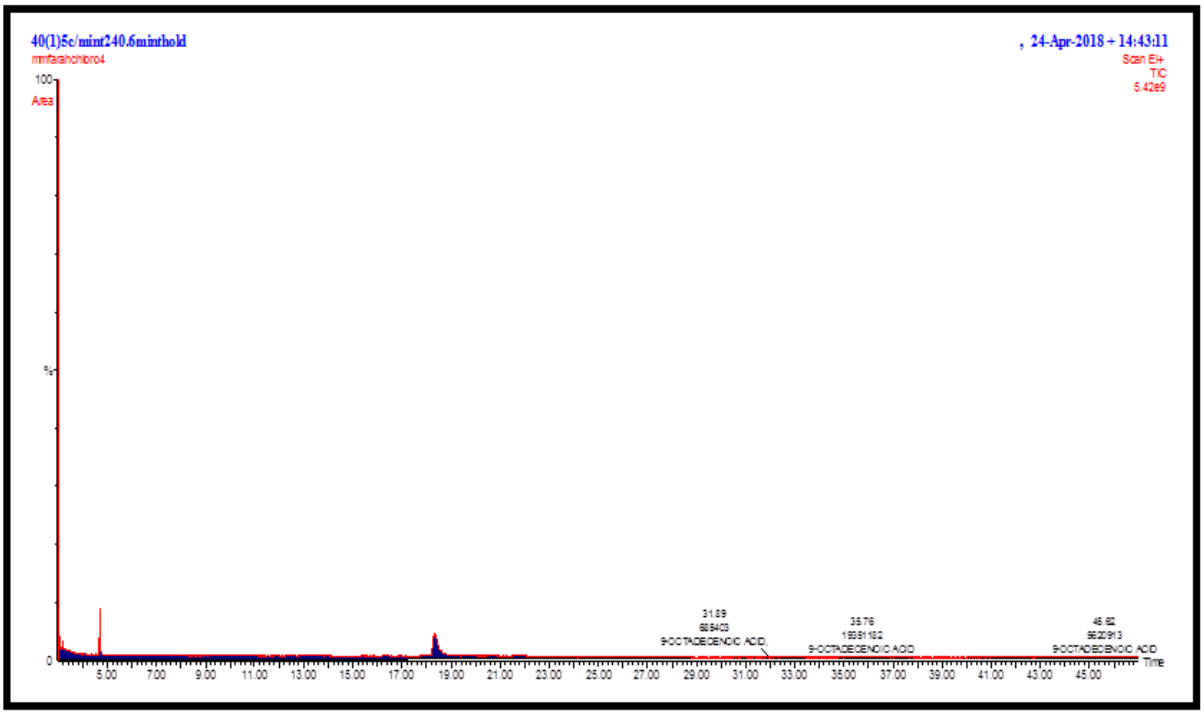

Fig. 1. GC- MS analysis of oil of *Olea Ferruginea*.

It was reported by Brighigna *et al.* (1989) that lower sugar and high oil content of olives from trees irrigated the water indicates greater metabolic activities.

Fatty acid profiling

Results of fatty acids shows similarity with fatty acids composition of oil reported by other research worker. The triglycerides fat/oil mainly depend on monounsaturated fatty acid (Oleic acid) along with minor quantity of linoleic acid (polyunsaturated fatty acid) and saturated fatty acids like stearic acid and palmitic acid (Aparicio and Aparicio-Ruiz, 2000). The results revealed that oil is good source of oleic acid. Whereas, linoleic acid (5.4- 12.8 %) and palmitic acid (6.3- 11.3 %) contents were lower but within the limit of European Community Regulation (2008). Furthermore these findings were also in agreement with results of the fatty acids reported earlier by other authors (Capote *et al.*, 2007). Fatty acid composition is considered as key parameter for purity and authentication of oil. High oxidative stability of virgin olive oil is related to high monounsaturated /polyunsaturated ratio.

This is an important factor in this finding that oil of *O. Ferruginea* has higher content of monounsaturated fatty acids but also composed of polyunsaturated fat. The variation in fatty acids composition of oil in current study could be due to ratio of rain fall genetic feature of the varieties and geographical growing area. Whereas, FT-IR analysis confirmed presence of important fatty acids in oil by showing various function groups and bonds of fatty acids in oil. The stretching absorption of (O-H) is stronger in oil, while stretching absorption (O-H) of intermolecular hydrogen bonding is for water (Garacia Gonza *et al.*, 2013; Rosenfeld, 2002).

Quality parameters

All quality parameters are within recommended limit of olive oil published in literature and recommended by International Olive Standards (IOC, 2006).Quality Parameters like Free acidity (FA), Peroxide value (PV) and UV absorption (K 270, K 232) of *O. Ferruginea* oil were analyzed to determine their oxidative stability. Free acid formation might be an important measure of rancidity of foods. Peroxide value is broadly used for measure of lipid oxidation and indicates amount of peroxide formed during oil oxidation. Similar results were also reported earlier

by (Ouni *et al.*, 2011). Saponification number of oil is a directory of average molecular weight of the triglyceride of any oil. Therefore if saponification number is exceeding 200 mg KOH/g of oil, it reveals the existence of fatty acid shaving low molecular weight. However, if saponification number is lower than 190 mg KOH/ g of oil , It expose availability of higher molecular weight of fatty acids in oil (Vekiari *et al.* 2007).

The variation in UV absorption of any oil at K 232 and K 270 nm are connected with changes in conjugated double and triple bonds produced by the oxidation of polyunsaturated fatty acids. Higher the percentage of polyunsaturated fatty acids in the oil, the higher will be levels of conjugated bonds (Borchani *et al.*, 2010). All sample of oil analyzed have higher oxidative stability ranges established for virgin olive oil as required by the European Community Regulation (2008). FA, PV and UV absorption values were lower in oil of *O. Ferruginea* from Barakhu area as compared to Murree and Kotlisattian areas , might be due to some geographical and climatic factors. PV of oil is measure of degree to which an oil will undergo primary oxidation, whereas, oil with higher degree of unsaturation are more susceptible to autoxidation .When oxidation of fatty acids takes place, the double bonds in the unsaturated fatty acids are attacked forming peroxides (Ouni *et al.*, 2011). According to the literature, hydro peroxides, the initial products of oxidation, comparatively unstable and very sensitive indicator of the early stages of oxidative deterioration of oil (Vekiari *et al.*, 2007).

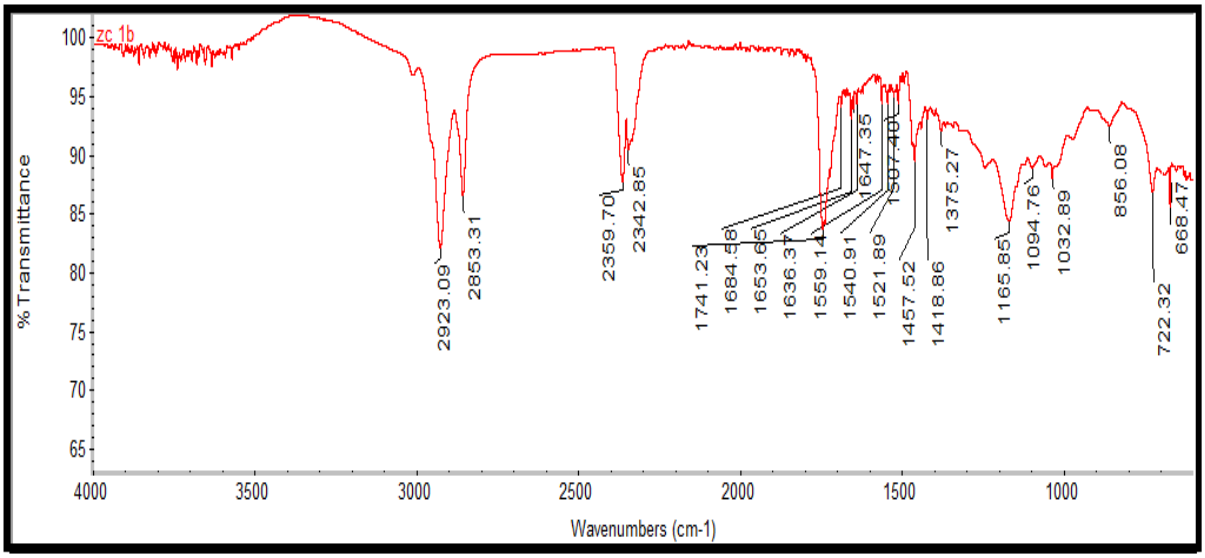

Fig. 2. FT- IR analysis *O. Ferruginea* oil.

Chlorophylls and carotenoids are considered as bioactive substances those contribute to olive oil color but also effects oxidative stability of olive oil due to their functional properties. Several studies indicate that pigment amount is independent of the olive variety and time of harvesting (Cerretani *et al.*, 2008). It has been recognized that polyphenols are substances with natural antioxidant properties and their presence in olive oils has been associated to their general quality, improving stability, nutritional value and sensorial properties (Fernandes –Silva *et al.,* 2013). Differences in Phenolic and O. phenols contents of oil from different locations are might be due to genetic variation, different climatic and geographical condition and also due to method of extractions of oil (Singleton and Rossi, 1965).

Iodine value depends directly on the number of double bonds present in oil (Dawodu *et al.* 2015). The oxidative stability of virgin olive oil is mainly depend on its characteristic pattern of triglycerides (Low unsaturation iodine value < 90) and also considered as polar antioxidant due to presence of α-tocopherol. Iodine value is identity characteristic of

natural oil and defined as the grams of iodine required to absorb 100 grams of samples. Whereas, saponification is chemical process in which oil is converted into glycerol and fatty acids when treated with alkali. It is defined as the milligrams of potassium hydroxide (KOH) required to saponify 1g of fat.

Bioactivity

Plant seeds oils are liquid mixture of different volatile compounds and possess antioxidant activities. Antioxidant work by giving damaged cells or free radicals that need to repair themselves by forming an intra-molecular hydrogen bond between the free hydrogen of their radicals. Virginal olive oil contents various types of phenols that act as antioxidants, which can lower the risk of heart disease and other similar infections in human body. Therefore assessment of DPPH radical activity of oil in this study exposed that *O. Ferruginea* oil is good source of antioxidant compounds could be useful to control lipid peroxidation and reduction of cholesterol level. It will also help to minimize risk of heart diseases to consumers (Moon and Shibanoto, 2009).

Conclusion

Results obtained in current study revealed that oil extracted from fruit of *O. Ferruginea* contained higher quantities of essential fatty acids (Oleic, linoleic and linolenic acids) and lower amount of non- essential fatty acids (palmitic and stearic acids), which is indication of suitability of this oil for edible proposes . Whereas, physical chemical parameters, oxidative stability and other quality indices of oils are comparable to European Community Regulation and International Olive oil standards. Although fruit size of *O. Ferruginea* is less as compared to *O. Europea* which can be improved by irrigation and by using modern horticulture techniques while currently these trees are growing naturally only in the forest.

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References

Abdul R, Janno H. 2018. Proceeding of 3rd Pakistan edible oil conference PECO, Karachi, Pakistan**.**

AOAC. 2003 Official Methods of the AOAC. 17th Ed. The Association of Official Analytical Chemists, Gaithersburg, MD.

Aparicio R, Aparicio-Ruiz R. 2000. Authentication of vegetable oil by chromatographic techniques. Journal of Chromatography **881,** 93–104.
https://doi.org/10.1016/S0021-9673(00)00355-1

Barranco D, Fernandez- Escobar R, Rallo L. 1996 .Olive growing (in spanish Junta de Andalusia, Consejeria de Agricultura pesca and ediciones mundi prensa Madrid, Barcelona, Mexico.

Borchani C, Besbes S, Blecker CH, Attia H. 2010. Chemical characteristics andoxidative stability of sesame seeds, sesame paste, and olive oils. Journal of Agricultural Science and Technology **12**, 585–596.

Boskou D. 1996. Olive oil chemistry and technology AOCS press. Champaigen. IL.USA.

Brighigna A, Marsilio V, Vlahov G, De Renzo ME. 1989. Le olive da tavola: caratteristicheed attitude inidellavarieta "Nocellara Messinese" coltivata in Calabria. Industrie alimentarie **28**, 1087-1092.

Caravita M, Benincasa A, De Rose C, Muzzalupo IF, Parise A. 2007. Omega 3/Omega 6 fatty acids ratio in olive oils from Italian olive varieties . Agro Food Industry Hi Tech **18**, 17- 18.

Cerretani L, Motila MJ, Romero MP, Bendini A, Lercker G. 2008. Pigment profile and chromatic parameters of mono varietal virginal olive

oils from different Italian varieties. Food research technology **226,** 1251-1256.

Capote FP, Jiménez JR, Castro MDL. 2007. Sequential (step-by step) detection identification and quantitation of extra virgin olive oil adulteration by chemome trictreatment of chromatographic profiles. Analytical and Bioanalytical Chemistry **388,** 1859-1865. https://doi.org/10.1007/s00216-007-1422-9

Christopoulou E, Lazaraki M, Komaitis K. 2004. Effectiveness of determinations of fatty acids and triglycerides for the detection of adulteration of olive oils with vegetable oils. Food Chemistry **84**, 463– 474. https://doi.org/10.1016/S0308-8146(03)00273-5.

Dawodu MO, Olutona GO, Obimakinde SO. 2015. Effect of temperature on chemical characteristics of vegetable oils consumed in Ibadan, Nigeria. Pakistan Journal of Nutrition.**14,** 698–707. https://doi.org/10.3923/pjn.2015.698.707.

Dridi-Gargouri O, Kallel-Trabelsi S, Bouaziz M, Abdelhèdi R. 2013. Synthesis of 3-O-methylgallic acid a powerful antioxidant by electrochemical conversion of syringic acid.Biochimicaet Biophysica Acta **1830**, 3643–3649. https://doi.org/10.1016/j.bbagen.2013.02.012.

European community Commission Regulation 640 /2008. 2008. Amending Regulation No.2568/91 /EEC. Official Journal of the European Community, July 4, L **178,** 11-16.

FAO. 2008.Food and Agriculture Organization. High food prices in Pakistan. Impact assessment and the way Forward .Inter Agency Assessment Mission FAO/UNDP/UNESCO/UNICEP/WFP/WHO Ministry of Food Agriculture and livestock Government of Pakistan Islamabad.

Fernandes-Silva AA, Gouveia JB, Vasconcelos P, Ferreira TC, Villalobos FJ. 2013. Effect of different irrigation regimes on the quality attributes of monovarietal virgin olive oil from cv. "Cobrancosa". Grasa y Aceite **64,** 41–49. https://doi.org/10.3989/gya.070512.

Firestone D. 1994.Determination of Iodine value of oilsant fats; Summary of collaborative study. Journal of AOAC International **77,**674-6.

Garcia-gonza DL, Sedman J, Van de Voort FR. 2013. Principles, performance and applications of spectral reconstitution (SR) in quantitative analysis of oils by Fouriertrans form infrared spectroscopy (FTIR). Applied Spectroscopy **67,** 448-455. https://doi.org/10.1366/12-06798.

Gutfinger T. 1981. Polyphenols in olive oil. Journal of the American oil Chemist's Society **58,** 966- 968. https://doi.org/10.1007/BF02659771.

Kalua CM, Allen MS, Bedgood DR, Bishop AG, Prenzler PD, Robards K. 2007.Olive oil volatile compounds, flavor development and quality: A critical review. Food Chemistry **100**, 573-575. https://doi.org/10.1016/j.foodchem.2005.09.059.

International Olive Council (IOC). 2006. Trade standard applying to olive oils and olive pomace oils COI/T.15/NC3 /Rev.2 EEC (1991).Regulation (EEC)No 2568/91 on the characteristic of olive oil and olive residue and on the relevant methods of analysis Official Journal of European Commission, L 248.

Minguez-Mosquera MI, Rejano L, Gandul B, Sanchez HA, Garrido J. 1991 Color pigment correlation in virgin olive oil. Journal of the American Oil Chemists. Society **68,** 332-336. https://doi.org/10.1007/BF02657688.

Moon JK, Shibamoto T. 2009. Antioxidant assays for plant and food components. Journal of agricultural and food chemistry **57,** 1655-1666.

https://doi.org/10.1021/jf803537k.

Ouni Y, Famini G, Douja D, Zaarrouk M. 2011. Journal of horticulture and Forestry **3,** 13-20.

Pervaiz A, Alessanddra B, Gulfraz M, Quershi R, Giuseppe VE, Saqlan NSM, Tullia GT. 2013. Characterization of olive oil obtained from wild olive trees (Olea Ferruginea Royle) in Pakistan. Food Research International.**54**, 1965-1971. https://doi.org/10.1016/j.foodres.2013.09.029.

Rosenfeld JM. 2002. Application of analytical derivatizations to the quantitative and qualitative determination of fatty acids. Analytical Chimica Acta **465,**93–100. https://doi.org/10.1016/S0003-2670(02)00467-1.

Singleton VL, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. American Journal of Enology Viticulture **16**, 144-158.

Vazquez JG, Falcón MSG, Gándara JS. 2003. Control of contamination of olive oil by sunflower seed oil in bottling plants by GC-MS of fatty acid methyl esters. Food Control **14,** 463– 467. https://doi.org/10.1016/S0956-7135(02)00102-0.

Vekiari SA, Papadopoulou P, Kiritsakis A. 2007. Effect of processing methods and commercial storage conditions on the extra virgin olive oil quality indexes. Grasas Y Aceites **58,** 237–242. https://doi.org/10.3989/gya.2007.v58.i3.178.