Qualification of the virgin olive oils sold in herbal stores of north of Iran

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Abstract

Background and objective: Olive oil has a lot of nutritional and medicinal benefits, which make it more valuable than other oils. Therefore, it is noticeable for adulteration by mixing with the cheaper oils. At this study, authenticity of virgin olive oils sold in herbal stores in north of Iran (Rasht) has been investigated.

Material and methods: During 2019, 26 samples of virgin olive oils were collected randomly from the herbal stores of Rasht city (Guilan, north of Iran). Fatty acid profile by gas chromatograph, peroxide value by calculation of milliequivalent of active oxygen per kilogram of oil, and acidity by titration of the samples with potassium hydroxide were determined in the laboratory and compared to the national regulation.

Results and conclusion: Approximately, 58% of the samples were out of the permitted range at least in one of the measured parameters. With regard to fatty acid profile, 77% of the samples were within the normal range. Predominant fatty acids were included to oleic acid (59.8 ±14.3%), linoleic acid (21.4 ±14.1%), palmitic acid (13.8 ±1.4%), stearic acid (2.7 ±0.8%), and palmitoleic acid (0.7 ±0.3%). In case of peroxide value, 92.3% of the samples were within the acceptable range. Based on acidity, different virgin olive oils were differentiated as extra virgin (n = 1, %3.8), first grade virgin (n = 11, 42.31%), ordinary virgin (n = 8, %30.77), and lampante virgin (n = 6, %23.08). Our investigation revealed that the olive oils sold in herbal stores as traditional market should be monitored restrictedly to assess their originality and storage condition. Although, they should be examined periodically to find any adulteration, if exists.

Keywords: Acidity, fatty acid, herbal store, peroxide, Rasht, virgin olive oil

1. Introduction

Olive oil is a valuable source of nutrients and is traditionally produced in the Mediterranean regions [1] especially Iran [2]. The oil is extracted from fruit of the olive tree, *Olea europaea*, belonging to the family of Oleaceae [3].

Approximately, 98% of olive oil is composed of triacylglycerol with a relatively high amount of oleic acid as a nonessential monounsaturated fatty acid. Palmitic acid, linoleic acid, stearic acid, and palmitoleic acid are other fatty acids in composition of the triacylglycerols in olive oil [4]. Oleic acid has anti-

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inflammation activity and shows beneficial roles in reduction of cardiovascular diseases by regulation of blood lipid profile through reduction of the low-density lipoprotein (LDL) cholesterol [5] and increasing the high density lipoprotein (HDL) cholesterol in the blood [6]. It has preventive effect against chronic diseases such as breast cancer and type 2 diabetes mellitus [7]. Phenolic compounds of olive oil have considerable antioxidant properties. Thus, consumption of extra virgin olive oils, which are rich of phenolic compounds, is recommended to delay ageing and reduce the risk of colon, breast, and skin cancers in people [8]. The beneficial health effect of the oil is the most important factor in determination of its high price compared to the other oils and makes it interesting for adulteration [9-10].

Virgin olive oil is obtained from fruit of the olive tree mainly by mechanical force under thermal condition which does not change the chemical composition of the oil. The process also includes washing, decantation, centrifugation, and filtration. The appropriate virgin olive oil for oral consumption is classified to extra virgin olive oil, first grade virgin olive oil, and ordinary virgin olive oil. Their acidity, expressed as oleic acid, should be less than 0.8, 2, and 3.3 g/100 g, respectively. In comparison, lampante virgin olive oil has acidity more than 3.3 g/100 g and is not appropriate for human consumption [11-12].

There is several research about authenticity of olive oils from different regions of Iran [13-16]. However, no data is available about the samples collected from the local herbal stores in which the storage condition might affect chemical composition of the oil, other than its intentional mixing with cheaper oils that is of concern in halal commerce. Therefore, the current study aimed to investigate chemical composition (fatty acid profile, peroxide value, and acidity) of virgin olive oils sold in herbal stores of Rasht city (Guilan province, north of Iran) in 2019.

2. Materials and methods

2.1. Chemicals and samples
All solvents/chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany). Pure fatty acids of palmitic acid, stearic acid, oleic acid, linoleic acid, and palmitoleic acid were purchased from Sigma Aldrich (USA). Twenty-six olive oil samples were randomly purchased from the herbal shops in Rasht city (Guilan province, north of Iran) during 2019. This study was approved in Ethics committee of Guilan University of Medical Sciences (Ethics code: IR.GUMS.REC.1398.369).

2.2. Gas chromatography
The fatty acids were esterified by methyl group and were analyzed by Shimadzu gas chromatograph (GC-14B, Kyoto, Japan) equipped with flame ionization detector (FID) and silica capillary Column BPX70 (60 m $\times$ 0.25 mm $\times$ 0.25 μm). Helium was used as carrier gas at flow rate of 1 ml/min. Thermal program was set to increase from 175 °C to 230 °C at rate of 3 °C/min. Temperature of injector and detector was 250 °C and 280 °C, respectively. Amount of fatty acids was determined by calculation of the peak area and expressed as percentage [11].

2.3. Peroxide value
Peroxide value of the samples, expressed as milliequivalent of active oxygen per kilogram of oil (meq O_2/kg oil), was determined by titration. Briefly, olive oil was dissolved in acetic acid/chloroform (3:2 v/v). The mixture was left to react with saturated potassium iodide in darkness. Then, deionized water was added followed by addition of starch dropwise. Finally, free iodine was titrated with 0.01 N sodium thiosulfate until the color disappeared [17].

2.4. Acidity
Free fatty acids content, expressed as oleic acid percentage, was determined by titration of oil solution dissolved in ethanol with 0.1 M potassium hydroxide ethanolic solution in the presence of phenolphthalein as reagent [18].

2.5. Sample size and statistical analysis

Cochran formula was used for determination of sample size. By considering a population size of 64 (based on a listed database available from Food and Drug deputy of Guilan University of Medical Sciences), excepted precision of 0.15, and proportion of population of 0.5, sample size of 26 was achieved.

$$n = \frac{N\,z^2\,p\,q}{N\,d^2 + z^2\,p\,q}$$

Where, N stands for population size, z stands for z-score for confidence interval 95%, p is proportion of population, q stands for (1-p), d is a desired level of precision, and n is sample size. All statistical analyses were performed by SPSS version 21 (SPSS Inc., Chicago, IL, USA). Normal distribution of data was checked by Kolmogorov-Smirnov test. Data are presented as mean ±standard deviation (SD).

3. Results and discussion

Our examinations revealed that 58% of the samples were not compatible with the determined levels in the regulations of olive oil at least in one of the measured parameters. Composition of fatty acid is one of the most important measures for qualification of edible oils especially olive oil [19]. As seen in Table 1, amount of some fatty acids in the samples was out of the range determined by Iranian regulation [11], and 73% of the samples were fully accepted in this regard. Predominant fatty acids were oleic acid (59.8 ±14.3%), linoleic acid (21.4 ±14.1%), palmitic acid (13.8 ±1.4%), stearic acid (2.7 ±0.8%), and palmitoleic acid (0.7 ±0.3%).

Oleic acid as the main monounsaturated fatty acid in olive oil [20] was between 29.5% and 74.5%. With respect to this fatty acid, approximately 27% of the samples were out of the acceptable range (55-83%), which reflects their poor nutritional quality [21]. In addition, linoleic acid, the main polyunsaturated fatty acid in olive oil, ranged from 10% to 53.3%, and 27% of our samples were

not in accordance to the acceptable range (3.5-21%). Palmitic acid, the main saturated fatty acid in olive oil [22], was found in range of 11% to 16.4%, which covered the acceptable range of 7.5-20% for olive oil. Similar result was observed for stearic acid and the calculated range of 1.1-4.3% was consistent with the acceptable range (0.5-5%). The minor fatty acid of palmitoleic acid was calculated from 0.1% to 1.3%, of which 19% of the samples were not in the acceptable range of 0.3- 3.5% [11]. Low amount of oleic acid and high amount of linoleic acid make the olive oil sensitive to oxidation [23]. Therefore, the sample no. 15 and the sample no. 9 with the least and the highest ratio of oleic to linoleic acid were the most sensitive and the most resistant samples to oxidation, respectively (Table 1).

Cultivar, climate, and fruit's maturity are factors affecting the fatty acid composition of olive oil [24], that is critical in monitoring and evaluation of adulteration [25-26]. The acceptable range of each fatty acid in olive oil is determined by considering all of these variables. Therefore, addition of inexpensive oils to olive oil changes its fatty acid profile out of the standard range [27-28].

Table 1- Fatty acid compositions of olive oil samples collected from herbal stores in Rasht city (Guilan province, north of Iran)

Sample	Fatty acid				
	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid
1	13.1	1.0	2.7	67.5	12.6
2	12.6	0.5	2.5	70.6	11.8
3	14.8	1.2	2.4	61.5	17.7
4	15.0	1.2	2.5	64.1	14.3
5	12.7	0.9	2.7	65.8	15.1
6	13.7	0.8	2.4	70.0	11.5
7	14.3	1.0	3.0	65.3	14.1
8	12.2	0.2*	4.2	29.5*	46.3*
9	11.8	0.7	2.6	72.8	10.0
10	15.2	1.3	2.0	70.4	10.8
11	15.1	1.0	2.8	65.5	15.3
12	14.4	1.0	2.6	67.7	14.1
13	14.3	1.0	2.4	67.8	14.3
14	15.5	0.2*	4.2	34.9*	44.9*
15	12.1	0.1*	3.6	30.7*	53.3*
16	16.4	1.1	2.7	67.6	11.8
17	14.3	0.2*	3.1	37.2*	44.9*
18	14.0	0.6	1.1	49.3*	30.9*
19	12.7	0.6	1.9	65.0	19.6
20	15.8	0.7	4.3	66.8	12.0
21	15.5	1.1	1.3	67.2	14.7
22	15.7	1.1	3.0	67.4	12.6
23	13.0	0.9	2.4	70.4	13.1
24	11.0	0.4	2.5	74.5	11.3
25	12.6	0.2*	3.9	34.1*	49.0*
26	11.8	0.9	1.9	51.8*	31.2*
Mean ±SD	13.8 ±1.4	0.7 ±0.3	2.7 ±0.8	59.8 ±14.3	21.4 ±14.1
Acceptable range	7.5-20	0.3-3.5	0.5-5.0	55.0-83.0	3.5-21.0

*Out of the acceptable range

The average acidity and peroxide value of the samples are listed in Table 2. Peroxide value varied between 2.3 to 43.2 meq O_2/kg, and 24 samples (92.3%) were within the acceptable range (20 meq O_2/kg for virgin olive oils) [11]. Peroxide value is a measure of hydroperoxides formed during lipid oxidation and its amount determines safety of edible oil for further consumption [29]. In addition, quality of olive oil is importantly affected by acidity. A lower acidity indicates higher quality of the virgin olive oil [13]. Indeed, acidity is reflected by amount of oleic acid released from triacylglycerols by activity of enzymes in the oil, which makes it susceptible to deterioration [30]. Comparison of acidity in our samples with permitted levels in the national regulation [11] helped us in differentiation of the samples. Accordingly, 1 extra virgin (3.8%), 11 first grade virgin (42.3%), 8 ordinary virgin (30.7%), and 6 lampante virgin (23.0%) olive oils were determined.

Table 2- Acidity and peroxide value of olive oil samples collected from herbal stores in Rasht city (Guilan province, north of Iran)

Sample	Acidity	Peroxide
1	2.5	12.7
2	1.7	16.9
3	1.9	11.5
4	3.1	14.3
5	2.9	13.5
6	3.6	13.8
7	1.9	15.1
8	1.5	12.4
9	2.8	43.2*
10	2.3	12.5
11	7.6	11.2
12	4.1	11.6
13	2.8	14.1
14	1.9	20.0
15	1.6	4.3
16	2.5	13.0
17	3.2	2.3
18	1.6	11.3
19	8.1	13.5
20	1.8	13.0
21	0.8	9.2
22	3.6	38.8*
23	5.9	5.0
24	1.1	16.4
25	1.4	4.1
26	0.6	12.7
Mean ±SD	2.8 ±1.8	14.0 ±8.9
Acceptable limit	Extra virgin: ≤0.8; First grade virgin: 0.8< ≤2; Ordinary virgin: 2< ≤3.3; Lampante virgin: >3.3	20

*Out of the acceptable limit

Although, all the samples were sold as virgin olive oil in the herbal stores, the storage condition such as room temperature, characteristic of the oils' bottles [31], and light [32] might affect

quality of the measured parameters. Importantly, use of industrial olive oils that are monitored periodically by the regulatory agencies is more recommended to avoid health and financial outcomes for the consumers.

4. Conclusion

This work was the first assessment about quality of virgin olive oils sold in the herbal stores in north of Iran (Guilan province, Rasht city). By considering all of the quality parameters (acidity, peroxide, and fatty acid composition), approximately 58% of the virgin olive oil samples were not compatible with the limits determined by the national regulations. It seems that the herbal stores as traditional market need a restricted surveillance to assure the olive oils' originality under appropriate storage. For this purpose, the oils should be monitored periodically each year to find any adulteration, if exists.

5. Acknowledgment

6. Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Andrikopoulos NK, Giannakis IG, Tzamtzis V. Analysis of olive oil and seed oil triglycerides by capillary gas chromatography as a tool for the detection of the adulteration of olive oil. Journal of Chromatographic Science. 2001; 39: 137-145. https://doi.org/10.1093/chromsci/39.4.137

2. Hamedi A, Zarshenas MM, Sohrabpour M, Barragan A. Herbal medicinal oils in traditional Persian Medicine. Pharmaceutical Biology. 2013; 51(9): 1208-1218. https://doi.org/10.3109/13880209.2013.777462

3. Uylaser V, Yildiz G. The historical development and nutritional importance of olive and olive oil constituted an important part of the Mediterranean diet. Critical Review in Food Science and Nutrition. 2014; 54: 1092-1101.

https://doi.org/10.1080/10408398.2011.626874

4. Gavahian M, Mousavi Khaneghah A, Lorenzo JM, Munekata PES, Garcia-Mantrana I, Carmen Collado M, et al. Health benefits of olive oil and its components: Impacts on gut microbiota antioxidant activities, and prevention of noncommunicable diseases. Trends in Food Science & Technology. 2019; 88: 220-227. https://doi.org/10.1016/j.tifs.2019.03.008

5. Gilmore LA, Walzem RL, Crouse SF, Smith DR, Adams TH, Vaidyanathan V, et al. Consumption of high-oleic acid ground beef increases HDL- cholesterol concentration but both high- and low- oleic acid ground beef decrease HDL particle diameter in normocholesterolemic men. Journal of Nutrition. 2011; 141: 1188-1194. https://doi.org/10.3945/jn.110.136085

6. Kwon H, Choi C. Comparison of fat content and monounsaturated fatty acid composition of beef by country of origin and marbling score. Journal of the Korean Society of Food Science and Nutrition. 2015; 44:1806-1812. https://doi.org/10.3746/jkfn.2015.44.12.1806

7. Foscoloua A, Critselisa E, Panagiotakosa D. Olive oil consumption and human health: A narrative review. Maturitas. 2018; 118: 60-66. https://doi.org/10.1016/j.maturitas.2018.10.013

8. Owen RW, Giacosa A, Hull WE, Haubner R, Wurtele G, Spiegelhalder B, et al. Olive-oil consumption and health: the possible role of antioxidants. Lancet Oncology. 2000; 1: 107–112. https://doi.org/10.1016/S1470-2045(00)00015-2

9. D'Imperio M, Dugo G, Alfa M, Mannina L, Segre AL. Statistical analysis on Sicilian olive oils. Food Chemistry. 2007; 102: 956-965. https://doi.org/10.1016/j.foodchem.2006.03.003

10. Flores G, Ruiz del Castillo ML, Herraiz M, Blanch GP. Study of the adulteration of olive oil with hazelnut oil by on-line coupled high performance liquid chromatographic and gas chromatographic analysis of filbertone. Food Chemistry. 2006; 97: 742-749. https://doi.org/10.1016/j.foodchem.2005.06.008

11. Iranian National Standardization Organization. Olive oil- specification and test methods. Standard no. 1446. 2011. 1-17.

12. International Olive Council. Trade standard applying to olive oils and olive pomace oils. Decision COI/T.15/NC No3/Rev. 6 November 2011.

13. Kharazi SH, Kenari RE, Amiri Z R, Azizkhani M.

Characterization of Iranian virgin olive oil from the Roodbar region: A study on Zard, Mari and Phishomi. Journal of the American Oil Chemists' Society. 2012; 89(7): 1241-1247.
https://doi.org/10.1007/s11746-012-2021-2

14. Najafi V, Barzegar M, Sahari MA. Physicochemical properties and oxidative stability of some virgin and processed olive oils. Journal of Agricultural Science and Technology. 2015; 17: 847- 858.

15. Movahed S, Khorshidpour B, Chenarbon HA. Evaluation of acidity and fatty acid compositions in Iranian and imported olive oils. International Research Journal of Applied and Basic Sciences. 2012; 3: 1140-1142.

16. Homapour M, Ghavami M, Piravi-Vanak Z, Hosseini SE. Chemical properties of virgin olive oil from Iranian cultivars grown in the Fadak and Gilvan regions. Grasas Y Aceites. 2014; 65(4): 1-9.
https://doi.org/10.3989/gya.0351141

17. Iranian National Standardization Organization. Animal and vegetable fats and oils- Determination of peroxide value- Iodometric (visual) endpoint determination. Standard no. 4179. 2017; 1-10.

18. Iranian National Standardization Organization. Animal and vegetable fats and oils- Determination of acid value and acidity test method. Standard no. 4178. 2011: 1-11.

19. Dag A, Kerem Z, Yogev N, Zipori I, Laveec S, Bendavi E. Influence of time of harvest and maturity index on olive oil yield and quality. Scientia Horticulturae. 2011; 127: 358-366.
https://doi.org/10.1016/j.scienta.2010.11.008

20. Ben Temime S, Wael T, Bechir B, Leila A, Douja D, Mokhtar Z. Changes in olive oil quality of Chetoui variety according to origin of plantation. Journal of Food Lipids. 2006; 13: 88-99.
https://doi.org/10.1111/j.1745-4522.2006.00036.x

21. Ayed RB, Ennouri K , Ercisli S , Ben Hlima H, Hanana M, Smaoui S, et al. First study of correlation between oleic acid content and SAD gene polymorphism in olive oil samples through statistical and bayesian modeling analyses. Lipids in Health and Disease. 2018; 17(1): 74.
https://doi.org/10.1186/s12944-018-0715-7

22. Aguilera MP, Beltran G, Ortega D, Fernandez A, Jimenez A, Uceda M. Characterization of virgin olive oil of Italian olive cultivars ''Frantoio'' and ''Leccino'' grown in Andalusia. Food Chemistry. 2005; 89: 387-391.

https://doi.org/10.1016/j.foodchem.2004.02.046

23. Lecce G, Piochi M, Pacetti D, Frega NG, Bartolucci E, Scortichini S, et al. Eleven monovarietal extra virgin olive oils from olives grown and processed under the same conditions: effect of the cultivar on the chemical composition and sensory traits. Foods. (2020) 9(7): 2-17.
https://doi.org/10.3390/foods9070904

24. Diraman H, Saygi H, Hisil Y. Classification of three Turkish olive cultivars from Aegean region based on their fatty acid composition. European Food Research and Technology. 2011; 233: 403-411.
https://doi.org/10.1007/s00217-011-1511-z

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

26. Di Bella G, Maisano R, La Pera L, Lo Turco V, Salvo F, Dugo G. Statistical characterization of sicilian olive oils from the Peloritana and Maghrebian zones according to the fatty acid profile. Journal of Agricultural and Food Chemistry. 2007; 55(16): 6568-6574.
https://doi.org/10.1021/jf070523r

27. Piravi Z, Ghasemi JB, Ghavami M, Ezzatpanah H. Detection and quantification of adulteration in olive oils by global method and extinction coefficient. Australian Journal of Basic and Applied Sciences. 2010; 4 (12): 6254-6259.

28. Pashaie A, Shavakhi F. Application of calorimeter for detection of adulteration of extra virgin olive oil mixed with rapeseed oil as a substitute for gas chromatography. Journal of Food Research. 2017; 27(3):139-149. (In Persian)

29. Cosio MS, Ballabio D, Benedetti S, Gigliotti C. Evaluation of different storage conditions of extra virgin olive oils with an innovative recognition tool built by means of electronic nose and electronic tongue. Food Chemistry. 2007; 101 (2): 485-491.
https://doi.org/10.1016/j.foodchem.2006.02.005

30. Grossi M, Palagano R, Bendini A, Ricco B, Servili M, Garcia-Gonzalez DL, et al. Design and in- house validation of a portable system for the determination of free acidity in virgin olive oil. Food Control. 2019; 104: 208-216.
https://doi.org/10.1016/j.foodcont.2019.04.019

31. Di Stefano V, Grazia Melilli M. Effect of storage on quality parameters and phenolic content of Italian extra-virgin olive oils. Natural Product Research. 2019: 78-86.
https://doi.org/10.1080/14786419.2019.1587434

32. Sadeghi E, Mahtabani A, Karami F. Considering the oxidative stability of cold-pressed sesame, sunflower and olive oils under different storage conditions. Annals of the Univiversity Dunarea de Jos of Galati Fascicle. VI Food Technology. 2019; 43(2): 70-83.
https://doi.org/10.35219/foodtechnology.2019.2.05