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Spectrophotometric method for quantification of flavonoids in olive oil supplied from Tehran market of Iran

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ABSTRACT

Olive oil is rich in antioxidants and it is a healthy fat for human consumption. It has a lot of flavonoid antioxidant compounds. The study aim was determination of flavonoids in crude and refined olive oils supplied in Tehran market of Iran using spectrophotometric method. Total flavonoid contents of olive oils were estimated by using the aluminum chloride colorimetric method as described by Willet. Results showed that the flavonoid contents of crude and refined olive oil were ranged from 3.61 to 1.56 mg rutin hydrate equivalent/g of sample. Compared to refined olive oil, flavonoid content of the crude olive oils was high. It was concluded that the olive oil, especially virgin olive oil can be a good source of flavonoids in human nutrition.

1. Introduction

Olive oil as an important component and main source of fat in the diet of Mediterranean people, is obtained by mechanical extraction from the fruit of the *Oleaeuropaea L*. tree, which belongs to the olive family, comprises some 400 species, and thrives in temperate and tropical climates (1). Olive oil is rich in antioxidants, especially vitamin E, long thought to minimize cancer risk. Among plant oils, olive oil has the highest monounsaturated fat mentioned as a healthy dietary fat that does not oxidize in the body (2).

Olive oil is recognized for its nutraceutical and organoleptic properties. Its unique and well balanced composition includes antioxidants such as hydrophilic phenols. This group of compounds has valuable antioxidant activity endowing the oil with high stability during storage, besides their effects on human health and on olive oil sensory characteristics (9).

Extensive scientific evidence shows that the Mediterranean diet prevents the onset and progression of coronary heart disease (CHD), metabolic disorders, and several types of cancer. These effects might in part be due to specific dietary factors such as consumption of fresh fruits, vegetables, legumes, cereals containing large amounts of fiber, antioxidants, minerals, vegetable proteins, vitamins, and concomitant low consumption of red meat. However, a clear understanding of the possible mechanisms underlying the cardio protective effects has not yet been reached.

Fruit and vegetable have biological effects in association with antioxidant components that can reduce risk of cancer and

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cardiovascular diseases. While these protective effects have been primarily attributed to beta-carotene and ascorbate, phenolic constituents may also play a role. Flavonoids are a broad class of low molecular weight; secondary plant phenolics characterized by the flavan nucleus (3). They are plant pigments that are synthesized from phenylalanine, generally display marvelous colors known from petals of flower (4). Flavonoids can be further classified into flavones (2-phenylchromen-4-one), flavonols (3-hydroxy-2-phenylchromen-4-one), flavanones (2,3-dihydro-2-phenylchromen-4-one), and isoflavones (3-phenylchromen-4-one) (5).Their daily consumption can reach tens and hundreds milligrams. Depending of diet the concentration of these compounds in blood considerably changes and can reach a few micromoles. The presence of flavonoids in diet is very important for human health because they reveal anti-inflammatory, antibacterial, antiviral and antifungal activities. Flavonoids are also known to prevent cardiovascular, cancer, and neurodegenerative diseases (6).

Food-derived flavonoids, in particular quercetin, critically modulate a variety of inflammatory processes and immune functions and these have been extensively reviewed (4).

A reason for the increasing interest in the flavonoids is that the pharmaceutical industry, true to its tradition, is always searching for new medical herbs, the functional compounds of which can serve as a starting point for the development of optimal derivatives. Another reason for the growing activity in the field of flavonoid biochemistry is the persistent claim by many lay medical practitioners of the beneficial effects of treatment with natural products, which proved to be rich in flavonoids (7). Olive oil has flavonoid compounds and it is important to know the content of flavonoid in consumed olive oil. The aim of this study was determination of flavonoids in olive oil supplied in Tehran market of Iran using spectrophotometric method.

2. Materials and Methods

2.1. Sample collection and preparation

Eight popular commercial olive oils including four brands (Sefidrood, Gilvan, Oila and Zidar) of crude and refined olive oil samples obtained from Tehran food market. The olive oils were acquired in a supermarket so that the amount of each selected variant had the same expiry date and a homogenous sample.

2.2. Determination of total flavonoids

Total flavonoid contents of olive oils were estimated using the aluminum chloride colorimetric method as described by Willet, with some modifications. Olive oil (0.5 mL) mixed with 10% aluminum chloride (0.1mL), 1M potassium acetate (0.1mL) and distilled water (4.3 mL). At room temperature it was incubated for 30 min (8). The absorbance was measured at 415 nm using Lambda 25, UV/VIS Spectrometer. In this study for greater homogenization of the oil in alcohol we used emulsifier (DMSO). Since water is polar and oil is non polar we used an emulsifier to avoid of two-phase problems. We also performed the same steps for emulsifier without oil and obtained absorption of flavonoids only (absorption of net flavonoid =absorption of oil-absorption of emulsifier). Rutin hydrate was used to make the calibration curve. The determination of total flavonoids in the olive oil was carried out in triplicate and the results were averaged.

3. Results

Figure 1 shows the calibration curve of flavonoid. At this study using the standard plot of rutin hydrate ($Y=0.334X+.055$, $R^2=.996$), the flavonoid contents of refined and crude olive oil were found ranging from 3.61 to 1.56 mg rutin hydrate equivalent/g of sample. Table 1 shows quantity of total flavonoid in crude and refined olive oils. Flavonoid profiles in olive oils show a variety between crude and refined samples. In four crude samples, Sefirood had 3.52 mg, Gilvan 2.73 mg, Oila 3.44 mg and Zidar 3.61 mg rutin hydrate

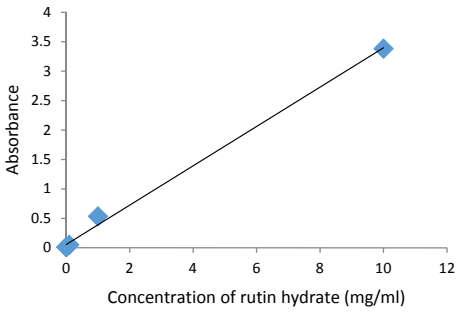

Figure 1. Calibration plot for flavonoid determination

Table 1. The optical absorbance rate and the flavonoid contents (mg rutin hydrate equivalent/g oil) in olive oil samples

Olive oil sample	Optical Absorbance Mean±Std Deviation	Concentration of total flavonoids
Refined Sefidrood	0.617±.249	2.934
Crude Sefidrood	0.715±.312	3.520
Refined Gilvan	0.471±.051	2.059
Crude Gilvan	0.584±.226	2.736
RifinedOila	0.388±.091	1.562
Crude Oila	0.702±.255	3.443
Refined Zidar	0.588±.270	2.760
Crude Zidar	0.731±.175	3.616
Emulsifier	0.072±.009	-

equivalent/g of sample while total flavonoids decreased in refined olive oil and acquired 2.93, 2.05, 1.56, 2.76 mg rutin hydrate respectively. The flavonoid content of the crude olive oils was higher than refined olive oils.

4. Discussion

Flavonoids are a broad group of secondary metabolites with varied and important roles in plant physiology. They have gained recent interest because of their broad pharmacological activity. Putative therapeutic effects of many traditional medicines may be ascribed to the presence of flavonoids (16).

In this study, the total flavonoids content of refined or crude of olive oils were compared and the results suggest that the total flavonoids has correlation with the refined or crude of olive oils. The total flavonoid contents (Table 1) in refined olive oils (1.56 – 2.93 mg/100g) are lower than those found in crude olive oils (2.73 – 3.61 mg/100g). Decreasing total flavonoid in refined sample compared with crude one shows the drastic changes in flavonoids from crude to refined samples. This was due to the hydrophilic nature of flavonoids which were decreased with the refined oil. Total flavonoid content of flax seeds as reported by Oomah et al. (1996) ranged from 30.2 to 83.5 mg/100 g(14). El-beltagi et al. (2007) found that total flavonoid in seed of different flax cultivars 12.9 – 20 mg rutin/100 g(15).

Zuk et al state that flavonoids due to their hydrophilic nature only very partially coextract with the oil during its production. Nevertheless, the accumulation of antioxidants in seedcake was expected to affect fatty acid composition. Indeed oil produced from transgenic seeds contains more unsaturated fatty acids and the total level of fatty acids was also increased. Thus the higher quantity of phenyl propanoid compounds in seedcake indirectly affects the fatty acids stability perhaps by protection them against oxidation during technological process of oil production (10).

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenols. These compounds possess abroad spectrum of chemical and biological activities including radical scavenging properties (11).

Ildiko et al. (12) reported that the concentration of flavonoids in vegetables is 0.25 to 0.18 g/g %. At present study, the flavonoid content of refined and crude olive oils ranged from 3.61 to 1.56 mg rutin hydrate equivalent/g of sample. The flavonoid content of the crude olive oils was high compared to that of refined olive oils. In a research, Sadighara et al. (13) reported that the plants with dark color have higher flavonoid than white color ones. Also, in our study, crude olive oil had dark color and it had more flavonoid compared with refined olive oil.

5. Conclusion

The present investigation revealed that the olive oil contain small amount of flavonoids. Crude olive oil has more flavonoid compared with refined olive oil. Refine processing can remove a part of flavonoid from the olive oil. It is recommended to consume crude olive oil for receiving more flavonoid from food.

Conflicts of interest

Authors have no conflict of interest.

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References

1. Shahidi F. Olive Oil. Bailey's Industrial Oil and Fat Products, Sixth Edition, Six Volume Set. Wiley. 2005. p. 3616.
2. Bogani P, Galli C, Villa M, Visioli F. Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. Atherosclerosis 2007; 190: 181–186.
3. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships .J Nutr Biochem 2002; 13(10): 572-584.

4. Madhavan P.N Nair, ChithanKandaswami, SupriyaMahajan, Kailash C Chadha, Ram Chawda, Harikrishnan Nair, Niranjan Kumar, Raji E Nair, Stanley A Schwartz .The flavonoid, quercetin, differentially regulates Th-1 (IFNγ) and Th-2 (IL4) cytokine gene expression by normal peripheral blood mononuclear cells .Biochimicaet Biophysica Acta (BBA) - Mol Cell Res 2002; 1593 (1):29-36. .
5. Huang Z, Fang F, Wang J, Wong C W. Structural activity relationship of flavonoids with estrogen-related receptor gamma .FEBS letters 2010; 584(1): 22–26 .
6. Yury S. Tarahovsky, Elena A. Yagolnik, Eugeny N. Muzafarov, Bolatbek S. Abdrasilov,Yuri A. Kim .Calcium-dependent aggregation and fusion of phosphatidylcholine liposomes induced by complexes of flavonoids with divalent iron. Biochimicaet Biophysica Acta (BBA)-Biomembranes 2012; 18(3): 695–702.
7. Bent H Havsteen. The biochemistry and medical significance of the flavonoids. Pharmacol Therap 2002; 96 (2–3):67–202.
8. Hossain MA, Shah MD, Gnanaraj C, Iqbal M. In vitro total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant Tetra stigma from Sabah. Asian Pacific J Trop Med 2011; 717-721.
9. El Riachy M, Priego-Capote F, Leon L, Rallo L, de Castro MDL. Hydrophilic antioxidants of virgin olive oil. Part 1: Hydrophilic phenols: A key factor for virgin olive oil quality: Review Article. Eur J Lipid Sci Technol 2011; 113: 678–691.
10. Zuk M, Kulma A, Dyminska L, Szołtysek K, Prescha A, Hanuza J, Szopa J. Flavonoid engineering of flax potentiate its biotechnological application. BMC Biotechnol 2011; 11:10.
11. Khatiwora E, Adsul VB, Kulkarni MM, Deshpande NR, Kashalkar RV. Spectroscopic determination of total phenol and flavonoid contents of Ipomoea carnea. Int J ChemTech Res 2010; 2(3): 1698-1701.
12. Ildiko S, Gabriela V, Alexandrina F, Simona B, Mirela TD. The quantitative analysis through spectrophotometery of flavonoids and polyphenols from vegetable products. HIBISCI TRIONI HERBA, radix and fructus. AnaleleUniversităţii din Oradea, Fascicula Protecţia Mediului 2012; 28.
13. Sadighara P, Gharibi S, Moghadam Jafari A, Jahed Khaniki GR,Salari S. The antioxidant and Flavonoids contents of Althaeaofficinalis L. flowers based on their color. Avicenna J Phytomed. 2012; 2(3): 113–117.
14. Oomah, B., G. Mazza, E. Kenaschuk. Flavonoid content of flaxseed. Influence of cultivar and environment, Euphytica 1996; 90: 163–167.
15. El-Beltagi H. S., Salama Z. A., El-Hariri D. M. Evaluation of fatty acids profile and the content of some secondary metabolites in seeds of different flax cultivars (Linum usitatissimul). Gen Appl Plant Physiol 2007; 33 (3-4): 187-202.
16. Sathishkumar T., Baskar R, Shanmugam S, Rajasekaran P, Sadasivam S and Manikandan.V. Optimization of flavonoids extraction from the leaves of Tabernaemontana heyneana Wall using L16 Orthogonal design. J Nat Sci 2008; 6(3): 1545-0740.