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Total Antioxidant Capacity and Total Phenol Contents of Turkish Edible Oils

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ABSTRACT

This study was designed to determine and compare total antioxidant capacity (TAC) and total phenol contents (TP) of some common edible oil samples (corn, sunflower, hazelnut, soybean, cotton, olive oil (virgin, riviera and extra virgin types) consumed in Turkey. Such data is of importance for the evaluation of nutritional and health impact of these oils. TAC of the samples were assessed by using three methods, two of them were more recently developed methods (TAC_1 and TAC_2) using Fe^{+2}-*o*-dianisidine complex and ABTS [2-2 azinobis (3-methybenzothiazoline-6-sulfonate)] radical respectively and one (TAC_3) was FRAP (ferric reducing ability of plasma) assay. Among the oils, extra virgin olive oil had the highest TAC and TP followed by virgin olive oil, whereas soybean oil and hazelnut oil had lower values. TAC results of the oils were correlated with TP of them and the correlation coefficients between TP and TAC_1, TAC_2, TAC_3 were found as r=0.70, r=0.65 and r=0.07 respectively.

Key Words: Antioxidant, Edible oils, Phenolic compounds

Türkiye'de Tüketilen Yemeklik Yağların Toplam Antioksidan Kapasitesi ve Toplam Fenol İçeriği

ÖZET

Bu çalışma, Türkiye'de yaygın tüketilen bazı yemeklik yağ örneklerinin (mısır, ayçiçeği, fındık, soya, pamuk, zeytin (naturel, riviera ve sızma çeşitleri) toplam antioksidan kapasite (TAC) ve toplam fenol içeriklerinin (TP) belirlenmesi ve karşılaştırılması amacıyla gerçekleştirilmiştir. Veriler, bu yağların beslenme ve sağlığa etkilerinin belirlenmesi açısından önemlidir. Örneklerin TAC değerleri, iki tanesi yakın zamanda geliştirilen ve sırasıyla Fe^{+2}-*o*-dianizidin kompleksi ve ABTS [2-2 azinobis (3-metilbenzotiazolin-6-sulfonat)] radikallerinin kullanıldığı (TAC_1 ve TAC_2) metotları ve bir tanesi de (TAC_3) FRAP (plazmanın ferrik indirgeme kabiliyeti) metodu olan üç yöntemle ölçülmüştür. En yüksek TAC ve TP içerikleri sızma zeytinyağında bulunmuş ve naturel zeytinyağı değerleri bunu takip etmiş, fakat soya ve fındık yağları daha düşük değerlere sahip bulunmuştur. Örneklerin TAC ve TP değerleri arasındaki ilişki incelenmiş ve TP ile TAC_1, TAC_2, TAC_3 değerlerinin sırasıyla r=0.70, r=0.65 ve r=0.07 korelasyon katsayıları ile ilişkili olduğu belirlenmiştir.

Anahtar Kelimeler: Antioksidan, Yemeklik yağlar, Fenolik bileşikler

INTRODUCTION

Human body has a number of defense systems to neutralize the harmful effects of free radicals and other reactive oxygen species. Normally, there is a balance between oxidant-antioxidant systems in an organism. There are internal and external defense systems of antioxidants against the reactive oxygen species produced depending on internal and external factors. Any insufficiency in the antioxidant defense system changes the balance in favor of oxidants [14]. The antioxidant compounds of the edible oils are important on account of the oxidant-antioxidant balance of the body. It is set forward for many disorders that the antioxidant level is decreased. High antioxidant level has an effective role of preventing atherosclerosis, cancer, early aging and lipid peroxidation [24]. It was observed in experiments *in vivo* and *in vitro* that vegetable oils reduce the incidence and severity of arrhythmias [18], have antithrombotic properties [13], affect lipid peroxidation and antioxidant parameters, and lead to favorable changes in the plasma lipid status [10, 25]. The antioxidant content of plant foods may contribute to the protection they offer from disease.

Individual antioxidant compounds do not act alone. They act in combination with other antioxidants, as interactions among them can affect total antioxidant capacity, producing synergistic or antagonistic effects [19]. Because plant foods contain many different classes and types of antioxidants, knowledge of their total antioxidant capacity (TAC), which is the cumulative capacity of food components to scavenge free radicals, would be useful for epidemiologic purposes [21] because the radical scavenging activity of vegetable oils can be interpreted as the combined action of different endogenous antioxidants [23]. To accomplish this, total phenol and total antioxidant capacity contents of eight Turkish edible oils were compared using different assays.

Previous studies revealed that antioxidant activities may differ by different measurement methods [20, 21]. Several methods were developed recently for measuring the total antioxidant capacity of food and beverages [4, 6, 7, 8, 28, 22] these assays differ in their chemistry (generation of different radicals and/or target molecules) and in the way end points are measured. Because different antioxidant compounds may act through different mechanisms, no single method can fully evaluate the TAC of foods.

In this study, total phenol contents were measured with Folin Ciocalteu method and total antioxidant capacity of the oil samples were analyzed using three different assays. These assays, based on different chemical mechanisms, were used to measure antioxidant capacity of compounds with wide variety and action range.

The purpose of this study was to investigate the total antioxidant capacity of eight edible oil samples commonly consumed in Turkey, to compare these total antioxidant determination methods for their convenience for oils related to their total phenol contents and so to find the most valuable oil for oxidative stress-induced disease preventing diets.

MATERIALS AND METHODS

Oil samples: Corn, sunflower, hazelnut, soybean, cotton, olive oil samples were purchased from local supermarkets in Sanliurfa, Turkey. Olive oil samples were virgin, riviera (mixed olive oil containing higher rate of refined and lower rate of virgin olive oil) and extra virgin olive oil. Before analysis the oil samples were mixed with methanol/water solution (1:1, v/v). The solution was vortex-mixed for an hour at room temperature. It was centrifuged (Universal 30 RF centrifuge, Hettich) at 5000 rpm for 20 minutes. An Aeroset model automatic analyser (Abbott) and UV/VIS spectrophotometer (Jasko V-530) was used for total phenol and antioxidant contents of the samples which were measured in methanol extracts of oil samples with two replications and three repeats.

Chemicals: Gallic acid, Folin-Ciocalteu reactive, *o*-dianisidine, ABTS radical, Trolox and TPTZ were purchased from Sigma (St. Louis, MO) and Na_2CO_2, methanol, KCl, H_2O_2, sodium acetate, acetic acid were purchased from Merc Co. with maximum purity.

Total phenol content determination: Total phenolic content was determined according to a modified method of Skerget et al. [26] that based on a colorimetric oxidation/reduction reaction. For this purpose 200 µL sample were taken and mixed with 1000 µL of 10 fold diluted Folin–Ciocalteu reagent, 800 µL of sodium bicarbonate solution (7.5%, w/w) were added, incubated at room temperature for 2 hours; then absorbance was read at 750 nm. Methanol-water solution (1:1, v/v) was used as blind sample. 1 mM gallic acid was used in standard preparation and results were expressed as mM gallic acid equivalents/L.

Determination of total antioxidant capacity: Total antioxidant capacity of samples was determined using three methods and the results were given as TAC_1, TAC_2 and TAC_3.

TAC_1 method: In this new method [6], a standardized solution of Fe^{+2}-*o*-dianisidine complex reacts with a standardized solution of hydrogen peroxide by a Fenton-type reaction, producing OH*. These potent reactive oxygen species (ROS) oxidize the reduced colorless o-dianisidine molecules to yellow-brown colored dianisidyl radicals and further oxidation reactions occur. Since the antioxidants in the sample suppress the oxidation reactions and color formation, this reaction can be monitored by a spectrophotometer. Trolox, a water soluble analogue of vitamin E, is used as the standard and the results are expressed as mM Trolox equivalents/L.

TAC_2 method: Total antioxidant capacity of the samples was measured by a new method developed by Erel [7]. This method is based on the decolorization of ABTS radical cation which stays more stable for a long time in the acetate buffer solution. While it is diluted with a more concentrated acetate buffer solution at high pH values, the color is spontaneously and slowly bleached. Antioxidants present in the sample accelerate the bleaching rate to a degree proportional to their concentrations which can be monitored spectrophotometrically and the bleaching rate is inversely related with the TAC of the sample. The reaction rate is calibrated with Trolox which is widely used as a traditional standard for TAC measurement assays, and the assay results are expressed in mM Trolox equivalents/L.

TAC_3 method: Total antioxidant capacity of the samples was measured by FRAP method developed by Benzie and Strain [4]. Twenty microliters of sample were mixed with freshly prepared study reactive (10 volume 300mM acetate buffer + 1 volume 10mM TPTZ solution), incubated at 37°C for 5 minutes and absorbance was read. Trolox which is a water soluble analogue of vitamin E was used as the standard and the results were expressed as mM Trolox equivalents/L.

Statistical analysis: Data were analysed using SPSS for Windows Release 10 (SPSS Inc.) by one-way

ANOVA and by Duncan's multiple range tests. Correlation analyses were also performed where appropriate. Statistical differences was calculated at $p<0.05$.

RESULTS AND DISCUSSION

The total antioxidant capacity of eight edible oils used in diet in Turkey was evaluated using three different assays. The total phenol contents of the oils were also evaluated. The oil samples had different antioxidant capacities in relation to the method applied; thus, the same item often ranked differently depending on the assay. The quality of oils is affected by a number of other factors like soil conditions, ripeness of the seeds, length of storage [9], and the large variability within the evaluation of the oil item and to the lack of standardization of the assays, therefore, it was expected that some results of our investigation could be different of other authors. For this discussion, the phenolic contents of the samples were compared with their TAC values and to understand the relationship between them, the correlation coefficients were compared. Iqbal et al. [12] reported that the estimate of TPC is a good measure of the antioxidant efficacy of the extracts and Awika et al. [3] reported that the phenolic compounds may contribute directly to antioxidant action. The overall TAC values were obtained from the mean values for each assay.

Among the oil samples, the highest amount of total phenol content was measured in the extra virgin olive oil as 1.596 mM gallic acid equivalents/L. Total phenol content was lower in virgin olive oil, riviera olive oil, corn oil, sunflower oil, hazelnut oil, cotton oil to soybean oil in order (Table 1).

Table 1. The total phenol and total antioxidant values of various edible oils (mean ± standard deviation)

Sample	Total Phenol (mM gallic acid equivalents/L)	TAC_1 (mM Trolox equivalents/L)	TAC_2 (mM Trolox equivalents/L)	TAC_3 (mM Trolox equivalents/L)
Sunflower oil	0.412±0.004	0.791±0.003	1.997±0.010	0.954±0.015
Hazelnut oil	0.360±0.003	0.218±0.013	0.357±0.007	0.115±0.014
Corn oil	0.448±0.004	0.853±0.003	1.856±0.004	1.915±0.032
Cotton oil	0.269±0.003	0.347±0.004	2.534±0.004	0.245±0.004
Riviera olive oil	0.684±0.012	0.307±0.005	1.178±0.006	0.905±0.011
Extra virgin olive oil	1.596±0.020	1.233±0.011	3.391±0.036	0.571±0.024
Olive oil	0.730±0.002	0.364±0.002	1.199±0.004	0.325±0.021
Soybean oil	0.133±0.002	0.326±0.007	0.142±0.002	0.175±0.009

The TAC_1 values of the analyzed oils are shown in Table 1. According to this assay, extra virgin olive oil had the greatest antioxidant capacity (1.233 mM Trolox equivalents/L) and it was followed by corn oil, sunflower oil, virgin olive oil, cotton oil, soybean oil, riviera olive oil and hazelnut oil respectively. As already observed by Mannino et al. [17], Trolox equivalent antioxidant capacity value of extra virgin olive oil was higher than that of virgin olive oil. The difference in their antioxidant capacities arises from the different manufacturing processes [5], leading to differences in the antioxidant composition. In particular, extra virgin olive oil is much richer in phenolic compounds than other types of olive oils and refined oils (obtained by solvent extraction), which are virtually devoid of phenols. Olive oil is a vaguely defined mixture of refined olive oil and extra virgin olive oil in which the amount of extra virgin olive oil may vary from 33 to 95% [2], thus affecting the amount of antioxidants present. It was previously reported [16] that the chemical composition of extra virgin olive oil contributes to daily requirements of essential fatty acids and active antioxidant nutrients in vitamin E deficiency. This particular and well-balanced situation [oleic acid (18:1n-9) and minor components in an ideal ratio] undoubtedly has a significant relevance in human clinical nutrition

According to the TAC_2 results (Table 1) the highest total antioxidant capacity value was obtained for extra virgin olive as 3.391 mM Trolox equivalents/L. Other oils were in the order of cotton oil, sunflower oil, corn oil, virgin olive oil, riviera olive oil, hazelnut oil and soybean oil having TAC_2 values from higher to lower values.

By the FRAP assay TAC_3 results of the samples were between 1.915 mM Trolox equivalents/L for corn oil and 0.115 mM Trolox equivalents/L for hazelnut oil. TAC_3 value for extra virgin olive oil was lower than those of corn oil, sunflower oil, riviera olive oil and higher than those of virgin olive oil, cotton oil, soybean oil and hazelnut oil.

TAC results of the oil samples were correlated with the total phenols of them. The correlation between the total phenols and antioxidant capacity, as determined by TAC_1 assay was the highest (r=0.70) and with TAC_3 test was the lowest (r=0.07). The correlation coefficient between the total phenol content and TAC_2 measurements was r=0.65 and comparable with the correlation coefficient. Because the most bioactive and oxidative stress reducing components of olive oils are their phenolic compounds [9]. Although, some authors claim that there is no correlation between the total phenolic content and the radical scavenging capacity [29] others who has shown that high total polyphenols content increases antioxidant activity and there is a linear correlation between phenolic content and antioxidant activity [1, 9, 11, 15, 27]. On account of the correlation test results of this investigation, the TAC_1 method was found more reliable than the other methods used in this study for the determination of the antioxidant capacity of oils and of the oils, extra virgin

olive oil has the highest total phenol, TAC_1 and TAC_2 values.

CONCLUSION

Results of this study provide information about nutritional and health impact of these oils to serve as dietary sources of natural antioxidants for health promotion and oxidative stress based disease prevention. In this respect, extra virgin olive oil was found to have the highest total phenol, TAC_1 and TAC_2 values. According to the results, TAC_1 method has a potential to be a more reliable method among the applied methods in this study for determination of the antioxidant capacity of the oils.

REFERENCES

[1] Abu-Amsha R, Croft KD, Puddey IB, Proudfoot JM, Beilin LJ., 1996. Phenolic content of various beverages determines the extent of inhibition of human serum and low-density lipoprotein oxidation in vitro: identification and mechanism of action of some cinnamic acid derivatives from red wine. *Clinical Science* 91: 449-458.

[2] Andrikopoulos NK, Hassapidou MN, Manoukas AG., 1989. The tocopherol content of Greek olive oils. *Journal of the Science of Food and Agriculture* 46: 503-509.

[3] Awika JM, Rooney LW, Wu X, Prior RL and Zevallos LC., 2003. Screening methods to measure antioxidant activity of Surghum (*Surghum bicolor*) and Surghum products. *Journal of Agriculture and Food Chemistry* 51: 6657-6662.

[4] Benzie FFI, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry*, 239, 70-76.

[5] Boskou D. 1996. Olive oil composition. (Boskou, D. ed.) Olive Oil: Chemistry and Technology, pp.52-83, AOCS Press, IL.

[6] Erel O., 2004a. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical Biochemistry* 37: 277-285.

[7] Erel O., 2004b. A novel automated method to measure total antioxidant response against potent free radical reactions. *Clinical Biochemistry* 37: 112-119.

[8] Ghiselli A, Serafini M, Maiani G, Azzini E, Ferro-Luzzi A., 1995. A fluorescence-based method for measuring total plasma antioxidant capability. *Free Radical Biology and Medicine* 18: 29-36.

[9] Gorinstein S, Belloso OM, Katrich E, Lojek A, Milan, C.Z., Miguel NG, Haruenkit R, Seo Park Y, Teck Jung' S, Trakhtenberg S., 2003. Comparison of the contents of the main biochemical compounds and the antioxidant activity of some Spanish olive oils as determined by four different radical scavenging tests. *Journal of Nutritional Biochemistry* 14: 154-159.

[10] Gustafsson IB, Vessby B, Ohrvall M Nydahl M., 1994. A diet rich in monounsaturated rapeseed oil reduces the lipoprotein cholesterol concentration and increases the relative content of n-3 fatty acids in serum in hyperlipidemic subjects. *American Journal of Clinical Nutrition* 59: 667-674.

[11] Holasova M, Fiedlerova V, Smrcinova H, Orsak M, Lachman J, Vavreinova S., 2002. Buckwheat - the source of antioxidant activity in functional foods. *Food Research International* 35: 207-211.

[12] Iqbal S., Bhanger MI, Anwar F. 2005. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. *Food Chemistry* 93: 265- 272.

[13] Karantonis HC, Antonopoulou S, Demopoulos C., 2002. Antithrombotic lipid minor constituents from vegetable oils. Comparison between olive oils and others. *Journal of Agricultural and Food Chemistry* 50: 1150-1160.

[14] Lee KY, Weintraub ST, Yu BP., 2000. Isolation and identification of a phenolic antioxidant from Aloe Barbadensis. *Free Radical Biology and Medicine* 28: 261-265.

[15] Litridou M, Linssen J, Schols H, Bergmans M, Posthumus M, Tsimidou M, Boskou D., 1997. Phenolic compounds in virgin olive oil: fractionation by solid phase extraction and antioxidant activity assessment. *Journal of the Science of Food and Agriculture*, 74: 169-174.

[16] Mangas-Cruz, M.A, Martínez-Brocca M, Ortiz-Leyba C, Garnacho-Montero J, Pereira Cunill JL, Garcia-Luna, P.P., (2004). Olive oil in clinical nutrition. *Grasas y Aceites* 55: 76-83.

[17] Mannino S, Buratti S, Cosio MS, Pellegrini N., 1999 Evaluation of the "antioxidant power" of olive oils based on a FIA system with amperometric detection. *Analyst* 124: 1115-1118.

[18] Mclennan P., 1993. Relative effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on cardiac arrhythmias in rats. *American Journal of Clinical Nutrition* 57: 207-212.

[19] Niki E, Noguchi N., 2000. Evaluation of antioxidant capacity. What capacity is being measured by which method? *Life* 50: 323-329.

[20] Pellegrini N, Re R, Yang M, Rice-Evans CA., 1999. Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying the 2, 2'-azobis(3-ethylenebenzothiazoline-6-sulfonic) acid radical cation decolorization assay. *Methods in Enzymology* 299: 379-389.

[21] Pellegrini N, Serafini M, Colombi B, Del Rio D, Salvatore S, Bianchi M, Brighenti F., 2003. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *Journal of Nutrition* 133: 2812-2819.

[22] Prior RL, Wu XL, Schaich K., 2005. Standardized methods for the determination of antioxidant

capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry* 53: 4290-4302.

[23] Ramadan FM, Moerse JT., 2006 Screening of the antiradical action of vegetable oils. *Journal of Food Composition and Analysis* 19: 838-842.

[24] Ou BX, Huang DJ, Hampsch-Woodill M, Flanagan JA, Deemer EK., 2002. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) ferric reducing antioxidant power (FRAP) assays: A comparative study. *Journal of Agricultural and Food Chemistry* 50: 3122-3128.

[25] Scaccini C, Nardini M, D'aquino M, Gentili V, Di Felice M, Tomassi G., 2005. Effect of dietary oils on lipid peroxidation and on antioxidant parameters of rat plasma and lipoprotein fractions. *Journal of Lipid Research* 33: 627-633.

[26] Skerget M, Kotnik P, Hadolin M, Hra A, Simonic M, Knez Z., 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chemistry* 89: 191-198.

[27] Velioglu YS, Mazza G, Gao L, Oomah BD., 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry* 46: 4113-4117.

[28] Wang H, Cao G, Prior RL., 1997. Oxygen radical absorbing capacity of anthocyanins. *Journal of Agricultural and Food Chemistry* 45: 304-309.

[29] Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M., 2002. Free radical scavenging properties of wheat extracts. *Journal of Agricultural and Food Chemistry* 50**:** 1619-1624.