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Original Article

ESTIMATING THE ANTIOXIDANT ACTIVITY FOR NATURAL ANTIOXIDANTS (TOCOCHROMANOL) AND SYNTHETIC ONE BY DPPH

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ABSTRACT

The tocochromanols (vitamin E) possesses powerful neuroprotective, anticancer, and cholesterol-lowering properties, current developments in vitamin E research clearly indicate that members of the vitamin E family are not redundant with respect to their biological functions.

Objective: the aim of this study was to determine the free radical scavenging capacity of different standard antioxidants (tocochromanols & synthetic) in term of hydrogen donating or radical scavenging activity using the stable DPPH method, dependent on (efficient concentration 50%, EC_{50}) parameter under same experimental conditions.

Methods: (3 ml) of standard antioxidant after proper dilution with methanol has been prepared in six tubes. Then 1ml of DPPH (200 µmol/L) added to achieve a standard serial chain of 2.5, 5, 7.5, 10, 12.5, 15 µmol/L studied antioxidant. The tubes were shaked and kept in dark until the end of the reaction. The remaining percentage of DPPH (%DPPH• rem) for different antioxidants in different concentrations was determined. All measurements of free radical scavenging activity were performed in triplicate and standard deviation was calculated. The relationship between measured sample and decreasing amount of (%DPPH• rem) have been determined.

Results: The results, shows; that the reaction time between DPPH and antioxidants was differed according to the antioxidant used and ranged from five to 110 minutes to completion. and scavenging activity of BHT were the most and similar to that of BHA, and higher than that of *α*-T and AP, while the scavenging activity for the rest of antioxidants were decrease in the following order: BHT> BHA> *α*-T> AP> *α*-T3> *β*-T> *γ*-T> *β*-T3> *γ*-T3> *δ*-T> *δ*-T3.

Conclusion: The study can guide to select suitable antioxidant depending on the scavenging activity of free radical

Keywords: Tocochromanols, Radical, Antioxidant, Scavenging, DPPH

INTRODUCTION

Tocochromanols (Tocopherols and tocotrienols) are a group of soluble lipid compounds recognized as a generic term for vitamin E, being well represented in vegetables, fruits, seeds, nuts, and oils [1]. In animal products, such as dairy foods [2,3], *α*-tocopherol is the major vitamin E vitamer found. Although cereals are a modest source of lipids, they are a good source of tocochromanols even if their concentration and composition vary considerably among the sources [4].

Naturally occurring vitamin E is composed of eight vitamers: tocopherols (*α-, β-, γ-* and *δ*-T); and their four corresponding unsaturated tocotrienols (*α-, β-, γ-* and *δ*-T3). Moreover, in certain cereals, together with the naturally occurring eight vitamers of tocochromanols, other components have been identified, such as plastochromanol-8 and novel tocotrienols [5,6].

Biological activities of tocochromanols are generally believed to be due to their antioxidant action by inhibiting lipid peroxidation in biological membranes. *α*-Tocopherol has been labeled as the most efficient antioxidant for breaking free radical driven chain reactions [7]. Tocotrienols were found to lower serum cholesterol levels in various animal models and humans [8, 9]. The number and localization of the methyl groups of their chroman rings influence their biological activities, *δ*-tocotrienol (8-methyl) being the most potent cholesterol inhibitor, followed by *γ*-tocotrienol (7,8-dimethyl) and *α*-tocotrienol (5,7,8-trimethyl) [10]. Furthermore, the tocotrienols differ substantially in their capacity to suppress tumor cell proliferation [11].

Tocochromanols, while protecting the fat, are generally themselves first oxidized to quinines and dimmers [12]. There is some difficulty in adequately evaluating the antioxidant activity of tocochromanols, because this activity is frequently influenced by their concentration, the types of oils and fats used as substrates, and the method of evaluation [13].

Antioxidant activity can be evaluated as total free radical-scavenging capacity, by spectrophotometrically measuring the disappearance of the free 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The DPPH• test has previously been successfully employed in assessing the antioxidant activity [14].

In the original method, a reaction time of 30 minutes was recommended, and this has been followed in more recent work [15]. Shorter times have also been used, such as 5 minutes [16], or 10 minutes [17]. However, in view of the fact that the rate of reaction varies widely among substrates [18,19], the best practice seems to be to follow the reaction until it has gone to completion ("plateau") [20-22]. The rate of reaction has also been proposed as a further parameter to characterise the antioxidant activity [21,23].

In our study, we have followed the reaction until it has gone to completion for each antioxidant, by studying absorption changes according to the time for each antioxidant.

The objective of this study was to assess the effect of nature antioxidants (tocochromanols) and the synthetic one (BHT, BHA, AP) on radical-scavenging activity, and to study the time required to complete reaction with DPPH by study of absorption changes according to the time for each.

MATERIALS AND METHODS

The Alpha Tocopherol [*α*-T], Beta Tocopherol [*β*-T], GammaTocopherol [*γ*-T], Delta Tocopherol [*δ*-T], Alpha Tocotrienol [*α*-T3], Beta Tocotrienol [*β*-T3], Gamma Tocotrienol [*γ*-T3] and Delta Tocotrienol [*δ*-T3] was obtain from (Chromadex®, Irvine California); the 1,1-Diphenyl-2-picrylhydrazyl [DPPH], Butylated hydroxyanisole [BHA], 2,6-Di-tert-butyl-4-methylphenol [BHT], Ascorbyle Palmitate

[AP] obtained from (Sigma-Aldrich, USA); the methanol p.a. from (Merck Chemicals, Germany).

Preparation standards

A series of working standard solutions were prepared by appropriate dilution with methanol. DPPH standard (0.02 mol/L) was prepared daily by dissolve (3.94) g in 50 ml of methanol then diluted to 500 ml; a 1/100 dilution was done to get (0.2 mmol/L).

Primary stock solutions (0.01 mol/L) of BHA, BHT, AP, *α*-T, *β*-T, *γ*-T, *δ*-T, *α*-T3, *β*-T3, *γ*-T3 and *δ*-T3 were prepared; by dissolve 0.18, 0.22, 0.414, 0.43, 0.416, 0.416, 0.402, 0.424, 0.41, 0.41, and 0.396 g from each antioxidant in order in methanol then diluted to 100 ml a 1/10 dilution was done to get (1 mmol/L) secondary stock solution of each antioxidant, and from the previous secondary stock solution was performed to get standards titrating at 2.5, 5, 7.5, 10, 12.5, 15 µmol/L in by taking the following volumes from secondary stock solution, 2.5, 5, 7.5, 10, 12.5, 15 ml in series of 1000 mL volumetric flasks respectively

Apparatus

Spectrophotometer UV-Vis (Thuramed T60, Germany), micropipette 100-1000 µl (Iso lab, Germany), sensitive balance (Sartorius, Germany), quartz Cuvette, volumetric flask (10, 25, 50, 100 ml), beaker (250, 500 ml), pipette (1, 2, 5, 10 ml).

Absorbance measurements

It is general practice to use a spectrophotometer to determine the absorbance with DPPH solutions, and the working wavelength of maximum absorbance, λmax, to be used for the absorbance measurements is given variously as 515 nm[16,18,19,21,24], 516 nm[17], 517 nm[20,25], 518 nm[26], and 520 nm[15]. However, in practice, given that the "peak" is a maximum, that is, round topped, and that the absolute absorbance values are not important, the wavelength can be set to that giving the maximum absorbance in the instrument that is used. Maximum absorbance of DPPH (200 micro mol/liter) recently prepared, has been determined. The solution had studied under different wavelength (400 to 650 nm), and λmax turned out to be (517 nm) for DPPH.

DPPH method

The molecule of 1,1-diphenyl-2-picrylhydrazyl (1,1-diphenyl-2-picrylhydrazyl; DPPH:1) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerism, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color, characterized by an absorption band in methanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced anther form with the loss of this violet color (although there would be expected to be a residual pale yellow color from the picryl group still present). Representing the DPPH radical by DPPH• and the donor molecule by AH, the primary reaction is:

DPPH• + AH = DPPH-H + A• {1}

Where DPPH-H is the reduced form and A• is free radical produced in this first step. This latter radical will then undergo further reactions, which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorized) by one molecule of the reductant. The reaction {1} is therefore intend to provide the link with the reactions taking place in an oxidizing system, such as the autoxidation of a lipid or other unsaturated substance; the DPPH molecule is thus intended to represent the free radicals formed in the system whose activity is to be suppressed by the substance AH. The substrate concentrations used should for definiteness, be those that would be in the reaction cuvette in the absence of any DPPH. Alternatively, the amount (moles) of substrate added to the reaction vessel may be used. sThree (ml) of standard antioxidant after proper dilution with methanol has been prepared in six tubes. Then 1ml of DPPH (200 µmol/L) added to achieve a standard serial chain of 2.5, 5, 7.5, 10, 12.5, 15 µmol/L studied antioxidant. The tubes were shaked and kept in dark until the end of the reaction. The measures were taking at the maximum wavelength and at the laboratory temperature. The remaining percentage of DPPH (%DPPH• rem) for different antioxidants in different concentrations was determined. All measurements of free radical scavenging activity were performed in triplicate and standard deviation was calculated. The relationship between measured sample and decreasing amount of (%DPPH• rem) have been determined. The ability to scavenge the DPPH radical was calculated using the following equation (1):

$$\%\text{DPPH• rem} = (A_F/A_{DPPH\bullet})*100 \quad \text{Equation (1)}$$

Where $A_{DPPH\bullet}$ is the absorbance of control, and A_F is the absorbance of the sample after addition of antioxidant and complete reaction, and %DPPH• rem is the remaining percentage of DPPH.

The total free radical-scavenging capacity of standard antioxidants were evaluated by measuring the disappearance of the stabilized 2,2-diphenyl-1-picrylhydrazyl artificial free radical (DPPH•) by measuring the absorbance at 517 nm according to Espin et al method[27]. The resulted were expressed as parameter that has been introduced for the interpretation of the results from the DPPH method, is the "efficient concentration" or EC_{50} value (otherwise called the IC_{50} value), which is the concentration of antioxidant that causes 50% loss of the DPPH activity (absorbance). This parameter was apparently introduced by Brand-Williams and his colleagues[18,19]. This parameter has the drawback that the higher the antioxidant activity ability of free radical scavenging activity), the lower is the value of EC_{50}. This is a disadvantage particularly when results are presented graphically as a bar chart[21] even if the same data are also available in numerical form[23]. Because that we had determined the antioxidant activity from the equation: ($1/EC_{50}$).

RESULTS AND DISCUSSION

After the (λmax = 517) of this solution was determined, the scavenging activity of each antioxidant was determined using EC_{50} parameter.

Time influence on the progress of the antioxidant reaction

The highest concentration tube (15 µmol/L) of studied antioxidants was measured and the absorbance of it was recorded every five minutes starting from (0) the starting point of the reaction. That is for defining the time needed to complete the reaction. The reaction development was evaluated by measuring the absorbance of the highest concentration (15 µmol/L) every five minutes, Figure 1, reveals the results obtained, and it indicate the reaction time for each antioxidant is different, according the absorbance changes.

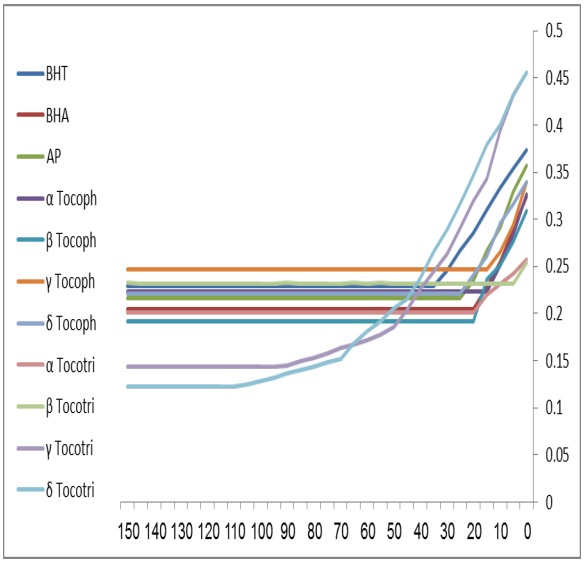

Fig. 1: It shows absorption changes of antioxidants per time

The needed time for reaction has differed among the antioxidants, and the antioxidants occupied the following order: (BHT: 110, BHA: 95, δ-T3: 35, β-T: 25, β-T3: 25, α-T: 20, δ-T: 20, γ-T3: 20, γ-T: 15, α-T3: 15, AP: 5) minute Figure 2.

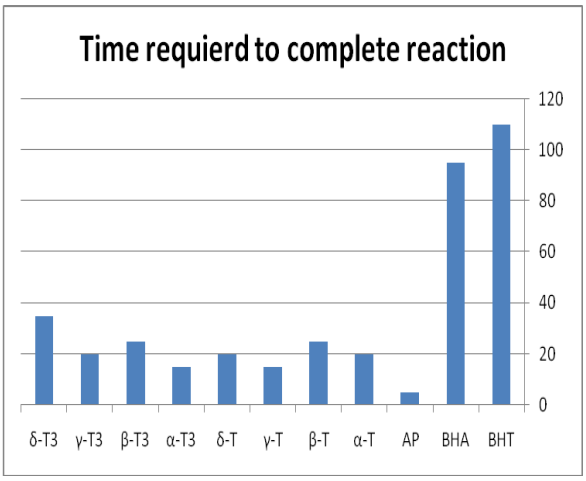

Fig. 2: It shows the differences of reaction time among different antioxidants

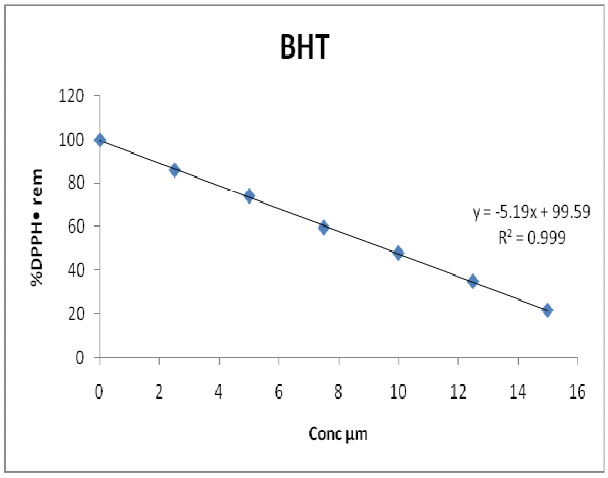

Fig. 3: It shows the changes of remaining percentages according to concentration changes

Determination of efficient concentration 50% of BHT

The series of standard concentrations prepared from standard solution of BHT were measured and the remaining percentage of DPPH was determined. All measurements of free radical scavenging activity were performed in triplicate. The Table 1, shows the results of measurement:

Applying statisticed analysis using SPSS.17 (on way test) there was a significant importance (P_{value}< 0.05) and the Figure 3, shows the correlation between the concentration and the remaining percentage and the equation of this liner relationship was (y= -4.285x +99.76)

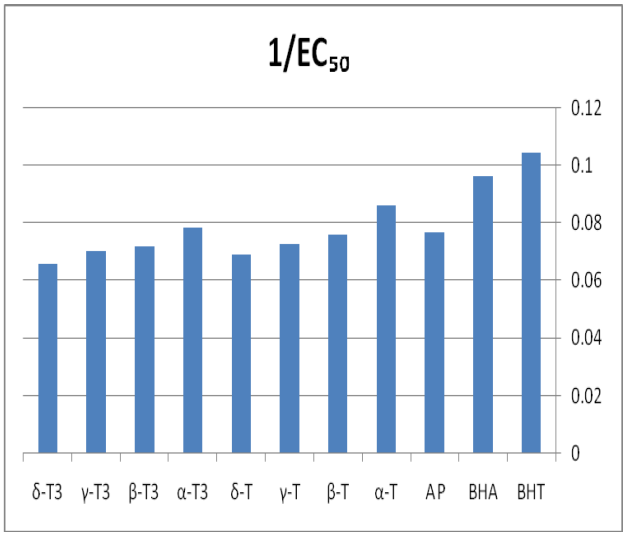

Fig. 4: It shows the free radical scavenging capacity of antioxidants in (µmol/L)

Following the previous steps, EC_{50} has been determined for the rest of the antioxidants, and Table 2, shows the equation of liner relationship between antioxidants concentration and the remaining percentage of DPPH.

The results, shows; that scavenging activity of BHT were the most and similar to that of BHA, and higher than that of α-T and AP, while the scavenging activity for the rest of antioxidants were decrease in the following order: BHT> BHA> α-T> AP> α-T3> β-T> γ-T> β-T3> γ-T3> δ-T> δ-T3. The Figure 4, shows the free radical scavenging capacity of antioxidants in (µmol/L).

Table 1: It shows results of remaining percentage of DPPH with BHT

Conc µmol/L	2.5	5	7.5	10	12.5	15
%DPPH • rem	86.097± 0.023	74.168± 0.016	59.72± 0.045	48.005± 0.018	35.01± 0.042	21.68± 0.039

Table 2: It shows the equation of liner relationship between antioxidants concentration and the remaining percentage of DPPH

Antioxidant	R^2	equation of linear relationship	EC_{50}
BHT	0.999	y = -5.19x + 99.59)	9.554
BHA	0.999	y = -4.814x + 99.88	10.361
AP	0.999	y = -3.877x + 100.3	12.973
α-T	0.999	y = -4.285x + 99.76	11.612
β-T	0.999	y = -3.834x + 100.4	13.145
γ-T	0.998	y = -3.633x + 100	13.762
δ-T	0.998	y = -3.493x + 100.4	14.428
α-T3	0.999	y = -3.950x + 100.2	12.708
β-T3	0.998	y = -3.620x + 100.4	13.922
γ-T3	0.997	y = -3.557x + 100.4	14.169
δ-T3	0.999	y = -3.347x + 100.2	14.998

The EC_{50} was differed for all studied antioxidant and ranged between 9.633 and 14.998, while ($1/EC_{50}$) was also differed and ranged between 0.1046 and 0.066 Table 3.

Table 3: It shows results of determination of the DPPH remaining percentage (%DPPH· rem)

Antioxidant	Concentration µml/L						EC_{50}	$1/EC_{50}$
	2.5	5	7.5	10	12.5	15		
BHT	86.097±0.023	74.168± 0.016	59.72± 0.045	48.005± 0.018	35.01± 0.042	21.68± 0.039	9.55	0.105
BHA	88.01± 0.017	75.51± 0.028	63.76± 0.036	51.15± 0.031	40.64± 0.018	27.36± 0.018	10.36	0.097
AP	90.301± 0.046	81.94± 0.048	71.28± 0.043	61.43± 0.049	51.85± 0.048	42.003± 0.042	12.97	0.077
α-T	89.07± 0.009	78.21± 0.035	67.11± 0.020	56.75± 0.050	46.97± 0.031	35.22± 0.036	11.61	0.086
β-T	90.59± 0.011	81.15± 0.050	73.105± 0.019	61.82± 0.032	52.29± 0.010	42.5± 0.012	13.14	0.076
γ-T	90.867± 0.032	81.63± 0.021	73.37± 0.025	64.023± 0.007	53.263± 0.008	46.153± 0.011	13.76	0.073
δ-T	91.52± 0.045	83.133± 0.022	75.173± 0.023	65.87± 0.015	55.48± 0.045	48.27± 0.029	14.42	0.069
α-T3	90.18± 0.051	80.33± 0.029	71.522± 0.010	60.76± 0.034	50.34± 0.016	40.902± 0.046	12.7	0.079
β-T3	91.22± 0.019	82.628± 0.041	74.29± 0.024	64.508± 0.026	53.681± 0.022	46.588± 0.033	13.92	0.072
γ-T3	91.4± 0.033	83.03± 0.036	74.82± 0.035	65.142± 0.040	54.14± 0.028	47.79± 0.015	14.16	0.071
δ-T3	91.79± 0.061	83.67± 0.040	75.53± 0.017	66.26± 0.012	58.42± 0.031	49.932± 0.022	14.99	0.066

CONCLUSION

It is obvious from the results that there were different between natural (tocochromanols) and synthetic one (BHT, BHA, AP) in antioxidant activity. There were similarities between BHT, and BHA as a scavenging activity of free radical. *α*-T, PA also showed similarities in results but less than the previous couple; whereas the rest of tocochromanols showed deviation in results. The time required completing the reaction between DPPH and antioxidant (tocochromanol, synthetic) was different for each antioxidant. Because that we suggest to make combination between these antioxidant and follows there synergistic effects.

REFERENCES

1. Bramley PM, Elmadfa I, Kafatos A, Kelly FJ, Manios Y, Roxborough HE, Schuch W, Sheehy PJA, Wagner KH. Vitamin E. J Sci Food Agric 2000; 80:913-938.
2. Panfili G, Manzi P, Pizzoferrato L. High performance liquid chromatographic method for the simultaneous determination of tocopherols, carotenes, and retinol and its geometric isomers in Italian cheeses. Analyst 1994; 119:1161-1165.
3. Manzi P, Panfili G, Pizzoferrato L. Normal and reversed phase HPLC for more complete evaluation of tocopherols, retinols, carotenes and sterols in dairy products. Chromatographia 1996; 43: 89-93.
4. Burton GW, Traber MG. Vitamin E: antioxidant activity, biokinetics, and bioavailability. Annu Rev Nutr 1990; 10:357-382.
5. Balz VM, Shulte E, Thier HP. Trennung von tocopherolen und tocotrienolen durch HPLC. Fat Sci Technol 1992; 94:209-213.
6. Qureshi AA, Mo H, Packer L, Peterson D. Isolation and identification of novel tocotrienols from rice bran with hypocolesterolemic, antioxidant, and antitumor properties. J Agric Food Chem 2000; 48:3130-3140.
7. Packer L. Nutrition and biochemistry of the lipophilic antioxidants; vitamin E and carotenoids. In Nutrition, Lipids, Health, and Disease; Ong A. S. H., Niki E., Packer L., Eds. American Oil Chemists' Society: Champaign, IL 1995; 8-35.
8. Qureshi AA, Burger WC, Peterson DM, Elson CE. The structure of an inhibitor of cholesterol biosynthesis isolated from barley. J Biol Chem 1986; 261:10544-10550.
9. Qureshi AA, Bradlow BA, Salser WA, Brace LD. Novel tocotrienols of rice bran modulate cardiovascular disease risk parameters of hypercholesterolemic humans. J Nutr Biochem 1997; 8:290-298.
10. Qureshi N, Qureshi AA. Tocotrienols, novel hypocolesterolemic agents with antioxidant properties. In Vitamin E in Health and Disease; Packer L, Fuchs J, Eds.; Dekker: New York 1993; 247-268.
11. He L, Mo H, Hadisusilo S, Qureshi AA, Elson CE. Isoprenoids suppress the growth of murine B16 melanoma in vitro and in vivo. J Nutr 1997; 127:668-674.
12. Hoffmann G. The chemistry of edible fats. In The chemistry and technology of edible oils and fats and their high fat products. London: Academic Press 1989; 1-28.
13. Yoshida H, Kajimoto G, Emura S. Antioxidant effects of α-tocopherols at different concentrations in oils during microwave heating. Journal of the American Oil Chemists Society 1993; 70:989-995.
14. Ramadan MF, Kroh LW, Morsel JT. Radical scavenging activity of black cumin (Nigella sativa L.), coriander (Coriandrum sativum L.), and niger (Guizotia abyssinica Cass.) crude seed oils and fractions. Journal of Agricultural and Food Chemistry 2003; 51:6961– 6969.
15. Kim JK, Noh JH, Lee S, Choi JS, Suh H, Chung HY, Song YO, Choi WC. The first total synthesis of 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether (TDB) and its antioxidant activity. Bull Korean Chem Soc 2002; 23(5):661-662.
16. Lebeau J, Furman C, Bernier JL, Duriez P, Teissier E, Cotelle N. Antioxidant properties of di-tert-butylhydroxylated flavonoids, Free Radic Biol Med 2000; 29(9):900-912.
17. Schwarz K, Bertelsen G, Nissen LR, Gardner PT, Heinonen MI, Hopia A, Huynh-Ba T, Lambelet P, McPhail D, Skibsted LH, Tijburg L. Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assays based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds. Eur Food Res Technol 2001; 212:319-328.
18. Bondet V, Brand-Williams W, Berset C. Kinetics and mechanisms of antioxidant activity using the DPPH• free radical method. Lebensmittel-Wissenschaft und - Technologie/Food Science and Technology 1997; 30: 609-615.
19. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und-Technologie/Food Science and Technology 1995; 28:25-30.
20. Lu Y, Foo LY. Antioxidant and radical scavenging activities of polyphenols from apple pomace. Food Chemistry 2000; 68:81-85.
21. Sańchez-Moreno C, Larrauri JA, Saura-Calixto F. Free radical scavenging capacity andinhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. Food Res Int 1999; 32:407-412.
22. Yepez B, Espinosa M, LOṕez S, Bolaños G. Producing antioxidant fractions from herbaceous matrices by supercritical fluid extraction. Fluid Phase Equil 2002; 194-197: 879-884.
23. Sańchez-Moreno C, Larrauri JA, Saura-Calixto F. New parameter for evaluation of free radical scavenging capacity of polyphenols. 2nd International Electronic Conference on Synthetic Organic Chemistry (ESCOC-2), http://www.ndpi.org/escoc/, September 15, 2005.
24. GOḿez-Alonso S, Fregapane G, Salvador MD, Gordon MH. Changes in phenolic composition and antioxidant activity of virgin olive oil during frying. J Agric Food Chem 2003; 51:667-672.
25. Zhu QY, Hackman RM, Ensunsa JL, Holt RR, Keen CL. Antioxidative activities of oolong tea. Food Chem 2002; 50: 6929-6934.
26. Leitão GG, Leitão SG, Vilegas W. Quick preparative separation of natural naphthoquinones with antioxidant activity by high-speed counter-current chromatography. Z Naturforsch 2002; 57c:1051-1055.
27. .Espıń JC, Soler-Rivas C, Wichers HJ. Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical. Journal of Agricultural and Food Chemistry 2000; 48: 648-656.