

Synergistic Effect of combined some natural and synthetic antioxidants to increase oxidative stability using DPPH test

Housam Haj Hamdo^{1*}, Warid Khayata¹, Zaid Al-Assaf²

¹Department of Analytical and Food Chemistry, Faculty of Pharmacy, Aleppo University, Aleppo, Syria.

²Department of Analytical and Food Chemistry, Faculty of Pharmacy, Damascus University, Damascus, Syria.

*Corres.author: housam.hamdo@gmail.com

Abstract: Binary combinations of natural antioxidants, named (Tocopherols: α -, β -, γ -, δ -T), and synthetic one named (BHA, BHT, AP) were prepared and their free radical scavenging activity were evaluated using DPPH test. Results indicate that the synergisms of the antioxidant pairs (synthetic: tocopherol) were different, with different synthetic antioxidants types. The combinations of ascorbyl palmitate showed the highest synergetic percentage, while combinations of tocopherols with BHA and BHT showed similar values, and these percentages were in low significant importance due to their low values. The (AP: δ -T) combination shows the highest percentage of synergism (21.66%) while (BHT: γ -T3) combination was the lowest (4.57%).

Keywords: synergistic, natural antioxidant, synthetic, free radical, scavenging activity, DPPH.

Introduction:

Active oxygen and, in particular, free radicals are considered to induce oxidative damage in bimolecular and to play an important role in aging, cardiovascular diseases, cancer, and inflammatory diseases^[1-3]. In addition, they are also well known to be major causes of material degradation and food deterioration.

Consequently, antioxidants are now known to be prospective protective or therapeutic agents. In the past few years, addition of synthetic antioxidants has begun to be restricted because of their health risks and toxicity. The importance of exploiting natural antioxidants from various sources and replacing synthetic antioxidants with natural ingredients has attracted increasing attention. At present, most of the natural antioxidants such as traditional nutrients, polyphenols, and flavonoids are obtained from plants.

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs. In both cases, there is a preference for antioxidants from natural rather than from synthetic sources^[4]. There is therefore a parallel increase in the use of methods for estimating the efficiency of antioxidants^[5-6].

Inhibition of oxidation through the use of antioxidants has been observed to increase the induction period (IP) of lipid to varying degrees^[7-9].

Cooperative effects (synergy) of antioxidants in fats and oils are documented in several studies^[10-15]. Miranova et al.^[10] reported that mixtures of α -T and myricetin produced a synergistic effect during the autoxidation of triglycerols of sunflower oil. Kinetic analysis demonstrated that α -T regenerates myricetin during autoxidation.

A study conducted by Becker et al.^[11] showed that binary combinations of four antioxidants (α -T, astaxanthin, quercetin and rutin) revealed factors that may affect the synergism and antagonism of antioxidant blends: structural organization of the lipid; solubility, polarity and the hydrophilic nature of the antioxidants. A transfer of hydrogen from BHT regenerated BHA resulting in higher antioxidant activity than the components used singly in soybean oil, lard and methyl oleate^[12]. Antioxidants (BHT, alkylated phenol/dithiophosphoric acid ester/diphenylamine and zinc diamyl dithiocarbamate) and anti-wear additives combinations were also reported to have synergistic effects in vegetable oil-based lubricants based on the FA profile (especially on the polyunsaturation) and the effectiveness of the inhibitors^[14,15].

Although a few studies^[7-9,16] reveal that antioxidants improve the oxidative stability of lipid, and reports have been made about the synergism of antioxidants in edible oils and fats^[10-13] and in lubricating oils^[14,15], the synergy of synthetic antioxidants in lipid has not been fully elucidated. The objectives of this paper is: to investigate the synergistic effects of synthetic antioxidants: BHA, BHT, AP when they are combined with natural one (tocopherols) in binary formulations.

1 Experiment

1.1. 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).

BHA, BHT, AP, α -T, β -T, γ -T and δ -T primary stock solutions (0.01 mol/L) were prepared, by dissolve 0.18, 0.22, 0.414, 0.43, 0.416, 0.416, 0.402, 0.424, 0.41, 0.41, 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. From the previous secondary stock solution was performed to get standards titrating at 2.5, 5, 7.5, 10, 12.5, 15 μ mol/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.

1.2. Materials

The Alpha Tocopherol [α -T], Beta Tocopherol [β -T], Gamma Tocopherol [γ -T] and Delta Tocopherol [δ -T] was obtain from (Chromadex[®], Irvine California); the 1,1-Diphenyl-2-picrylhydrazyl [DPPH], Butylated hydroxyanisole [BHA], 2,6-Di-tert-butyl-4-methylphenol [BHT] and Ascorbyle Palmitate [AP] from (Sigma-Aldrich, USA); the methanol p.a. from (Merck Chemicals, Germany).

1.3. Apparatus

Spectrophotometer UV-Vis (Thuramed T60, Germany), micropipette 100-1000 μ l (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).

1.4. Reaction time

In the original method a reaction time of 30 minutes was recommended, and this has been followed in more recent work^[17].

Shorter times have also been used, such as 5 minutes^[18], or 10 minutes^[6]. However, in view of the fact that the rate of reaction varies widely among substrates^[19,20], the best practice seems to be to follow the reaction until it has gone to completion ("plateau")^[21-23]. The rate of reaction has also been proposed as a further parameter to characterize the antioxidant activity^[22,24].

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

1.5. Absorbance measurements - wavelength and instrument used

The working wavelength of maximum absorbance, λ_{max} , to be used for the absorbance measurements is given variously as 515 nm^[18-20,22,25], 516 nm^[6], 517 nm^[21,26], 518 nm^[27], and 520 nm^[17]. 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.

It is general practice to use a spectrophotometer to determine the absorbance with DPPH solutions. Maximum absorbance of DPPH (200 micro mol/liter) recently prepared, has been determined. The solution has been studied under deferent wavelength (400 to 650 nm). λ_{max} turned out to be (517 nm) for DPPH.

1.6. DPPH radical–scavenging capacity

The antioxidant activity of different combinations were measured in term of hydrogen donating or radical scavenging capacity using the stable DPPH method according to the method proposed by Brand-Williams et al^[20]. (1 ml of DPPH solution (200 μ mol/L) to 3 ml of antioxidant solution found within appropriate dilution for getting diverse binary antioxidants survey, so that the total concentration of use was equivalent to (10 μ mol/L), and studied in pairs as follows:

(BHA: α -T), (BHA: β -T), (BHA: γ -T), (BHA: δ -T), (BHT: α -T), (BHT: β -T), (BHT: γ -T), (BHT: δ -T), (AP: α -T), (AP: β -T), (AP: γ -T), and (AP: δ -T).

The absorbance at 517 nm of the resulting solutions was measured. The antiradical activity was expressed in terms of the percentage reduction of the DPPH. The ability to scavenge the DPPH radical was calculated using the equation [1]:

$$\% \text{ Scavenging capacity} = [1 - A_F/A_0] \times 100 \text{ ----- Equation [1]}$$

Where A_0 is the absorbance of blank DPPH Solution, A_F is the absorbance of the solution after addition of antioxidant and access to the state of stability at 517 nm. All samples were analyzed in duplicates.

1.7. Synergistic Effect

To determine the existence of synergies between antioxidants, the existence of synergies between antioxidants in free radical scavenging capacity was determined for the preparation of bilateral combinations of different antioxidants.

The free radical scavenging capacity of combinations between antioxidants $SA_{(1,2)}$ and for each antioxidant $SA_{(1)}$ and $SA_{(2)}$ separately, were determined within the concentrations used in the combination within the same conditions, and the presence of synergy was assessed by the equation [2]:

$$S = SA_{(1,2)} - SA_{(1)} + SA_{(2)} \text{ ----- Equation [2]}$$

$S > 0$ this refers to the existence of synergies between antioxidants.

$S \leq 0$ this refers to the absence of synergy between antioxidants.

2 Results & Discussion

The effectiveness of eight natural antioxidants: The four known naturally occurring tocopherols termed α -, β -, γ - and δ -tocopherol and a synthetic antioxidant:[BHA], [BHT] and [AP] were studied by determined the free radical scavenging capacity.

The naturally occurring antioxidant, tocopherols, were found not to be an effective antioxidant, only slightly increasing the free radical scavenging capacity comparing with synthetic one. The study indicated that the synthetic antioxidants were more effective in increasing the free radical scavenging capacity and different combination resulted different synergism.

2.1. Combination between AP and Beta-Tocopherol (AP: β -T)

The free radical scavenging capacity were determined for binary $SA_{(AP: \beta-T)}$, and for each $SA_{(\beta-T)}$ and $SA_{(AP)}$ individually in the same concentration and within the same conditions. (Table 1) shows the difference between practical scavenging capacity of $SA_{(AP: \beta-T)}$ combination, and theoretical one resulting from the sum of the free radical scavenging capacity for each antioxidants mentioned $SA_{(\beta-T)} + SA_{(AP)}$. (Fig.1) curve showing the differences between actual free radical scavenging capacity of $SA_{(AP: \beta-T)}$ and the theoretical one of $SA_{(AP)} + SA_{(\beta-T)}$.

Table 1 the results of the partnership between AP and (β-T)

| (AP:β-T) μmol/L | SA _(β-T) | SA _(AP) | SA _(β-T) + SA _(AP) | SA _(β-T, PA) |
|-----------------|---------------------|--------------------|--|-------------------------|
| (0:10) | 38.18 | 0 | 38.18 | 38.18 |
| (1:9) | 34.614 | 3.92 | 38.534 | 42.24 |
| (2:8) | 30.44 | 7.88 | 38.32 | 42.454 |
| (3:7) | 26.632 | 11.61 | 38.242 | 43.752 |
| (4:6) | 23.09 | 15.69 | 38.78 | 44.3923 |
| (5:5) | 18.75 | 19.5 | 38.25 | 44.523 |
| (6:4) | 15.4 | 23.302 | 38.702 | 44.623 |
| (7:3) | 11.443 | 27.29 | 38.733 | 43.403 |
| (8:2) | 7.536 | 30.93 | 38.466 | 42.904 |
| (9:1) | 3.78 | 34.765 | 38.545 | 41.821 |
| (10:0) | 0 | 38.57 | 38.57 | 38.57 |

Results showed that there was a difference between the calculated values SA₍₁₎ + SA₍₂₎ and the practical one SA_(1,2) of free radical scavenging capacity, they were greater than zero (S>0) for all binary combinations with different values of synergism. The (Fig. 2) showed the difference between the practical values and theoretical one of free radical scavenging capacity of the combinations (BHA:α-T3), (AP:β-T). (Fig. 1) showing the differences between actual free radical scavenging capacity of SA_(AP:β-T) and the theoretical one of SA_(AP) + SA_(β-T).

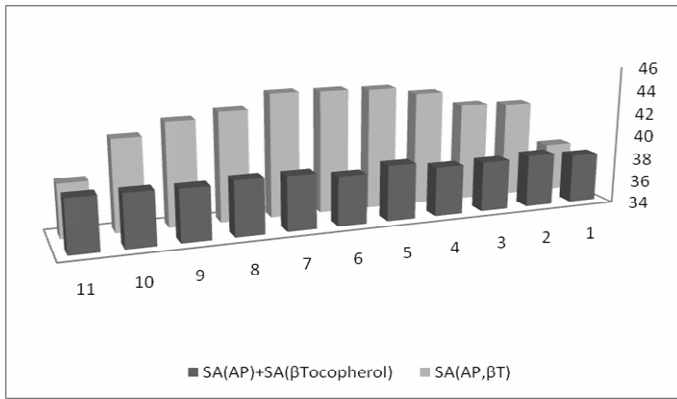


Fig. 2 comparison between theoretical and practical scavenging capacity of the combination between (AP) and (β-T)

Following the previous steps, the theoretical and the practical values calculated for all binary combinations (Table 2), shows the results of the combination between tocopherols and synthetic antioxidants.

Table 2 the results of the combinations between synthetic antioxidants and tocopherols

| Antioxidant | | | (X : Y) | | | | | | | | | | |
|-------------|-----|-------------|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| X | Y | | (10:0) | (9:1) | (8:2) | (7:3) | (6:4) | (5:5) | (4:6) | (3:7) | (2:8) | (1:9) | (0:10) |
| BHT | α-T | Theoretical | 51.95 | 51.15 | 50.81 | 50.01 | 48.77 | 47.71 | 46.85 | 45.84 | 44.99 | 44.63 | 43.25 |
| | | Practical | 51.95 | 52.79 | 52.5 | 52.24 | 51.23 | 50.56 | 49.26 | 48.01 | 46.65 | 46.05 | 43.25 |
| | β-T | Theoretical | 51.95 | 50.38 | 49.49 | 48.23 | 46.73 | 44.73 | 43.86 | 42.38 | 40.76 | 40 | 38.18 |
| | | Practical | 51.95 | 50.87 | 51.14 | 50.08 | 49.35 | 47.54 | 46.68 | 43.64 | 41.83 | 41.07 | 38.18 |
| | γ-T | Theoretical | 51.95 | 50.26 | 49.25 | 47.34 | 45.41 | 44.2 | 42.39 | 40.97 | 39.23 | 37.92 | 35.98 |
| | | Practical | 51.95 | 51.46 | 50.78 | 49.36 | 47.86 | 46.98 | 45.12 | 43.29 | 41.2 | 39.27 | 35.98 |
| | δ-T | Theoretical | 51.95 | 50.04 | 48.86 | 47.16 | 45.05 | 43.23 | 41.35 | 39.66 | 37.72 | 36.25 | 34.13 |
| | | Practical | 51.95 | 51.05 | 50.19 | 48.92 | 46.98 | 45.43 | 43.11 | 41.2 | 38.89 | 37.11 | 34.13 |
| BHA | α-T | Theoretical | 48.85 | 48.26 | 47.96 | 47.49 | 46.88 | 46.3 | 45.7 | 44.84 | 44.51 | 44.2 | 43.25 |
| | | Practical | 48.85 | 49.19 | 49.41 | 49.38 | 48.94 | 48.71 | 47.88 | 46.76 | 45.89 | 44.98 | 43.25 |
| | β-T | Theoretical | 48.85 | 47.49 | 46.64 | 45.72 | 44.84 | 43.32 | 42.71 | 41.38 | 40.28 | 39.56 | 38.18 |
| | | Practical | 48.85 | 48.12 | 47.65 | 47.31 | 46.63 | 45.3 | 44.11 | 42.7 | 41.3 | 40 | 38.18 |
| | γ-T | Theoretical | 48.85 | 47.37 | 46.4 | 44.82 | 43.52 | 42.79 | 41.24 | 39.97 | 38.75 | 37.49 | 35.98 |
| | | Practical | 48.85 | 48.33 | 47.71 | 46.68 | 45.61 | 44.74 | 42.99 | 41.59 | 40.18 | 38.18 | 35.98 |
| | δ-T | Theoretical | 48.85 | 47.15 | 46.01 | 44.64 | 43.16 | 41.82 | 40.2 | 38.66 | 37.24 | 35.82 | 34.13 |
| | | Practical | 48.85 | 47.93 | 47.17 | 46.39 | 45.1 | 43.95 | 41.75 | 40.4 | 38.41 | 36.4 | 34.13 |
| AP | α-T | Theoretical | 38.57 | 39.32 | 39.79 | 40.51 | 40.74 | 41.23 | 41.77 | 41.71 | 42.55 | 43.17 | 43.25 |
| | | Practical | 38.57 | 43.62 | 45.67 | 47.32 | 48.06 | 49.03 | 49.39 | 49.07 | 49.74 | 48.01 | 43.25 |
| | β-T | Theoretical | 38.57 | 38.55 | 38.47 | 38.73 | 38.7 | 38.25 | 38.78 | 38.24 | 38.32 | 38.53 | 38.18 |
| | | Practical | 38.57 | 41.82 | 42.9 | 43.4 | 44.62 | 44.52 | 44.39 | 43.75 | 42.45 | 42.24 | 38.18 |
| | γ-T | Theoretical | 38.57 | 38.43 | 38.23 | 37.84 | 37.38 | 37.72 | 37.31 | 36.83 | 36.79 | 36.46 | 35.98 |
| | | Practical | 38.57 | 42.95 | 43.14 | 43.19 | 43.35 | 44.13 | 43.69 | 42.71 | 42.12 | 41.17 | 35.98 |
| | δ-T | Theoretical | 38.57 | 38.21 | 37.84 | 37.66 | 37.02 | 36.75 | 36.27 | 35.52 | 35.28 | 34.79 | 34.13 |
| | | Practical | 38.57 | 44.48 | 44.47 | 44.77 | 44.57 | 44.71 | 43.92 | 42.93 | 42.37 | 41.35 | 34.13 |

In order to assess the fact that these differences statistically significant, the application of the (t-test paired) for comparison between practical scavenging capacity and the ability of theoretical scavenging capacity after merger process of the combination was applied. The $P_{\text{value}} < 0.05$ for all combinations was noted that there was substantial difference between the practical values and theoretical one of the merger and thus led to an increase in the scavenging capacity and there was synergistic partnerships between all different combinations and different concentrations have used.

To determine the importance of synergies resulting from the various combinations, the synergies percentages of antioxidants combinations were concluded, when using same concentrations for each antioxidant ($5\mu\text{mol/L}$) within binary combinations according to equation [3]:

$$\%S = [\text{SA}_{(1,2)} - (\text{SA}_{(1)} + \text{SA}_{(2)})] \times 100 / (\text{SA}_{(1)} + \text{SA}_{(2)}) \quad \text{equation [3]}$$

Where: %S: is the synergies percentages of antioxidants combinations, $\text{SA}_{(1,2)}$ is the practical free radical scavenging capacity of the blend, $\text{SA}_{(1)}$ and $\text{SA}_{(2)}$ is the free radical scavenging capacity of each antioxidant separately.

(Table 3) shows different synergies ratios of different combinations; also, Fig. 3 shows the difference between percentages of synergy for various combinations when used in equal concentrations.

Table 3 the percentage of synergies (S%) of various synergies combinations

| Participation of antioxidants | Synergies (S) | The percentage of synergies (S%) |
|-------------------------------|---------------|----------------------------------|
| (BHT, α -T) | 2.853 | 5.98 |
| (BHT, β -T) | 2.814 | 6.29 |
| (BHT, γ -T) | 2.781 | 6.29 |
| (BHT, δ -T) | 2.204 | 5.1 |
| (BHA, α -T) | 2.411 | 5.21 |
| (BHA, β -T) | 1.983 | 4.58 |
| (BHA, γ -T) | 1.955 | 4.57 |
| (BHA, δ -T) | 2.131 | 5.09 |
| (AP, α -T) | 7.799 | 18.91 |
| (AP, β -T) | 6.273 | 16.4 |
| (AP, γ -T) | 6.411 | 16.998 |
| (AP, δ -T) | 7.96 | 21.66 |

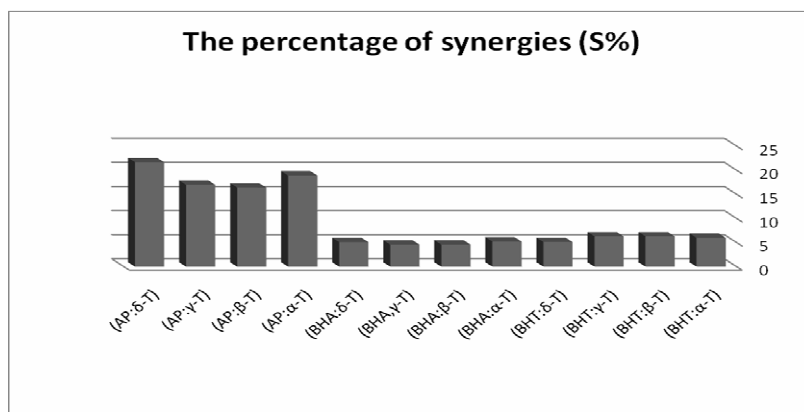


Fig. 3 shows the percentages of synergy for various combinations when used in equal concentrations.

3 Conclusion

The combinations of tocopherols with ascorbyl palmitate showed the highest synergetic percentage, and the best synergistic effect was observed with the (AP: δ -T) blend with all mixing proportions, suggesting a dependence on that ascorbyl palmitate donates hydrogen to regenerate tocopherols. While combinations with BHA and BHT showed similar values, and these percentages were in low significant importance due to their low values.

References

1. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing [J]. *J Nature*, 2000, 47: 239-408.
2. Bauerova K, Bezek A. Role of reactive oxygen and nitrogen species in etiopathogenesis of rheumatoid arthritis [J]. *Gen Physiol. Biophys*, 1999, 15-18.
3. Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause or consequence? [J]. *Lancet* 344, 1994. 721-4.
4. Abdalla AE, and Roozen JP, Effect of plant extracts on the oxidative stability of sunflower oil and emulsion [J]. *Food Chemistry*, 1999, 64: 323-329.
5. Sánchez-Moreno C. Review: methods used to evaluate the free radical scavenging activity in foods and biological systems [J]. *Food Sci. Tech. Int*, 2002, 8 (3): 121-137.
6. Schwarz K, Bertelsen G, Nissen LR, Gardner PT, Heinonen MI, Hopia A, Huynh-Ba T, Lambelet P, McPhail D, Skibsted LH, and 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 [J]. *Eur. Food Res. Technol*, 2001, 212: 319-328.
7. Domingos AK, Saad EB, Vechiatto WWD, Wilhelm HM, Ramos LP. The influence of BHA, BHT and TBHQ on the oxidation stability of soybean oil ethyl esters (biodiesel) [J]. *J Braz Chem Soc*, 2007, 18(2): 416-423.
8. Sendzikiene E, Makareviciene V, Janulis P. Oxidation stability of biodiesel fuel produced from fatty wastes [J]. *Pol J Environ Stud*, 2005, 14(3): 335-339.
9. Liang YC, May CY, Foon CS, Ngan MA, Hock CC, Basiron Y. The effect of natural and synthetic antioxidants on the oxidative stability of palm diesel [J]. *Fuel*, 2006, 85(5-6): 867-870.
10. Miranova E, Toneva A, Yanishlieva N. Synergistic antioxidant effect of α -tocopherol and myricetin on the autoxidation of triacylglycerols of sunflower oil [J]. *Food Chem*, 2008, 106: 628-633.
11. Becker E, Ntouma G, Skibsted L. Synergism and antagonism between quercetin and other chain-breaking antioxidants in lipid systems of increasing structural organization [J]. *Food Chem*, 2007, 103: 1288-1296.
12. Lundberg WO. *Autoxidation and Antioxidants*. vol 2. Wiley, New York, 1962.
13. Niki E, Saito T, Kawakami A, Kamiya Y. Inhibition of oxidation of methyl linoleate in solution by vitamin E and vitamin C [J]. *J Biol Chem*, 1984, 259: 4177-4182.
14. Sharma B, Perez J, Erhan S. Soybean oil-based lubricants: a search for synergistic antioxidants [J]. *Energy Fuels*, 2007, 21: 2408-2414.
15. Erhan S, Sharma B, Perez J. Oxidation and low-temperature stability of vegetable oil-based lubricants [J]. *Ind Crops Prod*, 2006, 24: 292-299.
16. Tang H, Wang A, Salley SO, Ng KYS. The effect of natural and synthetic antioxidants on the oxidative stability of biodiesel [J]. *J Am Oil Chem Soc*, 2008, 85(4): 373-382.
17. 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 [J]. *Bull. Korean Chem. Soc*, 2002, 23(5): 661-662.
18. Lebeau J, Furman C, Bernier JL, Duriez P, Teissier E, Cotellet N. Antioxidant properties of di-tert-butylhydroxylated flavonoids [J]. *Free Radic. Biol. Med*, 2000, 29(9): 900-912.
19. Bondet V, Brand-Williams W, Berset C. Kinetics and mechanisms of antioxidant activity using the DPPH• free radical method [J]. *Lebensmittel-Wissenschaft und -Technologie/Food Science and Technology*, 1997, 30: 609-615.
20. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity [J]. *Lebensmittel-Wissenschaft und-Technologie/Food Science and Technology*, 1995, 28: 25-30.
21. Lu Y, Foo LY. Antioxidant and radical scavenging activities of polyphenols from apple pomace [J]. *Food Chemistry*, 2000, 68: 81-85.
22. Sánchez-Moreno C, Larrauri JA, Saura-Calixto F. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents [J]. *Food Res. Int*, 1999, 32: 407-412.
23. Yopez B, Espinosa M, L Opez S, Bolaños G. Producing antioxidant fractions from herbaceous matrices by supercritical fluid extraction [J]. *Fluid Phase Equil*, 2002, 194-197: 879-884.

24. Sánchez-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.mdpi.org/escoc/>,September 1-30, 1998 [dp130]; http://ecsoc2.hcc.ru/DP_TOP1/dp130/dp130.htm., (1998).
25. Gómez-Alonso S, Fregapane G, Salvador MD, Gordon MH. Changes in phenolic composition and antioxidant activity of virgin olive oil during frying [J]. J. Agric. Food Chem, 2003, 51: 667-672.
26. Zhu QY, Hackman RM, Ensunsa JL, Holt RR, Keen CL. Antioxidative activities of oolong tea [J]. Food Chem, 2002, 50: 6929-6934.
27. Leitão GG, Leitão SG, Vilegas W. Quick preparative separation of natural naphthoquinones with antioxidant activity by high-speed counter-current chromatography [J]. Z.Naturforsch, 2002, 57c: 1051-1055.
