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Determination of the antioxidant activity based on the content changes in fatty acid methyl esters in vegetable oils

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Abstract: Free radicals, which are generated in several biochemical reactions in the body, have been implicated as mediators of many diseases, including cancer, atherosclerosis and heart diseases. Although the endogenous antioxidants can scavenge these free radicals, they are often insufficient to maintain the in vivo redox balance. The antioxidant activity (AOA) was examined by addition of each tested antioxidants [alpha-tocopherol (α-T), beta-tocopherol (β-T), gamma-tocopherol (γ-T), delta-tocopherol (δ-T), butylated hydroxyanisole (BHA), 2,6-di-*tert*-butyl-4-methylphenol (BHT), and ascorbyle palmitate (AP)] to four types of different vegetable oils (sunflower oil, soybean oil, corn oil and olive oil). Moreover, content changes in fatty acids were then investigated every 3 months during the storage period. The results showed that the AOA was different among the tested antioxidants. The AOA for BHA was the most for different types of oil compared with other antioxidants, whereas the δ-T possessed the lowest AOA.

Keywords: Antioxidant, Activity, Vegetable oil, FAMEs, Tocopherol

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1. Introduction

Antioxidants are widely used as ingredients in dietary supplements in order to maintain health and prevent diseases, such as cancer and coronary heart disease. In addition, these compounds have many industrial uses, such as preservatives in food and cosmetics. It has been known for many years that free radicals cause oxidation, which can be controlled or prevented by a range of antioxidant substances[1].

Lipid autoxidation reactions have been extensively investigated[2]. Since many vegetable oils and animal fats possess significant amounts of unsaturated fatty acids (UFA), oxidative stability is of concern, especially under long-term storage conditions at ambient temperatures, with exposure to air and/or light, and/or in the presence of some contaminants[3]. The main form of fatty acid methyl esters (FAMEs) in biodiesel is saturated C_{16} and saturated/unsaturated C_{18}. C_{18} contains one double-bond for oleic acid ($C_{18:1}$), two for linoleic acid ($C_{18:2}$) and three for linolenic acid ($C_{18:3}$).

Relative oxidation rates have been found to increase as the degree of saturation is increased[4]. The polyunsaturated fatty acid chains contain a higher total number of reactive bis-allylic sites compared with the monounsaturated ones, therefore, they are more prone to oxidation. In addition, dimerization and oligomerization can occur from peroxides, form from the reactions of radicals through oxidation and react with other fatty acids. Fang and McCormick[5] reported that the dimerization of peroxides is not the sole mechanism for molecular weight growth and deposit formation in lipids, but all possible mechanisms involve peroxide formation at the initiation of oxidation reaction. This emphasizes the importance of minimizing peroxide formation in lipid manufacturing and handling, leading to the need of antioxidants.

Inhibition of oxidation through antioxidants has been observed to increase the induction period (IP) of lipids to certain degrees[6–8]. Tang et al[9] studied the effectiveness of naturally occurring antioxidant α-tocopherol (α-T) and seven synthetic and one commercial antioxidants in short- and long-term storage. The naturally occurring antioxidant, α-T, has been found not to be an effective antioxidant, and only a slight increase in IP of lipids has been detected. This study indicated that the synthetic

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antioxidants are more effective in increasing the IP of lipids, and lipids from different feedstock have different IP improvements.

On the other hand, oxidation is one of the most common causes of flavor quality deterioration for oils and oil products. Deterioration occurs through rancidity resulting from oxidation, which takes place at the double-bond sites in the triacyleglycerol molecules. Oxidation causes great economic loses to the food industry[10]. Protection against the oxidation reaction is provided by the tocopherols, phenolic compounds and carotenoids present in the vegetable oils[11]. The addition of antioxidants is an efficient method to extend shelf life of oils and oils products. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have a restricted use in foods due to their carcinogenic properties[12]. Natural antioxidants are important for human health because they can reduce heart disease risks and posses anti-carcinogenic properties. In addition, they are also safer compared with synthetic antioxidants[13].

In the present work, we aimed to determine the content of FAMEs in vegetable oils with or without antioxidants, study the effect of antioxidant addition to the vegetable oils on the content of FAMEs and evaluate the antioxidant activity (AOA) for each antioxidant.

2. Methods

2.1. Materials

Antioxidants (α-, β-, γ-, and δ-T) were obtained from Chromadex® (Irvine, California, USA). BHA, BHT and AP were purchased from Sigma-Aldrich (USA). Fatty acid methyl esters (FAMEs), methyl Hexadecanoate ($C_{16:0}$), methyl Octadecanoate ($C_{18:0}$), methyl Oleate ($C_{18:1}$), methyl Linoleate ($C_{18:2}$) and methyl Linolenate ($C_{18:3}$), were supplied from Larodan (Larodan Fine Chemicals/ Malmö, Sweden). All other chemicals and reagents were provided by Sigma (St. Louis, MO, USA).

The samples of sunflower oil, corn oil and olive oil were collected from the local farming places in Syria. Soybean oil sample was purchased from a local factory. The saponification value, iodine value, relative density, melting point and peroxide value of the samples were calculated to assess their quality. Samples were tested during the first year of the collection and production. The oil samples were stored in glass containers at 20 ºC in nitrogen atmosphere.

2.2. Preparation of FAMEs

The samples of sunflower oil, corn oil and olive oil were collected from the essential local farming places in Syria (Hamah, Latakia, Aleppo). Soybean oil samples were purchased from a local factory (Homs, Alrqaa). The samples were tested during the first year of the collection and production. Subsequently, one sample from each type of oil was selected as identical to the standard specifications for the experiment. The oil samples were kept in glass containers at 20 ºC in nitrogen atmosphere.

The samples were prepared according to the recommended method of IUPAC (1987)[14] based on esterification by their interactions with potassium hydroxide solution. Moreover, the esterification was performed according the following steps. Briefly, 1 g of oil sample was placed in test tube, and 10 mL of hexane was then added into the tube. Subsequently, 0.5 mL of 2 N methanolic potassium hydroxide solution was also added into the tube, the cap of the tube was tightened, and the tube was vigorously shaken for 20 s. The mixture was stratified until the upper solution became clear. The upper layer containing the methyl esters was then collected.

2.3. Fatty acid analysis

Briefly, 2 μL FAMEs was analyzed with gas chromatograph (DANI MASTER GC HSS 86.50: DANI Instruments S.P.A. Italy) equipped with a flame ionization detector and a capillary column (model: CBP1-M100-025, 0.25 mm×100 m, Dani. Co., Italy). The oven temperature was set at 200 ºC. The temperatures of injector and detector were maintained at 290 ºC. The carrier gas was helium, the flow rate was 1 mL/min, and the split ratio was 1/6. FAME identification was determined based on the retention time compared with the standard FAME mixture. Results were expressed as percentage of the peak area without any corrections. Fatty acid analysis was performed in triplicate for each sample, and the average values were reported.

The FAME ratios for oil samples were determined using gas chromatography device. In order to determine

the quantitative decrease in UFA resulting from oxidation, area under the curve (*AUC*) of tested fatty acids was calculated based on the *AUC* of methyl hexadecanoate.

2.4. Determination of AOA

The AOA was examined by addition of an equal concentration (200 mg/kg) of each tested antioxidant (BHT, BHA, AP, *α*-T, *β*-T, *γ*-T, *δ*-T) to four different vegetable oil samples (sunflower oil, soybean oil, corn oil and olive oil). The samples were stored at 25 ºC for 9 months, and content changes in fatty acid as well as AOA were determined every 3 months. The AOA was determined from the equation as follows[15,16]:

$$AOA = \text{Non-Oxidized FAME}_{(AH)}/\text{Non-Oxidized FAME}_{(0)}$$

where Non-Oxidized $FAME_{(AH)}$ is the overall residual percentage of remaining fatty acids in the presence of antioxidant, and Non-Oxidized $FAME_{(0)}$ is the overall residual percentage of remaining fatty acids in the absence of antioxidant.

3. Results and discussion

3.1. Retention time

The retention time (R_t) of FAMEs was determined under the above-mentioned conditions. Table 1 indicates the R_t of the five FAMEs, which was determined using mixture of FAME standards. Figure 1 shows the chromatogram of the FAME standard mixture.

3.2. The content changes of the fatty acids

Oil samples were analyzed in the moment (0), and every 3 months over 9 months of storage period. Figure 2 shows the chromatogram of fatty acids in some studied samples, and Table 2 indicates the quantitative results of fatty acids according to different antioxidants.

The difference between the total remaining quantitative fatty acids, which were known as nonoxidized FAMEs, and the total initial FAMEs, which was assumed equivalent to 100, reflected the oxidized FAMEs. It is easier to determine the total loss of fatty acids due to oxidation as well as the individual loss of each UFA using this simple calculation[16].

Table 1. The average retention time of fatty acids in the standard mixture

The fatty acid	R_t (min)
$C_{16:0}$	18.035
$C_{18:2}$	27.527
$C_{18:1}$	27.830
$C_{18:3}$	28. 080
$C_{18:0}$	29.508

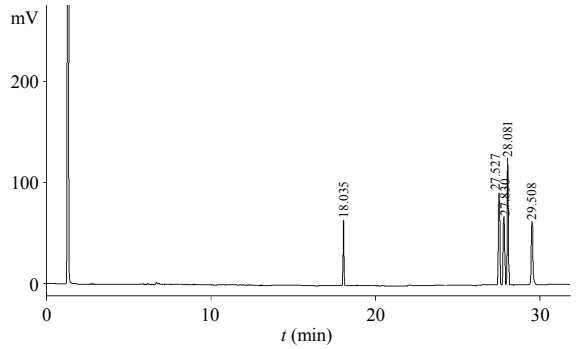

Figure 1. Chromatogram of FAME standard mixture.

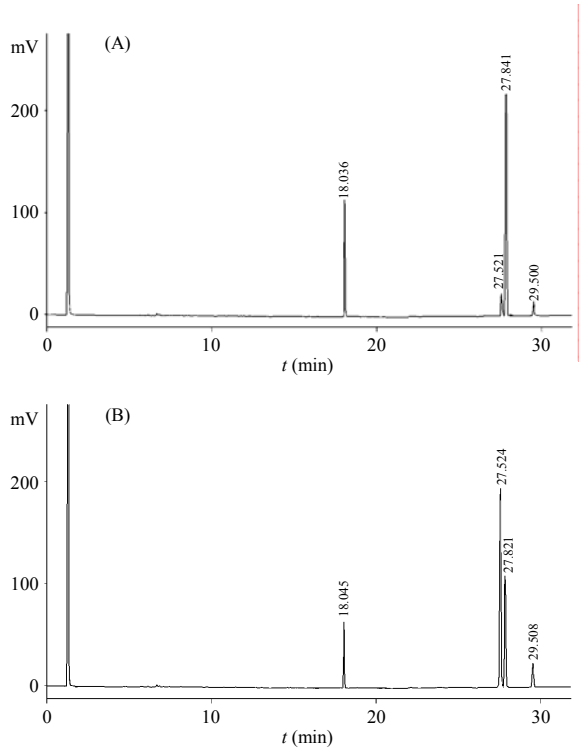

Figure 2. The chromatogram of olive oil and sunflower oil samples after 9 months of storage.

Results showed that the total content of UFA was decreased in blank samples. The overall percentage of oxidized fatty acids was (23.7%, 23.9%, 22.1% and 20.9%) for each oil sample (sunflower, soybean, corn, olive oil), respectively. This percentage of the samples supplied with antioxidants became less.

The AOA was determined based on the previous experimental data using the equation (1). Table 3 shows the results of AOA determination for different antioxidants in different oil samples.

Table 2. The content (%) changes of studied FAMEs in oil sample within storage period

Oil	Fatty acid	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Non-oxidized FAME			Oxidized FAME		
	Storage period (month)	9	9	9	9	9	3	6	9	3	6	9
Sunflower oil	Initial sample of sunflower	6.10	3.13	25.67	64.85	0.25		100			0	
	Blank sample of sunflower	6.10	3.13	17.51	49.60	0	92.00	84.49	76.34	8.00	15.50	23.70
	BHA	6.10	3.13	19.63	53.41	0.17	94.14	88.34	82.44	5.86	11.70	17.60
	BHT	6.10	3.13	19.31	53.07	0.06	93.93	87.88	81.67	6.07	12.10	18.30
	AP	6.10	3.13	19.02	52.85	0	93.66	87.80	81.10	6.34	12.20	18.90
	α Tocopherol	6.10	3.13	18.76	52.61	0	93.50	87.01	80.60	6.50	13.00	19.40
	β Tocopherol	6.10	3.13	18.52	52.20	0	93.33	86.63	79.95	6.67	13.40	20.10
	γ Tocopherol	6.10	3.13	18.33	51.76	0	93.07	86.37	79.32	6.93	13.60	20.70
	δ Tocopherol	6.10	3.13	17.97	50.76	0	92.71	85.49	77.96	7.29	14.50	22.00
Soybean oil	Initial sample of soybean	9.55	3.7	24.50	52.50	9.75		100			0	
	Blank sample of soybean	9.55	3.7	18.17	39.80	4.91	91.96	83.70	76.13	8.04	16.30	23.90
	BHA	9.55	3.7	19.39	42.70	6.78	94.08	88.08	82.12	5.92	11.90	17.90
	BHT	9.55	3.65	19.22	42.06	6.56	93.66	87.33	81.04	6.34	12.70	19.00
	AP	9.55	3.7	19.17	41.82	6.41	93.43	86.95	80.65	6.57	13.10	19.40
	α Tocopherol	9.55	3.7	19.12	41.37	6.29	93.31	86.62	80.03	6.69	13.40	20.00
	β Tocopherol	9.55	3.7	19.08	41.35	6.18	93.21	86.54	79.86	6.79	13.50	20.10
	γ Tocopherol	9.55	3.7	18.72	41.19	6.16	92.99	86.24	79.32	7.01	13.80	20.70
	δ Tocopherol	9.55	3.7	18.33	40.16	5.30	92.28	84.63	77.04	7.72	15.40	23.00
Corn oil	Initial sample of corn	14.25	1.65	27.10	57.00	0		100			0	
	Blank sample of corn	14.25	1.65	18.60	43.39	0	92.34	85.24	77.89	7.66	14.80	22.10
	BHA	14.25	1.65	20.31	46.81	0	94.31	88.70	83.02	5.69	11.30	17.00
	BHT	14.25	1.65	20.06	46.02	0	93.90	87.91	81.98	6.10	12.10	18.00
	AP	14.25	1.65	19.67	45.62	0	93.70	87.40	81.19	6.30	12.60	18.80
	α Tocopherol	14.25	1.65	19.55	45.36	0	93.68	87.16	80.81	6.32	12.80	19.20
	β Tocopherol	14.25	1.65	19.38	44.91	0	93.38	86.72	80.19	6.62	13.30	19.80
	γ Tocopherol	14.25	1.65	19.16	44.58	0	93.21	86.44	79.64	6.79	13.60	20.40
	δ Tocopherol	14.25	1.65	18.69	43.83	0	92.66	85.65	78.42	7.34	14.40	21.60
Olive oil	Initial sample of olive	13.10	2,53	73.75	9.66	0.96		100			0	
	Blank sample of olive	13.10	2.53	58.60	4.37	0.48	93.04	86.00	79.08	6.96	14.00	20.90
	BHA	13.10	2.53	62.04	6.25	0.73	94.88	89.87	84.65	5.12	10.10	15.40
	BHT	13.10	2.53	61.86	6.07	0.71	94.76	89.49	84.27	5.24	10.50	15.70
	AP	13.10	2.53	61.75	5.83	0.68	94.63	89.23	83.89	5.37	10.80	16.10
	α Tocopherol	13.10	2.53	61.24	5.77	0.66	94.46	88.88	83.30	5.54	11.10	16.70
	β Tocopherol	13.10	2.53	60.59	5.51	0.62	94.17	88.31	82.35	5.83	11.70	17.70
	γ Tocopherol	13.10	2.53	60.04	5.36	0.59	93.87	87.70	81.62	6.13	12.30	18.40
	δ Tocopherol	13.10	2.53	59.02	4.40	0.52	93.18	86.59	79.57	6.82	13.40	20.40

Table 3. The results of AOA determination for different antioxidants

Oil		Sun flower	Soybean	Corn	Olive
Antioxidant	BHA	1.0805	1.0791	1.0657	1.0702
	BHT	1.0704	1.0649	1.0524	1.0654
	AP	1.0629	1.0598	1.0422	1.0606
	α Tocopherol	1.0564	1.0516	1.0374	1.0531
	β Tocopherol	1.0478	1.0494	1.0294	1.0411
	γ Tocopherol	1.0396	1.0423	1.0223	1.0319
	δ Tocopherol	1.0218	1.0124	1.0067	1.0059

For comparison between natural and synthetic antioxidants in all types of oil, *t*-test was applied and Table 3 shows the results. The *P* value was less than 0.05, indicating that there was a statistically significant difference between natural and synthetic antioxidants in all types of oil.

The AOA values varied among different antioxidants. The AOA for BHA was the most compared with other antioxidants, and the *δ*-T had the lowest AOA, while the AOA for other antioxidants was in the following order: BHA, BHT, AP, *α*-T, *β*-T, *γ*-T, *δ*-T.

The AOA for each antioxidant varied among different oil types. The AOA for *β*-T and *γ*-T was the most in the corn oil compared with the rest types of oil, while it was the lowest for *δ*-T in sunflower oil.

4. Conclusions

The content changes of FAMEs in certain vegetable oils were related to the AOA of each studied antioxidant, which varied according to different antioxidants and different types of oil. This finding indicated that it is necessary to carefully study the chemical composition of the fatty acid content, especially the unsaturated one in oil. It might directly affect the AOA calculation of antioxidants.

References

[1] Bjelakovic, G.; Nikolova, D.; Gluud, L.L.; Simonetti, R.G.; Gluud, C. *JAMA*. **2007**, 842–857.

[2] Weisburger, J.H. *Am. J. Clini. Nutr*. **1991**, *53*, 226–237.

[3] Knothe, G. *Fuel Proc. Technol*. **2007**, *88*, 669–677.

[4] Cosgrove, J.P.; Church, D.F.; Pryor, W.A. *Lipids*. **1987**, *22*, 229–304.

[5] Fang, H.L.; McCormick, R.L. *SAE Technical Paper Series*. **2006**, 3301–3300.

[6] Domingos, A.K.; Saad, E.B.; Vechiatto, W.W.D.; Wilhelm, H.M.; Ramos, L.P. *J. Braz. Chem. Soc*. **2007**, *18*, 416–423.

[7] Sendzikiene, E.; Makareviciene, V.; Janulis, P. *Pol. J. Environ. Stud.* **2005**, *14*, 335–339.

[8] Liang, Y.C.; May, C.Y.; Foon, C.S.; Ngan, M.A.; Hock, C.C.; Basiron, Y. *Fuel*. **2006**, *85*, 867–870.

[9] Tang, H.; Wang, A.; Salley, S.O.; Ng, K.Y.S. *J. Am. Oil Chem. Soc*. **2008**, *85*, 373–382.

[10] Kim, H.J.; Hahm, T.S., Min, D.B. *J. Am. Oil Chem. Soc*. **2007**, *84*, 349–355.

[11] Blazĕvić, I.; Mastelić, J. *Food Chem*. **2009**, *113*, 96–102.

[12] Jayaprakasha, G.K.; Singh R.P.; Sakariah K.K. *Food Chem*. **2001**, *73*, 285–290.

[13] Emad, S.S. *LWT*. **2006**, *39*, 883–892.

[14] IUPAC. Standard Method 2.301, preparation of fatty acid methyl ester, in Standard methods for Analysis of Oils, Fats and Derivatives. 7th, 1987. Oxford, Blackwell.

[15] Marmesat, S.; Morales, A.; Velasco, J.; Ruiz-Mendez, M.V.; Dobarganes, M.C. *Grasasy Aceites*. **1999**, *60*, 155–160.

[16] Dobarganes, M.C.; Perez-Camino, M.C. *Rev. Franc. Corps Gras*. **1988**, *35*, 67–70.