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DETERMINATION OF THE ANTIOXIDANT ACTIVITY (AOA) BY STUDYING THE CHANGES IN CONTENT OF FAMES IN SOME 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 *in vivo* produced antioxidant compounds can scavenge these free radicals, the endogenous antioxidants are insufficient to completely remove them and maintain a balance. The antioxidant activity (AOA) was examined by addition an equal concentration (200 mg/Kg) of each studied antioxidants (BHT, BHA, AP, α-T, β-T, γ-T, δ-T) to four samples of different vegetable oils (sunflower oil, soybean oil, corn oil, olive oil), then studying changes in fatty acids content every three months along the storage's period. The results showed that there was difference in AOA depending on the used antioxidant. The AOA for BHA was the most for different oil types compared with other antioxidants, and the δ-T had the lower AOA.

Key words: Antioxidant activity, Vegetable oil, FAMEs, Tocopherol.

INTRODUCTION

Lipid autoxidation reactions have been investigated extensively[1-4]. Since many vegetable oils and animal fats possess significant amounts of unsaturated fatty acids (UFA), oxidative stability is of concern, especially under long periods in storage conditions above ambient temperatures, with exposure to air and/or light, and/or in the presence of some contaminants[5]. The main fatty acid methyl esters (FAMEs) in biodiesel are saturated C_{16}, and saturated and unsaturated C_{18}; C_{18} contain 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 were found to increase as the degree of saturation increased[6]. The polyunsaturated fatty acid chains contain a higher total number of reactive bis-allylic sites than the monounsaturated ones, and hence are more prone to oxidation. In addition, dimerization and oligomerization can occur from peroxides, formed from the reactions of radicals through oxidation, reacting with other fatty acids. Fang and McCormick[7] reported that dimerization of the peroxides is not the sole mechanism for molecular weight growth and formation of deposits in lipids, but all possible mechanisms involve peroxide formation at the initiation reaction of oxidation. This stresses the importance of minimizing peroxide formation in lipid manufacturing and handling, hence the need for antioxidants.

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Inhibition of oxidation through the use of antioxidants has been observed to increase the induction period (IP) of lipid to varying degrees[8-10]. Tang et al.[11] studied the effectiveness of naturally occurring antioxidant alpha tocopherol and seven synthetic and a commercial antioxidants in short- and long-term storage. The naturally occurring antioxidant, α-T, was found not to be an effective antioxidant, only slightly increasing the IP of the lipids. The study indicated that the synthetic antioxidants were more effective in increasing the IP of lipids, and lipids from different feedstock's showed different IP improvement.

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[12]. Protection against the oxidation reaction is provided by the tocopherols, phenolic compounds and carotenoids present in the vegetable oils[13]. The addition of antioxidants is method of increasing shelf life of oils and oils products. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have a restricted use in foods as they are suspected as being carcinogenic[14]. Natural antioxidants are important for human health because of decreasing heart disease risks and possessing anticarcinogenic properties and also they are safer than synthetic antioxidants[15].

The objectives of this paper are: (a) to determine the content of FAMEs in vegetable oils with and without antioxidant, to study the effect of addition antioxidant to the vegetable oil on the content of FAMEs; (b) to determine the AOA for each different antioxidant.

EXPERIMENTAL

Methods and 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 Butylated hydroxyanisole [BHA], 2,6-Di-tert-butyl-4-methylphenol [BHT], and Ascorbyle Palmitate [AP] obtained from Sigma-Aldrich, USA; A fatty acid methyl ester (FAME): Methyl Hexadecanoate [$C_{16:0}$], Methyl Octadecanoate [$C_{18:0}$], Methyl Oleate [$C_{18:1}$], Methyl Linoleate [$C_{18:2}$], Methyl Linolenate [$C_{18:3}$] was purchased from Larodan (Larodan Fine Chemicals/Malmö, Sweden). All the other chemicals and reagents for analysis were purchased from Sigma (St. Louis, MO).

Apparatus

Gas chromatography (DANI MASTER GC HSS 86.50: DANI Instruments S.P.A. Italy) equipped with a flame ionization detector, and capillary column (Model: CBP1-M100-025, 0.25 mm X 100 m; Dani. Co., Italy) No. 0305.102 072 was used, 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).

Sampling

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 local factory. The saponification value, iodine value, relative density, melting point, and peroxide value had been calculated for the samples to assess their quality; the samples had been tested during the first year of the collecting and production. The oil samples were kept in glass containers at 20^{o}C having nitrogen atmosphere.

Preparation of fatty acid methyl esters (FAMEs)

The sample were prepared according to the recommended method of IUPAC[16], which depending on esterification the glyceride by their interactions with potassium hydroxide solution, and the esterification was did according the following steps: weight 1 g of oil sample in test tube. Add to the sample 10 mL of hexane (high purity special for chromatographic analysis). Add 0.5 mL of 2 N methanolic potassium hydroxide solution, put on the cap, tighten the cap and shake vigorously for 20 seconds. Leave to stratify until the upper solution becomes clear. Decant the upper layer containing the methyl esters.

Fatty acid analysis

Two microliter of the FAME was analyzed with gas chromatograph equipped with a flame ionization detector, and A capillary column (Model: CBP1-M100-025, 0.25 mm X 100 m; Dani. Co., Italy) was used. The oven temperature: 200^{o}C. The injector and detector temperatures were each kept at 290^{o}C. The carrier gas helium, the flow rate 1 mL/min, and the split ratio was 1/6. FAME identification was based on the retention times as compared with those of 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 fatty acids methyl ester ratios for oil's samples were determined using gas chromatography device. Area under the carve (AUC) of examined fatty acids was calculated depending on the AUC of methyl hexadecanoate, to determine the quantitative decrease in unsaturated fatty acids resulting from oxidation.

The antioxidant activity

The antioxidant activity (AOA) was examined by addition an equal concentration (200 mg/Kg) of each studied antioxidants (BHT, BHA, AP, α-T, β-T, γ-T, δ-T) to four samples of different vegetable oils (sunflower oil, soybean oil, corn oil, olive oil), in addition to preparing a blank samples. The samples were stored at 25^{o}C for nine months for the propose of determining the antioxidant activity through studying changes in fatty acids content every three months along the storage's period, and the AOA was determined from the Equation (1)[17,18]:

$$AOA = \text{Non-Oxidized } FAME_{(AH)} / \text{Non-Oxidized } FAME_{(0)} \quad \ldots(1)$$

Where:

Non-oxidized $FAME_{(AH)}$: The overall residual percentage of remaining fatty acids in presence of antioxidant.

Non-oxidized $FAME_{(0)}$: The overall residual percentage of remaining fatty acids in absence of antioxidant.

RESULTS AND DISCUSSION

Retention time R_t

The retention time for FAME were determined within the conditions mentioned obviously which set on the GC device, the (Table 1) indicated to the retention time of the five FAMEs, which was determined by using FAME standards mixture, and (Figure 1) showed the chromatogram of the standards mixture of FAME.

Table 1: Retention time average of fatty acids in the standard mixture

Fatty acid	Retention time (R_t)
$C_{16:0}$	18.035
$C_{18:2}$	27.527
$C_{18:1}$	27.830
$C_{18:3}$	28. 080
$C_{18:0}$	29.508

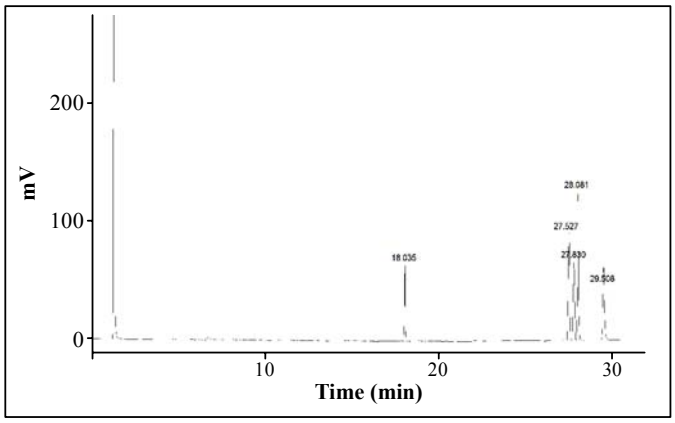

Fig. 1: Chromatogram of FAME standards mixture

The changes in content of the fatty acids

Oil samples were analyzed in the moment (0), and every three months over nine months of storage's period, the Figures (2, 3) showed the chromatogram of fatty acids in some studied samples, and the Table 2, showed the results of quantitative determination of fatty acid according to each different added antioxidant.

Table 2: Changes of the content 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	6.1	3.13	25.67	64.85	0.25		100			0	
	Blank sample of sunflower	6.1	3.13	17.51	49.6	0	92	84.49	76.34	8	15.5	23.7
	BHA	6.1	3.13	19.63	53.41	0.17	94.14	88.34	82.44	5.86	11.7	17.6
	BHT	6.1	3.13	19.31	53.07	0.06	93.93	87.88	81.67	6.07	12.1	18.3
	AP	6.1	3.13	19.02	52.85	0	93.66	87.8	81.1	6.34	12.2	18.9
	α Tocopherol	6.1	3.13	18.76	52.61	0	93.5	87.01	80.6	6.5	13	19.4
	β Tocopherol	6.1	3.13	18.52	52.2	0	93.33	86.63	79.95	6.67	13.4	20.1
	γ Tocopherol	6.1	3.13	18.33	51.76	0	93.07	86.37	79.32	6.93	13.6	20.7
	δ Tocopherol	6.1	3.13	17.97	50.76	0	92.71	85.49	77.96	7.29	14.5	22

Cont…

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
Soybean oil	Initial sample of soybean	9.55	3.7	24.5	52.5	9.75		100			0	
	Blank sample of soybean	9.55	3.7	18.17	39.8	4.91	91.96	83.7	76.13	8.04	16.3	23.9
	BHA	9.55	3.7	19.39	42.7	6.78	94.08	88.08	82.12	5.92	11.9	17.9
	BHT	9.55	3.65	19.22	42.06	6.56	93.66	87.33	81.04	6.34	12.7	19
	AP	9.55	3.7	19.17	41.82	6.41	93.43	86.95	80.65	6.57	13.1	19.4
	α Tocopherol	9.55	3.7	19.12	41.37	6.29	93.31	86.62	80.03	6.69	13.4	20
	β Tocopherol	9.55	3.7	19.08	41.35	6.18	93.21	86.54	79.86	6.79	13.5	20.1
	γ Tocopherol	9.55	3.7	18.72	41.19	6.16	92.99	86.24	79.32	7.01	13.8	20.7
	δ Tocopherol	9.55	3.7	18.33	40.16	5.3	92.28	84.63	77.04	7.72	15.4	23
Corn oil	Initial sample of corn	14.25	1.65	27.1	57	0		100			0	
	Blank sample of corn	14.25	1.65	18.6	43.39	0	92.34	85.24	77.89	7.66	14.8	22.1
	BHA	14.25	1.65	20.31	46.81	0	94.31	88.7	83.02	5.69	11.3	17
	BHT	14.25	1.65	20.06	46.02	0	93.9	87.91	81.98	6.1	12.1	18
	AP	14.25	1.65	19.67	45.62	0	93.7	87.4	81.19	6.3	12.6	18.8
	α Tocopherol	14.25	1.65	19.55	45.36	0	93.68	87.16	80.81	6.32	12.8	19.2
	β Tocopherol	14.25	1.65	19.38	44.91	0	93.38	86.72	80.19	6.62	13.3	19.8
	γ Tocopherol	14.25	1.65	19.16	44.58	0	93.21	86.44	79.64	6.79	13.6	20.4
	δ Tocopherol	14.25	1.65	18.69	43.83	0	92.66	85.65	78.42	7.34	14.4	21.6
Olive oil	Initial sample of olive	13.1	2,53	73.75	9.66	0.96		100			0	
	Blank sample of olive	13.1	2.53	58.6	4.37	0.48	93.04	86	79.08	6.96	14	20.9
	BHA	13.1	2.53	62.04	6.25	0.73	94.88	89.87	84.65	5.12	10.1	15.4
	BHT	13.1	2.53	61.86	6.07	0.71	94.76	89.49	84.27	5.24	10.5	15.7
	AP	13.1	2.53	61.75	5.83	0.68	94.63	89.23	83.89	5.37	10.8	16.1
	α Tocopherol	13.1	2.53	61.24	5.77	0.66	94.46	88.88	83.3	5.54	11.1	16.7
	β Tocopherol	13.1	2.53	60.59	5.51	0.62	94.17	88.31	82.35	5.83	11.7	17.7
	γ Tocopherol	13.1	2.53	60.04	5.36	0.59	93.87	87.7	81.62	6.13	12.3	18.4
	δ Tocopherol	13.1	2.53	59.02	4.4	0.52	93.18	86.59	79.57	6.82	13.4	20.4

The difference between the total remaining quantitative fatty acids, which was known as nonoxidized FAME and the total initial fatty acids methyl ester, which was assumed to be equivalent to 100, indicated to the oxidized fatty acids methyl ester. This simple calculation made it easier to determine the total loss of fatty acids during oxidation, also for individual loss of each unsaturated fatty acids[18].

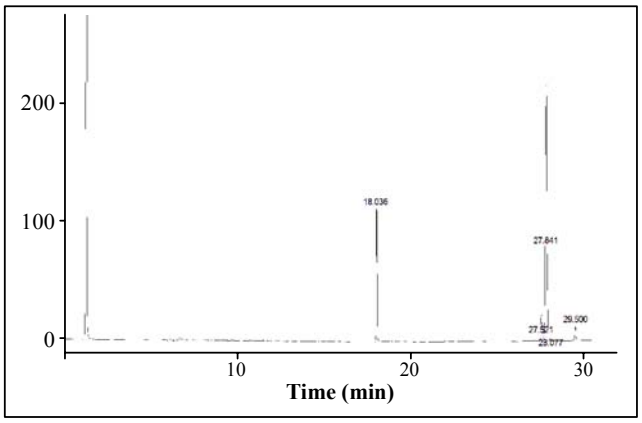

Fig. 2: The chromatogram of olive oil sample after 9 months of storage

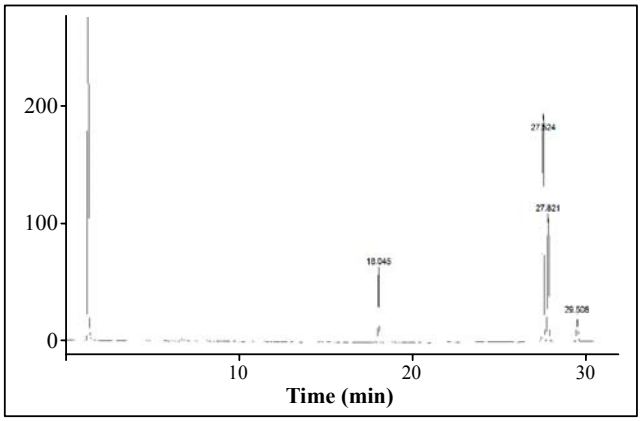

Fig. 3: The chromatogram of sunflower oil sample after 9 months of storage

Results showed that there were decreases in the total content of unsaturated fatty acids in blank samples, thru storage period at 25°C. Where the overall percentage of oxidized fatty acids was (23.7, 23.9, 22.1, 20.9) for each oil (sunflower, soybean, corn, olive) in order, and the percentage was less comparing to the rest of the samples supplied with antioxidants varied according to each different added antioxidants Table 2.

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

Table 3: Results for determination of AOA for different antioxidant

		AOA			
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

The AOA was represented for different antioxidants Figure 4, which showed that the AOA for BHA was the most for different oils compared with other antioxidants, and the δ-T had the lower AOA, while the rest antioxidants were in the following order: (BHA, BHT, AP, α-T, β-T, γ-T, δ-T).

The AOA for each antioxidant was differed in different oil types of oil, the AOA for β-T, γ-T was the most in the corn oil compared with the rest different oils types, while it was the lower for δ-T in sunflower oil.

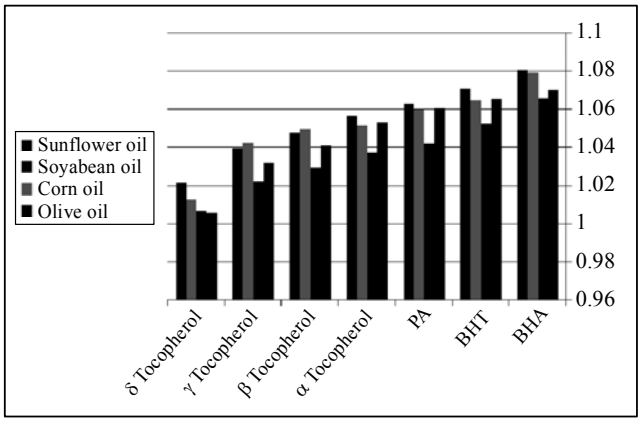

Fig. 4: Values of antioxidant activity for studied antioxidants

CONCLUSION

The changes in content of FAMEs in some vegetable oils were related to the AOA for each studied antioxidant, which differed according to different antioxidant, and different types of oil. This indicated the need for carefully studying the chemical composition of the fatty acid content, epically the unsaturated one in oil. It directly affects the AOA of the antioxidants.

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