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Utilization of Natural Antioxidants Extracted from by-products Using New Methods to Enhance the Oxidative Stability of Cooking Oils

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WITH the concern of adverse effects of lipid oxidation on food deterioration resulting off-flavor which led to lowering nutrition value. This study is chiefly concerned to improve the stability of cooking oils via addition of different concentrations of plant extracts. New assisted extraction methods (ultrasonic and microwave) with eco-friendly solvents (water, ethyl lactate, ethanol) were used to obtained natural components having antioxidative potency from tomato and grape by-products. The changes in oil oxidation stability in individual and treated oils were determined using the oven test at 60 °C. Moreover, the established analytical methods such as peroxide value, inhibition of oil oxidation, *p*-anisidine value and total oxidation value were used to evaluate the stability of treated oils. In addition, radical scavenging activity by DPPH• assay was used to evaluate the antioxidant activity. From the results, it was found that the addition of extracted natural antioxidants (from tomato &grape by-products) improved the oil samples stability. The effect of addition 200, 400 and 600 ppm of tomato extracts and 600 ppm of grape extract to soybean and sunflower oils mixture was found to be superior to the synthetic antioxidant. These results suggest that tomato and grape extracts from by-products are safe and vital sources of natural antioxidants.

Keywords: Natural antioxidants, Tomato and grape by-products, Microwave and ultrasonic extraction, oxidation stability.

Introduction

Lipid oxidation is one of the culprits of deterioration in fats and oils which affect the nutritional quality of the products leading to harmful human health (Womeni et al., 2016 and Sohaib et al., 2017). Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been widely used as food additives to increase the shelf life and to overcome the stability problems of oils and fats. But, recent reports revealed that these synthetic compounds may possess potential health risks corresponding to carcinogenesis and are negatively perceived by consumers (Womeni et al., 2016). Therefore, the synthetic antioxidants are not allowed for food application in many countries and have also been removed from the generally recognized as safe (GRAS) list of compounds. Concerning the food safety, the addition of natural antioxidants is needed to protect human body and preserve food products (Sohaib et al., 2017 and Rozman & Jersek, 2009) .

In Egypt, the food industries generate surpluses amounts of wastes or by-products annually. These wastes are an excellent source of nutraceutical, bioactive, inherently functional and possess many components that are good for human health. Therefore, the recovery of by-products is benefits for human health and economically (Helkar et al., 2016; Hassanien et al., 2014 and Abdel-Razek et al., 2016).

Natural components extracted from by-products can be applied in the food industry as safe, cheap and potent antioxidants with high nutritional value. In the present study, new assisted extraction methods (ultrasonic and microwave) with eco-friendly solvents (water, ethyl lactate, ethanol) were used to obtained natural components having antioxidative potency from tomato and grape by-products (Khaw et al.,2017 and El-Malah et al.,2015). Grape and tomato by-products produced in huge amounts contain a variety of antioxidants that have antioxidation merits. Grapes (*Vitis vinifera*) are a rich source of natural compounds (polyphenols). It mainly includes anthocyanins, flavonols, stilbenes and phenolic acids. Flavonoids and other plant

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phenolics reported to have antioxidant activity and antimicrobial activity (Al-Amrousi; 2016 and Klunklin & Savage, 2017). Tomato (*Solanum lycopersicum*) and tomato based products contain phytochemicals such as lycopene, folate, vitamin C, phenolics and flavonoids which are having health beneficial effect. Lycopene is the major carotenoid present in tomato and a highly potent antioxidant (El-Malah et al., 2015 and Agarwal & Rao, 2000). By virtue of accumulation of large amounts of by-products of grape and tomato from food industries, it was interesting to utilize these by-products in producing natural antioxidant components. The plant extracts were analyzed by HPLC in our previous work to show their main antioxidant components (Al-Amrousi, 2016). In addition the antioxidant activity was also evaluated by DPPH• scavenging and β-carotene-linoleic acid oxidation method (El-Malah et al., 2015). Soybean and sunflower oils are used widely as an essential fatty acid sources in nutrition (Poiana, 2012). The main deterioration process in these oils is the lipid oxidation of polyunsaturated molecules and generates toxic compounds causing off-flavor and color deterioration (Yanishlieva et al., 2006). Thus, the aim of this study was to evaluate protection efficiency of the added natural antioxidants to some cooking oils against oxidation.

Materials and Methods

Materials

Tomato and grape pomace were kindly supplied from Kaha Factory for Preserved Foods, Egypt and Ginaclis Factory, Alexandria, Egypt, respectively. Soybean and sunflower oils were provided by Cairo for Oil and Soap Company, Cairo, Egypt. All solvents are analytical grade, were purchased from Elnasr Pharmacutical Chemicals Co. (ADWIC), cairo, Egypt.

Methods

By-product treatment

Tomato and grape by products from food industry were subsequently air dried then homogenized in a domestic blender and ultimately ground in a laboratory mill (Janke & kunkel, IKA-labortechnik) and kept at 4ºC until needed (Strati and Oreopoulou, 2011).

Tomato water extract (TWE) was extracted using microwave assisted extraction method (MAE) according to El-Mallah et al., 2015 and Zheng et al., 2011.

TABLE 1. The levels of different types of the natural antioxidants added to the cooking oils.

Oil sample	TWE (ppm)	TEE (ppm)	GEE (ppm)
SFO	200	200	200
	400	400	400
	600	600	600
SBO	200	200	200
	400	400	400
	600	600	600
SFO:SBO (50:50 %)	200	200	200
	400	400	400
	600	600	600

While, tomato ethyl lactate extract (TEE) and grape ethanol extract (GEE) were obtained by ultrasound assisted extraction method (UAE) according to El-Mallah et al. (2015) and Navarro-Gonzalez et al. (2011). Ethanol and ethyl lactate were evaporated under vacuum. However, water was evaporated by freeze drying method.

Preparation of oil samples

200, 400, 600 ppm of each natural extract and 200 ppm of Butylated Hydroxytoluene (BHT) were dissolved in few drops of distilled water and Tween 20. The dissolved natural antioxidants then added to different oil samples of soybean oil (SBO), sunflower oil (SFO) and their admixture (50:50, wt:wt) and vortexed for 1 min.

Measuring antioxidant activity using DPPH• assay

DPPH• assay was used to measure the antioxidant activity of treated oils. Toluene solution of DPPH• was prepared freshly at concentration of 10^{-4} M. Different amounts of oil was weighted in test tubes (10, 20, 30, 40 mg) and completed to 4 ml by toluenic DPPH• solution and vortexed for 20 seconds. The decrease in absorption was measured at 515 nm after 30 min using blank of toluene and DPPH• solution as control. The radicals scavenging activity (R.S.A %) was calculated from the equation:

$$R.S.A\ \% = [(A\ control - A\ sample) / A\ sample] \times 100$$

The concentration was plotted against the R.S.A. % on excel program and the relation line was drown. Then, the resulting equation was used to calculate the EC_{50} (concentration of sample that

can scavenge 50 % of DPPH• radicals (Ramadan, 2013).

Evaluation of the thermal oxidative stability of the treated oil (oven test)

The treated and untreated oil samples were kept in an oven at 60 °C for 4, 8, 12 and 16 days. Then oil samples were removed from the oven every 4 days to carry out the oxidative stability tests (Spigno & DeFaveri, 2007 and Pimpa et al., 2009). Primary oxidation was measured by PV and secondary oxidation was measured by *p*-AV.

Determination of peroxide value (PV):"Primary Oxidation"

PV of oil samples was determined according to AOCS (1996). About 2 g of oil sample was weighed in 150 ml Erlenmeyer flask. The sample was dissolved in 25 ml (1:1.5) chloroform: glacial acetic acid. Saturated KI solution (0.5 ml) was added and shacked for 30 second. 30 ml of distilled water was added followed by 2 ml of starch solution before titration with (0.01 N) $Na_2S_2O_3$ until the blue color disappeared (AOCS., 1996).

The peroxide value was calculated from the equation:

PV = [ml of $Na_2S_2O_3$ × (0.01) N of $Na_2S_2O_3$ × 1000] / weight of the sample. PV was expressed as meq/Kg oil.

Inhibition of oil oxidation (IO %)

The percentage of inhibition of lipid oxidation of oil was calculated from the equation (Poiana, 2012):

IO % = [1- (PV increase of sample / PV increase of control)] × 100

Determination of p-anisidine value (p-AV): "Secondary Oxidation"

p-AV assay is based on the reaction between the amino group of *p*-anisidine and the carbonyl group of aldehydes or ketones to form Schiff base that absorbs at 350 nm (Poiana, 2012).

About 0.5 g oil was weighed in 25 ml measuring flask and dissolved in 25 ml *n*-Hexane. The absorbance (A_1) was measured at 350 nm against *n*-hexane as a blank. 5 ml of sample solution was transferred to 100 ml stoppered test tube and 1 ml *p*-anisidine solution was added to it and to the blank (*n*-hexane). After 10 min the absorbance (A_2) was measured at 350 nm against the blank. *p*-anisidine value was calculated from equation:

$$p\text{-AV} = 25\times(1.2A_2\text{-}A_1)/\text{weight of the sample}$$

Where A_1 = The absorbance before addition of *p*-anisidine, A_2 = The absorbance after addition of *p*-anisidine.

Total oxidation value (TOTOX value)

TOTOX value is the sum of both PV and *p*-AV which used to estimate the oxidative deterioration of oil (Poiana, 2012). It was calculated according to the equation TOTOX value = *p*-AV + 2 PV

Statistical analysis

Results are presented as the Mean ± Standard deviation from three replicates of each experiment. A p-value <0.05 was used to denote significant differences between mean values determined by the Analysis of Variance (ANOVA) with the assistance of Statistica 7.0 (Stat Soft Inc., Tulsa, OK) software

Results and Discussion

The effectiveness of different concentrations of tomato and grape by-products extracts (natural antioxidants), on retarding the lipid oxidation of SBO, SFO and their mixture was investigated in comparison with butylated hydroxytoluene (BHT) as a synthetic antioxidant.

In our previous work HPLC analysis of TWE, TEE and GEE antioxidants (Table 2) showed that phenolics were the predominant components in both water and ethanol extracts (hydrophilic solvent) whereas, carotenoids were the predominant components in ethyl lactate (hydrophilic and lipophilic solvent) (El-Malah et al., 2015 and Al-Amrousi, 2016).

The treated oils were found to have higher R.S.A % than that of the control samples which indicate the improvement of antioxidant activity of oils as a result of addition of the by-product extracts (Fig. 1).

EC_{50} (the concentration of antioxidant that reduces the DPPH• absorbance by 50%) of oil samples in Table 3 showed that control samples had the highest EC_{50} (60.3, 45.4 53.4 for SBO, SFO and SBO:SFO respectively), while addition of 200 ppm of BHT to oil samples gave lowest EC_{50} (36.7, 29.6, 33.4 for SBO, SFO and SBO:SFO respectively) and higher activity (Govindan and Muthukrishnan, 2013). Although the oil samples treated with synthetic antioxidant (200ppm BHT) gave the highest R.S.A % and the lowest EC_{50}, the

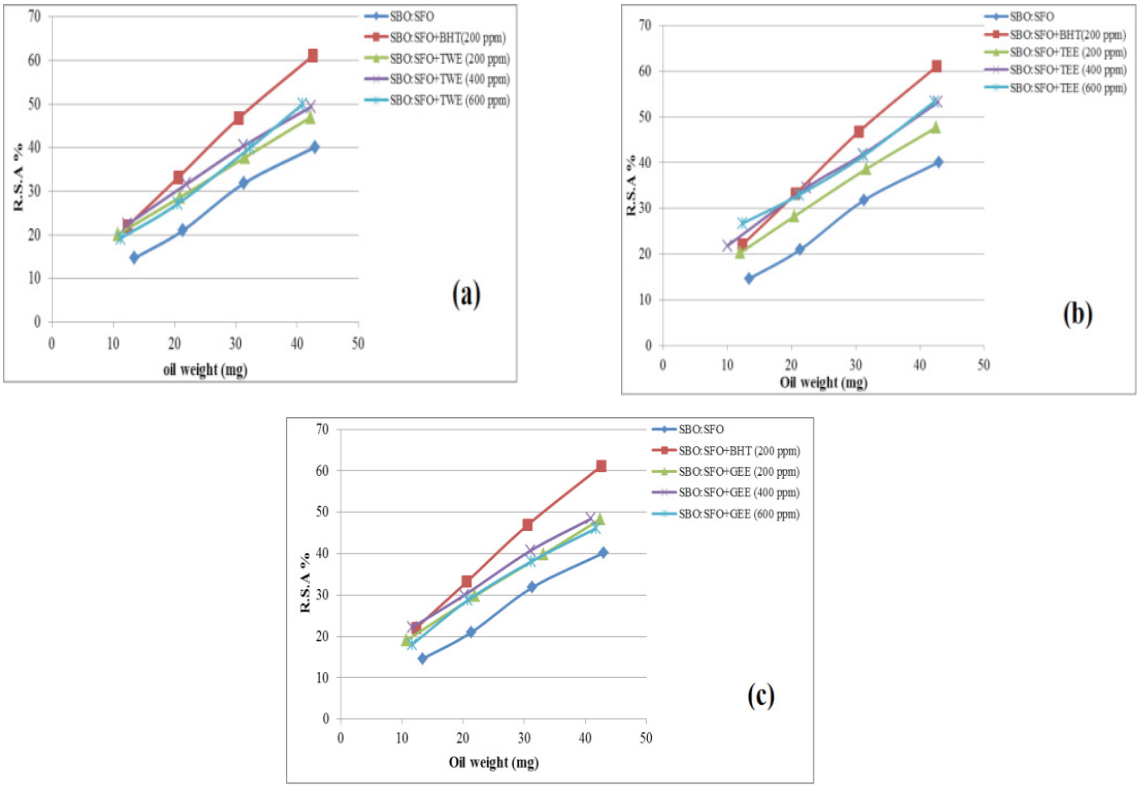

Fig . 1. Effect of addition of different natural extracts (a, b and c for TWE, TEE and GEE respectively) in comparison with BHT on R.S.A % of SBO:SFO mixture.

TABLE 2 . Representative HPLC of tomato and grape by-product extracts.

Peak NO	Antioxidant compound	Retention time (min)	TWE	TEE	Peak NO	Antioxidant component	Retention time (min)	GEE
1	Gallic acid	2.72	1.30	Nd	**1**	Gallic acid	2.72	4.50
2	Catechin	3.23	3.12	Nd	**2**	Catechin	3.23	12.04
3	Epicatechin	4.01	2.60	0.37	**3**	Epicatechin	4.01	4.50
4	Epichatechingallate	4.77	0.52	0.74	**4**	Epichatechingallat	4.77	7.50
5	Dihydro-querecetin	6.40	3.38	2.96	**5**	Rutin	9.87	1.50
6	Lutein	6.66	6.76	9.99	**6**	Resveratrol	10.35	0.90
7	Lycopene	9.30	6.50	18.50	**7**	Quercetin	10.82	15.60
8	Lycopene isomers	9.99	2.60	7.03	**8**	Kaempferol	11.42	1.50
9	Kaempferol	11.15	0.52	1.85	**-**	Total	-	48.04
10	β-carotene	11.82	3.12	10.36	**-**	Others	-	51.96
-	Total carotenoids	-	18.98	45.88				
	Total phenoplics	-	11.52	5.92				
-	Others	-	69.50	48.20				

by-product natural extracts still favorite to be used as it's safe and low cost than the synthetic one.

Data recorded in Table 4 express the effect of adding natural antioxidant extracts TWE, TEE and GEE on the PV change during heating of SBO, SFO and their mixture at 60 °C in thermal oven for 4, 8, 12 and 16 days. The lower the change in PV the higher the stability of the oil. Maximum PV was given after 16 days heating time. PV change of treated oils was found to be lower than that of control sample (SBO, SFO and their mixture). In case of treated SBO and SFO with natural plant extracts, PV changes are still higher than the oil treated with 200 ppm BHT. Meanwhile, addition of antioxidant extracts (TWE, TEE) at different concentrations and 600 ppm GEE to oil mixture (SBO: SFO), decreased PV change than that of both control and oil treated with BHT. It was noted that addition of TWE gave gave the higher oxidative stability followed by TEE then GEE in most cases.

The IO % of SBO samples were recorded in Table 5. The higher IO % values were found to be after 12 days heating time. These values were 40.34 % for 200 ppm of BHT; 31.6, 32.0 and 32.6 % for TWE; 30.5, 32.5 and 32.2 % for TEE and 12.7, 23.5 and 30.3 % for GEE at 200, 400, 600 ppm respectively. Concerning SFO, the higher IO% values were found to be after 12 days heating treatment the IO% were amounted to 34.1 % for 200 ppm BHT; 7.6, 21.0 and 25.7 % for TWE; 12.4, 15.6 and 17.8 % for TEE at 200, 400 and 600 ppm, respectively. It was noted that the SFO treated with 200 ppm BHT decreased gradually with prolonged heating treatment (36.8, 32.7, 34.1 and 24.9 at 4, 8, 12 and 16 heating days respectively). It was also observed that as the concentration of the used antioxidant extract increased the IO % increased and the oxidative stability of SFO also increased. The synthetic antioxidant was found to have the highest IO% values followed by TWE, TEE and GEE respectively in case of SBO and SFO. It was noted that 200ppm GEE gave negative results.

In case of SBO:SFO admixture TWE was found to have the highest IO% values followed by TEE, BHT and GEE respectively.

Generally, thermal treatment of oil samples leads to remarkable increase in PV due to primary oxidation but this effect was significantly reduced when natural antioxidant extracts are added at different concentrations to SBO, SFO and their mixture and improved the oxidation stability of investigated oil samples.

TABLE 3. EC_{50} treated and untreated of SBO and SFO and their mixture samples with different concentrations of TWE, TEE or GEE and 200 ppm BHT.

Antioxidant	EC_{50} of investigated oil samples								
	SBO			SFO			SBO:SFO (50:50 %)		
	Concentration of added antioxidant								
	200 ppm	400 ppm	600 ppm	200 ppm	400 ppm	600 ppm	200 ppm	400 ppm	600 ppm
BHT	36.7 ±0.55	---	---	29.6 ±0.41	---	---	33.4 ±0.30	---	---
TWE	56.5 ±0.60	54.2 ±0.45	54.5 ±0.60	37.7 ±0.45	37.5 ±40	37.7 ±0.36	45.8	42.5 ±0.40	41.4 ±0.45
TEE	56.8 ±0.61	49.7 ±0.50	48.4 ±0.60	38.1 ±0.37	31.6 ±45	31.2 ±0.30	44.6 ±0.45	39.3 ±0.39	39.4 ±0.35
GEE	58.6 ±0.56	55.7 ±0.60	53.9 ±0.56	37.8 ±0.52	35.5 ±35	38.4 ±0.40	44.1 ±0.40	42.1 ±0.35	44.9 ±0.36

SFO=sunflower oil, SBO= soybean oil, BHT=butylated hydroxytoluene, TWE= tomato water extract, TEE= tomato ethyl lactate extract, GEE= grape ethanol extract. EC_{50} for SBO, SFO and SBO:SFO were 60.3 ±0.65, 45.4 ±0.50 and 53.4 ±0.45 respectively

TABLE 4. Effect of addition of natural antioxidant extracts compared with BHT to SBO, SFO and their mixture on the PV after heating in oven test

Time (day)	a- PV(meq/kg oil) of SBO samples										
	control	Oil +BHT 200 ppm	Oil + TWE			Oil +TEE			Oil +GEE		
			200 ppm	400 ppm	600 ppm	200 ppm	400 ppm	600 ppm	200 ppm	400 ppm	600 ppm
0	3.4 **±0.01**	3.3 **±0.01**	4.4 **±0.1**	3.3 **±0.1**	3.4 **±0.1**	3.3 **±0.1**	4.5 **±0.2**	3.3 **±0.1**	3.0 **±0.1**	3.5 **±0.1**	3.8 **±0.02**
4	27.1 **±0.2**	21.6 **±0.02**	25.5 **±0.2**	23.7 **±0.2**	21.9 **±1.0**	25.5 **±0.2**	24.3 **±0.1**	24.9 **±0.1**	25.4 **±0.4**	25.3 **±0.7**	24.8 **±0.03**
8	52.5 **±0.4**	36.8 **±0.1**	42.8 **±0.8**	40.1 **±2.1**	40.4 **±0.2**	42.5 **±0.2**	45.2 **±0.9**	42.8 **±0.4**	48.0 **±0.5**	40.9 **±2.5**	40.0 **±0.3**
12	77.8 **±0.8**	48.2 **±0.2**	55.2 **±0.7**	53.8 **±2.2**	53.5 **±0.5**	55.1 **±0.2**	54.7 **±2.7**	53.6 **±3.0**	67.9 **±0.3**	60.4 **±0.7**	55.7 **±1.0**
16	88.8 **±3.6**	64.8 **±0.2**	74.8 **±0.9**	71.4 **±3.5**	70.2 **±0.6**	70.6 **±1.0**	70.2 **±0.9**	69.5 **±0.8**	82.4 **±1.7**	79.6 **±0.7**	70.0 **±0.5**
b- PV(meq/kg oil) 0f SFO samples											
0	3.5 **±0.1**	3.0 **±0.02**	3.0 **±0.01**	3.2 **±0.01**	3.3 **±0.06**	3.3 **±0.02**	3.2 **±0.01**	3.1 **±0.01**	3.3 **±0.02**	3.7 **±0.03**	3.4 **±0.01**
4	33.7 **±0.2**	22.1 **±0.08**	30.8 **±0.9**	30.9 **±0.3**	31.4 **±0.5**	32.8 **±0.5**	32.0 **±0.7**	32.3 **±0.6**	32.8 **±1.3**	32.3 **±0.7**	32.1 **±0.6**
8	62.4 **±0.4**	42.6 **±0.01**	54.7 **±0.9**	51.3 ±0.9	50.8 **±0.2**	52.8 **±0.02**	51.4 **±0.3**	49.9 **±1.1**	88.2 **±0.8**	60.0 **±4.0**	57.1 **±1.0**
12	82.6 **±1.3**	55.1 **±0.4**	76.1 **±0.2**	65.6 **±0.4**	62.0 **±0.8**	72.6 **±0.1**	70.0 **±0.2**	68.1 **±0.3**	135.0 **±0.6**	76.9 **±0.7**	75.1 **±0.5**
16	109.8 **±2.4**	82.8 **±3**	102.6 **±0.4**	98.1 **±0.4**	85.1 **±0.9**	94.4 **±3.0**	93.6 **±1.3**	92.7 **±0.2**	160.4 **±3.9**	100.9 **±4.5**	98.7 **±0.2**
c- PV(meq/kg oil) of SBO:SFO(50:50%) samples											
0	2.7 **±0.01**	3.2 **±0.06**	3.8 **±0.01**	3.5 **±0.01**	3.4 **±0.01**	3.3 **±0.07**	3.1 **±0.05**	3.3 **±0.05**	3.3 **±0.02**	3.6 **±0.09**	3.8 **±0.02**
4	45.9 **±1.7**	38.1 **±0.5**	28.2 **±0.6**	26.6 **±0.4**	29.3 **±0.3**	31.7 **±0.7**	29.9 **±0.4**	28.5 **±0.2**	39.6 **±0.09**	32.4 **±0.7**	32.2 **±0.5**
8	80.6 **±1.5**	64.2 **±0.3**	48.4 **±1.4**	44.7 **±1.5**	41.0 **±0.2**	47.8 **±0.5**	46.9 **±0.1**	45.0 **±0.4**	78.8 **±0.4**	64.3 **±0.6**	52.7 **±0.4**
12	115.1 **±1.3**	82.9 **±0.2**	64.2 **±0.5**	60.0 **±0.4**	53.4 **±0.1**	70.7 **±0.9**	69.6 **±0.2**	68.2 **±1.4**	108.8 **±0.5**	88.4 **±.08**	72.8 **±0.1**
16	165.1 **±1.3**	104.9 **±2.5**	84.5 **±1.0**	79.1 **±0.3**	74.4 **±1.1**	94.1 **±0.9**	93.0 **±1.3**	90.8 **±0.2**	152.0 **±1.1**	118.9 **±4.4**	85.8 **±0.7**

PV= peroxide value, SBO=soybean oil, SFO=sunflower oil, BHT=butylated hydroxytoluene, TWE= tomato water extract, TEE= tomato ethyl lactate extract, GEE= grape ethanol extract.

The natural antioxidant extracts have higher IO% than that of BHT in case of cooking oil mixture (SBO : SFO) in all interval heating times. That is indicated that their effect was surpassed the effect of the synthetic antioxidant.

Changes in *p*-AV during heating of treated and untreated SBO, SFO and their mixture samples were recorded in Table 6. It was found that, highest *p*-AV after 16 days heating (60 ^{O}C) were obtained by control samples. The addition of 600 ppm of TEE to SBO lower *p*-AV change than addition of 200 ppm BHT, which indicates the higher protection of TEE against secondary oxidation than the legal limit of BHT. As well as the addition of 400, 600 ppm TWE and 200 ppm TEE lowers *p*-AV change after 8 days heating (60°C) than addition of 200 ppm BHT.

From data in Table 6, it was noticed that addition of different concentrations of plant extracts (TWE, TEE) and 400, 600 ppm GEE to SFO lowering *p*-AV change than control and SFO supplemented by 200 ppm of synthetic antioxidant (BHT). After 16 days of heating the higher *p*-AVs were obtained by mixture control sample (32.7) followed by mixture oil sample contained 200 ppm of GEE (23.66) and oil sample contained 200 ppm BHT (17.95). Meanwhile, mixture oil samples treated with other antioxidant extracts gave lower *p*-AV change (11.0, 9.8 and 9.7 for TWE, 10.9, 12.1 and 13.4 for TEE at 200, 400 and 600 ppm respectively and 16.0, 10.5 for 400 and 600 ppm GEE respectively.

TABLE 5. The IO % of natural antioxidant extracts compared with BHT during heating of SBO, SFO and their mixtures in oven at 60°C.

Time (day)	IO %									
	Oil samples	200 ppm BHT	TWE			TEE			GEE		
			200 ppm	400 ppm	600 ppm	200 ppm	400 ppm	600 ppm	200 ppm	400 ppm	600 ppm
4	SBO	25.0	10.9	13.9	22.2	6.5	16.6	8.7	5.6	8.0	11.5
	SFO	36.8	7.9	8.0	6.8	13.2	4.5	3.1	1.9	4.9	4.6
	SBO:SFO	19.2	43.4	46.6	40.1	34.4	38.1	41.8	16.0	33.4	34.2
8	SBO	32.7	21.8	25.0	24.8	20.2	17.1	19.5	8.3	23.8	26.4
	SFO	32.7	12.2	18.2	19.2	16.0	18.1	20.5	-44.3	4.3	8.6
	SBO:SFO	21.7	42.8	47.1	51.7	43.0	43.9	46.5	3.1	22.2	37.2
12	SBO	40.3	31.6	32.0	32.6	30.5	32.5	32.2	12.7	23.5	30.3
	SFO	34.1	7.6	21.0	25.7	12.4	15.6	17.8	-66.5	7.4	9.3
	SBO:SFO	29.0	46.2	49.7	55.5	40.1	40.8	42.3	6.0	24.5	38.5
16	SBO	28.6	17.5	20.2	21.8	21.2	23.2	22.5	7.1	10.8	22.5
	SFO	24.9	6.3	10.7	23.0	14.3	14.9	15.7	-47.8	8.5	10.3
	SBO:SFO	37.3	50.2	53.4	56.2	44.1	44.6	46.1	8.4	29.0	49.5

IO %= the calculated inhibition of oil oxidation, BHT=butylated hydroxytoluene, TWE= tomato water extract, TEE= tomato ethyl lactate extract, GEE= grape ethanol extract. SBO= soybean oil, SFO= sunflower oil

TABLE 6. Effect of addition of natural antioxidant extracts compared with BHT to SBO, SFO and their mixture on the p-AV after heating in oven test.

Time (day)	a- *p*-AV of SBO samples										
	Control	Oil +BHT 200 ppm	Oil + TWE			Oil +TEE			Oil +GEE		
			200 ppm	400 ppm	600 ppm	200 ppm	400 ppm	600 ppm	200 ppm	400 ppm	600 ppm
0	0.4 **±0.06**	0.5 **±0.01**	0.8 **±0.02**	0.9 **±0.01**	1.1 **±0.02**	1.2 **±0.02**	1.1 **±0.02**	1.1 **±0.01**	1.06 **±0.05**	1.6 **±0.07**	1.2 **±0.06**
4	4.7 **±0.05**	2.9 **±0.1**	3.51 **±0.02**	3.5 **±0.02**	3.6 **±0.05**	3.7 **±0.02**	2.7 **±0.1**	2.5 **±0.05**	2.5 **±0.06**	2.7 **±0.1**	3.1 **±0.06**
8	8.4 **±0.2**	6.0 **±0.2**	6.1 **±0.02**	5.4 **±0.1**	5.4 **±0.2**	5.9 **±0.1**	6.4 **±0.02**	6.5 **±0.02**	6.6 **±0.07**	7.8 **±0.01**	8.0 **±0.05**
12	12.2 **±0.2**	8.7 **±0.2**	10.0 **±0.3**	10.2 **±0.1**	9.9 **±0.08**	9.2 **±0.01**	9.2 **±0.02**	8.6 **±0.05**	13.5 **±0.1**	10.5 **±0.1**	11.6 **±0.1**
16	14.3 **±0.2**	10.8 **±0.4**	12.9 **±0.2**	13.3 **±0.2**	13.4 **±0.05**	11.3 **±0.4**	11.0 **±0.1**	10.4 **±0.05**	16.2 **±0.08**	12.7 **±0.08**	13.5 **±0.1**
b- *p*-AV of SFO samples											
0	1.4 **±0.02**	1.3 **±0.03**	1.6 **±0.02**	1.3 **±0.02**	1.8 **±0.07**	2.7 **±0.07**	2.8 **±0.02**	2.4 **±0.01**	3.1 **±0.02**	2.4 **±0.02**	2.0 **±0.01**
4	6.7 **±0.1**	5.8 **±0.08**	4.4 **±0.5**	3.5 **±0.2**	3.2 **±0.02**	3.3 **±0.05**	2.9 **±0.1**	2.6 **±0.08**	5.0 **±0.1**	2.1 **±0.08**	3.7 **±0.03**
8	12.7 **±0.3**	9.4 **±0.08**	6.9 **±0.06**	5.8 **±0.2**	4.8 **±0.02**	4.7 **±0.02**	4.5 **±0.1**	3.8 **±0.05**	9.6 ±0.05	5.4 **±0.1**	5.4 **±0.03**
12	17.1 **±0.2**	13.3 **±0.2**	9.1 **±0.08**	7.3 **±0.01**	6.2 **±0.02**	6.5 **±0.3**	8.1 **±0.02**	6.2 **±0.1**	12.6 **±0.05**	8.4 **±0.05**	6.8 **±0.02**
16	24.1 **±0.7**	16.5 **±0.2**	11.7 **±0.1**	8.7 **±1.4**	6.4 **±0.05**	9.3 **±0.05**	10.6 **±0.1**	10.2 **±0.2**	18.5 **±0.08**	12.2 **±0.08**	10.2 **±0.02**
c- *p*-AV of SBO: SFO(50:50%) samples											
0	1.07 ±0.09	0.7 ±0.01	1.2 ±0.1	1.1 ±0.1	1.0 ±0.08	2.0 ±0.08	1.3 ±0.08	1.5 ±0.09	1.9 ±0.1	1.4 ±0.05	1.6 ±0.1
4	5.3 ±0.08	4.3 ±0.05	3.8 ±0.4	3.3 ±0.02	3.5 ±0.1	2.6 ±0.4	2.4 ±0.02	2.8 ±0.07	5.0 ±0.2	4.8 ±0.1	4.1 ±0.1
8	15.0 ±1.3	8.9 ±0.1	6.7 ±0.5	6.2 ±0.3	5.4 ±0.4	3.9 ±0.1	4.6 ±0.4	4.3 ±.07	10.4 ±0.1	7.0 ±0.05	7.7 ±.02
12	25.2 ±0.1	13.3 ±0.1	8.0 ±0.1	7.6 ±0.1	8.2 ±0.3	6.5 ±0.1	7.9 ±0.2	8.1 ±0.2	17.2 ±0.5	12.7 ±0.2	9.2 ±0.3
16	32.7 ±0.05	17.9 ±0.05	11.0 ±0.02	9.8 ±0.1	9.7 ±0.07	10.9 ±0.1	12.1 ±0.1	13.4 ±0.1	23.6 ±0.08	16.0 ±0.08	10.5 ±0.3

p-AV = *p*-anisidine value, SBO=soybean oil, SFO=sunflower oil, BHT=butylated hydroxytoluene, TWE= tomato water extract, TEE= tomato ethyl lactate extract, GEE= grape ethanol extract.

These data indicated that:

The addition of BHT, TWE, TEE, and GEE significantly decreased the rate of peroxide decomposition comparing to untreated SBO sample. These results are in agreement with the results reported by Poiana (2012).

TEE was the most efficient in inhibiting the decomposition rate of the hydroperoxide in SFO followed by TWE, GEE and BHT.

The rate of decomposition of hydroperoxides in SBO:SFO control and oil samples containing 200 ppm of BHT and GEE was higher than that of the other antioxidant extracts, while oils contain-

ing different concentrations of TWE showed the low rate of hydroperoxides decomposition.

The addition of tomato and grape by-product extracts to the oil mixture of SBO:SFO (1:1) protected the oil from the secondary oxidation. Furthermore, the effect of natural extracts in retarding this stage of oxidation was superior to the effect of legal limit of synthetic antioxidant (200 ppm BHT).

Considering total oxidation value (TOTOX value), it was found that after 16 days of heating the highest TOTOX value was obtained by control sample in SBO and SFO while the lowest value was obtained by oil sample supplemented by 200 ppm BHT. Meanwhile, the TOTOX value of SBO and SFO treated with natural antioxidant extracts were in the range between those of the control and sample with the BHT. Total oxidation values showed that the addition of TWE, TEE and GEE to the SBO or SFO improved their oxidative stability and decreased the rate of formation of total oxidation products.

The TOTOX values of SBO:SFO mixture treated with both TWE and TEE at 200, 400 and 600 ppm and 600 ppm GEE were found to be lower than the control and mixture oil treated with 200 ppm BHT (Fig. 2). The higher oxidative stability of these oil samples illustrate the efficiency of tomato by-product extracts as strong antioxidants which were superior to the effect of synthetic one (BHT).

Generally, TWE and TEE have higher protective effect against oxidation process than GEE. This may be attributed to the presences of high amounts of lycopene in tomato extracts which strengthen the activity of the other antioxidants in the extract (synergistic effect) (Ciriminna et al., 2016). Moreover, 200 ppm of GEE gave negative values of IO% and higher PV, *P*-AV and TOTOX than that of SFO control samples This may be suggested to the pro-oxidative effect of this concentration. This result agrees with Spigno and Defaveri 2007 and Shaker 2009 (Spigno and DeFaveri, 2007; Shaker, 2006). Although the effect of plant extracts was lower than the synthetic antioxidant in some cases, but they are preferable as they are safer and have no health risks even in high concentrations.

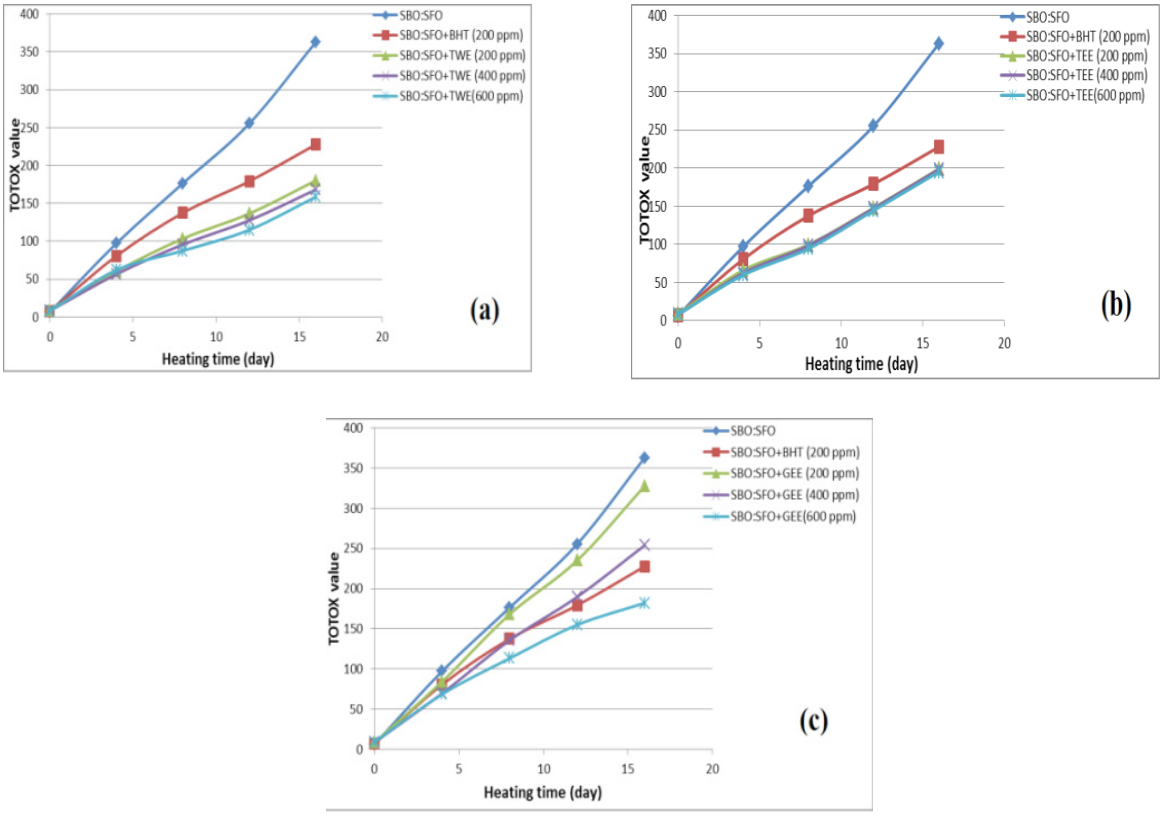

Fig. 2. Effect of addition of different natural extracts (a, b and c for TWE, TEE and GEE respectively) in comparison with BHT on TOTOX value of SBO:SFO mixture.

Conclusions

Plant extracts from food industrial by-products have attracted great attention mainly for role in food preservation especially for prevention of lipid oxidation. The addition of tomato and grape by-product extracts exhibited a significant inhibitory power against thermal oxidation of cooking oils. The effect of addition 200, 400 and 600 ppm of TWE and TEE and 600 ppm of GEE to SBO:SFO mixture was found to be superior to that of the synthetic antioxidant (BHT). The extracts from tomato by-product were found to be more effective than that from grape by-product. Therefore, tomato and grape by products can be recommended as a cheap, green and potent source of natural antioxidants that can inhibit the formation of free radicals in the initiation step and retarding its deterioration to improve the oxidative stability of cooking oils that can be replace the carcinogenic synthetic antioxidants.

Conflict of interest: The authors declare that they have no conflicts of interest.

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استخدام مضادات اكسدة طبيعية مستخلصة من النواتج الثانوية للصناعات الغذائية بطرق حديثة لتحسين ثبات الزيوت الغذائية ضد التأكسد

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١ قسم الزيوت والدهون- شعبة الصناعات الغذائية والتغذية- المركز القومى للبحوث
٢ قسم الكيمياء العضوية- كلية العلوم- جامعة بنها

من المعروف ان أكسدة الليبيدات تؤدي الي فساد الاطعمة الدهنية وتغيير رائحتها وتقليل جودتها وسلامتها و قيمتها الغذائية مما يؤثر سلبا علي صحة الانسان. لذلك تهدف هذه الدراسة الي تحسين ثبات بعض زيوت الطعام٠ و ذلك عن طريق إضافة تركيزات مختلفة من بعض المواد الفعالة النباتية (المستخلصة من النواتج الثانوية لصناعة الطماطم والعنب) ومقارنتها باضافة مضاد اكسدة صناعي. وقد تم تحليل مدى التغيير فى ثبات الزيوت المعاملة لتحسين ثباتها ضد التأكسد وذلك باستخدام الطرق القياسية التسخين في الفرن عند درجة حرارة ٦٠ م° مقارنة بالعينات الكنترول. حيث تم قياس نواتج الأكسدة الاولية عن طريق تقدير رقم البيروكسيد وقياس نواتج الأكسدة الثانوية وذلك بتقدير رقم البارانزيدين وكذلك حساب نسبة منع التأكسد و الاكسدة الكلية للزيوت.
بالإضافة الى ذلك تم قياس فاعلية الزيوت المعالجة كمضادات اكسدة باستخدام تجربة DPPH لصيد الشوارد الحرة .
من النتائج وجد أن اضافة مستخلصات مضادات الأكسدة الطبيعية (المستخلصة من النواتج الثانوية لصناعة الطماطم والعنب) قد أدت الى تحسين ثبات عينات الزيوت ضد التأكسد. ووجد ايضا ان اضافة ٢٠٠، ٣٠٠، ٤٠٠ جزء في المليون من مستخلص الطماطم و اضافة ٦٠٠ جزء في المليون من مستخلص العنب لخليط زيت الصويا و زهرة الشمس(٥٠ : ٥٠٪) اعطى ثباتية تفوق ثباتية اضافة مضاد الاكسدة الصناعي. ومن النتائج وجد ان المستخلصات النباتية المستخرجة من النواتج الثانوية لصناعة الطماطم والعنب تعتبرمصدر امن و حيوي كمضادات اكسدة طبيعية ساعدت علي ثبات الزيوت الغذائية وكذلك هي مصدر لزيادة محتوي الزيوت الغذائية من المواد الفعالة الطبيعية .