# UTILIZATION OF EXTRACTS FROM POMEGRANATE AND SOUR ORANGE WASTES AS NATURAL ANTIOXIDANTS IN RETRADING COTTON SEED OIL OXIDATION EI-Bagoury, A. A.

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# ABSTRACT

Total polyphenolic compounds extracted by methyl alcohol from pomegranate peel and flavedo layer of sour orange peel were found to be 23.251 and 3.562 (mg/g dry weight), respectively. GC profiles of the two methyl extracts showed that the highest peak for pomegranate extract was recorded at retention time of 0.556 min, whereas the highest peaks for sour orange extract were recorded at retention times of 0.548,0.606,1.053,1.44 and 11,271 min. The presence of eliagic acid, tertgalilc acid, gallic acid, caffeic, p-coumaric, ferulic acid, sinapinic acid in pomegranate peel extract and ferulic acid, sinapinic acid, neoeriocitrin, narirutin, naringin, hesperidin, caffeic acid and p-coumaric in sour orange extract have been reported. Oven storage test (at 100 °C up to 72 hrs) was carried out to evaluate antioxidant activity of the two extracts on the stability of cotton seed oil at three concentrations (200,400,600 ppm) compared with synthetic antioxidants (BHA, BHT, 200ppm). Iodine value for all oil samples was decreased and the highest decrease was recorded for the control sample (without addition of any antioxidant). As a result of accelerated storage, all oil samples showed gradual increases in peroxide value, acidity, thiobarbituric acid, and absorbances at 400 and 450 nm vs time. Addition of pomegranate extract, sour orange extract, BHA or BHT retarded these changes, whereas pomegranate extract recorded the best results. On the other side, as a result of accelerated storage, the unsaturated fatty acids were decreased whilst saturated ones were increased. Thus, the ratio between total unsatured fatty acids and total saturated ones (TWTs) decreased, especially for the control oil sample. The overall results showed that both pomegranate peel and flavedo layer of sour orange peel extracts have antioxidant property, and may be exploited as biopreservative in food applications.

# INTRODUCTION

Autoxidation of lipids as well as enzymatic oxidation during storage and processing is the major reaction in fats, oils and fat-containing foods responsible for the deterioration in food quality. It affects the color, flavor, texture and especially the nutritive value of the foods. Also, it may lead to formation of toxic compounds associated with aging, membrane damage, heart disease and cancer (Van Ruth *et al.* 2001 and Matthaus 2002). Autoxidation is a complex process, but model studies have revealed that the rate of autoxidation is affected by fatty acid composition, degree of unsaturation, the presence and activity of pro- and antioxidants, partial pressure of oxygen, the surface exposed to oxygen (dispersed system) and the storage conditions (light, temperature, moisture content) (Krings and Berger 2001).

To avoid or delay the autoxidation process, antioxidants have been used for over 50 years (Marie *et al.*1994). They can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chaln reactions (Zheng and Wang 2001). In general, there are two basic categories of antioxidants: natural and synthetic. Synthetic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and tert – butyl hydroquinone (TBHQ) are widely used. They have some critical disadvantages: highly volatile, unstable at elevated temperature, restricted by legislative rules and above all, they are suspected to be carcinogenic and have some toxic properties (Singh *et al.* 2002). Therefore, the interest in replacing synthetic antioxidants with natural components from oil seeds, spices and other plant materials has increased considerably. Environmental consciousness and cost factors have led to extraction of natural antioxidants from easily renewable sources, including plant wastes (Van Ruth *et al.* 2001).

Polyphenols are the major plant compounds with antioxidant activity, although they are not the only ones (Moure et al. 2001). Their antioxidant activity is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Zheng and Wang 2001).

Pornegranate peel (a byproduct of juice extraction) contains substantial amounts of polyphenoles such as ellagic tannins, ellagic acid and gallic acid. It has been used in the preparation of tinctures, cosmetics, therapeutic formula and food recipes (Negl et al. 2003). Also, peels and seeds of citrus fruits are an interesting source of phenolic compounds, which include phenolic acids and flavonoids. The citrus flavonoids have been found to have health-related properties, which include anticancer, antiviral and antiinflammatory activities, effects on capillary fragility and an ability to inhibit human platelet aggregation (Bocco et al. 1998).

Accordingly, the present study was carried out to investigate antioxidants effectiveness of natural antioxidants, namely methyl extracts of pomegranate peel and flavedo layer of sour orange peel (a by-product of jam made) at three concentrations (200,400,600 ppm) compared with that of synthetic antioxidants (BHA, BHT, 200ppm) on the quality of cotton seed oil during accelerated storage at 100 °C up to 72 hrs.

# MATERIALS AND METHODS

#### Materials

Pomegranate (Punica granatum, L) and sour orange (Citrus a urantium) were obtained from a local market in Tanta city (Egypt). The fruits were washed well with distilled water and dried with a piece of cotton. Sour orange flavedo layer was obtained by a grater and peel of pomegranate was collected by a knife. Both the flavedo layer and the peel were (separately) dried at 40°C over night in an air draft-drying oven. The two samples were ground into fine powder in a Moulinex Coffee grinder (made in France). The pulverized samples that passed through 60-mesh sieve were kept in glass jars at 4°C until used.

### Antioxidants, oil and other chemicals

Butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) were obtained from Sigma Chemical Company. Fresh refined cotton seed oil

(without addition of any antioxidants) was obtained from Tanta Company for Oils and Soaps, Tanta, Egypt. All other chemicals were supplied from El-Gomhoria Company for chemists, Egypt.

## Methods

#### 1 - Extraction of phenolic compounds:

Phenolic compounds from pomegranate and sour orange powders were extracted according to the procedure of Abou Rayan *et al.* (1998) using methanol alcohol 80% as follows: - One gram sample was extracted by shaking for 1 hour at room temperature with 50 ml of methanol (80%). The mixture was centrifuged (15 minutes) at 3500 rpm and then filtered through Whatman No.1 filter paper. The extraction process was carried out twice and the filtrates were collected for quantitative analysis. All analyses were carried out in triplicate.

### 2 - Quantitive determination of total polyphenolic compounds:

Total polyphenolic compounds were quantitatively determined according to the method of Price et al. (1978). A standard curve was prepared using catechin as a standard and total polyphenolic compounds were expressed in mg catechin equivalents.

# 3 – Gas chromatography

The analysis was run in the Laboratory of Special Nutrition Department, Food Technology Institute, Agriculture Research Center, Giza, Egypt. The two methanolic extracts were prepared as outlined by Zang and Pawliszyn (1994) and run in a gas chromatography (HP 5890) on HP1 column with helium as the carrier gas. The temperature program was 135°C for 2 min, and then increased to 250°C with a rate of 15 °C/min. The compounds were identified through comparison with the retention times obtained.

 Antioxidative activity of pomegranate and sour orange extracts (Oven storage test of antioxidant activity)

The obtained methenolic extracts were evaporated in a rotary evaporator (type 350P made in Poland) at 45°C to dryness. The dried extracts were mixed well with cotton seed oil using an ultrasonic water bath (UP 200 Ultrasonic Processor dr.hielscher, GmbH) to form an emulsion (which remained completely, dispersed in the oil throughout the storage period) at levels of 200,400,and 600 ppm (w/w), respectively. BHA and BHT were mixed well with cotton seed oil at levels of 200mg/kg oil as recommended by Egyptian Organization for Standardization (Anonymous 1993). Also, an oil sample was kept without any addition to serve as control. The samples were stored in the dark in an oven at 100°C for 72 hours. Oil samples were periodically withdrawn at the end of 8,24,32,48,56 and 72 hrs of heating periods and stored in brown bottles at -20°C until analysis.

Acid value (as % oleic acid), peroxide value (millequivelant  $O_2$  per kg oil) and iodine value (Hannus method) were determined according to the official methods of the American Oil Chemists Society (A.O.C.S. 1990). Thiobarbituric acid (TBA) value was measured as the method explained by Kirk and Sawyer (1991). Color absorbance of 1% oil in chloroform was measured at 400 and 450 nm (Abdel-Aal and Karara 1986). Methyl esters of faity acids were obtained according to A.O.A.C. (1990) in the Central

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Laboratory, Fac. of Agric., Alex. Univ. The fatty acids were determined by Gas Liquid Chromalography (Shimadzu-GC4CM Japan) equipped with flame ionization detector.

# **RESULTS AND DISCUSSION**

## Total polyphenolic compounds and gas chromatography

Epidemiological studies show that consumption of fruits and vegetables with high phenolic content was correlated with reduced cardio- and cerebrovascular diseases and cancer mortality (Gil et al. 2000). The presented results in Table (1) Illustrated that the highest concentration of polyphenols was found in the pomegranate peel extract, whereas the flavedo layer of sour orange peel extract recorded the lowest concentration. The phenolic contents of methanol extract were found to be 23,251 and 3,562 (mg/g dry weight) for pomegranate peel and flavedo layer of sour orange peel, respectively.

# Table (1): Total polyphenolic compounds in methanol extracts of nomegranate neel and flavedo laver of sour grange

Methanol extract	Polyphenols*
Pomegranale peel	23.252
Flavedo layer of sour orange peel	3.562
· (As ma catechiniam dry weight)	

(As mg catechin/gm dry weight).

The GC profiles of the two methanol extracts are shown in Fig.1 and 2. Concerning pomegranate extract, the highest peak was recorded at retention time of 0.556 min.; other peaks were recorded at retention times of 0.35,7.75,8.832 and 8.966 min., respectively. On the other side, the highest peaks for sour orange were recorded at retention times of 0.548,0.606,1.053,1.440 and 11,271 min., respectively. While the peak at 11.271 for sour orange was not found in the pomegranate extract, the peaks at 7.75, 8.832 and 8.966 for pomegranate extract were not found in the sour orange extract. This clearly shows the differences between the two extracts in their contents of phenolics. Moreover, the phenolic pattern of pomegranate peel extract includes additional phenolics to those present in the flavedo layer of sour orange peel extract. The presence of polyphenols such as ellagic acid, tertgallic acid, and gallic acid, caffeic, p-coumaric, ferulic, and sinapinic acids in pomegranate peel extract have been reported (Gil et al. 2000 and Singh et al. 2002). On the other hand, ferulic acid, sinapinic acid, neoeriocitrin, narirutin, naringin and hesperidin, caffeld acid, p-coumarid were identified in the methyl extract of sour orange peel (Bocco et al. 1998 and Moure et al.2001). Many plant polyphenols such as ellagic acid, catechin, chlorogenic, caeffic and ferulic acids, as well as their dietary sources have been shown to act as potent antimulagenic and anticarcinogenic agents (Negi et al. 2003).

Additives		Pomegranate extract			S	our orange e	BHA	BHT	
Storage time (hrs)	Control (without antioxidants)	200 ppm	400 ppm	600 ppm	200 ppm	400 ppm	600 ppm	200 ppm	200 ppm
0	108.580	108.580	108.580	108.580	108.580	108.580	108.580	108.580	108.580
8	101.828	105.870	106.788	107.804	103.064	103.804	131.258	132.084	132.084
24	99.168	104.448	105.434	106.308	102.702	103.032	124.063	127.239	127.239
32	95.534	103.523	104.476	105.604	99.817	101.976	121.102	124.044	124.044
48	92.901	102.704	103.330	104.828	96.808	98.576	118.392	123.755	123.755
56	88.750	99.818	101.534	102.520	93.556	94.566	116.778	121.032	121.032
72	86.169	93.080	96.942	98.114	89.824	91.274	114.868	120.055	120.055

Table (2): lodine value<sup>®</sup> of cotton seed oil containing pomegranate peel and sour orange extracts and Butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA) during storage at 100 °C up to 72 hrs.

"Hennus method(A.O.C.S. 1990).

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Table (3): P eroxide v alue (Pv) of c otton seed oll containing pomegranate peel and sour orange extracts and Butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA) during storage at 100°C up to 72 hrs.

Additives		Pomeg	ranate extract	Sour	BHA	BHT			
Storage time (hrs)	Control (without antioxidants)	200 ppm	<b>4</b> 00 ppm	600 ррт	200 ppm	<b>400</b> ppm	600 ppm	200 ppm	200 ppm
0	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05
8	15.610	6.731	5.505	4.672	14.286	14.010	11.764	11.374	11.389
24	63.420	50.000	41.176	38.238	57.416	55.779	53.465	50.249	50.691
32	66.505	55.093	50.697	42,365	60.562	58.903	56.250	55.392	55.501
48	74.257	68.462	62.264	60.784	71,427	70.588	69.901	68.545	68,797
58	76.555	71.356	64.356	61.275	75.301	73,779	72.507	71.144	72.228
72	81.198	75.980	68.454	63.235	78.228	77.490	76.529	76.050	76.300

#### lodine value:

Table (2) showed a gradual decrease in the iodine value for all oil samples during the accelerated storage period at 100°C up to 72 hrs. The highest decrease was observed for the control sample. On the other side, addition of natural or synthetic antioxidants, led to reduce the decrements in the iodine value of all treated oil samples, especially the oil sample treated with 600 ppm of methyl pomegranate extract. The decrease in i odine value may be due to the consumption of double bonds by oxidation and polymerization (Alexander 1978 and Chang *et al.* 1978).

### Peroxide value:

Effect of accelerated storage period at 100°C up to 72 hrs on peroxide value of cotton seed oil samples is shown in Table (3). The peroxide value of the control sample oil (without addition of any antioxidant) at the beginning of the storage period, was lower than that recommended by the Egyptian Standard (10 mEq O<sub>2</sub> / Kg oil, Anonymous 1993). As a result of accelerated storage at 100°C, all cotton seed oil samples showed a gradual increase in Pv vs time. This increase was higher for the control sample than the other samples, which reached a maximum of 81.198 mEq  $O_2$  / Kg oil after 72 hrs at 100°C. The change in peroxide value was due to hydroxyperoxide formation (Arya et al. 1969). Addition of BHA, BHT or pomegranate and sour orange extracts, retarded the changes in peroxide value during accelerated storage period at 100°C. Also, the results showed that peroxide values after 8 hrs of accelerated storage of cotton seed oil samples containing pomegranate extract at the different concentrations used (200,400,600) were lower than the maximum level in the Egyptian Standard (Anonymous 1993). On the other side, the efficiency of BHA or BHT (200ppm) Inhibiting cotion seed oil oxidation was higher than that of sour orange extract at the three concentrations used (200,400,600 ppm). Farag at al. (1989) reported that phenolic compounds act as hydrogen donors to the oxidation systems and therefore they decrease formation of hydroperoxides.

### Acid value:

It could be noticed that the acid value (Table: 4) at the start and up to 8 hrs of accelerated storage at 100°C remained below 0.2 (as oleic acid) that recommended by Egyptian Standard for the oil (Anonymous 1993). Acid value was increased in parallel with Increasing the heating time, especially the control oil sample (without addition of any antioxidant). This increase is due to the oxidation of aldehydes and ketones-formed during heating-to acids (Abdel-Aal and Karara 1986; Badway *et al.*1990; Mostafa *et al.*1994 and El-Adawy and Taha 1999). On the other hand, a ddition of antioxidants to the cotton seed oil had delayed the changes in acid value during storage. Also, pomegranate extract was more effective on acid value than sour orange extract. In addition, increasing the concentrations of antioxidants resulted on a decrease in the acid value.

#### Thiobarbituric acid (TBA value):

Results prensted in Table (5) showed that TBA value was gradually increased during accelerated storage at 100°C up 72 hrs for all oil samples.

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Table (4): Acid value* of cotton seed oil containing pomegranate and sour orange extracts and Butylated hydroxy
toluene (BHT) , butylated hydroxy anisole (BHA) during storage at 100°C up to 72 hrs.

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Additives	Control (without antioxidants)	Pomegranate extract			5	our orange	BHA	8HT	
Storage time (hrs)		200 ppm	400 ppm	600 ppm	200 ppm	400 ppm	800 ppm	200 ppm	200 ppm
0	1.26	1.26	1.26	1.26	1.26	1.25	1.26	1.26	1.26
8	1.98	1.65	1.56	1.46	1.99	1.86	1.81	1.70	1.70
24	3.01	2.36	2.26	2.11	2.66	2.58	2.46	2.36	2.41
32	3.31	2.71	2.61	2.41	3.02	2.86	2.81	2.76	2.76
48	3.86	3.16	3.02	2.86	3.57	3.41	3.26	3.21	3.21
56	4,12	3.47	3.22	3.02	3.62	3.71	3.61	3.52	3.56
72	4.32	3.67	3.47	3.27	4.07	3.92	3.82	3.72	3.76
As a oleic acid / k	0.08								

Table (5):	Thiobarbituric acid (TBA)*	of cotton seed oll	containing pomegranat	e and sour orange	extracts and
	Butylated hydroxy toluene	(BHT), butylated i	nydroxy anisole (BHA) d	uring storage at 10	0°C up to 72
	hrs.	-	-		·

Additives	Control	Pome	egranate e	ktract	So	BHA	BHT		
Charles and	(without	200	400	600	200	400	600	200	200
Storage lime (ms)	antioxidants)	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
0	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029
8	0.098	0.084	0.078	0.076	0.092	0.088	0.086	0.085	0.086
24	0.130	0.114	0.098	0.092	0.120	0.118	0.116	0.114	0.115
32	0.137	0.121	0.111	0.103	0.127	0.124	0.123	0.121	0.122
48	0.148	0.129	0.122	0.110	0.139	0.136	0.132	0.130	0.131
56	0.150	0.133	0.128	0.116	0.143	0.138	0.135	0.134	0.135
72	0.188	0.148	0.140	0.130	0.155	0.151	0.150	0.149	0.150

\*As mg malonaldehyde / kg oll.

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The increase In TBA value may be due to the formation of carbonyl compounds and oxidation of unsaturated fatty acids as reported by Owon (1991) and Ackoh (1998). On the other side, it could be observed that TBA value of the control oil sample was higher than those of treated ones. Moreover, pomegranate extract was the superior in reducing TBA values at the three concentrations used (200,400,600ppm), while sour orange extract was the inferior.

## The Absorbance:

Extinction at 400 and 450 nm simultaneously increased during accelerated storage period at 100°C up to 72 hrs (Table: 6). Nawar (1984) attributed these changes to oil oxidation and formation of dimmer components. Addition of pomegranate extract, sour orange extract, BHA or BHT retarded the changes in the color, whereas pomegranate extract recorded the best result in this respect.

# Table (6): Absorbance values of cotton seed oil containing pomegranate and sour orange extracts and Butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA) during storage at 100°C up to 72 hrs at 400 and 450 nm.

Additives		Pomag	ranate	anate extract Sour o extr			ange ci	BHA	внт				
Storage time (hrs)	Control (without antioxidants)	200 . ppm	400 ppm	600 ppm	200 քբտ	400 ppm	600 ppm	200 ppm	200 ppm				
		Wave length at 400 nm											
0	0.027	0.027	0.027	0.027	0.027	0.027	0.027	0.027	0.027				
8	0.035	0.029	0.028	0.028	0.031	0.030	0.029	0.0297	0.029				
24	0.044	0.035	0.033	0.031	0.041	0.040	0.039	0.036	0.036				
32	0.051	0.040	0.038	0.036	0.048	0.046	0.044	0.041	0.042				
48	0.059	0.049	0.048	0.044	0.056	0.052	0.049	0.049	0.049				
56	0.060	0.052	0.051	0.049	0.059	0.057	0.056	0.055	0.055				
72	0.065	0.058	0.056	0.054	0.063	0.061	0.060	0.059	0.059				
		Wa	ve leng	th at 4	50 nm								
0	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019				
8	0.023	0.020	0.020	0.020	0.021	0.021	0.020	0.020	0.020				
24	D.027	0.021	0.020	0.020	0.023	0.022	0.022	0.021	0.021				
32	0.027	0.022	0.021	0.021	0.028	0.024	0.024	0.022	0.023				
48	0.028	0.023	0.022	0.022	0.026	0.025	0.025	0.023	0.023				
56	0.029	0.024	0.023	0.022	0.027	0.026	0.028	0.024	0.024				
72	0.033	0.025	0.024	0.023	0.029	0.027	0.027	0.025	0.025				

### Faity acids composition:

According to the illustrated results in Table (7), cotton seed oil contained higher concentrations of linoleic acid (52.095%) followed by oleic acid (24.672%). Also, palmitic acid (19.177%) represented the major saturated fatty acid, whereas linoleic acid was the main unsaturated one. As a result of accelerated storage at 100°C for 72 hrs, the unsaturated fatty

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acids were decreased while saturated ones were increased. Thus, the ratio between total unsatured fatty acids and total saturated ones (Tu/Ts) was decreased, especially for the control oil sample. Addition of pomegranate, sour orange extracts, BHA or BHT reduced the decrements of Tu / Ts, especially pomegranate extract at 600 ppm level. Nawar (1979) attributed the decrease in the Tu/Ts ratio to the oxidation and decomposition of unsaturated fatty acids especially Lionlenic acid (C18:3).

Thus, overall results showed that both pomegranate peel and flavedo layer of sour orange peel extracts have antioxidant property, and may be exploited as bio-preservatives in food applications.

Table (7) : Fatty acid composition of cotton seed oil containing extracts of pomegranate and sour orange and Butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA) as a result of storage at 100 °C for 72 hrs.

	Control		Pomegranate extract			Sourc	Mange	BHA	BHT	
Fatty acids (%)	Fresh	After72 hrs at100*C	200 ppm	400 ppm	600 ppm	200 ppm	400 ppm	600 ppm	200 ppm	200 ppm
Myristic (C14:0)	00.308	00.542	00.471	00.460	00.458	00.454	00.418	00.417	00,455	00.455
Palmetic (C 16: 0)	19.177	24.707	20.422	20.059	19.269	22.317	21.475	21.506	21.375	21,454
Palmitololo(C18:1)	01.018	00.278	00.536	00.545	00.808	00.323	00.407	00.410	00.454	00.439
Steartc (C18: 0)	01.192	01.874	01.252	01.231	01.213	01.516	01.412	01.359	01.279	01.326
Oleic (C 18: 1)	24.672	22.773	24.739	24.979	25.115	24.775	24.516	24.365	24,691	24.952
Lionleic (C18::2)	52.095	49.031	51.317	51.453	51.774	49.806	50.798	51.015	50,668	50.328
Lionlenic (C18:3)	01.538	00.795	01.263	01.273	01.383	00.809	00.904	00.928	01.078	01.046
Arachidic (C20:0)	20.677	27.123	22.145	21.750	20.940	24.287	23.375	23.282	23.109	23.235
Ts	79.323	72.877	77.855	78.250	79.060	75.713	76.625	76.718	76.891	78.765
โบ	03.838	2.687	03.516	3.598	3.776	3.117	3.278	3.295	3.327	3.304
т́и / Тs	00.048	0.037	0.045	0.046	0.048	0.048	0.043	0.043	00.043	0.043
TEFA	53.633	49.826	52.580	52.726	53.137	50.615	51.702	51.943	51.746	51.374

TS=Total Saturated fatty acids= Myristic+Paimitic+Stearic

TU=Total Unsaturated fatty acids =Paimitoleic+Oleic+Linoleic+linolenic

TEFA=Total essential fatty acids=Linoleic+linolenic

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استخدام مستخلصات مخلفات الرمان والنارنج كمضادات أكسدة طبيعية لإعاقة أكسدة زيت بذرة القطن

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كانت كمية المركبات الفينولية المعديدة الكلية المستخلصة بكحول المونيل من المسرة الارسان وطبقة الفلاتيدو لقشرة النارنج ٢٣,٢٥١ و ٣,٥٦٢ ملجم/جم وزن جاف على الترتيب وسجلت أعلمي لمسة فسي التحليل الكروماتوجرافي الفازي لمستخلص الرمان عند زمن لمتجاز ٥٠,٥٥١ من النائية بيلما سجلت أعلمي القم لمستخلص النارنج علد أزمنة احتجاز ٥٩٨ و و١٩، و ١٩، و ٢٥، و١٤، و١٤، و ١٩، ٢٢ نقوة، ولقد نكر وجود لحماض الايلاجيك وترتجاليك والجاليك والكافيك وبي حويمارك والفيريس ولسك والسينابينوك فسي مستخلص الأمرة الرمان بينما وجد كل من حمض الفريوليك و المسينابينيك ونيوريوس ترين ولساريروتين ونارنجين و مسيريدين وحمض الكافيك وحمض بي حكويمارك في مستخلص طبقة الفلافيو التاريج و ونارنجين و مسيريدين وحمض الكافيك وحمض بي حريمارك في مستخلص طبقة الفلافيو التاري النارنج و

وكد لجرى لغتبار تخزين زيت بذرة القطن في درجة حرارة ١٠٠ درجة مئوية لمدة ٢٢ ساعة وذلك لتكبيم نشاط مضادلت الأكمدة لكلا المستخلصين على ثبات للزيت وباستخدام ثلاثة تركيزات (٢٠٠ و ٤٠٠ و ٦٠٠ جزء في العليون) مع المقارنة بمضادين أكمسة مستاعيين و معاييوتيلاتيــد هيدروكمـــي للومسول وبيرتيلاتيد هيدروكسي تولوين (٢٠٠ جزء في العليون) وعينة كنترول (بدون أي إضافة).

رتبين من الاختبار نقص الرقم اليودي لكل الحينات وكان اكثر الحينات لتفقاضا هي الحينة الكنترول وكنتيجة لمعلية التخزين فلقد أظهرت كل العينات زيادة تدريجية في كل من أرقام الحموضة و البير وكمسيد وحمض الثيوبابيرتريك والامتصاصية علد طولي الموجة ٥٠٠ و ٤٥٠ ناتوميتر مع الوقت و أدت إضافة كل من مستخلصي الرمان والذارنج وأيضا مضادين الأكسدة الصناعيين إلى التقليل من هذه التغيرات حيث كسان أنضلهم في ذلك مستخلص الرمان .

رمن ناحية أخرى نتج من صلية التغزين الخفاض الأحماض الدهلية الغير مشبعة وزيادة الأحماض الدهنية. المشبعة مما ترتب عليه الخفاض نسبة الأحماض الدهنية الغير مشبعة السي الأحمساص الدهنية العشسيمة وبخاصة في عينة الزيت الكنترول.

وبصفة عامة أظهرت النتائج أن مستخلصي تشرة الرمان وطبقة الفلاقيدو لتشرة النارنج الهمسا فعمل مضاد للأكسة ويمكن استخدامهما كمواد حافظة حيوية في التطبيقات الغذانية ·