

## **EFFECT OF NATURAL ANTIOXIDANTS OF ORANGE PEEL ON SUNFLOWER OIL DURING STORAGE**

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### **ABSTRACT**

Extracts of orange peel (Balady Var.) with different organic solvents (methanol, petroleum ether, ethanol diethylether, hexan and acetone) were studied. Methanol proved the best solvent for yeild extract. Antioxidant activity of sunflower oil treated with different concentrations of methanolic extracts of orange peel (MEOP) were also studied by determining peroxide value (POV), free fatty acid (FFA) and iodine value (IV) during 5 months storage at room temperature ( $20 \pm 3$  °C)., *Result indicated that:* Orange peel is considered as a good source of polyphenols. Methanol proved the best solvents of yeild extracts and also polyphenol contents.

The higher concentration of MEOP, the Lower value of (POV) and FFA) and the higher value of IV was observed during storage at the end of storage. Sunflower oil treated with 1500 and 2000 ppm of MEOP showed a significant ( $p \leq 0.05$ ) lower value of POVs (6.90 and 6.77 meq/ kg<sup>-1</sup>); FFA (0.280%, 0.275%) and higher IVs (67.0 and 70.3). Meanwhile, refined oil (POVs 77.3 meq/ kg<sup>-1</sup>, FFA 0.710 and 1V, 51.2), respectively. Addition of BHT and BHT with 200 ppm showed POVs(10.4 and 8.80 meq/ kg<sup>-1</sup>); FFA (0.251 and 0.270) and IVs(71.3 and 71.0), respectively.

MEOP proved their strong efficiency as a natural antioxidant activity as compared to BHT and BHA, therefore it is suggested that it can safety be used instead of synthetic antioxidants for preservation of oils and fats from peroxidation and rancidity.

**Keywords:** orang, citrus peel extract, sunflower oil, flavanone polyphenols, antioxidant activity, flavonoids.

### **INTRODUCTION**

Free radicals or reactive oxygen species (ROS) are produced in vivo from biochemical reactions and also from the respiratory chain as a result of occasional leakage. These free radicals are the main agents in lipid peroxidation (*Cheeseman and Scater, 2003*).

Phenolic compounds exhibit a wide range of physiological properties such as anti-allergenic, anti-atherogenic cardio protective, antithrombic; anti-inflammatory, anti-bacterial and antioxidant effects (*Heim et al., 2000, Manach et al., 2005 and Balasundram et al., 2006*). Recent scientific study has proved that antioxidants are capable of protecting cells from radical damage (*Saint-Grick , et al., 1999*). Furthermore, anticarcinogenic effects (*Carrol, et al., 1999 and Kawaii et al., 1999*).

Fruits are the major sources of phenolic compounds in the human diet and could be valuable natural sources of antioxidants, some of these by-products have been the subject of investigations and have proven to be effective source of phenolic antioxidants (*Balasundram et al., 2006*). Fruits and vegetables are considered as a good sources of natural antioxidants such as vitamins, carotonoids, flavonoids and other phenolic compounds

(Minussi *et al.*, 2003, Zhang and Hamazu.,2004 and Abdel-Rahman *et al.*, 2009).

Fruits, vegetables and beverages are the major sources of phenolic compounds in the human diet. The food and agricultural products processing industries generate substantial quantities of phenolic rich by products, which could be valuable natural sources of antioxidants as reported by (Nagendran *et al.*, 2005) and (Balasundram *et al.*, 2006). Citrus fruits are rich in naturally occurring flavonoids, which are primarily found in peel.

Citrus peel is known to have different antioxidative compounds and rich in phenolic acids, flavonoids, and flavonals as reported by (Alexandra, *et al.*, 1998) and (Abdel-Rahman *et al.*, 2009). The citrus industry produces large quantities of peels and seed residue, which may account for up to 50% of the total fruit weight (Bocco *et al.*, 1998). Citrus industry by-products is considered as the major source of phenolic compounds as the peels, in particular, have been found to contain higher amounts of phenolic compared to the edible portions (Gorinstein *et al.*, 2001).

Fats and oils undergo pronounced oxidative changes at elevated temperature during storage. The oxidative changes decrease the nutritional quality of fats and oils. However, addition of suitable antioxidant retard the oxidation process. Synthetic antioxidants, especially butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) are commonly used to prevent lipid oxidation (Rehman, 2003). But these are known to have toxic and carcinogenic effects on humans. Dietary antioxidants protect against free radicals such as reactive oxygen species (ROS) in the human body (Nilsson *et al.*, 2004). Therefore, there is great interest in finding new and safe antioxidants from natural sources, as alternative synthetic antioxidants (Alexander *et al.*, 1998 and Abdel-Rhman *et al.*, 2009).

The present study aimed to investigate the utilization of orange peel extract, as a source of natural antioxidants, in refined sunflower oil during subsequent storage period at room temperature ( $20\pm 3^{\circ}\text{C}$ ). Also, the comparative study between orange peel antioxidants as natural antioxidants and synthetic ones (BHT and BHA) was carried out.

## **MATERIALS AND METHODS**

### **Materials:**

Orange samples (*Citrus Sinesis*) Balady Var. were obtained from Benha local market, in Benha city, Egypt. Refined sunflower oil samples, free from any additives were obtained from Tanta Company of Oils, Tanta city, Egypt.

### **Methods:**

#### **Preparation of orange peel powder:**

Orange fruits were washed, hand peeled, the peels were cut into small slices, then dried in solar energy and ground into fine powder with a mortar and pestle.

#### **Extraction of phenolic compounds:**

Total phenolic compounds of orange peel powder were extracted by different organic solvents (petroleum ether, ethanol, methanol, hexane, diethyl

ether and acetone). Extraction was carried out using a shaking filtration watman no. 1 filter paper. The residue was re-extracted in the same manner and the two filtrates were combined (Sobhy, and Abdalla, 2009). The extract obtains after evaporation below 40°C of organic solvent was used as natural antioxidant.

#### **Preparation of oil samples:**

Each 100ml refined sun flower oil sample was placed in a 250ml brown glass bottle. The oil samples were mixed with 1000, 1500 and 2000ppm from methanolic extract of orange peel powder, (MEOP) also synthetic antioxidant (BHT and BHA) with concentration 200ppm were mixed in oil samples as the legal limit according to *Rehman, (2006)*. All sunflower oil samples of each treatment were prepared triplicate the oil samples of each treatment. Determination of chemical changes were studied during subsequent storage at room temperature 20±3C°. Total phenolics were determined by using folin-ciocalteu micro method according to the method described by *Slinkard and Singleton, (1977)*. The phenolic content was expressed as mg/g gallic acid equivalents.

#### **Analytical methods**

Peroxide value (POV) meq/kg<sup>-1</sup> of sunflower oil samples was determined according to the method described by *Pearson, (1973)*. Free fatty acids (FFA) as oleic acid percentages and iodine value (IV) of sunflower oil were determined according the method described by *AOAC, (2000)*. Statistical analysis of data was carried out by two ways analysis of variance (ANOVA, F Test) and least significant differences (L. S. D) according to *Scnedecor and Cochran, (1980)*

## **RESULTS AND DISCUSSION**

#### **Extraction of phenolic compounds**

Total polyphenols were extracted from orange peel powder using different organic solvents petroleum ether, diethyl ether, ethanol, methanol, acetone and hexane. the percentage of yield extract and total polyphenols are shown in table (1) and fig (1) Results, indicate the significant differences ( $p \leq 0.05$ ) of extracts of orange peel powder. Methanolic extracts proved that the best solvent for yield extracts (10.53%), followed by petroleum ether (9.3%), diethyl ether (9.03%), ethanol (7.73%), acetone (6.4%) and hexane (6.03%). Extraction yield of extracts depended on the solvents due to the different polarity of organic solvents (*Marinova and Yanishlivea, 1997*).

Table (1) and figure (1) illustrate also the total polyphenols content of different organic solvents. It is evident from results that, polyphenols content of different organic solvents extract from orange peel powder (mg/g gallic acid equivalents on dry weight basis) showed that varying of total polyphenols due to different solvents. However, methanol extract recorded the highest value of polyphenols (1.70 mg/g) followed by petroleum ether (1.40 mg/g), diethyl ether (1.13 mg/g), ethanol 1.03, acetone (0.94 mg/g) and hexane (0.80 mg/g). There were significant difference ( $p \leq 0.05$ ) of polyphenols content among all extracts. Also, there was a strong positive relationship between percent yield extracts of different organic solvents as shown in Table

(1) and total polyphenols of orange peels. These results are in agreement with those found by (Abdel Rahman et al., 2009).

**Treatment of sunflower oil with MEOP during storage**

Sunflower oil was treated with methanolic extract of orange peel powder (MEOP) compared with synthetic antioxidants (BHA and BHT) the oil was stored at  $20 \pm 3^\circ\text{C}$  for 8 months. Some chemical properties of oil (PV, FFA and IV) were determined monthly. The results are illustrated in Table (2, 3 and 4). Results in table (3) showed that the higher concentration of MEOP, the lower value of POV was observed. This may be due to orange peel is a rich source of polyphenols as shown in the previously table (2). The same trend of results is in agreement with those obtained by (John, 2004 and Rehman, 2006).

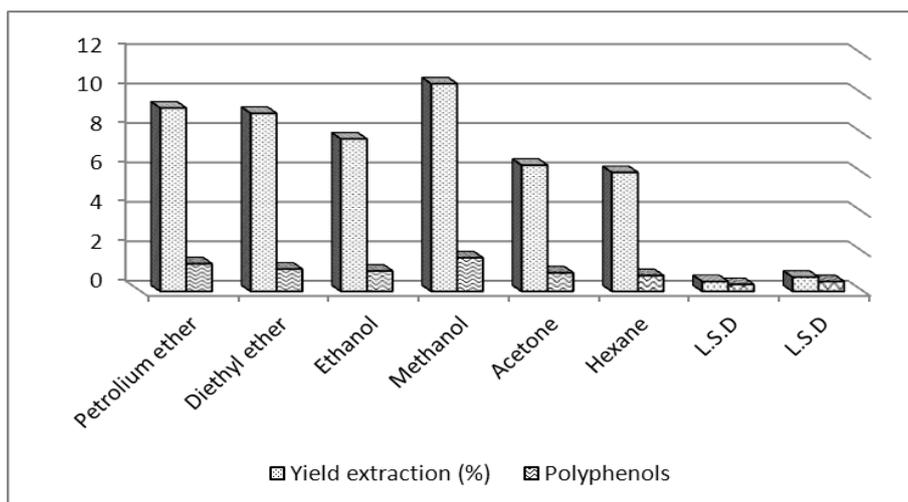
Peroxide value of sunflower oil was significantly reduced ( $p \leq 0.05$ ) by the addition of natural MEOP and synthetic antioxidants. However, BHT showed the better results than BHA. These results are consistent with those findings of (Rehman, 2003).

At the end of storage period, (POV) of refined sunflower oil (control) recorded the highest value ( $77.3 \text{ meq/kg}^{-1}$ ), meanwhile the lowest value was found in oil treated with 2000 ppm of MEOP ( $6.77 \text{ meq/kg}^{-1}$ ); followed by 1500 ppm ( $6.90 \text{ meq/kg}^{-1}$ ); BHT ( $8.80 \text{ meq/kg}^{-1}$ ) and BHA ( $10.4 \text{ meq/kg}^{-1}$ ).

Lipid peroxides were significantly reduced by the addition of antioxidants in oils (Abdel Rhman et al., 2009). Antioxidants decreasing the localized oxygen concentrations; preventing chain initiation by scavenging radicals; binding catalysis, prevent initiating radical generation and decomposing peroxides so they cannot be reconverted to initiating radical (Dorman, et al., 2003).

**Table (1): Yield extract and total poly phenols from orange peel powder using different organic solvents.**

Solvents	Petroleum ether	Diethyl ether	Ethanol	Methanol	Acetone	Hexane	L.S.D (0.05%)	L.S.D (0.01%)
Yield extraction (%)	9.30	9.03	7.73	10.53	6.40	6.03	0.502	0.723
Polyphenols (mg/g)	1.40	1.13	1.03	1.70	0.94	0.80	0.350	0.498



**Fig. (1): Yield extract and total poly phenols from orange peel powder using different organic solvents.**

**Table (2): Peroxide value of sun flower oil treated methanolic extracts of orange peels (meq/ kg) during storage period.**

Antioxidants \ Storage period	Refined oil (control)	BHA 200 ppm	BHT 200 ppm	MEOP 1000 ppm	MEOP 1500 ppm	MEOP 2000 ppm
Zero time	0.62	0.62	0.62	0.62	0.62	0.62
1 month	14.3	5.30	4.17	6.07	5.53	5.07
2 Months	27.0	6.40	5.77	7.0	5.70	5.40
3 Months	46.3	8.33	7.40	9.0	7.13	6.80
4 Months	63.0	9.53	7.80	10.9	8.20	8.27
5 Months	77.3	10.4	8.80	11.2	6.90	6.77

BHT: Butylated hydroxy toluene.

BHA: Butylated hydroxy anison.

MEOP: Methanolic extract of orange peel.

LSD (0.05%)= 1.96

LSD (0.01%)= 2.58

Table (3) illustrates free fatty acids (FFA<sub>s</sub>) of sunflower oil treated with synthetic antioxidants (BHT and BHA and methanolic extracts from orange peel (MEOP) during storage at room temperature. Results revealed that the addition of BHT and BHA to sunflower oil decreased FFA<sub>s</sub>. However, BHT showed the higher reduction of FFA<sub>s</sub> than BHA. On the other hand, the higher concentration of methanolic extract, the higher inhibitory effects of FFA<sub>s</sub> was observed during storage. These results were in agreement with those found by Rehman, (2006). It is evident from these result. that refined oil was significantly higher ( $p \leq 0.05$ ) than other samples. Moreover, the addition of both synthetic antioxidants (BHT and BHA) and natural MEOP to sunflower oil retarded the development of rancidity.

**Table (3): Free fatty acid of sunflower oil treated with methanolic extracts of orange peel during storage period.**

Antioxidants	Refined oil (control)	BHT 200 ppm	BHA 200 ppm	MEOP 1000 ppm	MEOP 1500 ppm	MEOP 2000 ppm
Storage period						
Zero time	0.035	0.035	0.035	0.035	0.035	0.035
1 month	0.150	0.072	0.08	0.091	0.090	0.082
2 Months	0.235	0.121	0.136	0.129	0.145	0.129
3 Months	0.450	0.155	0.167	0.175	0.169	0.165
4 Months	0.680	0.196	0.199	0.210	0.201	0.200
5 Months	0.710	0.251	0.270	0.286	0.280	0.275

BHT: Butylated hydroxy toluene.

LSD (0.05)= 2.15

BHA: Butylated hydroxy anison.

LSD (0.01)= 2.83

MEOP: Methanolic extract of orange peel.

At the end of storage, sunflower oil treated with BHT showed a reduction of FFA<sub>s</sub> by about 64.6%, followed by BHA 62.0%. Meanwhile, addition of MEOP with 1000, 1500 and 2000 ppm decreased FFAs by about 59.7%, 60.6% and 61.3%, respectively as compared to refined sunflower oil (without any addition). Results proved that polyphenols of orange peels is considered as a good source of natural antioxidants which prevent oil from peroxidation and rancidity. Moreover, their efficiency as antioxidative activity when compared to BHA and BHT.

Table (4) shows iodine value (IVs) of sunflower oil treated with synthetic antioxidants (BHT and BHA) and natural antioxidants (MEOP) during subsequent storage at room temperature. Results indicated that (IVs) of oil were decreased as the time of storage increased. Moreover, addition of both synthetic antioxidants (BHT and BHA) and natural MEOP retarded the decreasing trend of IVs of oil during storage. This decrease indicates the development of rancidity (FFA) due to formation of secondary oxidation products during storage of oil as shown in previously tables (3 and 4). Besides, increase of PV and FFA of sunflower oil, a marked decrease in iodine value was observed during storage period.

**Table (4): Iodine value of sunflower oil treated with methanolic extracts of orange peel during storage (meq/kg).**

Antioxidants	Refined oil (control)	BHT 200 ppm	BHA 200 ppm	MEOP 1000 ppm	MEOP 1500 ppm	MEOP 2000 ppm
Storage period						
Zero time	177	107.7	107.7	107.7	107.7	107.7
1 month	88.0	104	105	97.0	93.0	94.0
2 Months	77.0	90.7	93.7	89.0	90.3	91.3
3 Months	64.7	85.3	88.3	77.6	80.0	83.7
4 Months	57.0	78.7	81.0	73.0	75.7	80.3
5 Months	51.2	71.3	71.0	63.3	67.0	70.3

BHT: Butylated hydroxy toluene.

LSD (0.05%)= 1.90

BHA: Butylated hydroxy anisone.

LSD (0.01%)= 2.88

MEOP: Methanolic extract of orange peel.

Iodine value could be attributed to breaking the double bonds of unsaturated fatty acids. These results, were in agreement with those found by

Rehman, (2006). Antioxidantive effect was observed in orange peel due to the presence of phenolic compounds flavonones, and flavone (Johne, 2004). At the end of storage, IV of refined oil was 51.2 meanwhile, addition of BHA to oil were 71.3 and 71.0, respectively. Moreover oil treated with MEOP (1000, 1500 and 2000 ppm) were 63.3, 67.0 and 70.3 Meg/Kg, respectively. Iodine value of refined oil showed a higher significant decrease ( $P \leq 0.05$ ) among all oil treatments.

It could be concluded that MEOP is considered as a good source of polyphenols. Also, it exhibited strong antioxidant activity, which was almost equal to BHT and BHA. However, the concentration of MEOP was about from 7 and 10 times higher than that of BHT and BHA. Moreover, synthetic antioxidants can be replaced by MEOP to control peroxidation and rancidity of fats and oils .

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## تأثير مضادات الأكسدة الطبيعية في قشور البرتقال على زيت عباد الشمس خلال فترة التخزين

عمر أحمد إمام و غادة محمود البسيوني

قسم الإقتصاد المنزلي- كلية التربية النوعية - جامعة بنها - مصر

تم استخلاص المركبات الفينولية من قشور البرتقال البلدي باستخدام بعض المذيبات العضوية (الميثانول، الإيثانول، الإثير البترولي، الداى إيثيل إثير، الهكسان، الأسيتون). وقد تم دراسة النشاط المضاد للأكسدة وذلك عن طريق تقدير رقم البروكسيد، الأحماض الدهنية الحرة، الرقم اليودي لزيت عباد الشمس حيث تم استخدام كل من مضادات الأكسدة الصناعية (بيوتيلاتيد هيدروكسي تولوين، بيوتيلاتيد هيدروكسي أنيسون بتركيز ٢٠٠ جزء في المليون) والمستخلص الميثانولي بتركيزات ١٠٠٠، ١٥٠٠، ٢٠٠٠ جزء في المليون وتخزين الزيت لمدة ٥ شهور على درجة حرارة الغرفة  $20 \pm 3$  م. وقد دلت النتائج على ما يلي:

- أثبت الميثانول بأنه أفضل المذيبات المستخدمة من حيث نسبة الاستخلاص الناتجة وكذلك الفينولات الكلية.
- لوحظ أنه كلما زادت تركيزات المستخلص الميثانولي كلما أدى إلى إنخفاض رقم البيروكسيد وكذلك الأحماض الدهنية الحرة بينما زاد الرقم اليودي خلال مدة التخزين.
- وفي نهاية فترة التخزين أظهرت عينات الزيت المعاملة بتركيزات (١٥٠٠، ٢٠٠٠) جزء في المليون/ إنخفاض معنوي عند مستوي معنوية ٥% في كل من رقم البروكسيد (٦.٩٠، ٦.٧٧ ملليمكافى/ كجم زيت) والأحماض الدهنية الحرة (٠.٢٨٠، ٠.٢٧٥%) وزيادة الرقم اليودي (٦٧، ٧٠.٣) على التوالي وذلك بالمقارنة بالزيت الخام (بدون أي إضافات) حيث كان رقم البروكسيد (٧٧.٣ ملليمكافى / كجم زيت) الأحماض الدهنية الحرة (٠.٧١٠%) والرقم اليودي (٥٢.٢).
- أدت إضافة مضادات الأكسدة الصناعية (BHA, BHT) إلى إنخفاض كل من رقم البروكسيد (١٠.٤، ٨.٨٠ ملليمكافى / كجم زيت) والأحماض الدهنية الحرة (٠.٢٥١، ٠.٢٧١%) وزيادة الرقم اليودي (٧١.٣ / ٧١) على التوالي وقد أعطي BHT نتائج أفضل من BHA.
- وبالتالي فقد أثبت المستخلص الميثانولي لقشور البرتقال كفاءة عالية كمضاد أكسدة طبيعي بالمقارنة بمضادات الأكسدة الصناعية لذا فإنه يوصى باستخدامه كمضاد أكسدة طبيعي بدلاً من الصناعي في حفظ الزيوت من الأكسدة والتزنخ.