Utilization of Some Vegetables and Fruits Waste As Natural Antioxidants

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ABSTRACT

Natural antioxidants are in great demand today due to both consumer preference and health concerns associated with the use of synthetic antioxidants, so this study was carried out to investigate the extraction, identification of antioxidant compounds in some vegetables and fruits waste and to evaluate its extract as natural antioxidants. Total phenolics were determined in methanolic extract. Phenolic compounds in each methanolic extract were screened by TLC and identified by HPLC. Antioxidant activities of these waste materials have been measured by PV (Peroxide Value), CDH (Conjugated Diene Hydroperoxide), TBA (Thiobarbituric Acid Value), AV (Anisidine Value) and TV (Totox Value) methods. TLC plates showed that vegetables, and fruit waste, contained antioxidant components. The percentage of total phenolic content can be summarized as follows: olive leaves > tomato peel > orange peel > cucumber peel > water melon peel > potato peel. All extracts exhibited antioxidant activity. Tomato extract (600 ppm) has high antioxidant activity which was lower than the control sample and had the same activity as TBHQ (200 ppm) during storage period of sunflower oil; this extract also exhibited antioxidant activity which was higher than cucumber peel and water melon peel. From the economical point of view, vegetables and fruits waste as natural source of antioxidants may play an important role in industry.

Key words: natural antioxidant, health concerns, synthetic antioxidant, phenolic compounds, conjugated diene hydroperoxide, TBHQ.

INTRODUCTION

Many of the antioxidants other than vitamin C, vitamin E and carotenoids, occur as dietary constituents (Robards *et al*., 1999). Moreover, Kalt *et al*. (1999) found strong antioxidant activity in fruits for example; antioxidants with important activity have been found in berries, and cherries. Strawberry showed high antioxidant capacity, total phenolics, and anthocyanins (Ayala-Zavala *et al*., 2004). Pumpkin seeds oil contains antioxidative components that are polar (phenolic) compounds (Fruhwirth *et al*., 2003). The antioxidant activities were shown in several citrus peel and seed extracts (lemon, bergamot, sour and sweet orange) that were obtained either by methanol extraction or alkaline hydrolysis. Several studies have analyzed the antioxidant potential of a wide variety of vegetables and particularly, of cacao beans, potato, spinach, legumes such as *Phaseolus vulgaris* (Moure *et al*., 2001), and tomato, which contains lycopene concentrate (LC). Lycopene is thus reported to have the potential to be used in anti-cancer medicines or healthcare products (Wenli *et al*., 2001).

Antioxidants play an important role in preventing undesirable changes in flavour and nutritional quality of foods. Antioxidants protect the cells against tissue damage associated with various human diseases (Shahidi *et al*., 1992, Jang *et al*., 1997, Arai *et al*., 2000). Synthetic antioxidants are widely used as food additives, but their application has been reassessed because of possible toxic or carcinogenic components formed during their degradation (Namiki, 1990). Consequently, the search for endogenous protective ingredients in accepted foods has been intensified, as their utilization will require only manipulation of food formulations. Identification of polyphenolic compounds in apple, and grape pomace (Abou Rayan *et al*., 1998, Lu, & Foo, 2000), citrus seeds and peels (Bocco *et al*., 1998), carrot pulp waste (Chen & Tang, 1998), old tea leaves (Zandi & Gordon, 1999), cocoa byproducts (Azizah *et al*., 1999), white grapefruit and its hybrid (Gorinstein *et al*., 2004), sunflower hull "Vedoc" (Gamal & Fakhriya, 2005), non-volatile residues from orange essential oil (Vargas-Arispuro *et al*., 1998), and soybean molasses (Hosny & Rosazza, 1999) have also been reported.

Agricultural and industrial residues are attractive sources of natural antioxidants. Natural compounds with antioxidant property were isolated

from common vegetable by-products (tomato seeds, seeds of green pepper, the outer leaves of yellow onion, peels of green beans and potato peel waste, rape of olive, olive mill waste waters, and grape seeds) (Hemaida, 1994, Larrosa *et al*., 2002).

In Egypt, there are many sources of vegetables and fruits waste but there is a lack of information about its content and activity of antioxidant compounds. Therefore, the objective of this study was to investigate the extraction, identification of antioxidant compounds in some vegetables and fruits waste, as well as, to evaluate these wastes as natural antioxidants.

MATERIALS AND METHODS

Materials:

Plant Materials: Vegetables and fruits used in this study were obtained from local grocery at Alexandria. Waste materials used were namely carrot peel (*Daucus carota*), cucumber peel (*Cucumis sarivus*), potato peel (*Solanum tuberosum* L.), tomato peel (*Lycopersicon esculentum*), water melon peel (*Citrullus vulgaris*), olive leaves (*Olea europea* L.).

Oil: Refined, bleached, and deodorized (RBD) sunflower oil used in the present study without any additives was obtained from Sila Company, at Fayoum, Egypt. As sunflower oil is easily oxidized, it was chosen for testing the antioxidant activity of the dried powder and the extracts of each plant material (Crapiste *et al*., 1999).

Methods:

Vegetables and fruits waste were obtained by peeling vegetables or fruits, then peels were rinsed with distilled water and dried at room temperature (25±2ºC), then overnight (40±2ºC) in an air draft drying oven (WT-binder labortechnik GMBH) until the moisture content became 12% or less.

Then they were ground and sieved through 60-mesh sieve, and finally cooled or kept at 4ºC for further treatments and/or analysis.

Extraction of antioxidant compounds: The antioxidant compounds were extracted according to the method described by Adegoke & Gopala Krishna (1998) with some modifications as follows: Firstly, one sample from each species was chosen to select the optimum solvent: from vegetables waste (tomato peel); and from fruits waste (water melon peel). The antioxidant compounds were extracted with different solvents at ratio of 1:5 w/v (methanol, ethanol, diethyl ether, acetone, chloroform, and hexane) in order to determine which solvent will give the highest amount of extracted yield. According to this primary study methanol gave the highest amount of extracted yield.

The powder of each dried sample (100g each) was extracted using methanol (500ml), with constant stirring for 24 hours at room temperature (25±2ºC). The extracts were filtered with Whatmann No. 1 filter paper. The filtered material was re-extracted to maximize the antioxidant extract. The filtrate was evaporated under vacuum in a rotary evaporator at 45ºC and weighed to determine the extracted yield of each plant material. The colours of the methanolic extracts were described visually.

Isolation and identification of antioxidant compounds: The antioxidant compounds were isolated and identified using thin-layer chromatography (TLC) plates (10 × 20 cm) coated with silica gel G to 0.3 mm thickness. The plates were spotted with 20 µl of each antioxidant extract (1% methanol solution used for extract preparation). The plates were then developed in the upper phase of chloroform / ethanol / acetic acid (98: 2: 2). The TLC plates were sprayed with $FeCl_3$ to identify the phenolic components as described by Pratt & Miller (1984) and Xing & White (1997).

Determination of total phenolic content: Total phenol (TP) contents of the extract were assayed colorimetrically using the Folin-Ciocalteu method (Gamez-Meza *et al*., 1999), where an aliquot (1ml) of the extract was mixed with diluted Folin-Ciocalteu reagent (0.5 ml) and 2% ethanol amine (1 ml) at room temperature. The absorbance was measured at 750 nm using a Shimadzu 160 1 PC UV – visible spectrophotometer.

Identification of phenolic compounds with HPLC: To identify the compounds of the methanolic plant extracts used in this study, HPLC system was carried out according to Lin *et al*. (1998) using a Waters 600 E system controller. The Waters 484 tunable absorbance detector was used to detect phenolic compounds constituents at 280 nm, and all peaks were plotted and integrated by a Waters 745 data module. The HPLC method used a Cosmosil (C18-MS packed column 5 µm, 46 mmi.d. x 250 mm) (Nacalai Tesque, Inc., Kyoto, Japan). The plant material extracts were filtered through a 0.45

µm filter disk, and then 20 µl was injected into the column. Each authentic standard compound was injected. The mobile phase was methanol/ distilled water / formic acid (19.5: 80.2: 0.3, v/v/v) and run by an isocratic elution at a flow rate of 1 ml/min. For the gradient elution, the solvent systems that were used: mobile phase A, methanol/ formic acid/ water (20: 0.3: 79.7, v/v/v; mobile phase B, methanol/ formic acid (99.7: 0.3, v/v). The gradient HPLC was performed as follows: 100% A for 10 min, to 90% A and 10% B for 15 min, and to 70% A and 30% B for 35 min in a linear gradient mode; elution was continued for 15 min. In all cases, the flow rate was 1.0ml/min and continuous bubbling with helium gas degassed both mobile phase flasks.

Identification of the phenolic compounds was based on the comparison of the retention times of unknown peaks to those reference authentic standards. The amount of each constituent in the plant material extract was estimated by the integrated datum provided by the Waters data module.

Determination of the antioxidant activity: The antioxidant activity of each of tomato peel, cucumber peel and water melon peel was tested in both dried powder and methanolic extract powder, the extracts were added separately to 50 g of sunflower oil. At the same time, TBHQ as a synthetic antioxidant (200 ppm food grade) was added to sunflower oil, as a control sample. The oxidation effect of sunflower oil containing no additives was measured for reference purposes.

Oil and additives were placed in 100 ml beakers and thoroughly mixed by ultrasonic waves using a Soniprep 150.

Beakers were transferred to a drying oven set at 60±2ºC for up to 18 days. Peroxide values (PV), and anisidine value (AV) were determined at zero time, 6, 9, 12, 15 and 18 days. The obtained data were used to calculate, the totox value (TV) according to AOCS official methods (1989).

RESULTS AND DISCUSSION

Separation of phenolic compound by TLC: Extracts of some vegetables and fruits waste collected as natural sources for antioxidants, were screened by TLC to identify their content of phenolic compounds. The TLC plates showed that vegetables, and fruits waste, contained antioxidant components as they produced clear colour bands on the TLC plates (Fig 1a, b). It is clear from the TLC plates that there are great variations among the studied samples materials for their antioxidant components.

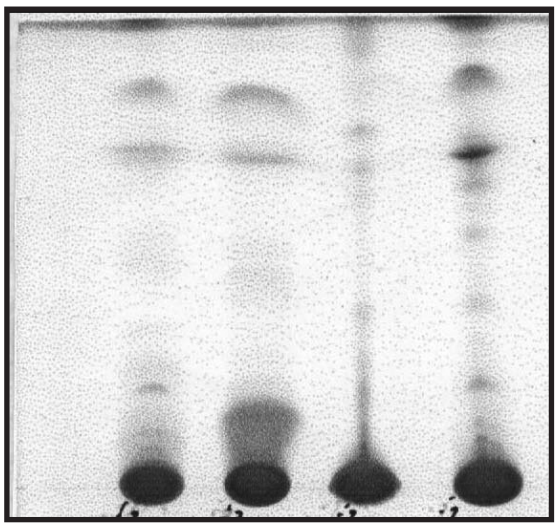

Fig. (1a): Separation of phenolic compound by TLC for some vegetables and fruits waste

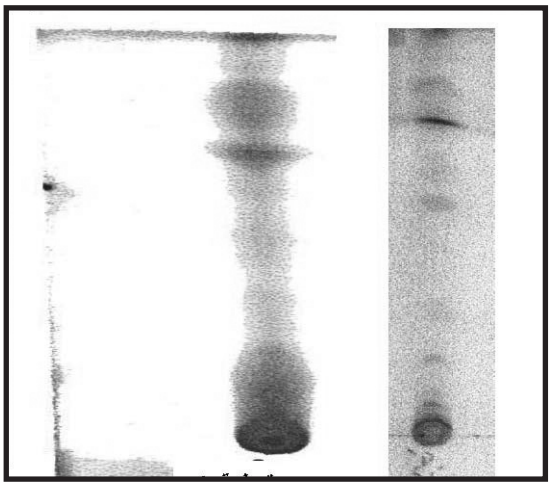

Fig. (1b): Separation of phenolic compound by TLC of some vegetables and fruits waste

Extracted yield and total phenolic contents of different plant materials:

The total phenolic content appeared to be proportional to the extracted yield (%). It was noted that the watermelon peel phenolic content (9.86 g/kg), is very low when compared to tomato peel phenolic content (68.79 g/kg) (Table 1). These variations in total phenolic content could be attributed to the specific nature of the plant type. Kim *et al*., (1994) reported that the antioxidant activity of extracts produced from herbs was dependent on the type of herb rather than the solvent use. The percentage of total phenolic content can be summarized as follows: olive leaves > tomato peel > orange peel > cucumber peel > water melon peel > potato peel.

Table 1: Extracted yield and total phenolic contents of different plant materials

Plant Material	Methanolic extracted yield (%)	Total phenolic compounds (%)
Potato peel	0.421	0.039
Tomato peel	8.142	6.879
Cucumber peel	4.080	1.121
Orange peel	4.250	2.335
Watermelon peel	8.166	0.986
Olive leaves	12.441	12.098

Identification of phenolic compounds with HPLC: Table (2) shows the percentage of each phenolic compound in tomato peel, cucumber peel, potato peel, watermelon peel and orange peel extracts. There was a great variation among the components identified in the methanolic extract of each plant material.

Phenolic compounds are widely distributed in nature. It is suggested that their antioxidant activity is related to their conjugated rings and hydroxyl groups (Decker, 1995).

The major phenolic compounds in potato peel (Table 2) were identified as chlorogenic acid, gallic acid, caffeic acid, pretocatecheic, vanillic acid and ρ-hydroxybenzoic acid with amounts ranging from 0.04 – 1.8 mg/g. Lyon & Barker (1984), Malmberg & Theander (1985), Ramamurthy *et al*. (1992) and Onyeneho & Hettiarachchy (1993) all reported the same phenolic acids in the potato peel.

At the same time, seven phenolic compounds were identified in olive leaves, namely oleuropein, apigenin 7-glucoside, rutin, vanillin, vanillic acid, caffeic acid and hydroxytyrosel with amounts ranging from 0.15–71.61 mg/g. These data are in agreement with data obtained by Benavente – Garcia *et al*. (2000), who studied the antioxidant activity of phenolics extracted from *Olea europeae* L. leaves. They found the same main phenolic compounds in olive leave extracts.

Cis-lycopene and trans- lycopene were the major components amounted to 58.4 mg/g of the tomato peel. Lycopene is one of the most effective singlet oxygen quenchers (Zhao *et al*., 1989). Also Wenli *et al*. (2001) concluded that lycopene is effective in scavenging such reactive oxygen species as superoxide anion, hydroxyl radical, singlet oxygen, and lipid free radical. This finding favorably supported the significant role of lycopene rich foods in the prevention of chronic diseases and cancer, which have been observed in cell culture, animal experiments, and clinics (Rao & Agarwal, 1999).

Table 2: Composition and content of phenolic compounds in methanolic extracts of various plant materials as determined by HPLC

Plant material	Compound	Compound content (mg/g)
Tomato peel	Cis-lycopene	22.02
	Beta carotene	6.87
	Trans-lycopene	36.49
	Lutein	1.08
	Ascorbic acid	12.27
	Quercetin	2.89
	Kaempferal	7.2
Cucumber peel	Chlorophyll	3.46
	Pheophytin	1.95
	Phellandrene	1.21
	Caryophellene	1.49
Water melon peel	Chlorophyll	5.28
	Diosmetin	1.57
	Pheophytin	1.27
	Mlvidin 3,5 diglycoside	1.23
Potato peel	Gallic acid	0.16
	Pretocatecheic	1.84
	p- Hydroxybenzoic	0.26
	Caffeic acid	0.19
	Vanillic acid	0.04
	Chlorogenic acid	0.28
Orange peel	P-coumaric	1.02
	Ferulic acid	0.91
	Syingic acid	7.71
	Naritutin	1.21
	Nazirgin	3.83
	Ascorbic acid	14.9
Olive Leaves	Oleuropein	71.61
	Apigenin 7-glucoside	4.1
	Rutin	0.15
	Vanillin	0.15
	Vanillic acid	1.87
	Caffeic acid	1.02
	Hydroxytyrosel	3.29

Chlorophyll and pheophytins were identified with the highest amount in both cucumber and water melon peel (Table 2). Frankel *et al*., (1997) reported that chlorophyll and pheophytin may act as photosensitizers.

Assessment of antioxidant activity of the studied plant materials in oil using different measures:

Peroxide Value: Peroxide value (meq O_2/ kg) was determined during accelerated storage of sunflower oil at 60±2ºC as primary products of autooxidation to evaluate the antioxidant activity of the dry powder and methanolic extract of each studied plant material.

Tomato peel was chosen due to its phenolic content as a representative of the waste that contains red pigments. Meanwhile, cucumber peels as well as water melon peel were chosen as waste materials that contain green pigments.

Figure (2) shows the antioxidant activity of the tomato peel powder, which was added to sunflower oil. The peroxide values of sunflower oil having tomato peel powder as additive, was always lower than the control sample (no addition), and higher than TBHQ (200 ppm) during storage for 18 days at 60±2ºC. The antioxidant extract had the same antioxidant activity equivalent to TBHQ (200 ppm) during the storage period at the acceleration temperature. Activity of tomato peel extract was evaluated using the same previously reported methods. It is clear from Fig. (3) that 600 ppm of tomato peel extract had the same antioxidant activity equivalent to TBHQ (200 ppm) during the storage period at accelerated temperature used.

Figure (4) also shows the effect of cucumber peel powder on the oxidative stability of sunflower oil. In spite of the fact that cucumber, even where using as high concentration as 800 ppm of both powder and extract, had lower antioxidant activity

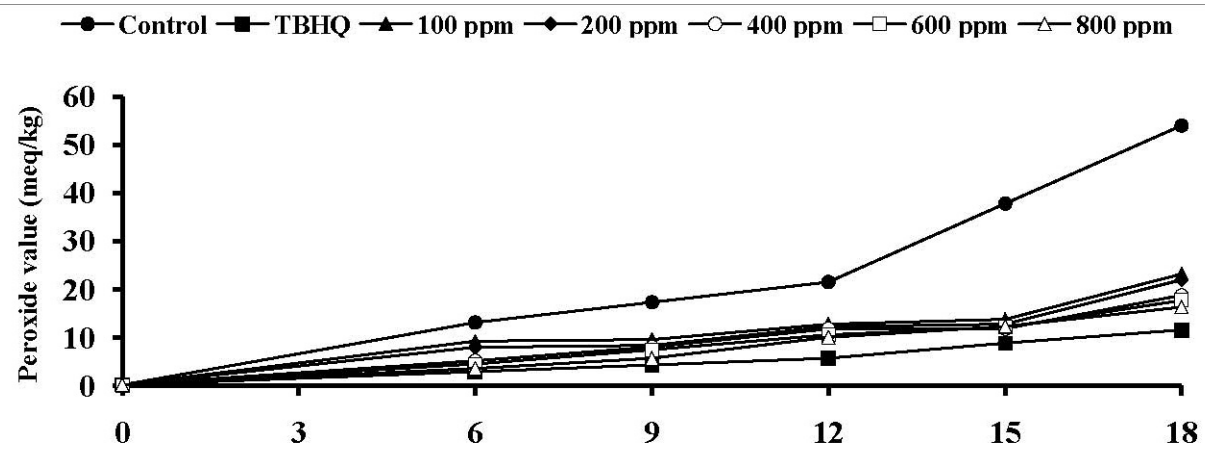

Fig. 2: Peroxide value of sunflower oil with added powder of tomato peel during different storage time at 60±2°C

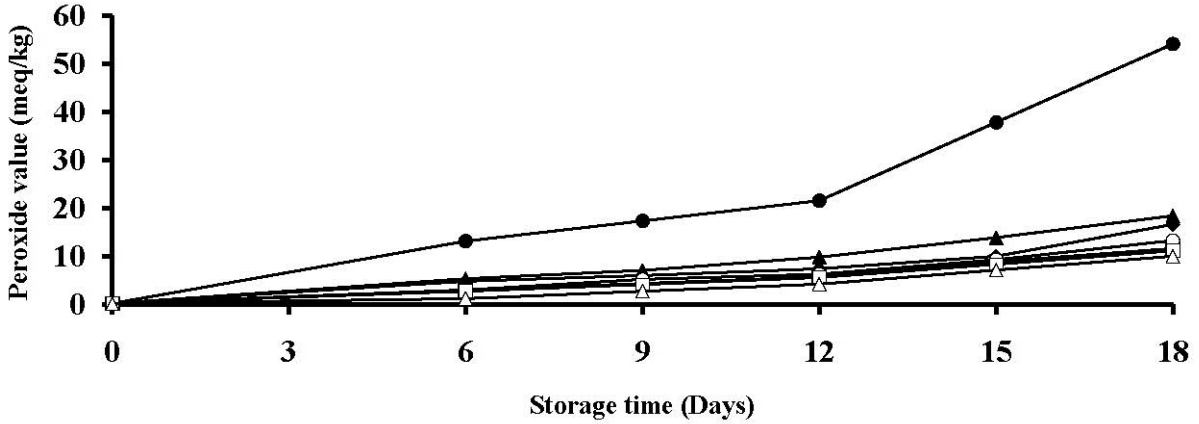

Fig. 3: Peroxide value of sunflower oil with added methanol extract of tomato peel during different storage time at 60±2°C

than that produced by 200 ppm additives of TBHQ (Fig. 5). It still showed an antioxidant activity when compared to the control sample without addition. The same trend was obtained for water melon peel powder and its methanolic extract. Figures (6 & 7) revealed that the antioxidant activity of water melon peel was nearly the same as the antioxidant activity of cucumber peel. Referring to the results of sunflower oxidative stability with adding different plant materials extract, there were great variations

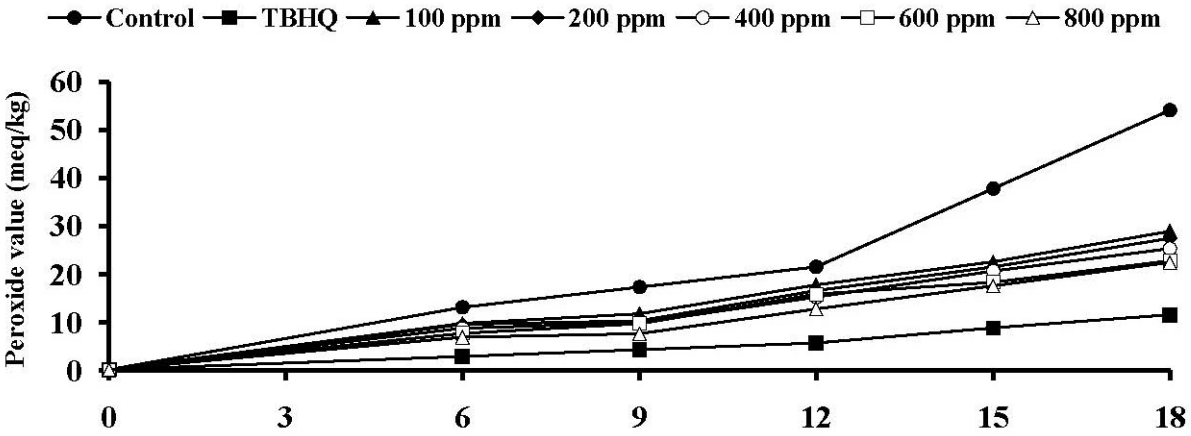

Fig. 4: Peroxide value of sunflower oil with added powder of cucumber peel during different storage time at 60±2°C

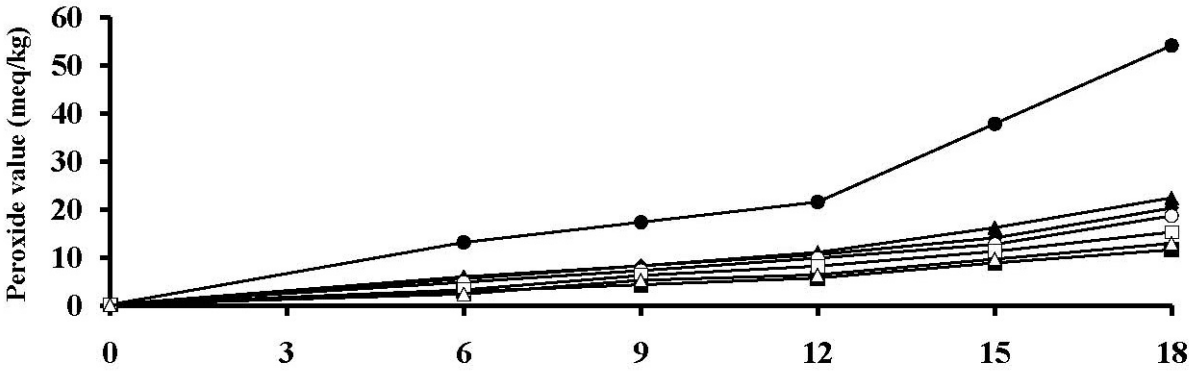

Fig. 5: Peroxide value of sunflower oil with added methanol extracts of cucumber peel during different storage time at 60±2°C

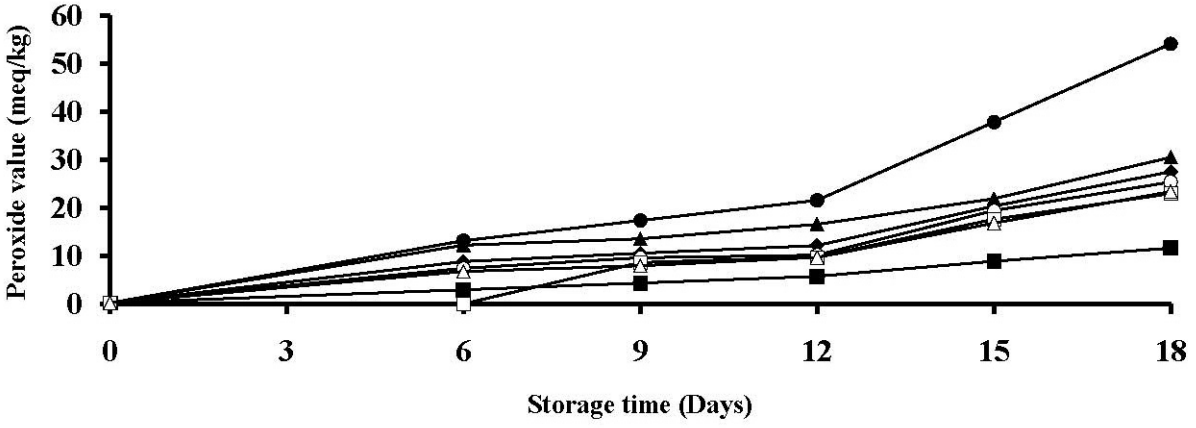

Fig. 6: Peroxide value of sunflower oil with added powder of watermelon peel during different storage time at 60±2°C

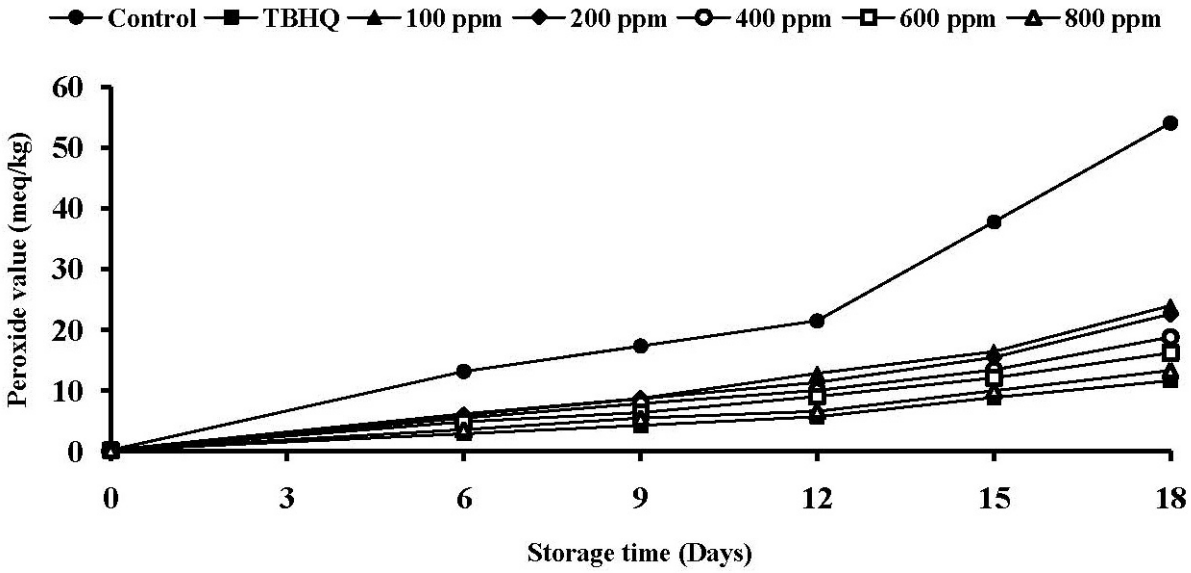

Fig. 7: Peroxide value of sunflower oil with added methanol extract of watermelon peel during different storage time at 60±2°C

in antioxidant activities among the different plant materials tested as antioxidants.

The antioxidant activity can be summarized in the following order: tomato peel > cucumber peel > water melon peel. These variations could be attributed to their phenolic content and their phenolic composition beside other components, which differ in their antioxidant activity rather than its synergistic or antagonistic effects such as chlorophylls. Moreover, the extract form proved to contain much more phenolic compounds than those are in the powder form of the plant material. The highest antioxidant activity of methanolic extracts can be partially attributed to the activity of the present phenolic compounds (Shahidi *et al*., 1992). Many researches found that methanolic extracts contained the most effective antioxidants when produced from different plant sources (Tsuda *et al*., 1993, Yen & Duh, 1993, Mehta *et al*., 1994 & Onyeneho & Hettiarachy, 1988).

The results obtained for anisidine value (Table 3), thiobarbituric acid (Table 4), totox value Table 5) and conjugated diene hydroperoxide (Table 6) are completely corresponding to the data obtained for peroxide value. Measurement of antioxidant activity of sunflower oil by these different methods and different concentrations of each added powder or extract from tomato peel, cucumber peel and water melon peel showed similar trends to those produced by the peroxide value.

Table 3: Anisidine value of sunflower oil with added vegetable and fruit powder and methanolic extract during storage time at 60±2°C

Storage time/days	Control*	TBHQ 200ppm	Anisidine value			Anisidine value		
			200 ppm powder			200 ppm extract		
			Tomato peel	Cucumber peel	Water melon peel	Tomato peel	Cucumber peel	Water melon peel
0	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
6	2.55	1.91	2.20	4.27	5.74	2.13	3.72	4.54
9	5.97	4.03	5.64	6.93	8.09	5.42	6.27	7.10
12	9.56	5.17	6.39	8.94	9.75	6.36	7.48	8.62
15	11.89	7.81	8.37	10.05	11.30	8.73	9.74	10.12
18	14.22	10.40	11.74	11.89	12.66	11.26	11.40	12.07

*Control: sunflower oil without any addition

Table 4: The TBA value of sunflower oil with added vegetable and fruit powder and methanolic extract during storage time at 60±2°C

Storage time/days	Control*	TBHQ 200ppm	TBA (mg malonaldehyde / Kg oil) 200 ppm powder			TBA (mg malonaldehyde / Kg oil) 200 ppm extract		
			Tomato peel	Cucumber peel	Water melon peel	Tomato peel	Cucumber peel	Water melon peel
0	1.00	1.00	1.00	1.00	1.00	4.42	4.96	5.07
6	6.34	3.03	4.64	5.47	5.63	5.94	6.16	6.32
9	12.29	5.02	6.34	7.02	7.26	8.16	8.46	8.54
12	15.13	7.77	8.36	8.65	8.76	8.80	9.02	9.17
15	18.30	8.77	9.12	9.46	9.74	9.60	9.92	9.90
18	21.48	9.78	9.90	10.02	10.27	4.42	4.96	5.07

*Control: sunflower oil without any addition

Table 5: Totox value of sunflower oil with added vegetable and fruit powder and methanolic extract during storage time at 60±2°C

Storage time/days	Control*	TBHQ 200ppm	Totox value 200 ppm powder			Totox value 200 ppm extract		
			Tomato peel	Cucumber peel	Water melon peel	Tomato peel	Cucumber peel	Water melon peel
0	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06
6	28.81	7.75	20.04	23.51	23.38	11.77	14.92	16.54
9	40.61	12.63	25.10	28.53	29.11	17.44	22.75	24.40
12	52.60	16.59	36.57	42.18	33.99	21.20	28.32	31.34
15	87.43	25.59	47.81	53.09	51.90	28.77	37.86	41.08
18	122.22	33.60	64.94	66.73	40.12	44.50	51.92	57.27

*Control: sunflower oil without any addition

Table 6: Effects of vegetable and fruits powder and methanolic extract on the formation of conjugated diene hydroperoxides in sunflower oil during storage time at 60±2°C

Storage time/days	Control*	TBHQ 200ppm	Conjugated diene hydroperoxide 200 ppm powder			Conjugated diene hydroperoxide 200 ppm extract		
			Tomato peel	Cucumber peel	Water melon peel	Tomato peel	Cucumber peel	Water melon peel
0	0.014	0.010	0.015	0.015	0.017	0.014	0.015	0.015
6	0.025	0.014	0.020	0.053	0.054	0.016	0.019	0.020
9	0.070	0.040	0.070	0.112	0.115	0.056	0.064	0.064
12	0.109	0.068	0.095	0.137	0.140	0.079	0.094	0.098
15	0.191	0.092	0.137	0.162	0.171	0.116	0.124	0.129
18	0.287	0.167	0.192	0.210	0.213	0.193	0.224	0.228

*Control: sunflower oil without any addition

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الاستفادة من مخلفات بعض الخضروات والفواكه كمصادر لمضادات الأكسدة الطبيعية

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مضادات الأكسدة الطبيعية موضع طلب كبير اليوم بسبب فوائدها العديدة للصحة العامة. وقد تم التوجه إلى استخدام العديد من مثبطات الاكسدة النباتية كمصدر طبيعي وآمن يمكن استخدامها في التصنيع الغذائي، لذا أجريت هذه الدراسة لاستخلاص والتعرف على المركبات الفينولية من مخلفات بَعْض الخضر والفاكهةِ، واختبار نشاطها كمواد مضادة للأكسدة.

تم استخلاص المركبات الفينولية من تلك المخلفات بواسطة الميثانول، ثم تم التقدير الكلي لتلك المركبات الفينولية في المستخلصات وفصل مكونات المستخلصات النباتية باستخدام الفصل الكروماتوجرافي على الطبقة الرقيقة، ثم التعرف على مجموعة المركبات الفينولية المختلفة في جميع المستخلصات باستخدام HPLC. وتقدير الروابط الزوجية المترافقة ورقم الأنسيدين وحمض الثيوبربيتيورك والتوتكس ورقم البروكسيد كمقياس لنشاط مضاد الأكسدة.

أظهر نظام الفصل الكروماتوجرافي على الطبقة الرقيقة أن هناك تبايناً شديداً في كمية الفينولات الكلية بين المصادر المختلفة.

وأوضحت النتائج المتحصل عليها أن نسبة المركبات الفينولية في مستخلصات المخلفات النباتية التي تمت دراستها كانت على النحو التالي: أوراق الزيتون > قشر طماطم > قشر البرتقال > قشر الخيار > قشرة البطيخ > قشر البطاطس. أثبتت النتائج أن جميع المستخلصات لها نشاط مضاد للأكسدة، وتدرجت فعاليتها كالتالي قشر الطماطم < قشر الخيار < قشر البطيخ.

وأن استخدام ٦٠٠ جزء في المليون من مستخلص قشر الطماطم كان له تأثير مماثل لتأثير مضاد الأكسدة الصناعي بتركيز ٢٠٠ جزء فى المليون أثناء فترات تخزين زيت عباد الشمس. بينما أظهر استخدام مسحوق ومستخاص قشر الخيار والبطيخ انخفاض تأثير مضاد الأكسدة مقارنة بمستخلص قشر الطماطم، لذا يمكن الاستفادة من مخلفات تلك النباتات واستخدامها في مجال الصناعات الغذائية كمواد مانعة للأكسدة الطبيعية.