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THE DIETARY FIBER, TOTAL PHENOLIC CONTENT, AND ANTIOXIDANT ACTIVITY OF ORANGE PEELS

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Abstract: This investigation was carried out on the dried orange peels. High dietary fiber content was prepared from orange peels. The dietary fiber composition, total phenolic compounds and antioxidant activity of dried orange peels were studied. Methanolic orange peel extract as a natural source of antioxidant was evaluated during 6 months storage of refined sunflower oil at ambient temperature.

The total dietary fiber content in orange peels was 70.95%, with an appreciable amount of soluble fiber (21.64%). Insoluble dietary fiber was the predominant fraction in orange peels (49.31%). The studied orange peels contained the best ratio of soluble/insoluble fractions (1.0-2.28).

The total phenolic compounds in dietary fiber were 21.24 mg/g. The antioxidant activity of total extractable polyphenols was studied, using β-carotene/linoleic acid antioxidant assay. The polyphenols showed high antioxidant activity, largely preventing the bleaching of β-carotene which indicates a good capacity for reduction of the radicals generated by the oxidation of linoleic acid.

Antioxidant activity of methanolic orange peels extract was assessed by measuring free fatty acid content, peroxide value and iodine value during 6 months storage of sunflower oil containing 2000 ppm orange peel extract. The treated samples showed lower FFAs content (0.968%) and PV (4.71 meq/kg^{-1}) and higher iodine value (98.0) compared to control sample. Therefore, the use of orange peels extract is recommended as a natural antioxidant to suppress development of rancidity in oils and fats.

Key words: soluble fiber, dietary fiber, phenolic content, antioxidant.

Introduction

Food processing industries create large quantities of by-products, which are difficult to dispose of as they have a high biological oxygen demand. Plant material wastes from these industries sometimes contain high levels of phenolic compounds that can have an adverse environmental impact. Positive impacts of phenolic compounds on human health include inhibition of oxidation of low-density protein thereby reducing the risk of heart disease (Meyer *et al*., 1997; Williams and Elliot, 1997). These

phenolic compounds are known to comprise of an antioxidant activity (Shahidi, 1997). The oxidative changes in food are responsible for the development of off flavors by formation of compounds that result in a decrease in its sensory and nutritional quality. The antioxidants are added to food to prevent these changes. Most of the antioxidants currently employed are synthetic including butylated-hydroxyanisole, butylated-hydroxytoluene (BHA, BHT) and studies have shown that these are sometimes toxic (Burlow, 1990). Studies have implicated these synthetic antioxidants such as BHA in promoting the development of cancerous cells in rats (Ito *et al*., 1986). These findings have reinforced interest in natural antioxidants and there also is consumer preference for natural foods and food ingredients that are believed to be safer, healthier, and less hazardous than their synthetic counterparts (Farag *et al*., 1986; Cozzi *et al*., 1997). Thus, isolation of antioxidative compounds from by-products of the food processing industries can result in value addition (Moure *et al*., 2001).

Citrus processing by-products represent a rich source of naturally occurring flavonoids. The peel which represents roughly half of the fruit weight contains the highest concentrations of flavonoids in the citrus fruit (Manthley & Grohmann, 1996&2001). As far as the peel is concerned, extracts from this part of the fruit were found to have a good total radical antioxidative potential (Gorinstein *et al*., 2001). Isolation of functional compounds from citrus peel can be of interest to the food industry as they can retard oxidative changes in food and thereby improve the quality and nutritional value of food. Fernandez-Lopez *et al*. (2004) reported that the presence of functional dietary fiber and antioxidants in citrus by-products allow their application in food processing to obtain healthy products.

Citrus peel has been reported to be a good source of pectin and dietary fiber in general, with an equilibrated proportion of soluble and insoluble fractions (Baker, 1994).

Recent approaches to the development of products with increased dietary benefits from citrus peel have placed emphasis not only on the recovery of carbohydrates and pectin (Baker, 1994) but also on the production of potentially important secondary metabolites, such as polyphenols (Manthley & Grohmann, 1996). Fiber associated polyphenols, which are known to exert important health promoting effects (Middleton & Kandaswami,

1994), have not been studied in detail.

The aim of this study was to characterize the high dietary fiber from orange peels, determining their antioxidant capacity, and identifying the associated polyphenols that could be responsible for their antioxidant properties. The antioxidant activity of methanolic extract of orange peels in refined sunflower oil during storage at ambient temperature was studied as well.

Materials and Methods

Materials:

Balady variety of orange fruits was obtained from the Experimental Farm in Faculty of Agriculture, Assiut University during 2006 season. Orange fruits were washed with tap water, peeled off in order to collect the peels and then dried in a hot air oven at 80°C for 24 hrs.

The dried peels were ground into a fine powder in a mill (Tecator-Cemotec 1090 samples mill, Hogans, Sweden). The material that passed through an 80-mesh sieve was retained for use.

Refined, bleached and deodorized sunflower oil was obtained from El-Nile Company for Oils and Detergents, El-Minia, Egypt. Whereas, synthetic antioxidants, namely butylated hydroxytoluene (BHT) was purchased from Sigma Chemical Company.

Methods:

Analysis of dietary fiber:

Total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) were determined as described by Prosky *et al*. (1988).

Determination of total polyphenols:

Dietary fiber orange samples (500 mg) were sequentially extracted with 40 ml methanol: water (50:50, v/v) and 40 ml acetone: water (70:30, v/v) at room temperature for 1 h, in each case. After centrifugation at 2500 x g for 15 min, the combined supernatants from the two previous extractions were concentrated in a vacuum rotatory evaporator at 50°C and dispersed in absolute ethanol. A spectrophotometric test was used on the total extractable polyphenols, following the Folin-Ciocalteau method, with Gallic acid as standard (Larrauri *et al*., 1997).

Determination of antioxidant activity:

Antioxidant activity of polyphenolic extracts from orange peels was determined by a β-carotene bleaching method as described by Matthaus (2002). 1 ml of β-carotene solution (1 mg/1 ml chloroform), 40 µl of linoleic acid and 200 mg of Tween 80

were transferred into a flask. Chloroform was removed at 40°C under vacuum. 50 ml of distilled water was added slowly to the residue and vigorously agitated to form a stable emulsion. To an aliquot of 5 ml of this emulsion, 200 µl of an antioxidant solution was added. To the control, 200 µl of distilled water was added. Absorbance was measured immediately at 470 nm. The tubes were placed in a water bath at 50°C and the absorbance was measured every 30 min up to 120 min.

Antioxidant index (AI) was reported as percentage protection of β-carotene protection against oxidation and it was calculated as:

$AI = As_{(120)} / As_{(0)} \times 100$,

Where: $As_{(120)}$ = Absorbance of sample after 120 min.

$As_{(0)}$ = Absorbance of sample at 0 min.

Orange peel extracts preparation:

Methanolic extract of ground orange peels was prepared according to the method described by Zia-ur-Rehman (2006). The extract obtained after evaporation of organic solvent was used as natural antioxidant.

Application of orange peel extract to sunflower oil:

Refined sunflower oil, (free of additives), was used as the substrate for oxidation studies. Sunflower oil samples containing 2000 ppm methanolic orange peel extract were separately prepared. Each 200 ml prepared oil sample was placed in a 250 ml brown air-tight glass bottle. Synthetic antioxidant (BHT) was mixed in with the oil for comparative study at the legal limit of 200 ppm (Duh and Yen, 1997). Control samples of sunflower oil without antioxidant were also placed under identical conditions. All oil samples were stored at ambient temperature for 6 months. The oil samples of each treatment were withdrawn periodically after one month intervals to evaluate the antioxidant activity of orange peel extract.

Evaluation of antioxidant activity:

Orange peel extract as antioxidant was evaluated by determination of free fatty acids (FFAs), peroxide value (PV) and iodine value (IV) during storage of sunflower oil at ambient temperature as described in AOAC (1990).

Results and Discussion

Dietary fiber contents:

Soluble, insoluble and total dietary fiber contents of orange peels are shown in Table (1).

Table(1): Dietary fiber composition of orange peels (g/100 g dry weight).

Soluble dietary fiber		Insoluble dietary fiber			Total dietary fiber
UA*	NS**	UA	NS	L***	
15.60	6.04	13.50	29.60	6.21	70.95

* Uronic acids ** Neutral sugars. *** Lignin.

Fiber must have a balanced composition of soluble and insoluble fractions in order to retain all its properties. Soluble dietary fiber content of orange peels was 21.64% of the total dietary fiber contents (Table 1). Uronic acid was the major constituent in soluble dietary fiber (15.60%).

Insoluble dietary fiber was the predominant fraction in orange peels. The major components of insoluble dietary fiber were neutral sugars (NS) being 29.60% of the total dietary fiber. The content of lignin (6.21%) is quite similar to the values of other citrus fruits, as in the case of orange and lime peels with 4.6 to 5.1% (Larrauri *et al*., 1996). Lignin is related to the hypercholesterolemia effect associated with fiber consumption due to its capacity to absorb bile acids. The ratio of soluble to insoluble fractions in dietary fiber must be within the range of 1.0-2.3 to be able to exert the physiological effect associated with both fractions in dietary fiber (Grigelmo *et al*., 1999). According to what was previously mentioned, the studied orange peels contain the best SDF to IDF ratio (1.0-2.28).

Soluble to total dietary fiber ratio was comparable to those reported for citrus fruit fiber, 30.50% (Anon, 1987), but lower than values reported by Wisker *et al*. (1994) for citrus fiber concentrate, 51%. This could be due to the differences in citrus by-products, analytical techniques used and location of harvest.

These results indicate that the dietary fiber in orange peels may confer benefits from a nutritional and health standpoint.

Saura-Calixto, (1998) reported that the nutritional value of dietary fiber concentrates is considerable, due to the presence of significant amounts of bioactive compounds, such as flavonoids and carotenoids. The high fiber content, the soluble/insoluble dietary fiber ratio, and the low energy value also play important roles in its nutritional quality.

Total extractable polyphenols and antioxidant activity:

The contents of total phenolic compounds in dietary fiber extract obtained from orange peels (as mg of Gallic acid per gram of dietary fiber) were 21.24 mg/g. It is likely that the main polyphenol components of orange fiber extract are hesperidins, ferulic aid, caffeic acid, naringin and myricetin (Larrauri *et al*., 1996).

The oxidative destruction of β-carotene by the products of linoleic acid degradation is measured by the decrease in absorbance at 470 nm. The decrement in absorbance might be due to the coupled oxidation of β-carotene and linoleic acid which generates free radicals. The linoleic acid free radical formed upon abstraction of a hydrogen atom from one of its diallylic methylene groups attacks highly unsaturated β-carotene molecules.

As β-carotene molecules loss their double bond, the system losses, its characteristic orange color, which can be monitored spectrophotometrically (Wettasinghe and Shahidi, 1999).

The presence of an antioxidant hinders the extent of bleaching by neutralizing the linoleate free radical formed in the system. Progress of discoloration process with time in differently treated samples was monitored spectrophotometerically. It was observed that control samples decolorized very rapidly. Meanwhile, orange peels extract gave very significant protection (Table2).

Table(2): Progress of β-carotene bleaching in β-carotene/linoleic acid system at 50°C in absence and presence of orange peel extract*.

Heating time (min)	Control sample	Treated sample
30	73.33	97.78
60	51.11	95.56
90	17.78	77.78
120	13.33	71.11

* Antioxidant index (AI %).

The tabulated data showed that the calculated antioxidant indexes (AI) of control sample after 30, 60, 90 and 120 min were 73.33, 51.11, 17.78 and 13.33%, respectively. Meanwhile, the

calculated values for orange peels extract were 97.78, 95.56, 77.78 and 71.11%. The orange peels extract showed high antioxidant activity, largely preventing the bleaching of β-carotene which indicates a good capacity for reduction of the radicals generated by the oxidation of linoelic acid.

These results are in an accord with Grigelmo & Martin (1999) and Kang *et al*. (2006), findings.

In conclusion, the results of this study suggest that orange peels extracts and its dietary fiber had high polyphenolic content and also possessed some flavonoids with a potent antioxidant activity. Other bioactive compounds that could be present in these samples, such as carotenoids and monoterpenes may also play an important role in the antioxidant properties of the peels. These findings confirm that natural antioxidant could be prepared from orange peels.

Citrus fruits have a high content of phenolics, dietary fiber, ascorbic acid and trace elements (iron, copper and manganese). These compounds are effective in preventation and treatment of atherosclerosis and its complications (Gey *et al*., 1993).

Free radicals attack the saturated fatty acids in the biomembrane. They cause lipid per oxidation, permeation decrease and protein membrane damage, resulting in cellular inactivation. DNA is also subject to mutations which lead to cancer. An important correlation of cancer prevention, antimutation, and antioxidant properties exists (Yen & Hsieh, 1998). Antioxidants act as breakers of chain-reactions caused by free radicals.

Noteworthy are the natural sources of dietary fiber that combine antioxidant properties with the physiological effects of the fiber itself. The progress in the development of nutraceutical products from orange peels underlines the importance of secondary metabolites such as flavones. The outstanding features of flavonoid compounds are in the antioxidant properties that are useful for obtaining natural ingredients that can replace synthetic antioxidants.

Free fatty acids (FFAs), peroxide value (PV) and iodine value (IV) were determined to evaluate the antioxidant activity of the methanolic extract of ground orange peels in sunflower oil during storage at ambient temperature for 6 months.

Table (3) showed the effect of synthetic (BHT) and natural (orange peel extract) antioxidants on the free fatty acids of sunflower oil during storage periods at ambient temperature. The data revealed that the

hydrolysis in sunflower oil was significantly affected by storage periods. A gradual increase in free fatty acids was observed during storage of sunflower oil. The changes in free fatty acids were more pronounced in sunflower oil without antioxidant (control). Initially, the FFAs contents of control sample were 0.142 (% oleic acid). After 6 months of storage, FFAs contents were 10.23%.

Table(3): Effect of synthetic and natural antioxidants on free fatty acids (%as oleic acid) of sunflower oil during storage periods at ambient temperature.

Storage periods (months)	Sunflower oil samples		
	Control	Oil + BHT*	Oil + orange peel extract
0	0.142	0.142	0.142
1	1.630	0.500	0.460
2	3.750	0.824	0.600
3	5.620	0.965	0.740
4	6.980	1.260	0.820
5	8.450	1.480	0.900
6	10.230	1.890	0.968

* Butylated hydroxytoluene.

It is clear from Table (3) that addition of BHT and orange peels extract significantly retarded the development of hydrolysis in sunflower oil. On the other hand, methanolic orange peels extract showed better results than BHT. The FFAs values were reduced from 10.23% (control) to 1.890 and 0.968% after 6 months of storage as a result of addition of BHT and orange peels extract, respectively.

The decrease in FFAs values clearly indicate that the autoxidation of sunflower oil was greatly inhibited by addition of orange peels extract at concentration of 2000 ppm. These results confirm the findings of earlier workers, who identified phenolic and flavonoid antioxidative compounds in the non-volatile fraction of methanolic extract of citrus peel (Alexandra *et al*., 1998).

The changes in peroxide values during storage of sunflower oil at ambient temperature after the addition of BHT and methanolic orange peels extract are listed in Table (4). The peroxide value in the stored samples tended to increase to a maximum value (19.63 meq/kg oil) after 6 months of storage at ambient temperature. Moreover, the data revealed that the rate of peroxide formation in samples contained BHT and orange peels extract was considerably lower than those without antioxidant (control).

These results are consistent with the findings of other workers who reported that lipid peroxides were significantly reduced by the addition of antioxidants in fats and oils (Kiyomi & Yasuko, 1995; Yanping *et al*., 1999).

Table(4): Effect of synthetic and natural antioxidants on peroxide value (meq/kg oil) of sunflower oil during storage periods at ambient temperature.

Storage periods (months)	Sunflower oil samples		
	Control	Oil + BHT*	Oil + orange peel extract
0	0.56	0.56	0.56
1	4.20	1.82	1.12
2	7.96	2.56	1.45
3	10.51	2.98	2.01
4	13.80	4.21	2.70
5	16.04	5.63	3.80
6	19.63	8.04	4.71

* Butylated hydroxytoluene.

The effect of synthetic (BHT) and natural antioxidants on iodine values of sunflower oil during storage periods at ambient temperature are shown in Table (5).

The iodine value decreased gradually in all samples during storage, which could be attributed to breaking of double bonds of unsaturated fatty acids during storage of sunflower oil (Noor & Augustin, 1984). In fact, a decreasing trend in iodine value indicates the development of rancidity due to the formation of secondary oxidation products

during storage of fats and oils. Table (5) pointed out that addition of synthetic antioxidants (BHT) and methanolic orange peels extract separately retarded the decreasing trend of iodine value during storage of sunflower oil. Addition of BHT or methanolic extract to sunflower oil, showed iodine values of 110 and 98, respectively, during 6 months of storage, whereas iodine value of oil without antioxidants (control) was 88.4.

Table(5): Effect of synthetic and natural antioxidants on iodine values of sunflower oil during storage periods at ambient temperature.

Storage periods (months)	Sunflower oil samples		
	Control	Oil + BHT*	Oil + orange peel extract
0	127.31	127.31	127.31
1	118.00	126.00	125.00
2	110.60	124.60	122.00
3	108.00	120.00	116.00
4	102.60	119.20	114.00
5	96.30	113.00	108.00
6	88.40	110.00	98.00

* Butylated hydroxytoluene.

This increase in iodine values clearly indicate that autoxidation of sunflower oil was greatly inhibited in the presence of methanolic orange peels extract. These results confirm the findings of earlier workers, who identified phenolic and flavonoid antioxidative compounds in methanolic extract of citrus peel (Alexandra *et al*., 1998; Kaehkoenen *et al*., 1999 and John, 2004).

In general, it could be concluded that the addition of methanolic extract of orange peels showed a strong antioxidant activity during storage of sunflower oil, which could be attributed to the presence of different phenolic compounds in orange peels. However, natural antioxidant extract of orange peels would be preferred over synthetic antioxidants to minimize the adverse health effects.

Therefore, this investigation confirms that, the higher the total

polyphenolic content, the greater is the antioxidant capacity.

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محتويات قشور البرتقال من الألياف الغذائية والفينولات الكلية ونشاطها المضاد للأكسدة

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أجرى هذا البحث على قشور البرتقال المجففة. وتم تقدير الألياف الغذائية، والفينولات الكلية ونشاطها المضاد للأكسدة.

وتم تقييم المستخلص الميثانولى لقشور البرتقال كمصدر طبيعي لمضادات الأكسدة خلال ٦ شهور من التخزين على درجة حرارة الغرفة لزيت عباد الشمس المكرر. وقد بلغت نسبة الألياف الغذائية في قشور الموالح ٧٠,٩٥% وكانت نسبة ملموسة من الألياف الذائبة ٢١,٦٤% بيد أن الألياف غير الذائبة كانت هي السائدة في قشور الموالح بنسبة ٤٩,٣١%. وكانت قشور البرتقال المدروسة محتوية على أفضل نسبة من الألياف الذائبة: الألياف غير الذائبة (١: ٢,٢٨).

ومن الجدير بالذكر أن ارتفاع نسبة الألياف ونسبة الألياف الذائبة: الألياف غير الذائبة في قشور الموالح تلعب دوراً غذائياً وصحياً هاماً.

كما أظهرت النتائج أن نسبة الفينولات الكلية في الألياف كانت ٢١,٢٤ مجم / جم.

وقد تناول البحث دراسة النشاط المضاد للأكسدة للبولي فينولات الكلية المستخلصة باستخدام طريقة تقدير البيتا كاروتين / حامض اللينوليك . وقد أظهرت البولي فينولات درجة عالية للنشاط المضاد للأكسدة، حيث منعت إزالة لون البيتا كاروتين مما يعطى دلالة على كفاءتها الجيدة لاختزال الأصول المتولدة نتيجة أكسدة حامض اللينوليك.

وقد تم تقييم درجة نشاط مضادات الأكسدة لمستخلص قشور البرتقال الميثانولى بقياس نسبة الأحماض الدهنية الحرة، ورقم البيروكسيد، الرقم اليودي خلال ٦ شهور تخزين لزيت عباد شمس المحتوى على ٢٠٠٠ جزء في المليون من مستخلص قشور البرتقال.

وقد أعطت العينات المعاملة نسبة منخفضة من الأحماض الدهنية الحرة (٠,٩٦٨%) ، ورقم البيروكسيد (٤,٧١) ملليكافئ / كجم$^{-1}$، رقم يودى مرتفع (٩٨,٠٠) أعلى من عينة الكنترول.

وتأسيساً على ما تقدم فإن استخدام مستخلص قشور البرتقال يوصى به كمضاد أكسدة طبيعي مانع لتزنخ الزيوت والدهون. ويفضل استخدامه عن مضادات الأكسدة الصناعية لتقليل الآثار الصحية الجانبية.

ومن جهة أخرى فإن احتواء قشور البرتقال على نسبة عالية من الألياف، نسبة الألياف الذائبة: نسبة الألياف غير الذائبة وارتفاع محتوياتها من البولي فينولات تلعب دوراً هاماً في التغذية كمضاد للأكسدة.

ويمكن استخدام مستخلص قشور البرتقال كمادة مضافة غذائية مضادة للأكسدة كبديل لمضادات الأكسدة الصناعية.