Evaluation of Mandarin and Navel Orange Peels as Natural Sources of Antioxidant in Biscuits

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ABSTRACT

The use of navel orange and mandarin peels as natural antioxidant in biscuits production was investigated. Chemical composition showed that peels had high contents of ash, ether extract and crude fiber and low protein content. Navel orange and mandarin peel powders were used in production of hard biscuits (Marie) at three levels of wheat supplementation (5, 10 and 15%). Sensory evaluation and chemical analysis of biscuits indicated that supplementation with 5% and 10% levels were well accepted in all organoleptic properties as compared to the control sample, but at 15% level, low scores indicated unacceptable product. Addition of peel powders increased crude fiber, ash and ether extract contents of biscuits. The results indicated that the mandarin and navel orange peel extracts have antioxidant activity. Mandarin peels had the highest total phenols (780mg/100g dry matter) and the highest antioxidant activity. Also, data indicated that the addition of navel orange and mandarin peels powder increased the shelf life of biscuits as compared to the control. Addition of peels inhibited lipid oxidation as indicated by the peroxide values of navel orange and mandarin biscuits. The peroxide values after 6 months of storage at 25ºC and 40ºC were (8.9 and 10.3 meq./kg fat) and (8.2 and 12.5 meq./kg fat) for navel orange and mandarin biscuits respectively as compared to control (29.5 and 35 meq./kg fat). Consequently, it could be concluded that navel orange and mandarin peel powders might be used instead of synthetic antioxidants, as the addition of peels at 10% level had no adverse effect on the organoleptic properties of biscuits. Moreover, the biological results indicated that the substitution with peels up to 10 % improved the weight gain of rats, decreased the levels of serum total cholesterol, liver lipid and liver cholesterol and glucose blood. Finally, addition of peels as a source of natural antioxidant can be recommended to increase the shelf life of food products containing fats and oils, which is safe and can impart health benefits to the consumer.

Keywords: mandarin and navel orange peels, antioxidants, chemical composition, biological tests, organoleptic evaluation, biscuits

INTRODUCTION

Biscuits are of the most popular bakery products consumed nearly by all levels of society. This is mainly due to its ready to eat nature, good nutritional quality, availability in different varieties, affordable cost and long shelf life. The keeping quality of baked foods such as biscuits is of great economic importance since this product contains fats and oils which are exposed to oxidation during long storage. Various oxidation products cause rancidity and deterioration of the sensory properties. Oxidation of fats and oils may be prevented or even delayed by antioxidants. Synthetic antioxidants have been used as antioxidants for certain foods, however, this has begun to be restricted to some extent because of their toxicity and carcinogenic effects on human health (Ito *et al.*, 1986, Gazzani *et al.*, 1998). Recently, natural plants have received much attention as source of biologically active substances including antioxidants, antimutagens and anticarcinogens. In addition, demand for health-oriented products such as high fiber products is increasing to overcome health problems such as hypertension, diabetes and colon cancer (Sudha *et al.*, 2007). Food processing industries create very large quantities of waste, which are difficult to dispose of as they have a high biological oxygen demand. In citrus and mandarin juice processing, peels remain as primary waste and give rise to serious environmental pollution (Liu *et al.*, 2006). Therefore, new by-products applications should be investigated to have a positive environmental impact or to turn them into useful products. AccordingIy, the functional properties of some peel components such as, pectin, flavonoids, carotenoids, limonene and polymethoxy flavones shoud be considered (Braddock, 1999, Li *et al.*, 2007). Citrus peel components were found to provide many health benefits, among which are, the effects of pectin on glycamic control, serum cholesterol concentration, cancer prevention and control of mineral balance (Larrauri *et al.*, 1995), the effect of limonene on cancer pre-

vention (Girard & Mazza, 1998), and the vitamin activity of carotenoids (Cerezal & Pinera, 1996, Zheng & Wang, 2001, Xu *et al.*, 2008, Wang *et al.*, 2008). The peel, which represents roughly half of the fruit mass, contains the highest concentrations of flavonoids in the citrus fruit. Methanolic extract of citrus peel is known to have different antioxidative compounds (Alexandra, *et al.*, 1998). Now adays, there is a strong need for effective antioxidants from natural sources to prevent deterioration of fats and oils (Samarth *et al.*, 2008) The present study is an attempt for utilization of navel and mandarin orange peel powders as natural source antioxidants, falvouring agents, fiber and minerals in hard biscuits (Marie). Biological tests as well as sensory and chemical compostion of biscuits and effect of storage were investigated.

MATERIALS AND METHODS

Materials

Fresh mature navel oranges were purchased in January 2007, from a local market in Fayoum, Egypt. The whole peels (flavedo and albedo) were directly separated from the washed fresh fruits, cut into small pieces, dried at room temperature (25 °C ±2) then ground in a laboratory mill to fine powder and sieved through 60 mesh sieve.. The peel powder was packed into polyethylene bags and stored under cooling at 4°C for further uses. Soft wheat flour (72% extraction) was obtained from the Middle Egypt Mills Co., Fayoum Governorate, Egypt. The solvents (Analytical Grade) used in the present study were purchased from Merck, DPPH (2, 2-diphenyl 1-1 picrylhdrazyl radical) was purchased from Sigma Chemical Company (USA). Kits for serum total lipids, total cholesterol and high-density lipoprotein-cholesterol were purchased from Wiener laboratories, Rasario, Argentina.

Methods

Processing of biscuits

Marie type biscuits were prepared according to a commercial formula and baking practice in Bisco Misr Company. The base recipe is given in Table (1). Wheat flour was replaced with navel orange and mandarin peel powders at levels of 5%, 10% and 15%. After baking at 220ºC for 12 minutes and cooling for 30 minutes, the biscuits were packed in polyethylene bags for chemical and sensory evaluation.

Table 1: Marie biscuits dough recipe

Ingredient	%
Wheat flour (72%)	100g
Shortening	10g
Sucrose	26g
Glucose syrup	6g
Milk powder	2g
Vanillin	0.04
Sodium bicarbonate	0.04
Ammonium bicarbonate	0.08
Water	21ml

Chemical Composition

Orange peels, wheat flour (72%) and biscuits samples were analyzed for moisture, protein, ash, crude fiber and ether extract contents according to AOAC (1990). Carbohydrate content was calculated by difference .The total phenolic content was determined and calculated as tannic acid according to AOAC (1990).

Radical DPPH scavenging capacity was determined according to Yu *et al.* (2003).The free radical scavenging capacity of citrus peel extracts was determined using the stable 2, 2-diphenyl 1-1 picrylhdrazyl radical (DPPH) The percentage of the remaining DPPH against µg dry peel extract/µg DPPH was plotted to obtain the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% (EC50) and the antiradical efficiency (AE) or (ARP) was calculated as follows: AE=1/ EC50

Determination of peroxide value

Biscuits samples were stored for 6 months at 25±3°C and 40±3°C. About fifty grams of biscuits were ground, extracted three times with 150 ml n-hexan, the extract was filtered over anhydrous sodium sulphate. From this extract 10 ml were pipetted into weighed dried dish and the solvent was evaporated at 100°C, then the residue was weighed and percentage of fat in 10ml extract was calculated. Peroxide value was determined as milliequivelant peroxides per kilogram of fat according to the method described in AOAC (1990)

Organoleptic evaluation

The organoleptic evaluation of biscuits was carried out, using a panel taste according to Sudha *et al.* (2007). The panelists were asked to evalu-

ate the biscuits for colour, surface character, crumb colour, mouth feel, odour and taste. The results were subjected to the statistical analysis according to Snedecor & Cochran (1980). The least significant difference test (LSD) at P<0.05 level was used to verify the differences among treatments.

Biological test

Adult male albino rats weighing about 104±5g were used in the biological assay. Rats were divided into three groups each of five animals. The rats were fed on a casein diet for one week. Feed and water were added *ad-libitume*. Total body weight of each group was recorded at the beginning, weekly intervals and at the end of experimental period (4 weeks). The biscuits contained 10% orange peel were ground to fine meal and used for preparation the diets. Diet ingredients used in this study consisted of crude protein (10%), corn oil (5%), wheat bran (5%), minerals mix. (4%) and vitamin mix. (1%). The experimental diets which contained navel orange and mandarin biscuits are presented in Table (2)

Determination of serum cholesterol and lipid

By using commercially available assay kits, concentration of total cholesterol was determined according to Richmond (1973). High-density lipoprotein cholesterol (HDLC) content in the serum samples was performed as described by Gordon *et al.* (1977). The serum total lipids were enzymatically determined according to Knight *et al.* (1972)

Determination of liver cholesterol and lipid

Total liver lipids were extracted from 1-2 g of liver with chloroform: methanol mixture (2:1v: v) according to the method of Folch *et al.* (1957). Cholesterol in the liver lipids extract was then determined calourimetrically at 490 nm (Searcy & Bergquist (1960). The content of total lipid in the liver was quantified gravimetrically by evaporating the solvents from the liver lipid extract at 50ºC under vacuum.

Liver functions

The concentration of serum glutamic pyruvic (GPT) and glutamic oxaloacetic (GOT) transaminase were calourimetrically determined using transaminase kit according to the method of Reitman & Frankel (1957).

Determination of blood glucose

Blood glucose was determined by enzymatic colourimetric method described by Trinder (1969).

RESULTS AND DISCUSSION

Chemical composition

The mean values of proximate chemical composition (moisture, fat, protein, crude fiber, ash and carbohydrates) of wheat flour, orange peel powders and biscuits samples are presented in Tables (3 and 4). Data in Table (3) show that the peels had high ash, ether extract and crude fiber contents (4.24 % and 4.06%) (9.52% and 11.15%) and (13.38% and 7.14%) for navel orange and mandarin peels, respectively as compared to wheat flour, on the other hand, the peels had low protein contents 2.67% and 2.16%, respectively as compared to the high protein content of wheat flour being 14.42%. The previous results also indicate that navel orange peels had higher crude fiber content meanwhile, mandarin orange peels had higher ether extract. These

Table 2: composition of experimental diets* (g/100 g)

Ingredients	Control diet	Diet with navel orange biscuits	Diet with mandarin biscuit
Casein**	11.26	---	---
Corn oil	5	---	---
Salt mix***	4	4	4
Vitamin mix****	1	1	1
Wheat bran (fiber)	5	2.86	2.29
Biscuits	---	91.07	83.47
Starch	73.74	1.07	9.23
Total	100	100	100

*All diets contained 10% total protein, **casein contains 88.8 % protein,
*** Salt mix. and **** Vitamin mix. (A.O.A.C, 1990).

Table 3: Chemical composition (% dry weight basis) of wheat flour, navel orange and mandarin peels

Composition	Wheat flour (72%)	Navel orange peels	Mandarin peels
Protein	14.42	2.67	2.16
Ether extract	0.82	9.52	11.15
Ash	1.07	4.24	4.06
Crude fiber	0.87	13.38	7.14
Carbohydrates	82.82	70.19	75.49

findings may be attributed to the large thickness of albedo layer in navel orange peel as compared to the mandarin orange peel. Finally, from the aforementioned results it could be concluded that the use of orange peels in some bakery products will increase its contents of fiber and ash.

From Table (4) it could be noticed that the chemical composition of biscuits was affected by the addition of orange peels powder. Ash, crude fiber and ether extract of biscuits samples increased with increasing orange peels powder level in biscuits as compared to the control. Meanwhile, a slight decrease could be traced in protein content of biscuits containing peels. Generally, from these results it could be concluded that the utilization of orange peels in some biscuits results in increase of its contents of fiber and ash.

Organoleptic evaluation

Table (5) summarizes the results of the sensory evaluation of biscuits samples. Data indicate that there are significant differences between the control and the biscuits samples. Biscuits containing Navel orange and mandarin peels at level of 5 % were well accepted and exhibited the maximum score of sensory properties. Similar trend was also seen for 10% level followed by control sample. Meanwhile, at 15% level, the mean scores were lower which indicate that the samples were unacceptable as compared to the 5% and 10%. Also, as shown in Table (5) the acceptability of biscuits containing 5% peels powder had higher total scores (73.69 and 73.30) for navel and mandarin powders, respectively followed by 10 %. These results indicate that the orange peels powder could be added in

Table 4: Chemical composition (dry weight basis) of biscuits containing navel orange and mandarin peels

Composition	Control*	Orange peel %			Mandarin peels %		
		5%	10%	15%	5%	10%	15%
Ether extract	11.10	12.24	12.36	13.43	13.25	14.64	15.35
Protein	12.35	11.52	10.98	10.19	12.67	11.90	11.23
Crude fiber	1.04	2.56	3.15	3.29	2.67	2.75	2.92
Ash	1.17	1.68	2.12	2.53	1.81	2.09	2.21
Carbohydrates	74.34	72.06	71.39	70.56	69.60	68.62	68.29

* Control: Biscuits made from wheat flour

Table 5: Organoleptic evaluation of biscuits samples containing mandarin, and orange peels

Parameter %	Control*	Orange peels powder %			L.S.D.	Mandarin peels powder %			L.S.D.
		5%	10%	15%		5%	10%	15%	
Colour (10)	7.80	8.90	8.20	7.60	n.s**	8.80	8.20	6.50	0.73
Surface character (10)	8.10	8.89	8.10	6.50	0.55	8.90	8.30	6.40	0.80
Crumb colour (10)	7.90	8.80	8.90	6.00	0.45	9.00	8.79	6.50	0.77
Mouth feel (10)	8.20	9.20	9.00	6.90	0.32	9.00	8.90	6.40	0.41
Odour (20)	17.20	18.90	18.80	15.10	0.66	18.70	18.50	14.60	n.s*
Taste (20)	17.50	19.00	18.50	14.20	0.76	18.90	18.00	14.10	1.32
Total scores (80)	66.70	73.69	71.50	56.30		73.30	70.69	54.50	

* Control : biscuits made from wheat flour n.s**: non significant (at P<0.05).

amount up to 10 % in the formula of biscuits without adversely affecting sensory characteristics of biscuits but to improvement colour and flavour of biscuits because of yellowish colour resulting from the natural pigments present in peels.

Total phenolic compounds and antioxidant properties

In the present study, 80 % methanol extracts of navel and mandarin orange peels were used for determination of total phenols content (expressed as mg tannic acid/100 g dry matter) and antioxidant activity expressed as antiradical power (ARP) and results are presented in Table (6) and Fig. (1a, b). Data indicate that the mandarin orange peel had the higher total phenol content (780 mg/100gm), as compared to orange peels which had 670 mg/100gm .Also, mandarin orange peel extracts had high antiradical efficiency (0.239) as compared to navel extract (0.205). From these results, it could be concluded that the mandarin peels extract is more effective as an antioxidant than navel peels. These data are in agreement with that obtained by Brand – Williams *et al.* (1995).

Keeping quality of biscuits

Oxidative rancidity is a serious quality problem confronting the biscuits because of its fat content which upon rancidity develops off-flavour. Oxidative rancidity of fats extracted from the control samples and the different treatments was evaluated by monitoring changes in peroxide values as a measure of lipid deterioration and loss in quality of biscuits during storage. The results are illustrated in Fig. (2) Which shows that the oxidative rancidity took place during storage as a result of oxidation of fat and is affected by both of the peels addition and the storage temperature. Fats extracted from control samples stored at 40°C showed the highest peroxide values followed by those stored at 25°C .On contrary, the addition of navel orange and mandarin peels powder to biscuits reduced the extent of the oxidative rancidity which is attributed to the antioxidant effect of peels orange. The antioxidant property was observed in orange peel may be due to the presence of phenols, including numerous flavanones, flavone glycocides, poly-methoxyylated flavones, hydroxyl cinnamates and other miscellaneous phenolic glycosides and amines (John, 2004). The illustrated data in Fig.(2) indicate that the biscuits containing mandarin peels containing biscuits stored at 25°C showed the lowest peroxide values. This finding may be attributed to the high total phenols content

Table 6: Total phenols content and DPPH scavenging capacity of navel orange and mandarin orange peels

Parameters	Navel peels	Mandarin peels
Total phenols (mg/100g)	670	780
EC50 *	4.88	4.19
AE**	0.205	0.239

* The amount of antioxidant necessary to decrease the initial DPPH concentration by 50%

** The antiradical efficiency (AE)

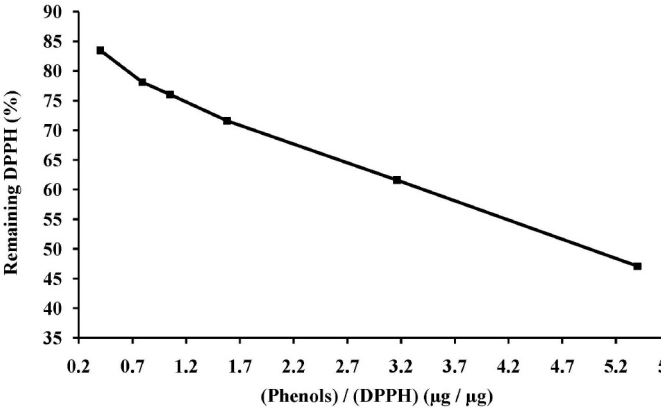

Fig. 1a: Exponential curve of the percentage of remaining DPPH as function of µg sample per µg DPPH of the navel orange peels

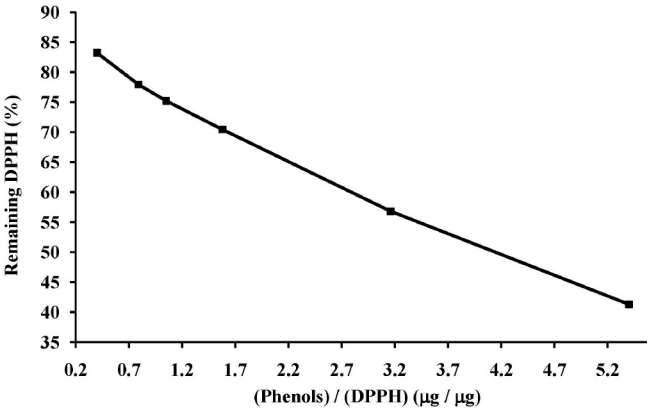

Fig. 1b: Exponential curve of the percentage of remaining DPPH as function of µg sample per µg DPPH of the mandarin peels

and the high antiradical power of mandarin peels than navel orange peels (Table 6). The radical scavenging activity of various extracts and fractions of navel orange peels make it useful for utilization as antioxidant in food (Anagnostopoulou *et al.*, 2006). Finally, the high peroxide values of samples stored at 40°C may be attributed to the effect of relatively high temperature which accelerated the rate of oxidative rancidity and peroxides formation.

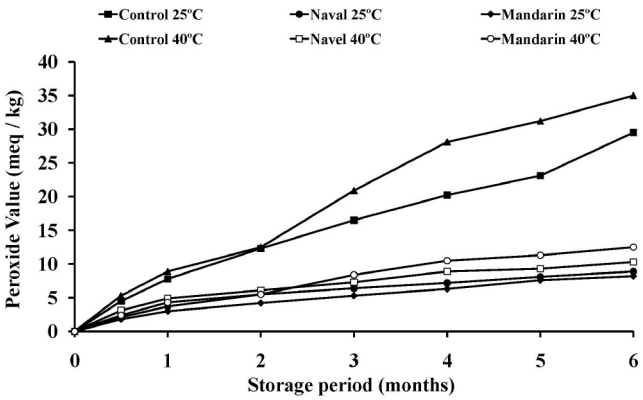

Fig. 2: Influence of 10% peels as substitution of wheat flour on oxidative rancidity of biscuits

Biological tests

Table (7) and Fig. (3) show the effect of addition of biscuits that contain 10 % orange peels on biological factors. Data show that the body weight gain increased gradually during the period of experimental (4 weeks).The rat groups fed on supplemented diets showed the highest % growth rate as compared to the group fed on control diet. This finding may be attributed to some extent to the fat content of supplemented diets (12.36 and 14.64 %) for navel and mandarin, respectively (Table 4) which is slightly higher as compared to 11.10 % fat content of control diet. On the other hand , the results shown in Table (7) indicate that the feeding on diets contained orange peels caused reduction of the all tested parameter .The rats group fed on mandarin supplemented diet had the highest reduction values 32.35 % ,33.58 %,23.81 % and 26.66 % of total cholesterol , liver total lipid ,GPT and GOT, respectively . Meanwhile, the rats group fed on navel – supplemented diet had highest reduction values (38.50 %and 20.53 %) of liver cholesterol and glucose blood, respectively. The reduction in glucose blood may be attributed to the fiber content of sample which lower the rate of carbohydrates absorption within the gastrointestinal tract (Cummings *et al.*, 1987). However, these results indicate that there was no harmful liver damage occurred as a result of supplementation with navel orange and mandarin peels. Daily observations showed that all animal remained healthy, active and no signs of abnormalities were observed.

Conclusions: The addition of orange and mandarin peel powders to biscuits formulations have many advantages as an antioxidant to increase the shelf-life of biscuits and to enhance the organoleptic properties of the biscuits. It can reduce the synthetic antioxidants. Concerning the biological activity *in vivo*, the results indicated that replacement of wheat flour with navel and mandarin orange peels in biscuits up to 10% led to high reduction on some biological parameter such as total cholesterol, blood glucose, and improving the liver functions.

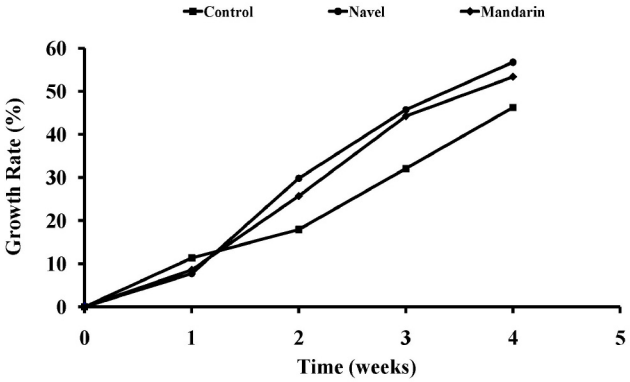

Fig. 3: Effect of feeding on peels powder of navel orange and mandarin containing diets on body weight of rats

Table 7: Effect of navel orange and mandarin peel powders on some biological parameters

Parameter	Groups				
	Control	Navel orange	± %*	Mandarin	± %*
Total cholesterol (mg/dI)	272	202	-25.73	184	-32.35
HDL cholesterol (mg/dI)	82.79	82.55	-0.290	83.37	+0.700
Liver total lipids (g/100g)	2.65	2.33	-12.07	1.76	-33.58
Liver cholesterol (g/100g)	8.31	5.11	-38.50	5.3	-36.22
Liver weight (g)	5.66	5.79	+2.297	5.87	+3.710
Liver/body weight ratio	0.036	0.036	0.00	0.036	0.00
GPT (U/mL)	42	35	-16.66	32	-23.81
GOT (U/mL)	30	25	-16.66	22	-26.66
Serum glucose blood (mg/100ml)	112	89	-20.53	94	-16.07

* As compared to control

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إستخدام قشور اليوسفى و البرتقال أبو صره كمضادات أكسدة طبيعية فى البسكويت

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تهدف هذه الدراسة إلي بحث إمكانية إستخدام قشور البرتقال أبو صره واليوسفي كمضادات أكسدة طبيعية في البسكويت حيث أظهرت نتائج التحليل الكيماوي إرتفاع محتوي القشور من الرماد والمستخلص الأيثيري والألياف الخام وإنخفاض محتواها من البروتين. أستخدم مطحون قشور البرتقال أبو صره واليوسفي في إستبدال دقيق البسكويت الجاف بثلاث مستويات مختلفة (٥ و ١٠و١٥٪) من الدقيق المستخدم وتم إجراء التقييم الحسي والتحليل الكيماوي للبسكويت الناتج وأظهرت النتائج أن العينات المحتوية على ٥ و ١٠٪ من مطحون القشور كانت مقبولة علي مستوى جميع الصفات الحسية مقارنة بالكنترول ولكن عند إستخدام مستوي ١٥٪ إستبدال أظهرت النتائج عدم قبول العينات حسيا .

أدت إضافة مطحون القشور إلي زيادة محتوي البسكويت من الألياف الخام والرماد والمستخلص الأيثيري كما أظهرت النتائج أن قشور البرتقال أبو صرة و اليوسفي لها تأثير كمضادات أكسدة طبيعية ووجد ان قشور البرتقال أبو صرة واليوسفي تحتوي علي النسبة الأعلي من الفينولات الكلية (٧٨٠ مجم / ١٠٠ مجم مادة جافة) و كان لها التأثير الأكبر كمضاد أكسدة . أظهرت النتائج أن إضافة مطحون قشور البرتقال أبو صره واليوسفي أدت إلي زيادة فترة صلاحية البسكويت المنتج مقارنة بالكنترول كما أدت إضافة مطحون القشور إلي تأخير معدل أكسدة الدهون حيث وجد أن رقم اليروكسيد بعد ٦ أشهر من التخزين علي درجة ٢٥ م° ، ٤٠ م° كان ٨,٩ و١٠,٣ ملليمكافئ / كجم دهن ، ٨,٢ و ١٢,٥ ملليمكافئ / ١كجم دهن لكل من البسكويت المضاف إليه قشور البرتقال أبو صره و اليوسفي علي الترتيب بينما كان رقم البيروكسيد لعينات الكنترول المخزنة تحت نفس الظروف ٢٩,٥ و ٣٥ ملليمكافئ / ١كجم دهن .

بينت الدراسة أنه يمكن إستخدام قشور البرتقال أبو صره و اليوسفي كمضادات أكسدة طبيعية بدلا من مضادات الأكسدة الصناعية وأن إضافة مطحون قشور البرتقال أبو صره و اليوسفي بنسبة ١٠٪ من الدقيق المستخدم لم تؤد إلي أى تغيرات غيرمرغوبه في الصفات الحسية للبسكويت الناتج .

أظهرت نتائج التجربة البيولوجية أن إستخدام البسكويت المضاف الية مطحون قشور البرتقال ابو صرة و اليوسفى بنسبة ١٠٪ قد أدى إلي زيادة في الوزن المكتسب لحيوانات التجارب وإنخفاض في مستوي كوليستيرول الدم ودهون و كوليستيرول الكبد بالإضافة إلي إنخفاض مستوي الجلوكوز في الدم مقارنة بالكنترول. أخيرا توصى الدراسة بإستخدام قشور البرتقال أبو صره و اليوسفي كمضاد أكسدة طبيعى لزيادة فترة صلاحية المنتجات الغذائية المحتوية على زيوت ودهون لأنها آمنة ويمكن ان تعود بفوائد صحية على المستهلك.