EXTRACTION OF POLYPHENOLIC COMPOUND FROM TOMATOES PEEL AS NATURAL ANTIOXIDANT TO BE KEEPING CAKE QUALITY DURING STORAGE.

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(Manuscript received 29 July 2008)

Abstract

Tomato peel was treated by ethanol 70% (v/v) to extract flavonoids (kaempferol, quercetin, lutolin, narginine and rutin) and phenolic acids (*P*-coumaric, ferulic and chlorgenic acids) were identified by paper charomatographic technique. Natural and synthetic (butylated hydroxyanisol, BHA) antioxidants exhibited strong and close antioxidative activities 85.85 and 92.60%, respectively. The tomato peel extract and BHA were added to sunflower oil at levels 100, 150 and 250 ppm to keep its quality during heating at 180^0C for 32 hours. Moreover, the natural and synthetic antioxidants were added to cake made up by sunflower oil at the same levels. Cake was stored at room temperature for four weeks and the lipids were extracted every four days.

The results showed that the addition of tomato peel extract as natural antioxidant to sunflower oil and cake delayed the lipid peroxidation during heating oil and storage of cake.

INTORDUCTION

Tomatoes (Lycopersum esculentum) are widely consumed either raw or after processing and can provide significant properties of total antioxidants in the diet. This is largely in the form of carotenes and polyphenolic compounds (Clifford,1999).
Moreover, tomato- processing by product, also known as tomato pomace, consisting of peel and seeds and represents around 4 % of the fruit weight. If these wastes remain unused, they not only add to the disposal problem but also aggravote environmental pollution (Valle *et al*.,2006).Tomatoes peel contains flavonoids mainly naringenin chalcone and the flavonol rutin , quercetin glycoside. Flavonols are very potent antioxidants and their dietary intake is correlated with a reduced risk of cardiovascular diseases (Bovy *et al*., 2002).

Flavonoids from a large group of polyphenolic compounds that occur naturally in plants. Based on their core structure. The aglycone, they can be grouped into different classes, such as chalcons, flovanones, dihydriflovnols, flavonols and anthocyanins. To over 4000 different flavonoids have been identified. This large diversity is attributable to single or combinatorial modifications of the aglycone, such as glycosylation, methylation and acylation. As a group, flavonoids are involved in many aspects of plant growth and development, such as pathogen resistance,

pigment production , ultraviolet light protection, pollen growth and seed coat development (Harborne, 1986).

There is increasing evidence to suggest that flavonoids , in particular those belonging to the class of flavonols (such as kaemplerol and quercetin) are potentially health –protecting components in the human diet as a result of their high antioxidant capacity (Duthie and Crozier , 2000) and their ability , invitro, to induce human protective enzyme systems (Shih *et al*., 2000).

Lipid peroxidation in fats and fatty foods not only causes chemical spoilage in foods but also produces free radicals active oxygens such as peroxyl and radicals (Tagi, 1987). Free radical attack the unsaturated fatty acids in the biomembrane, resulting in memberane lipid peroxidation, decrease in memberane fluidity , loss of enzyme and receptor activity and damage to memberane protein leading to cell inactivation. Also , free radical attack DNA cause mutation leading to cancer (Diplock *et al*., 1994). Likewise, Duh and Yen (1997) cited that the addition of the antioxidants to food is effective in retarding fat oxidation. It is impressive that many substance have been identified which prevent lipid peroxidation. Some of these compounds are synthetic antioxidants and others occur as natural dietary constituents.

The present investegation aimed to isolate and identify flavonoid and phenolic compounds from tomatoes peel as a waste of low cost. Also, to study the effect of nartural antioxidants (flavonoid and phenolic compounds) to prevent liped peroxidation in sunflower oil and cake and to compare their effect to butylated hydroxyanisol (BHA).

MATERIALS AND METHODS

Materials :

Tomatoes peel were obtained from Kaha Company for Preserved Foods, Kalubeia, Egypt. The peels were dried in an oven at 60^{o}C and finely ground.

Butylated hydroxyanisol (BHA) was obtained from Noarden International Company Holland. Whilest, authentic flavonoid and phenolic compounds were purchased from Sigma Chemical Company, Deisenhofen Germany.

Sunflower oil was obtained from Arma Food Company, EL- Asher of Ramodan , Egypt.

Mathods :

Extraction, isolation and purification of polyphenolic compounds from tomatoes peel:

Air dried tomatoes peel (500g) was finely powdered and extracted with petroleum ether (40-60^{0}C) to remove fats and resinous materials. The residue was exchustively

extracted with two liters 70% ethanol by heating on a boiling water bath for six hours. Extraction was repeated until a color extract then the extracts were combined and concentrated to obtain aqueous ethanolic extract. A brown product was obtained upon evaporation of ethanol to dryness and kept for flavonoid and phenolic compounds investigation according to Mebry *et al.*, (1970).

The brown extract of tomatoes peel were tested by paper chromatographic technique in order to identify the major flavonoid and phenolic compounds as described by Markham and Mabry (1968).

The tomatoes peel extract and authentic samples were spotted on one dimensional Whatman no., 1 paper chromatoghraphy. The eluting solvents were butanol: acetic acid: water (4: 1: 5) and acetic acid 15% (ACOH). The different spots (major flavonoid and phenolic compounds and authentic samples) were located by color reaction and R_F values under U.V. lights with and without the presence of NH_3 fumes were calculated according to Markhan and Mabry (1968).

Determination of Antioxidant activity :

Flavonoid and phenolic compounds were evaluated as antioxidant activity of the previous extract from tomatoes peel and compared with butylated hydroxyanisol (BHA) by thiocyante method as described by Tsuda *et al.*, (1993).

Addition of antioxidant to sunflower oil:

Sunfower oil was used as a substrate for oxidations studies. Natural antioxdant extracted from tomatoes peel and synthetic antioxidant (BHA) were added to oil at 100, 150 and 250 ppm on a dry weight basis to test thier antioxidant effectiveness according to Buford (1988). Control sample without additive was prepared under the same conditions.

Sunflower oil with and without antioxidant (natural or synthetic) was heated in 500 ml glass beaker at $180^0C \pm 5^0C$ for 32 h. (total heating hours) intermittent heating period was 4 h/ day.The oil samples after heating were taken periodically and stored in glass bottles at -10^0C till analysis.

Preparation of cake:

The ingredients of oil cakes are given in Table (1) according to Mizukoshi , *et al.*, (1979) with little modification, the foaming agent was substaituted by baking powder and vanillia. Natural and synthetic antioxidants were added to the oil cakes at 100, 150 and 250 ppm levels. Suger , whole egg, vanillia , paking powder and water were mixed for 5 min flour was added and mixed for 10 min in a mixer. The product was baked at 191^0C for 25 min in an electric oven and the cake was stored in refrigerator at 5.0 C and packaged in polyethylene bags for four weeks.

Table 1. Ingredients of cake made using sunflower oil

Ingredients	Weight / g
Flour	200
Sugar	250
Whole egg	150
Vanillia	1
Baking powder	13
Water	40
Sunflower oil	100

Extraction of the oil from cake:

Oil was extracted from cake samples every four days by soaking in n-hexan at room temperature for 48h. The extract was filterated and evaporated to dryness. The extracted oils were kept in the deep-freezer for further investigations.

Physico-chemical characteristics of oil :

Peroxide value ml.equivlnts/kg oil was determined in heated sunflower oils and in the extracted oils from cakes according to AOAC. (2000).

Statistical analysis:

The results from heated sunflower oils and extracted oils were statistically analyzed according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Chromatographic analysis of tomatoes peel extract:

The components of tomatomes peel extract were identified with paper chromatographic techniques and compared with authentic samples. The solvents used, (were butanol: acetic acid: water) (4: 1: 5) and acetic acid (ACOH, 15%). The color and R_F values of the flavonoid and phenolic compounds are shown in Table (2). The ethanolic extract was found to contain five flavonoid compounds (kaempferal , quercetin, lutolin, nargenin and rutin) and three phenolic acid compounds (*P*. coumaric, ferulic and chlorogenic acids). These results are agreemented with Martinez-Valvarde *et al*., (2002), who found that the content of phenolic compounds in tomatoes peel were characterized as flavonoids (quercetin, kaemferol and nargenin) and hydroxycinnamic acids (caffeic , chlorogenic , ferulic and p-coumaric acids).

Antioxidant activity of tomatoes peel extract:

The efficiency of natural antioxidants of tomatoes peel extract was compared with synthetic antioxidant (butylated hydroxyanisol, BHA) measured by thiocyanate method

and the results are reported in Table (3). The natural and synthetic antioxidant showed strong antioxidant activety 85.85 and 92.60%, respectively. Lipid per oxidation occurred by oxidation of fatty acids in the presence of enzymes and by exposure to reactive oxygen species and to transition metal ions in a free radical chain reaction. Phenolic compounds , because of their structure are very efficient scavengers of free radicals (Gazzani *et al.*, 1998).

Table 2. polyphenolic compounds of tomatoes peel extract

Compounds	R_F value		Without NH_3 Fumes	With NH_3 Fumes
	BAW	ACOH 15%	U.V	U.V
Flavonoids				
Kaempferol	82	1	Yellow	Bright yellow
Quercetin	65	4	Yellow	Bright yellow
Lutolin	87	67	Dark brown	Yellow
Narginin	88	3	Deep purple	Greenish purple
Rutin	44	56	Deep purple	Yellow
Phenolic acids				
p-Coumaric acid	69	92	Faint	Violet
Ferulic acid	74	54	Blue – violet	Green
Chlorognic acid	62	62	Blue	Green

BAW Butanol : Acetic acid : Water

ACOH Acetic acid

U.V. Ultra violet light

Table 3. Antioxidant activity of tomatoes peel extract compared with BHA.

	Absorbance at 500 nm.	% lipid peroxidation	Activity %
No additive	0.65	100	0.00
Tomato peels extracts	0.092	14.15	85.85
BHA	0.048	7.38	92.60

BHA Butylated hydroxyanisol.

Peroxide values in heated and extracted oils:

The peroxide value is a good index for the quality of fat. Refined fats should have peroxide value of less than 1 millequivalant / kg. Fats that have been stored for some period of time after refining may have peroxide value up to 10 millequivalent / kg oil (Rossell, 1983).

Table (4) showed the treatment of sunflower oil by heating at 180^0C for 32 h. with natural and synthetic antioxidants at different levels. From the results it could be observed that the natural and synthetic antioxidants at level 250 ppm effectively inhibited the increase in peroxide value for a peroid of 32 hours heating from 0.26 to 12.1 and 9.5 mileque./ kg , respectively. Little close effects were observed for the addition of BHA and natural antioxidants at 250 ppm. This means that tomatoes peel extract contained antioxidants (flavonoids and phenolic compounds) to retard lipid peroxidation during continous heating.

Table 4. Effect of antioxidants and heating temperature on peroxide value of sunflower oil.

Heating Period (hr)	Control	Sunflower oil containing andtixoidants					
		Natural			BHA synthetic		
		100 ppm	150 ppm	250 ppm	100 ppm	150 ppm	250 ppm
Zero	0.26						
4	7.5	4.2	3.3	3.2	3.3	3.2	3.0
8	9.7	5.3	4.0	3.7	4.1	3.4	3.2
12	29.0	8.7	5.2	4.3	4.7	4.4	3.9
16	33.4	15.4	6.3	5.1	7.1	5.1	4.5
20	40.7	17.0	7.2	5.6	9.2	6.3	5.0
24	56.4	19.7	9.1	6.7	12.3	8.1	6.1
28	63.2	22.5	10.7	9.7	14.5	9.3	8.3
32	73.1	25.3	13.1	12.1	17.8	11.5	9.5
L.S.D at 5%	0.03	0.07	0.04	0.09	0.05	0.12	0.16

Control sunflower oil free antioxidants

Peroxide value of oils extracted from sunflower cake was determined every four days up to twenty eight days and the results are given in Table (5). From the results, it could be noticed that 250 ppm of the tomatoes peel extract and BHA effectively inhibited the peroxide formation for a period of four days (p.v.2.4 to 2.7 and 2.3 to 2.6 millequ./ kg). Then the peroxide value increased to 15.2 in natural antioxidant and 12.1 millequ/kg at the end of storage period. Very close results were observed for the addition of natural and BHA at 250 ppm. It is worth to mention that 250 ppm from tomatoes peel extract also decresed the peroxide value.

From the aformationed results, it could be suggested that the addition of natural antioxidant from tomatoes peel at 250 ppm delayed the peroxide value.

Table 5. peroxide value of sunflower oil extracted from cake after baking as affected with tomatoes peel extract and BHA.

Time /day	Control	Sunflower oil containing andtixoidants					
		Natural			BHA synthetic		
		100 ppm	150 ppm	250 ppm	100 ppm	150 ppm	250 ppm
Zero	3.4	2.4	2.4	2.4	2.4	2.3	2.3
4	5.1	3.5	3.1	2.7	3.3	3.1	2.6
8	8.3	5.7	4.4	3.9	5.5	4.0	3.2
12	11.7	7.2	6.7	5.7	6.8	6.2	4.8
16	13.5	10.4	9.5	8.4	11.2	9.8	7.6
20	15.2	13.7	12.3	9.7	12.5	11.8	9.3
24	17.3	15.3	14.7	11.5	14.2	13.2	10.1
28	20.2	17.0	15.2	13.1	16.5	13.7	12.1
L.S.D at 5%	0.683	0.476	0.580	0.535	0.436	0.692	0.478

Before backing cake peroxide value of sunflower oil was 3.2 mel equ. /kg

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استخلاص المركبات البولي فينولية من قشور الطماطم كمضادات أكسدة طبيعية لحفظ جودة الكيك أثناء التخزين

رأفت نجيب سندق ، عبد المنعم صالح ابراهيم الشاذلي ، سهير طاهر الحديدي

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تم استخلاص المركبات الفلافونيدية والأحماض الفينولية من قشور الطماطم بواسطة كحول الايثايل ٧٠% لاستخدامها كمضادات أكسدة طبيعية وتم تفريد المركبات الفلافونيدية (كامفيرول – كيرستين – ليوتين – نارجينين – ريوتن) والأحماض الفينولية (بارا كيوماريك – فيريوليك – كلوروجينك) بواسطة التحليل الورقي الكروماتوجرافي – مضادات الأكسدة الطبيعية والمخلقة صناعيا BHA مثبطات قوية للأكسدة ونشاطها ٨٥.٨٥ و ٩٢.٦٠ % علي التوالي. تم إضافة مستخلص قشور الطماطم و BHT إلي زيت عباد الشمس بتركيزات ١٠٠ و ١٥٠ و ٢٥٠ جزء في المليون بغرض الحفاظ علي جودة الزيت أثناء التسخين علي ١٨٠° م لمدة ٣٢ ساعة وأيضا تم إضافة هذه التركيزات أثناء تصنيع الكيك من زيت عباد الشمس بغرض الحفاظ علي جودة الكيك أثناء التخزين لمدة أربعه أسابيع مع استخلاص الزيت من الكيك كل أربعه أيام.

أوضحت النتائج أن إضافة مضادات الأكسدة الطبيعية إلي زيت عباد الشمس يؤخر الأكسدة ويحسن صفات الزيت كما يؤخر أيضا من عملية أكسدة الليبيدات أثناء تخزين الكيك.