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EFFECT OF NATURAL ANTIOXIDANT EXTRACTED FROM HULLS OF *Vicia faba* ON CAKE SHELF LIFE.

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ABSTRACT

Hulls of *Vicia faba* were treated by ethanol 70% (v/v) to extract favonoids (kaempherol, quercetin, catechin, narginine and rutin) and phenolic acids (P-coumaric, ferulic and chlorgenic acids) then were identified by paper chromatographic technique. Synthetic antioxidant (butylated hydroxytoluene, BHT) and natural antioxidant exhibited strong and close antioxidative activities (91.70% and 86.88%, respectively). The hulls of *Vicia faba* extract and BHT were added to sunflower oil at levels 100, 150 and 200 ppm to keep its quality during heating at 180$\pm$5^{o}C for 32 hours. Moreover, the synthetic and natural 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 reported that the addition of *Vicia faba* hulls extract as natural antioxidant to sunflower oil and cake delayed the lipid peroxidation during heating oil and storage of cake.

Keywords: *Vicia faba*; antioxidants activity, sunflower oil, BHT

INTRODUCTION

Antioxidant play an important role in manufacturing packaging and storage of fats and fatty foods.

Consumers allover the world are becoming increasing conscious of the nutritional value and the safety of their food and its ingredients. At the same time, there is an increase preference for natural foods and food ingredients which are generally believed to be safer, more healthy and less subject to hazards than foods containing artificial food additives (Allen and Hamilton, 1993 and Farag *et al.*, 1989).

Plant seeds and leaves contain effective antioxidants, such as tea and barley leaves contain strong antioxidants (Osawa *et al.*, 1992). An isoflavonoid isolated from young barley leaves inhibited malonaldehyde formation from squalene by almost 100% upon ultra violet (UV) irradiation at the level 10 µmol (Nishiyama *et al.*, 1993).

There is a great need for safe, natural antioxidants, not only to prevent oxidative deterioration in foods but also to inhibit oxidative damages caused by lipid peroxidation. Therefore, antioxidants in foods have recently attracted special interest because they can protect the human body from free radicals which may cause various diseases including carcinogenesis and aging (Osawa *et al.*, 1987 and Catlar, 1992).

Addition of synthetic antioxidants can control lipid oxidation in foods. However, use of such compounds has been related to health risks resulting in strict regulations over their use in food products (Hettiarachchy *et al.* 1996).

There has been some discussion recently of the undesirable use of synthetic antioxidants, for example dietary administration of butylated hydroxytoluene (BHT) to rats caused fatal hemorrhages in the pleural and peritoneal cavities and in organs such as epididymis tests and pancrease. BHT caused also changes in rat thyoide stimulation of DNA synthesis and induction of enzymes. BHT had toxic and carcinogenic effects since some of synthetic antioxidants had toxigenic, mutogenic and carcinogenic effects and some natural antioxidants were effective in enhancing the shelf life of food, but less effective than synthetic antioxidants, there is a great demand for the use of new natural antioxidants in food (Nanditha and Prabhasankar, 2009 and Hussein *et al.*, 2009).

The present investigation aimed to isolate and identify flavonoid and phenolic compounds from *Vicia faba* hulls as a waste of low cost. Also, to study the effect of natural antioxidants (flavoid and phenolic compounds) to prevent lipid peroxidation in sunflower oil and cake comparing with the effect of butylated hydroxy toluene (BHT).

MATERIALS AND METHODS

Materials:

Hulls of *Vicia faba* was obtained from the Field Crops Research Institute, Agric. Res. station at Sakha (Kafr El-Sheikh governorate, Egypt). Hulls of *Vicia faba* was finely ground then passed through 40 mesh sieve to separate the bran.

Wheat flour, 72% extraction was obtained from south Tanta, Middle East Mills Co.

Ethanol and Butylated hydroxytoluene (BHT) was obtained from El-Nasr Pharmaceutical chemical, El-Ameriea, Cairo, Egypt. While synthetic antioxidant as flavonoids and phenolic compounds were purchased from Sigma ChemicalCo., St. Louis, Mo, USA.

Sunflower oil was obtained from Tanta Company for Oils and Soaps, Tanta Egypt.

Sugar, egg, vanilla and backing powder were obtained from local markets in Tanta city.

Methods:

Extraction, isolation and purification of polyphenolic compounds from hulls of *Vicia faba*:

Air dried hulls of *Vicia faba* (600 g) was finely powdered and extracted with petroleum ether (50-60^{o}C) to remove fats and resinous materials. The residue was exhaustively extracted with two liters 70% ethanol by heating on a boiling water both for six hours. Extraction was repeated until a color extract become colorless then the extracts were combined and concentrated to obtain aqueous ethanolic extract. A brown product was obtained upon evaporation of ethanol of dryness and kept for flavonoid and phenolic compounds investigation according to Merby *et al.* (1970).

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

The hulls of *Vicia faba* extract and authentic samples were spotted on one dimensional whatman No. 1 paper chromatography. 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 value under UV 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 hulls of *Vicia faba* and compared with butylated hydroxytoluene (BHT) by thiocyanate method as described by Tsuda *et al.* (1993).

Addition of antioxidant to sunflower oil:

Sunflower oil was used as a substrate for oxidations studies.. Natural antioxidant extracted from hulls of *Vicia faba* and synthetic antioxidant (BHT) were added to oil at 100, 150 and 200 ppm on a dry weight basis to test their 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±5^{o}C 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^{o}C 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 substituted by baking powder and vanilla. Natural and synthetic antioxidantns were added to the oil cakes at 100, 150 and 200 ppm levels. Sugar, whole egg, vanilla, baking 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^{o}C for 25 min and electric oven and cake was stored in refrigerator at 5 ^{o}C and packaged in polyethylene bags for four weeks.

Table (1): ingredients of cake made using sunflower oil

Ingredients	Flour	Sugar	Whole egg	Vanilla	Baking powder	Water	Sunflower oil
Weight (g)	200	250	150	1	13	40	100

Extraction of oil from cake:

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

Physico-chemical characteristics of oil:

Peroxide value ml-equivalents/kg oil was determined in heated sunflower oil and in the extracted oil 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

Paper chromatography of *Vicia faba* hulls extract:

Hulls of *Vicia faba* extract were identified with paper chromatographic technique and compared to authentic samples. Two solvents system were used (BAW 4:1:5) and ACOH (15%) color reaction and R_f values of the flavonoids and phenolic acids compounds are shown in Table (2). The ethanolic extract was found to contain five flavonoid compounds (Kampferol, quercetin, catechin, narginine and rutin) and three phenolic acid compounds (P. coumaric, ferulic and chlorogenic acids). These results are in agreement with Elfallah *et al.* (2011) who found that the content of phenolic compounds in hulls *Vicia faba* were characterized as flavonoids (Kaempherol, quecetin, catechin, marginine and rutin) and phenolic acids (P. coumaric, ferulic and chlorogenic acids).

Table (2):Polyphenolic compounds of *Vicia faba* hulls extract

Compounds	R_f value		Without NH_3 fumes	With NH_3 fumes
	ACOH 15%	BAW	UV	UV
Kampferol	1	82	Yellow	
Quecetin	4	63	Yellow	Bright yellow
Catechin	67	85	Dark brown	Yellow
Narginine	3	86	Deep purple	Greenish purple
Rutin	55	43	Deep purple	Yellow
Phenolic acids	-			
P-coumaric	91	67	Faint	Violet
Ferulic acid	52	72	Blue-violet	Green
Chloragnic acid	63	63	Blue	Green

ACOH = Acetic acid
UV = Ultra violet light
BAW = butanol Acetic acid: water

Antioxidantn activity of *Vicia faba* hulls:

The efficiency of natural antioxidant of *Vicia faba* hulls extract was compared with synthetic antioxidant (butylated hydroxyl toluene, BHT) measured by thiocyanate method and the results are reported in Table (3).

The natural and synthetic antioxidant showed strong antioxidant activity 86.88% and 91.70%, respectively. Lipid oxidation deterioration was the most cause of vegetable oils spoilage lipid oxidation do not only lower quality and nutritional value of foods, but also is associated with aging, membrane damage, heart diseases and cancer (Cosqrove *et al.*, 1987).

Table (3):Antioxidant activity of *Vicia faba* hulls extract compared with BHT

	Absorbance at 500 nm	% lipid peroxidation	Activity %
No additive	0.66	0.99	0.00
BHT	0.045	7.33	91.70
Hulls *Vicia faba* extracts	0.090	14.30	86.88

BHT: Butylated hydroxytoluene:

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 milli-equivalent /kg oil (Egan *et al.* 1989).

Table (4) reported the treatment of sunflower oil by heating at 180±5°C 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 200 ppm effectively inhibited the increase in peroxide value for a period of 32 hours heating from 0.24 to 11.7 and 9.1 meq/kg, respectively. Little close effects were observed for the addition of BHT and natural antioxidants at 200 ppm. This means that hulls of *Vicia faba* extract contained antioxidants (flavonoids and phenolic compounds) to retard lipid peroxidation during continuous heating.

Table (4): Changes in peroxidase values of sunflower oil extracted from cake after baking as affected with hulls of *Vicia* extract and BHT

Heating period	Control	Antioxidants level					
		Natural			BHT synthetic		
		100 ppm	150 ppm	200 ppm	100 ppm	150 ppm	200 ppm
Zero	0.24	0.24	0.24	0.24	0.24	0.24	0.24
4	7.8	4.0	3.1	2.9	3.1	3.1	3.0
8	9.7	5.1	4.0	3.2	4.0	3.2	3.1
12	29.0	8.1	5.0	4.0	4.2	4.0	3.8
16	33.0	14.5	5.9	5.0	6.9	4.8	4.5
20	41.0	16.0	6.8	5.2	9.0	6.0	5.0
24	55.4	18.7	8.8	6.4	11.8	7.9	6.0
28	63.2	22.0	10.8	9.1	14.1	9.0	8.2
32	73.5	24.0	12.7	11.7	16.9	11.5	9.1
LSD 5%	0.03	0.5	0.03	0.07	0.04	0.11	0.15

The 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).

Table (5): Peroxide value of sunflower oil extracted from cake after baking as affected with hulls of *Vicia faba* extract and BHT.

Storage period (days)	Control	Antioxidants levels					
		Natural			BHT synthetic		
		100 ppm	150 ppm	200 ppm	100 ppm	150 ppm	200 ppm
Zero	3.2	2.2	2.2	2.2	2.2	2.3	2.3
4	4.9	3.3	3.0	2.8	3.1	3.0	2.5
8	8.1	5.5	4.3	3.7	5.5	4.0	3.1
12	11.2	7.0	6.5	5.5	6.4	6.2	4.5
16	13.2	10.0	9.1	8.2	11.0	9.7	7.6
20	15.0	13.2	12.0	9.2	12.3	11.3	9.0
24	16.9	15.3	14.5	11.3	14.0	13.0	10.1
28	20.0	17.1	15.0	13.1	16.3	13.5	12.0
LSD 5%	0.68	0.47	0.59	0.52	0.43	0.68	0.47

From the results, it could be noticed that 200 ppm of the hulls of *Vicia faba* extract and BHT effectively inhibited the peroxide formation for a period of four days (P.V 2.2 to 2.8 and 2.3 o 2.5 meq/kg). Then the peroxide value increased to 15.0 in natural antioxidants and 12.0 meq/kg at the end of store period. Very close results were observed for the addition of natural and BHT at 200 ppm. It is worth to mention that 200 ppm from hulls of *Vicia faba* extract also decreased the peroxidase value. Suggested that the addition of natural antioxidant from hulls of *Vicia faba* at 200 ppm delayed the peroxide value.

From the aforementioned results, it could be suggested that addition of natural antioxidant from hulls of *Vicia faba* at 200 ppm delayed the peroxide value.

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تأثير مضادات الأكسدة الطبيعية المستخلصة من قشور الفول البلدى على إطالة فترة الصلاحية للكيك

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تم إستخلاص المركبات الفلافونيدية والأحماض الفينولية كمضادات أكسدة طبيعية من قشور الفول البلدى بإستخدام كحول الإيثايل ٧٠% وتم تفريد هذه المركبات بواسطة التحليل الكروماتوجرافى.

مضادات الاكسدة المخلقة صناعيا ، والطبيعية مانعات قوية للأكسدة ودرجة نشاطها ٩١.٧٠ ، ٨٦.٨٨% على التوالى وتم إضافة مضادات الأكسدة الصناعية BHT والطبيعية المستخلصة من قشور الفول البلدى إلى زيت عباد الشمس بتركيزات ١٠٠ ، ١٥٠ ، ٢٠٠ جزء فى المليون وذلك بهدف المقارنة والحفاظ على جودة الزيت أثناء التسخين على درجة ١٨٠°م لمدة ٣٢ ساعة ثم تم إضافة هذه التركيزات أثناء تصنيع الكيك على الزيت بهدف الحفاظ على جودة الكيك أثناء فترة التخزين لمدة أربع أسابيع وبعد ذلك تم استخلاص الزيت من الكيك كل أربعة أيام.

أظهرت النتائج أن إضافة مضادات الأكسدة الطبيعية إلى زيت عباد الشمس تمنع عملية الأكسدة وتحسن من صفاته كما تمنع عملية أكسدة الليبيدات أثناء تخزين الكيك وذلك مقارنة BHT الذى أعطى نفس النتائج.