ADDITION OF NATURAL ANTIOXIDANT TO RETARD CAKE RANCIDITY

SANDAK, R.N.

Food Technology Research Institute, Agriculture Research Center, Giza, Egypt.

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ABSTRACT

Bran from *sorghum vulgare* cultivar Assiut 14, was treated by ethanol 70% (v/v) to extract flavonoids and phenolic compounds as natural antioxidants. Flavonoids (Kaemfherol, quercetin, catechin fisetin, myricetin and narginin) and phenolic acids (p coumaric, ferulic, and chlorogenic) were identified by paper chromatographic technique using butanol: acetic acid: water (BAW 4:1:5) and acetic acid 15% as solvent system. Antioxidative activites of sorghum bran extract and synthetic antioxidant (butyulated hydroxyanisol, BHA) were determined using a linoleic acid system and measurement at 500 n.m. Natural and BHA antioxidants exhibited strong and close antioxidative activities of 86.43 and 88.57% respectively. The natural antioxidant extract was added to two types of cake, made up by French butter and sunflower oil, at levels of (500,100 and 2000 ppm) and results compared to BHA at (1000 and 2000 ppm). A control experiment was carried out without addition of any antioxidants. Cake was stored at room temperature for eight weeks. Every two weeks lipids were extracted, from the two types of cake by n-hexan solvent and their phyiso-chemical characteristics (refractive index, acid value, iodine value and peroxide value) were determined.

The results showed that addition of natural antioxidant extract and BHA at 1000 and 2000 ppm inhibited lipid peroxidation during storage of cake made up by butter. While in case of cake made by sunflower oil, 2000 ppm of antioxidant extract or BHA were effective.

INTRODUCTION

Sorghum is an important grain for human consumption in some parts of Asia and Africa and according to estimates reported by Agency for International Development, more than 300 million people depend on sorghum as their principal food. Brown-coated sorghum grains are proved to be more resistant to weathering and to be attacked by birds than other varieties with light-colored seed coats. The resistance of these sorghums had been attributed to their higher tannin content (Strumeyer and Malin, 1975).

Osuntogun *et al*, (1989) found that tannin, in the seeds of 15 Nigerian sorghum cultivars were between 0.25 - 2.92% (catechin equivalent). Total poly phenol con-

tent ranged from 0.32-2.7% (tannic acid equivalent). Total polyphenol content ranged from 0.32 to 2.7% (tannic acid equivalent).

Yossef *et al.*, (1989) extracted tannin and non-tannin polyphenols with methanol from defatted samples of ground sorghum (Sorghum vulgaare), and separated them by funnel technique using H2O to remove tannin while ethyl acetate was applied to remove non-tannin polyphenols.

Fenz *et al.*, (1992) investigated the phenolic compounds in milled grains of zea mays, 9 peaks were obtained in an HPLC chromatogram after clean up with polyamide. The structures of these substances were established using chemical and spectroscopic methods: Vanillic acid, coffic acid, p-coumaric acid and ferulic acid were identified by U.V spectra and retention times.

Duh *et al*, (1992) mentioned that the methanol extraction of peanut hulls produced a higher yield of a component having strong antioxidant activity than other organic solvents. The efficiency of solvents in extraction was in order of methanol > ethanol>acetone> chloroform >n-hexane. The antioxidant activity of methanolic extract was equal to butylated hydroxyanisol (BHA).

Tsuda, *et al.*, (1993) evaluated the antioxidative activity of pea bean hull by using a linoleic acid system as measured by the thiocyanate method. The methanol extract exhibited strong antioxidant activity.

Chen *et al*, (1996) examined the antioxidative activity of ethanol extracts from six major groups of tea (green, yellow, white, black, dark-green and oolong tea) against the oxidation of heated canola oil at at 100^{o}C. The ethanol of green, yellow and white tea, strongly inhibited oxidation of canola oil compared to butylated hydroxytoluene, probably due to the presence of natural antioxidants.

Khalil *et al*, (1997) extracted the broad bean hull with four separate solvents, methanol 70%, ethanol 95%, ethyl acetate and water to determine which solvent was the most effective for antioxidant extraction. The antioxidant activity of methanolic, ethanolic, ethyl acetate and water were, 95.0, 88.3, 80.0 and 75.0%, respectively compared to α-tocopherol and BHA, which were 85 % and 91.66%, respectively.

Flavonoids are polyphenolic antioxidant naturally present in vegetables, fruits and beverages. In vitro, flavonoids inhibit oxidation of low-density liporotein, re-

duce thrombotic tendency and atherosclorosis complications in human (Hertog *et al.*, 1993 and Jovanovic *et al.*, (1996).

Flavonoids are potent antioxidants with free radical scavengers, and metal chelators and inhibit lipid peroxidation. The structure requirements for antioxidants and free radical scavening functions of flavonoids include a hydroxyl group in carbon number four, and poly hydroxylation of the A and B aromatic rings (Cook and Samman, (1996) and Giese, (1996).

Culter (1984) cited that the lipid peroxidation is one of the major factors in the deterioration of food during storage and processing. The addition of antioxidants have become popular as means of increasing the shelf life of food products and improving the stability of lipid and lipid containing foods by preventing loss of sensory and nutritional quality.

Huage *et al.*, (1996) added tea polyphenol to fried noodles at 0-250 ppm and determined the peroxide value of the treated noodles for 3-day intervals during storage at 50°C. Results showed that peroxide value was 0.02-0.048, 0.018-0.053 and 0.014-0.042% in noodles containing 50,100 and 250 ppm tea polyphenols while it was 0.025-0.072% in untreated controls during storage respectively for 3-27 days. The optimum dose of tea polyphenols was 50 ppm. Tea polyphenols caused no taste or color changes in treated food.

Duh and Yen, (1997) cited that the addition of the antioxidants to food is effective in retarding fat oxidation. It is impressive that many substances have been identified which prevent lipid peroxidation. Some of these compounds are synthetic antioxidants and others occurs as natural dietary constituents.

Okezie, *et al.*, (1997) indicated that the antioxidants such as vitamin E, C and flavonoids are important dietary antioxidant factors that may help to protect against some diseases. In addition there is a growing interest in the use of natural antioxidant in food preservation.

The aim of this investigation was to study the effect of natural antioxidants (flavonoids and phenolic compounds extracted from Sorghum vulgare) to prevent lipid oxidation in cake and to compare their effect to BHA.

MATERIALS AND METHODS

Materials

Sorghum (*sorghum vulgare*) cultivar Assiut 14 was obtained from the Field Crop Research Institute Agric. Res. Center, Giza Egypt.

Sorghum was finely ground then passed through 40 mesh sieve to separate the bran.

Wheat flour, 72% extraction, was obtained from South Cairo Mills Co., Egyptian Ministry of Supply and Trade.

Butylated hydroxyanisol (BHA) was obtained from Naarden International Company, Holland. While synthetic antioxidant as flvonoids and phenolic compounds were purchased from Sigma Chemical company, Deisenhofen, Germany.

French butter, sunflower oil, vanillia and baking powder were purchased from local market.

Methods:

Extraction of antioxidant from sorghum bran.

Air dried sorghum bran (500g) was finely grcund and extracted with petrolum ether (B.P. 40-60^{o}C) in Soxhelt apparatus to remove lipids and resinous materials. The residue was exhaustively extracted with 2 liters 70% ethanol by heating on a boiling water bath for 6 hours. Extraction was repeated until a colorless extract was obtained and the extracts were combined. A brown product was obtained after evaporation of ethanol to dryness and kept for flavonoids investigation according to Mabry *et al*, (1970).

Determination of antioxidant acttivity

The antioxidant activity of the previous extract of sorghum bran was compared to BAH and was determined as reported by Tsuda *et al*, (1993). A dried antioxidant sample (2.0 mg) was added to a solution mixture of linoleic acid (0.13 ml) ehtanol (10 ml) and 0.2 M phosphate buffer (pH 7.0, 10 ml) and the total volume was adjusted to 25 ml with distilled water. The solution was mixed in a conical flask and incubatad at 37^{o}C. At intervals during incubation, the degree of oxidation was measured in duplicate by thiocyanate method and reading the absorbance at 500 n.m. after coloring with FeCl2 and thiocyanate.

Isolation and purification of flavonoid compounds

The brown extract of sorghum bran was tested by paper chromatography technique in order to identify the major flavonoids and phenolic acids as described by Markham and Mabry, (1968).

Isolated compounds and authentic flovonoids sample were spotted on one dimensional whatman No. I paper. The used solvent was butanol : acetic acid: water (BAW 4: 1:5) and acetic acid 15% (ACOH). The different spots (major flavonoid compounds and authentic samples) were located by color reaction and RF values under U.V. lights with and without the presence of NH3 fumes according to Markham and Mabry, (1968).

Preparation of butter and oil cake

The ingredients of butter and oil cakes are given in Table 1 according to Mizukoshi, *et al.*, (1979) with little modefication where as the foaming agent was replaced by baking powder and vanillia. Flavonoid as antioxidant extracted from sorghum bran was added to the butter or oil cakes at 500, 1000 and 2000 ppm and (BHA at 1000 and 2000 ppm.) Sugar, whole egg, vanillia, 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. in an electric oven and the cake was stored at room temperature for eight weeks.

Extraction of the lipid from cake

Cakes made up from butter and oil were extracted every two weeks by soaking in n-hexan at room temperature for 48 hours. The extract was filtrated and evaporated to dryness. Butter and oil were kept in the deep freezer for further investigation.

Physico-chemical characteristics of lipid

Refractive index at 25^{o}C, acid value, iodine value and peroxide value ml-equi/kg were determined in butter and oil according to AOAC (1990).

RESULTS AND DISCUSSION

Properties of flavonoids

Flavonoids inhibit lipid peroxidation in vitro at initiation stage by acting as

scavengers of superoxide anions and hydroxyl radicals. It had been proposed that flavonoids terminate chain radical forming a flavonoid radical. The flavonoid radical in turn reactts with free radicals thus terminating the propagating chain. In addition to their antioxidative properties, some flavonoids act as metal chelating agents and inhibit the superoxide-driven Fenton reaction, which is an important source of active oxygen radicals. However ther is no clear evidence of the antioxidant and free radical scavenging effects of flavonoids in vivo (Cook and Samman, 1996).

Antioxidant activity of crude extract

The antioxidant activity of sorghum bran extracted by ethanol 70% was compared to BHA and measured by thiocyanate methods. The results are shown in Table 2. The ethanolic extract showed strong antioxidant activity (86.43%) while that of BHA had a value of (88.57%).

Onyeneho and Hettiarachchy (1991) reported that Navy bean hull extract with ethanol 95% was acting as antioxidant in soy and sunflower oils and it was stronger antioxidant than butylated hydroxyanisol-butylated hydroxytoluene (BHA-BHT) mixture.

Paper chromatography analysis of sorghum bran.

Sorghum bran extract was identified with paper chromatography technique and compared to authentic samples. Two solvents system were used (BAW 4:1:5 and ACOH 15%) color reaction and Rf values of the flavonids and phenolic acids are summarized in Table (3). Three phenolic acids, P-coumaric, ferulic, and chlorgenic acid, and six flavonoids, (kaemfherol, qurectine, catechin, fisetin, myricetin narginin) were identified.

Vekiari *et al*, (1993) reported that flavonol Kaemfherol was an effective antioxidant. Dihydroflavonols had the same antioxidant activity as the corresponding flavonols. Conversion of dihydroflavonols to flavonols took place while the compounds were in contact with the oxidizing lipids and that the conversion migh account for the aantioxidant activity.

Antioxidant activity of the flavonoids increased as the number of phenolic hydroxyl groups was increased. Antioxidant activity was in order : myricetin > quercetin > catechin > Kaemfherol The glycons rutin and narginin were consistently weaker antioxidant than the aglycones (pekkarinen, 1996).

Physio-chemical characteristics of the cake

Butter type cake

The refractive index, acid value and iodine value did not change during storage. Therefore, the peroxide value as an indicator of rancidity was used.

For the cake made up from French butter, natural antioxidant extracted from sorghum bran was added at 500, 1000 and 2000 ppm levels and was compared to BHA at 1000 and 2000 ppm. Physio-chemical characteristics of the lipids extracted from butter cake (refractive index, acid value, iodine value and peroxide value) were determined every two weeks up to 8 weeks. The results are given in table 4.

From Table 4 it can be observed that 2000 ppm of the sorghum bran extract effectively inhibited the increase in peroxide value for a period of two weeks (p.v.2.3 to 2.7). Then the peroxide value increased to 3.9, 5.0 and 6.5 after 4,6 and 8 weeks respectively. Very close results were observed for the addition of BHA at 2000 ppm. This means that the sorghum bran extract contained high amounts of antioxidants. It is worth to mention that 1000 ppm extract also decreased the peroxide value.

The peroxide value is a good index for the quality of a fat. A refined fats should have peroxide value of less than I milliequivalent/kg. Fats that have been stored for some period of time after refining may be found to have peroxide value of up to 10 milliequivalents/kg. (Rossell, 1983).

Oil type cake

Antioxidant extract was added at 500, 1000 and 2000 ppm to the oil type cake and was compared to BHA at (1000 and 2000 ppm). The physio-chemical characteristics of the oil extracted from the cake were determined every two weeks for up to 8 weeks, and the results are presented in table 5. The results revealed that the addtion of 2000 ppm of the extract was effective as antioxidant for up to two weeks. Then the peroxide value recorded 4.3, 6.1 and 7.8 after 4, 6 and 8 weeks. Similar results were observed in case of BHA. It is worthy to mention that 1000 ppm of antioxidant extract was very less effective than 2000 ppm of BHA.

Table 1. Ingredients of two types of cake made using frensh butter and sunflower oil

Ingredients	Weight/g.
Flour	200
Sugar	250
Whole egg.	150
Vanillia	1
Bakinng powder	13
Water	40
Frensh butter Or sunflower oil	100

Table 2. Antioxidant activity of sorghum extract as compared to BHA

Antioxidant	Absorbance at 500 nm.	% Lipid Peroxidation	Activity %
No additive	0.70	100	0
Sorghum extract	0.095	13.57	86.43
BHA	0.08	11.43	88.57

*The antioxidant activity of extract was determined by the thiocyanate method, and reported as percentages inhibition of peroxidation of linoleic acid.

Table 3. Color reaction and RF values of flavonoid and phenolic acid compounds extracted from sorghum bran.

Compounds	Color reactetion		RF valves	
	U.V.Light	U.V.NH_3	BAW	ACOH 15%
Flavonoids				
Kaemfherol	Yellow	Bright-yellow	84	1
Qurcetine	Yellow	Bright-yellow	65	3
Catechin	Faint	Dark	63	39
Fisetin	Yellow	Fluorescent-Yellow	56	5
Myricetin	Yellow	Yellow	29	2
Narginin	Deep-purple	Greenish purple	88	3
Phenolic acids				
P. coumaric acid	Faint	Violet	69	92
Ferulic acid	Blue-vilot	Green	74	54
Chlorogenic acid	Blue	Green	62	62

BAW: Butanol: Acetic acid: Water (4:1:5) upper layer.
ACOH : Acetic acid 15%.

Table 4. Physio- chemical Characteristics of butter cake treated with NAE and BHA antioxidants.

Storage perod in weeks	*Treatment*	*Aeid value*	*Iodine value*	*Iodine value*
Zero time	Contrl	1.4627	43.2	3.4
	Contrl+ 500 ppm NAE	1.4628	44.0	2.4
	Contrl+ 1000 ppm NAE	1.4628	44.1	2.4
	Contrl+ 2000 ppm NAE	1.4627	44.2	2.3
	Contrl+ 1000 ppm BHE	1.4628	44.1	2.4
	Contrl+ 2000 ppm BHE	1.4627	44.2	2.3
Two weeks	Contrl	1.4618	35.4	5.8
	Contrl+ 500 ppm NAE	1.4620	37.6	3.4
	Contrl+ 1000 ppm NAE	1.4622	39.2	3.1
	Contrl+ 2000 ppm NAE	1.4624	40.8	2.7
	Contrl+ 1000 ppm BHE	1.4623	39.2	3.0
	Contrl+ 2000 ppm BHE	1.4625	41.5	2.6
Four weeks	Contrl	1.4611	29.5	10.9
	Contrl+ 500 ppm NAE	1.4615	33.3	6.7
	Contrl+ 1000 ppm NAE	1.4618	35.6	4.4
	Contrl+ 2000 ppm NAE	1.4620	37.1	3.9
	Contrl+ 1000 ppm BHE	1.4619	36.9	4.0
	Contrl+ 2000 ppm BHE	1.4623	38.5	3.2
six weeks	Contrl	1.4605	24.2	14.8
	Contrl+ 500 ppm NAE	1.4611	29.7	8.2
	Contrl+ 1000 ppm NAE	1.4612	30.6	6.1
	Contrl+ 2000 ppm NAE	1.4616	34.1	5.0
	Contrl+ 1000 ppm BHE	1.4615	31.2	5.5
	Contrl+ 2000 ppm BHE	1.4618	35.5	4.3
eight weeks	Contrl	1.4603	19.3	19.7
	Contrl+ 500 ppm NAE	1.4605	24.7	11.5
	Contrl+ 1000 ppm NAE	1.4606	25.6	8.3
	Contrl+ 2000 ppm NAE	1.4610	27.1	6.5
	Contrl+ 1000 ppm BHE	1.4608	26.9	7.9
	Contrl+ 2000 ppm BHE	1.4612	28.4	6.0

NAE: Natural antioxidant extracted from sorghum bran.
BHA: Butylated hudroxyansl.

Table 5. Physio- chemical Characteristics of oil cake treated with NAE and BHA antioxidants.

Storage perod in weeks	Treatment	Refractive index	Iodne value	Iodne value	Iodine value
Zero time					
	Contrl	1.4729	0.17	131.2	3.3
	Contrl+ 500 ppm NAE	1.4734	0.15	136.0	2.8
	Contrl+ 1000 ppm NAE	1.4735	0.13	136.2	2.6
	Contrl+ 2000 ppm NAE	1.4735	0.12	136.6	2.0
	Contrl+ 1000 ppm BHE	1.4735	0.13	136.2	2.4
	Contrl+ 2000 ppm BHE	1.4735	0.12	137.7	2.0
Two weeks	Contrl	1.4722	1.81	125.1	5.8
	Contrl+ 500 ppm NAE	1.4731	1.16	133.2	3.4
	Contrl+ 1000 ppm NAE	1.4732	0.92	133.8	3.1
	Contrl+ 2000 ppm NAE	1.4733	0.75	134.9	2.7
	Contrl+ 1000 ppm BHE	1.4733	0.85	133.9	3.0
	Contrl+ 2000 ppm BHE	1.4734	0.73	135.2	2.6
Four weeks	Contrl	1.4711	2.73	116.1	10.9
	Contrl+ 500 ppm NAE	1.4726	1.53	128.3	6.7
	Contrl+ 1000 ppm NAE	1.4728	1.27	130.7	4.4
	Contrl+ 2000 ppm NAE	1.4730	1.06	132.0	3.9
	Contrl+ 1000 ppm BHE	1.4728	1.19	131.1	4.0
	Contrl+ 2000 ppm BHE	1.4730s	0.98	132.4	3.2
six weeks	Contrl	1.4699	3.93	105.2	14.8
	Contrl+ 500 ppm NAE	1.4706	2.17	120.1	8.2
	Contrl+ 1000 ppm NAE	1.4713	1.73	122.3	6.1
	Contrl+ 2000 ppm NAE	1.4717	1.46	128.4	5.0
	Contrl+ 1000 ppm BHE	1.4714	1.67	123.7	5.5
	Contrl+ 2000 ppm BHE	1.4719	1.32	129.0	4.3
eight weeks	Contrl	1.4686	5.10	94.3	19.7
	Contrl+ 500 ppm NAE	1.4706	3.18	111.3	11.5
	Contrl+ 1000 ppm NAE	1.4713	2.57	117.6	8.3
	Contrl+ 2000 ppm NAE	1.4717	2.13	120.8	6.5
	Contrl+ 1000 ppm BHE	1.4714	2.43	118.4	7.9
	Contrl+ 2000 ppm BHE	1.4719	2.05	121.1	6.0

NAE: Natural antioxidant extracted from sorghum bran.
BHA: Butylated hudroxyansl.

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إضافة مضادات الأكسدة الطبيعية لحماية الكيك من التزنخ

رأفت نجيب سندق

قسم بحوث تكنولوجيا المحاصيل - معهد بحوث تكنولوجيا الأغذية مركز البحوث الزراعية - جيزة - مصر.

تم استخلاص المركبات الفلافونيدية والأحماض الفينولية من قشور الذرة الرفيعة صنف (أسيوط ١٤) بواسطة كحول الإيثايل ٧٠٪ لأستخدامها كمضادات أكسدة طبيعية وتم تفريد المركبات الفلافونيدية(كامفيرول-كيرسيتين-كاتشين-فيزتين-ميرستين-نارجتين) والأحماض الفينولية (بارا كيوماريك -فيريوليك -كلورو جينك) بواسطة التحليل الورقى الكروماتوجرافى مع استخدام المذيبات (كحول البيوتيل : حامض الخليك : الماء بنسبة٤: ١:٥) وحامض الخليك ١٥٪. تم تقدير نشاط مضادات الأكسدة الطبيعية المستخلصة من قشور الذرة الرفيعة ومضادات الأكسدة الصناعية (BHA) باستخدام حامض اللينوليك وقياس النشاط بطريقة الثيوسينات أوضحت النتائج أن مضادات الأكسدة الطبيعية والصناعية(BHA) لها تأثير مثبط قوى على منع الأكسده بلغ ٨٦,٤٣٪ و٨٨,٥٧٪ على التوالى. تم إضافة مستخلص مضادات الأكسدة الطبيعية على نوعى من الكيك المصنع من السمن الفرنسى وزيت عباد الشمس بتركيزات ٥٠٠، ١٠٠٠، ٢٠٠٠ جزء فى المليون ومقارنتها بمضادات أكسدة صناعية BHA بتركيزات ١٠٠٠ ، ٢٠٠٠ جزء فى المليون اما عينة الكيك القياسية لا تحتوى على أى إضافات من مضادات الأكسدة وتم تخزين الكيك على درجة حرارة الغرفة لمدة ثمانى أسابيع وقد تم إستخلاص الزيوت والسمن من نوعى الكيك كل أسبوعين بواسطة مذيب الهكسان وتقدير الخواص الكيماوية والطبيعية (معامل الأنكسار -رقم الحامض -الرقم اليودي- رقم البروكسيد) للزيت والسمن.

أوضحت النتائج أن إضافات مضادات الأكسدة الطبيعية وBHA بتركيزات ١٠٠٠ . ٢٠٠٠ جزء فى المليون تعمل على حماية الليبدات من الأكسدة أثناء تخزين الكيك المصنع من السمن بينما الكيك المصنع من الزيت تتم حماية الليبدات على تركيز ٢٠٠٠ جزء فى المليون من مضادات الأكسدة الطبيعية وBHA .