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DETERMINATION OF ANTIOXIDANT ACTIVITY OF UNDERUTILIZED PLANT EXTRACTS AND SYNTHETIC ANTIOXIDANT FOR ITS APPLICATION IN BAKERY PRODUCT

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Antioxidants have gained considerable interest in recent years for their role in preventing the auto oxidation of fats, oils and fat containing food products. In the present study, plant foods viz., amla (*Emblica officianalis*), Guduchi (*Tinospora Cordifolia*) and Barnyard millet (*Echinochloa frumentacea*) were used as sources of natural antioxidants. The two extracts exhibited a high antioxidant activity which was evaluated using DPPH method. Biscuits treated with natural antioxidants (Barnyard millet) received higher sensory score during storage, than control and BHA. Biscuits were analyzed for the stability during storage at an interval of one week for the period of five weeks at ambient temperature by determining antioxidant activity, free fatty acid, and peroxide value. Addition of plant extracts gave an excellent antioxidant effect on the biscuit, as peroxide and acid values were lower than that of the control samples after storage period. The AOA was found to be decreased during storage period.

Keywords: Antioxidant, barnyard extract, biscuit, free fatty acid, peroxide value

INTRODUCTION

The changing food habits of people due to rapid urbanization, the demand of processed and ready to eat food is also increasing rapidly. Among the processed foods, bakery products, particularly biscuits command wide popularity in rural as well as urban areas among all the age groups. Now a days people are becoming health conciousnous and desire to have a healthier diet (Hilliam, 1995) without changing their conventional dietary patterns due to change in their life style (Becker and Kyle, 1998). Cereals are important part of daily diet across the world, still the phytochemicals and the bioactive antioxidants present in them does not receive attention as fruits and vegetables have, although they contains a unique blends of phytochemicals and bioactives present in them. Auto-oxidation of fat may be the primary cause of deterioration in quality of food products which results in decrease of organoleptic value of foods and hence imparts rancid and unpleasant flavors to the raw and processed fat products, thus making them unacceptable to consumers (Min and Lee, 1998). Lipid oxidation can be slowed down by adding appropriate quantity of antioxidants. Traditionally, chemically synthesized antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are added in oil/fat products to prevent oxidation of them thus minimize deterioration of the sensory properties of the food products (Whysner *et al.,* 1994). Present scenario necessitates exploring the possibility of incorporating novel ingredients in commonly consumed foods rather than developing new food product (Reddy *et al.,* 2005). With the current status nutritional quality of biscuits and the growing demand for nutritious foods, it seems worthwhile to make efforts in enhancing the nutritional value of biscuit (Reddy, *et al.,* 2005). Auto oxidation of fats and oils in processed

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foods may be prevented by the use of oxidation inhibitors or antioxidants (Coulter, 1988; Adegoke *et al.*, 1998; and Reddy *et al.*, 2005). Synthetic antioxidants such as Butylated Hydroxyl Anisole (BHA) and butylated hydroxy toluene (BHT) have been used as antioxidants for food products, since the beginning of this century (Jan Pokorny, 1991; and Byrd, 2001). The use of synthetic antioxidants has began to be restricted because of their toxicity and ill effect produced during long term consumption (Branen, 1975; and Gazzani *et al.*, 1998). Plant extracts obtained from some fruits and vegetables have been reported to be effective antioxidants (Al-Saikhan *et al.*, 1995; Cao *et al.*, 1996; Vinson *et al.*, 1998; and Dillard and German, 2000). Additions of freeze-dried extracts from fenugreek seeds and ginger rhizomes to beef patties are reported to be effective in controlling lipid oxidation during cold storage (Pokorny, 1991; Mansour and Khalil, 2000; and Reddy *et al.*, 2005). *Tinospora cordifolia* an indigenous plant used in Ayurvedic medicine is commonly known as (Gulancha) belonging to the family Menispermaceae has chemopreventive (Chaudhary *et al.*, 2008), anti osteoporetic (Kapur *et al*, 2008), hepatoprotective (Panchabhai *et al.*, 2008), immuno modulatory (Bishayi *et al.*, 2002), Antihperglycaemic (Umamaheshwari and Mainzen Prince, 2007), antitumor (Jagetia and Rao, 2006) and antiallergic (Badar *et al*., 2005) properties. They were effective in scavenging superoxide anion radical and inhibited deoxyribose degradation by scavenging hydroxyl radical directly, barnyard millet possess antioxidant properties as they contain good amount of phenolic compound (Dykes and Rooney, 2007; Hodzic *et al.*, 2009; Sreeramulu *et al.*, 2009; and Rao *et al*., 2011). Millets cointains mineral like magnesium and phosphorous, vitamins B, bioactive components and phytochemicals, including phytic acid (Shashi *et al*., 2007) which is proving to reduce cholesterol level and reduces the risk of cancer while amla is one of the richest source of Vitamin C known. The present study is an attempt to evaluate the utilization of some plant foods which are considered underutilized as a source of antioxidant in biscuit for the study of stability of fats in it.

MATERIALS AND METHODS

Three plant food sources namely barnyard millet (*Echinochloa frumentacea*), tinospora stem and dehydrated amla (*Emblica officinalis*) were used as sources of natural antioxidants. They were obtained in bulk from local market and were subjected to different processing treatments prior to extraction. Amla of *chakiya* variety was procured from the local market of Allahabad, Uttar Pradesh, India. The powder was prepared by drying the grated Amla in a hot air oven at 50 °C for 8 h, followed by grinding in a mixer grinder. The barnyard grains were purchased from local market and were grinded in a laboratory mixer; while tinospora stem was obtained from botanical garden of Botany Department, University of Allahabad, Allahabad. The tinospora stem was dried in vaccum oven at 55 °C at 12.5 mmHg for 10 h, followed by grinding in a mixer grinder. All the obtained powders were stored in air-tight containers at 5 °C refrigerated temperature until used for preparation of extract. All the chemicals used for the analysis were AR grade procured from Science Corporation Allahabad.

Preparation of Plant Extracts

Dry powders of amla, tinospora stem and barnyard were separately passed through a 60 mesh screen. About 100 g of each food sample was shaken with 90% ethanol in an orbital shaker for 6 h and filtered. The residues obtained after filtration were dried overnight and were extracted twice with 90% ethanol by shaking for 1 h and filtered. The combined extracts were concentrated by evaporating the solvent in a rotary evaporator. The obtained extracts were stored in amber colored air-tight containers at 4 °C, for future use.

Determination of Antioxidant Activity

The antioxidant activities of native and processed raw materials were also measured by the DPPH radical scavenging method (De Ancos *et al.*, 2002). An aliquot (0.10 ml) of sample extract in methanol was mixed with 2 ml of methanolic 0.1 mM DPPH solution and the volume was made upto 5 ml with distilled water. The mixture was thoroughly vortex-mixed and kept in dark for 30 min. The absorbance was measured at 515 nm. The result was expressed as percentage of inhibition of the DPPH radical. The percentage of inhibition of the DPPH radical was calculated according to the following equation:

%inhibition of DPPH = [(Abs control-Abs sample)/(Abs control)] x 100

where, Abs control is the absorbance of the DPPH solution without the extracts.

Preparation of Biscuits

Biscuits were prepared as per the prescribed method by Manohar and Rao (1999). Sugar (90 g) and fat (60 g) were creamed for 3-4 min in a laboratory mixer. The extracts were blended with the fat and the emulsion was mixed with sugar.

Dough water containing sodium and ammonium bicarbonate (1.5 and 3 g), and sodium chloride (3 g) was added to the above cream for 5 min to obtain a homogenous dough. Refined flour (300 g) sieved twice with baking powder (0.9 g) was added and mixed for 3 min. The dough was sheeted to a thickness of 3.5 mm and cut into circular shapes using 45-mm cutter and placed on an aluminium tray, baked at 160 ^{0}C for 10 min and then cool at room temperature. The biscuits were stored in air-tight containers at ambient temperature. Five variations of biscuit were prepared. Control treatment was prepared without antioxidant extract addition. The other variations were prepared by adding synthetic antioxidant (BHA 200 ppm) and natural antioxidant extracts (1% amla and Tinospora, 3% Barnyard). Initially biscuits were prepared with addition of different levels (0.5%, 1%, 2% and 3%) of extracts, and were subjected to sensory acceptability. The data indicated that the threshold level of amla and tinospora extract in biscuits was 1%, while that for barnyard extract was 3%. Hence, for further studies biscuits were prepared accordingly.

Sensory Studies

Sensory evaluation of biscuits (freshly prepared and stored) was conducted to determine the acceptability of the product prepared by antioxidant extract addition. Seventy five panelists were selected from among the postgraduate students in the Center of Food Technology, University of Allahabad, on the basis of their willingness to participate and also a sweet threshold test. Panelists were presented with the product on two occasions to familiarize them with the quality attributes. Five differently coded samples were served to the panelists. The samples were evaluated for sensory attributes using a 9 point Hedonic rating (9-like extremely and 1-dis like extremely) (Murray *et al.*, 2001) for color, flavor, texture and overall acceptability.

Chemical Analysis

The ground biscuits were used for the study. Stability of defatted biscuit were followed periodically at intervals of 1 week during storage for 5 weeks at ambient temperature, by determining AOA (as given in Section 2.2), Peroxide Value (PV), Free Fatty Acid (FFA), was determined according to the methods described by I.S: 548 (Part 1) – 1964. The above analyses were carried out in two replicates.

Statistical Analysis

The sensory data were subjected to ANOVA followed by Duncan's new multiple range test at 0.05% probability.

RESULTS

Chemical analysis

Antioxidant activity of the extracts obtained from food sources are given in Table 1. The AOA in the extracts of amla, tinospora and baranyard were higher than BHA. AOA was also determined in dry powders of the samples. All the extracts showed higher AOA then their powders. The higher antioxidant activity in extracts may be due to the presence of antioxidant compounds (vitamin C, E and beta-carotene) in concentrated form. Biscuits were analyzed for its antioxidant activity, FFA and peroxide value, to determine the stability during storage (Table 2). The AOA was found to decrease during storage period in all the five variations. The decrease was marginal in BHA biscuits and there was a moderate decrease in biscuits containing amla, tinospora and barnyard extracts. However, the mean AOA after 5 weeks was significantly higher ($P<0.05$) in B5 compared to the rest. The results indicate that maximum lipid stability, at ambient temperature, was exhibited by the addition of barnyard millet extract as compared to synthetic antioxidants – BHA, after 5 weeks. Changes occurring in the PV and FFA values of the biscuit variations during storage are given in Table 3. In general, all the four antioxidants used slowed down the rate of peroxide formation, since PV of all samples which contained synthetic (BHA) or natural antioxidants

Table 1: Antioxidant Activity in Extract

	Antioxidant Activity %	
Sample	Extract	Powder
Amla	85±1.2	71±0.42
Tinospora	73.56±0.85	66.49±0.51
barnyard	51.74±0.83	35.67±0.33
BHA	92±1.1	92±0.68

Table 2: Antioxidant Activity (%) in Biscuit Extract

Biscuit Sample	Initial (0 day)	21st day	35th day
B1 (control)	29	13	9
B2 (BHA)	71	66	60
B3 (Amla)	62	59	47
B4 (Tinospora)	51	42	35
B5 (Barnyard)	64.54	58	50

Table 3: Free Fatty Acid and Peroxide Value in Biscuit

Biscuit Sample	Initial	3rd week	5th week
Peroxide value (g equiv. of O_2/100 g)			
B1 (control)	0.74	0.9	2.8
B2 (BHA)	0.5	0.76	1.5
B3 (Amla)	0.62	1.07	2.3
B4 (Tinospora)	0.4	0.9	1.9
B5 (Barnyard)	0.3	0.82	1.8
Free fatty acid value (% of stearic acid)			
B1 (control)	7.1	9.79	12.08
B2 (BHA)	0.34	1.07	1.63
B3 (Amla)	0.86	2.54	2.94
B4 (Tinospora)	0.6	1.64	2.42
B5 (Barnyard)	0.34	1.38	2.11

Table 4: Mean Scores for the Acceptability of Biscuits

Biscuit Sample	Initial	3rd week	5th week
B1 (control)	6.73[a]	5.83[f]	5.34[g]
B2 (BHA)	7.40[b]	6.16[b]	5.7[b]
B3 (Amla)	6.65[c]	6.00[c]	5.76[c]
B4 (Tinospora)	6.03[d]	5.83[d]	5.17[d]
B5 (Barnyard)	7.00[e]	6.66[e]	6.36[e]

were lower than that of control sample, during storage. PV of lipids extracted from the control sample after baking (at zero time) was 0.74 which did not differ considerably from those of other variations, which ranged between 0.3 and 0.62. The increase in PV was observed in all the biscuit samples; however control biscuits had the highest value of 2.8 after 5 weeks and the PV of the other biscuit samples, ranged from 1.5 to 2.3. The values obtained are with in acceptable range and significantly lower in B4 and B5 after 5 weeks. The changes occurred in PVs of biscuit lipids during storage confirmed those reported for antioxidant activity of biscuit lipids during storage at ambient temperature. An increase in FFA value was observed in all the biscuit samples, on storage. The increase was considerably higher in biscuits (control) prepared without the addition of antioxidant compared to biscuit samples, in which synthetic (B2) or natural antioxidants (B3, B4, and B5) were incorporated. In B2 and B5 samples, initially FFA was not detected, a gradual increase (upto1.63% and 2.11 respectively) was observed indicating the potency of antioxidants in inhibiting the formation of FFA. The FFA in B3, and B4 were comparable to that of B5 values.

Sensory Studies

Table 4 summarizes the results of sensory analysis of the biscuits and gives the mean scores for over all acceptability scores (during the storage period and between the variations). In the fresh samples, it was observed that B3, B4, and B5 samples were well accepted in terms of flavor, crumb texture, taste and mouth feel compared to the control and standard variation. In B1 sample, which did not contain any antioxidant the mean scores assigned by the panelists did not differ on initial, 3rd and 5th week. Similar trend was also seen in B2 samples prepared with BHA.

In B3 (amla), B4 (tinospora) and B5 (barnyard extract), it was interesting to note that the overall acceptability did not differ during the storage period, which indicates that all the samples containing natural antioxidant samples were acceptable during 5 weeks of storage evaluation. Biscuits treated with natural antioxidants extracted from barnyard millet received higher (P60.05) scores on 3rd and 5th week than control, BHA, and amla extract incorporated biscuits. Incorporation of different extracts did not have any significant influence on the surface color characteristics and texture of the biscuits, owing to their level of addition, i.e., 1% and 3%. However, incorporation of amla and tinospora (B3 and B4) extract in biscuits imparted greenish color, despite this, the biscuits were well accepted (Table 4).

DISCUSSION

Three different plant materials (amla, tinospora and barnyard) were used in the present study as sources of natural antioxidants. The samples were analyzed for percent antioxidant activity, all three sources exhibited a high percentage of antioxidants, and the activity was higher in amla extract compared to barnyard and tinospora extract. Biscuits were prepared by the incorporation of extracted antioxidants from Sensory evaluation of prepared biscuits was judged on 9 point hedonic rating to determine the acceptability and efficacy of incorporated antioxidants over

a storage period of 5 weeks. The effects of antioxidants on the stability of the added fat and the product were determined by monitoring AOA, FFA and PV periodically under normal storage conditions used for the storage of bakery products. Biscuits incorporated with extract from Barnyard appeared to possess stronger antioxidant activity than the other two extracts which may be due to higher percentage of volume of extract added as compared to other two. In order to obtain maximum benefit from the use of antioxidants in food products, several points need to be considered in their selection and use. The form of antioxidant (powder or solution), method and time of incorporation are particularly important for the dispersion of antioxidant and ultimately stabilization of the product (Prevention of Food Adulteration, 1996). In the present study, the antioxidants extracted from the selected sources were in the solution form. They were added in small amounts (1-3%) to the product, i.e., biscuits.

The extracted antioxidants were added to the fat/lipid medium hence could ensure uniform dispersion by acting as a solvent. It is well known that naturally occurring antioxidants could be significantly lost as a result of processing and storage (Jonsson, 1991). Among the various processing methods used for the product development, thermal treatments are reported to influence antioxidant activity the most. The selected food product, i.e., biscuits were baked at 160-180 °C, the incorporated antioxidant in the three variations (B3, B4 and B5) were not affected by temperature as indicated by the antioxidant analysis. The increased in antioxidant activity can be explained by the formation of non enzymatic browning like mallard and caramelization reaction, which increases the antioxidant activity during thermal processing which is known to alter the antioxidant profile and generate more antioxidants which contribute in antioxidant activity (Sharma *et al.*, 2012). Increase in antioxidant activity during thermal processing has been widely reported (Dewanto *et al.*, 2002; and Sharma *et al.*, 2012). In addition, the antioxidants were not affected by storage over a period of 5 weeks, as the AOA (%) was significantly ($P<0.05$) higher in B3 and B5 samples. Shelf-life is a major consideration in developing, producing and marketing food products. It refers to the time during which a product remains acceptable to a consumer in terms of sensory characteristics. Many factors influence the shelf-life of a product viz., moisture loss, and spoilage due to micro organisms, enzymatic changes and oxidation (Adegoke *et al.*, 1998). Oxidation is of particular significance in biscuits as they contain added fat. The PV was estimated in the products as an indication of the degree of oxidation. The results suggest that antioxidant extracts of both B3 and B5 inhibited the process of lipid oxidation as the PV of B4 and B5 samples were significantly lower (P60.05) than the other samples even after 5 weeks. In biscuits, addition of purified extracts of marjoram, mint and basil is reported to have an excellent antioxidant effect compared with the effect of BHA (Bassiouny *et al.*, 1990). In this study, the sensory scores of biscuits prepared with the extract of barnyard millet were accepted well during the storage study, which indicates the efficiency of antioxidants in preventing the onset of rancidity.

CONCLUSION

The addition of extracts of the three plant materials, gave an excellent antioxidant effect on the biscuits compared with the effect of synthetic antioxidant BHA. The higher efficiency of the plant extracts could be due to the stability of this natural antioxidant during baking. Results of sensory evaluation reveal that the selected plant extracts at concentrations of 1% and 3% may be used in place of synthetic antioxidants, since these extracts had no effect on the organoleptic properties of the biscuit. Addition of natural antioxidants can increase shelf-life of food products containing fats and oils. In addition, natural antioxidants are safe and impart health benefits to the consumer.

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