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Food Chemistry 90 (2005) 317–321
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Evaluation of antioxidant activity of some plant extracts and their application in biscuits

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Received 1 October 2003; revised 22 May 2004; accepted 22 May 2004

Abstract

Natural 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, three plant foods viz., amla (*Emblica officianalis*), drumstick leaves (*Moringa oleifera*) and raisins (*Vitis vinifera*) were used as sources of natural antioxidants. All the three extracts exhibited a high percentage of antioxidant activity evaluated using β-carotene–linoleic acid in vitro system, compared to synthetic antioxidants. Biscuits prepared by addition of natural extracts were subjected to sensory studies and chemical analysis. Biscuits treated with natural antioxidants, extracted from raisins (B4) and drumstick leaves (B5) received higher ($P \leqslant 0.05$) panel scores during storage period of 6 weeks, than control (B1), butylated hydroxyl anisole (BHA) (B2) and amla (B3) extract incorporated biscuits. Addition of plant extracts from the three plant foods gave an excellent antioxidant effect on the biscuit compared with the effect of BHA, as the % increase in both peroxide and acid values after 6 weeks were lower than that of the control and BHA treated samples. Extracts from drumstick leaves and amla were more effective in controlling lipid oxidation during storage.

Keywords: Natural antioxidants; Biscuits; Lipid oxidation; Storage

1. Introduction

Processed foods containing fats and oils, oxidize slowly during storage, various oxidation products cause rancidity and deterioration of the sensory properties of the food products. Autooxidation of fats and oils in processed foods maybe prevented by the use of oxidation inhibitors or antioxidants (Adegoke et al., 1998; Coulter, 1988). Synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxy toluene (BHT) have been used as antioxidants for foods, since the beginning of this century (Byrd, 2001; Jan Pokorny, 1991). The use of these synthetic antioxidants, however, has begun to be restricted because of their toxicity (Branen, 1975 and Gazzani, Papetti, Massolini, & Daglia, 1998).

Recently, natural plants have received much attention as sources of biologically active substances including antioxidants, antimutagens and anticarcinogens (Dillard & German, 2000). Plant extracts obtained from some fruits and vegetables have been reported to be effective antioxidants (Al-Saikhan, Howard, & Miller, 1995; Cao, Sofic, & Prior, 1996; Vinson, Hao, Su, & Zubik, 1998). 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 (Mansour & Khalil, 2000).

Natural aromatic plants and spices have been widely used in many food products such as meat and meat products, dairy and bakery products (Jan Pokorny,

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doi:10.1016/j.foodchem.2004.05.038

1991). The keeping quality of baked foods such as crackers, cookies and biscuits is of great economic importance since these products are widely used and are often stored for extended periods before consumption. The spices most commonly used in bakery products are cinnamon, mint, nutmeg, mace cloves, poppy and sesame seeds (Bassiouny, Hassanien, El-Razik Ali, & El-Kayati, 1990).

The present study is an attempt to evaluate the utilization of some plant foods as sources of natural antioxidants. The effects of addition of the plant extracts on the stability of fat in biscuits was studied.

2. Materials and methods

Three plant food sources namely drumstick leaves (*Moringa oleifera*), dehydrated amla (*Emblica officinalis*) and raisins (*Vitis vinifera*) 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. Fresh drumstick leaves (DL) were washed and blanched in 0.2% potassium meta-bisulphate, followed by oven drying at 50 °C for 8 h. The dried leaves were finely powdered and stored in air-tight containers at −4 °C until used for preparation of extract. The raisins were deseeded and cut into small pieces, kept in the oven at 50 °C for 4 h.

β-Carotene was obtained from Sigma chemicals (USA), linoleic acid from Sisco Research laboratory (Bombay, India), Tween 40 from ICN Pharmaceuticals (USA) and BHA from British Drug House Ltd. (England). All other chemicals used were of analytical grade.

2.1. Preparation of plant extracts

Dry powders of amla and DL were separately passed through a 60-mesh screen. The partially dehydrated raisin pieces were ground to a paste and passed through 60 mesh. About 100 g of each food sample was shaken with 90% ethanol in a metabolic shaker for 6 h and filtered. In case of raisins, a mixture of ethanol and water (1:1) was used for extraction. 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, until further use.

2.2. Antioxidant activity test

β-Carotene–linoleic acid emulsion system was used to determine the antioxidant activity of the extracts obtained from the three samples (Taga, Miller, & Pratt, 1984). β-Carotene (2 mg) was dissolved in chloroform (20 ml). To an aliquot (3 ml) of the solution was added linoleic acid (40 mg) and Tween 40 (400 mg). Chloroform was removed using a rotary evaporator at 50 °C, made up to 100 ml with oxygenated water mixed well. Aliquots of the β-carotene–linoleic acid emulsion were mixed with antioxidant extract (40 μl) and incubated at 50 °C, for 60 min. The absorbance of the oxidized emulsion was measured in a spectrophotometer at 470 nm. Distilled water was used as control instead of antioxidant extract. The degradation rate of extracts was calculated as follows. Sample degradation rate = ln $(a/b)\times 100$, where ln is the natural log, a is the initial absorbance at time zero, b is the absorbance at 60 min, t is the time. Antioxidant activity (AOA) was expressed using the following equation:

$$\text{Antioxidant activity} = \frac{\text{Degradation rate of control} - \text{degradation rate of sample}}{\text{Degradation rate of control}} \times 100.$$

2.3. Preparation of biscuits

Biscuits were prepared as per the following method (Sai Manohar & Haridas Rao, 1999). Sugar (90 g) and fat (60 g) were creamed for 3–4 min in a Hobart 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 and for 5 min to obtain a homogenous dough. Wheat 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 °C for 10 min and then allowed to cool. The biscuits were stored in air-tight containers at ambient temperature.

Biscuits were prepared to provide five variations. 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 DL, 2% raisin). 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 DL extract in biscuits was 1%, while that for raisin extract was 2%. Hence, for further studies biscuits were prepared accordingly.

2.4. Sensory studies

Sensory evaluation of biscuits (freshly prepared and stored) was conducted to determine the acceptability of the product prepared by antioxidant extract addition. Fifteen panelists were selected from among the post-graduate students in the Department of Food science and Nutrition, 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. Sensory scores for different attributes like color, flavor, texture, taste and overall quality were obtained. The samples were evaluated for sensory attributes on four occasions.

2.5. Chemical analysis

The lipids of ground biscuits were extracted using *n*-hexane. Stability of biscuit lipids were followed periodically at intervals of 1 week during storage for 6 weeks at ambient temperature, by determining AOA, (as given in Section 2.2), free fatty acid (FFA), peroxide value (PV) according to the methods described by Taga et al. (1984) and AOCS methods (1993). The above analysis were carried out in two replicates.

2.6. Statistical analysis

The sensory data were subjected to ANOVA followed by Duncan's new multiple range test (Steele & Torrie, 1980).

3. Results

3.1. Chemical analysis

Antioxidant activity of the extracts obtained from food sources are given in Table 1. The AOA in extracts from amla and raisins were comparable, while the extract from DL appeared to possess stronger activity than the synthetic antioxidants – BHA. AOA was also determined in dry powders of the samples. It was interesting to note that both amla and DL exhibited antioxidant in powder form, although it was lesser than the activity in extracts. The higher activity in extracts maybe attributed to the presence of antioxidant compounds (vitamin C, E and β-carotene) in concentrated form.

Table 1
Antioxidant activity in extracts

Sample	Antioxidant activity (%)	
	Extract	Powder
Amla	87	72
Drumstick leaves	98	87
Raisins	88	25
BHA	85	85
BHT	92	92

Table 2
Antioxidant activity (%) in biscuit lipids

Biscuit sample	Initial	2nd week	4th week	6th week
B1	30	13	13	9
B2	70	68	62	60
B3	73	62	52	50
B4	57	50	45	36
B5	95	89	78	68

B1, control; B2, BHA; B3, amla; B4, raisins; B5, drumstick leaves.

Lipids extracted from biscuits were analyzed for 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 and raisin extracts. In DL biscuits, the activity at zero time was very high (95%) and declined to 68% after 6 weeks. However, the mean AOA after 6 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 DL extract as compared to synthetic antioxidants – BHA, after 6 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 were lower than that of control sample, during storage. PV of lipids extracted from the control sample after baking (at zero time) was 0.7 which did not differ considerably from those of other variations, which ranged between 0.1 and 0.6. The increase in PV was observed in all the biscuit samples, however control biscuits had the highest value of 3.2 after 6 weeks and

Table 3
Peroxide value and free fatty acid in biscuit lipids

Biscuit sample	Initial	2nd week	4th week	6th week
Peroxide value (gequiv. of O_2/100 g)				
B1	0.7	0.9	2.6	3.2
B2	0.5	0.7	1.4	1.5
B3	0.6	1.8	2.0	2.6
B4	0.1	0.5	0.6	1.1
B5	0.3	0.4	0.58	0.8
Free fatty acid value (% of stearic acid)				
B1	7.1	8.79	12.08	14.10
B2	0.8	2.47	2.94	3.31
B3	0.34	1.07	1.19	2.69
B4	0.34	1.42	2.10	2.78
B5	0.6	1.37	1.39	2.64

B1, control; B2, BHA; B3, amla; B4, raisins; B5, DL biscuits.

the PV of the other biscuit samples, ranged from 0.7 to 2.6. The values obtained are with in acceptable range and significantly lower in B4 and B5 after 6 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 (upto 3.3%) 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.

3.2. 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 2nd, 4th and 6th week. Similar trend was also seen in B2 samples prepared with BHA. In B3 (amla) and B5 (DL extract), it was interesting to note that the over all acceptability did not differ during the storage period. However in B4 sample (raisin), the mean scores after 6 weeks were significantly lower ($P \leqslant 0.05$), which indicates that B4 samples were not as acceptable compared to 0th, 2nd and 4th week of evaluation. Biscuits treated with natural antioxidants extracted from raisins and DL received higher ($P \leqslant 0.05$) scores on 2nd and 4th 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 2%. However, incorporation of DL (B5) extract in biscuits imparted greenish color, despite this, the biscuits were well accepted (Table 4).

Table 4
Mean scores for the acceptability of biscuits

Sample	Initial	2nd week	4th week	6th week	SEM*
B1	6.83^{bl}	5.83^{al}	5.5^{am}	5.33^{am}	0.23
B2	7.0^{bl}	6.16^{al}	6.16^{am}	5.66^{al}	0.15
B3	6.66^{al}	6.0^{al}	5.66^{am}	5.66^{al}	0.18
B4	7.0^{al}	6.83^{am}	6.0^{alm}	5.83^{bl}	0.11
B5	6.16^{am}	6.66^{am}	6.5^{al}	6.16^{al}	0.23
SEM*	0.14	0.15	0.21	0.22	–

Values carrying different super scripts l,m..., in columns (comparison between samples) and a,b..., in rows (comparison between storage period), differ significantly ($P \leqslant 0.05$).

* Standard error of the mean at 25 degrees of freedom.

4. Discussion

Three different plant materials (amla, raisins and DL) were used in the present study as sources of natural antioxidants. The samples were analyzed for % antioxidant activity, all three sources exhibited a high percentage of antioxidants, however, the activity was higher in DL extract compared to raisins and amla.

Biscuits were prepared with addition of extracted antioxidants. Sensory evaluation of prepared biscuits was conducted to determine the acceptability and efficacy of incorporated antioxidants over a storage period of 6 weeks. The effects of antioxidants on the stability of the added fat and the product was determined by monitoring AOA, FFA and PV periodically under actual storage conditions. Biscuits incorporated with extract from DL appeared to possess stronger antioxidant activity than the other two extracts.

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 (1–2%) to the product i.e., biscuits. The extracted antioxidants were added to the fat medium which acts as a solvent and hence could ensure uniform dispersion.

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 (blanching, slicing sterilization, dehydration, freezing and storage) thermal treatments are reported to influence antioxidant activity. 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. In addition, the antioxidants were not affected by storage over a period of 6 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, 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 raisins and DL inhibited the process of lipid oxidation as the PV of B4 and B5 samples were significantly lower ($P \leqslant 0.05$) than the other samples even after 6 weeks. Raisin containing bakery products are reported to stay fresher for a longer period. Addition of raisins at 12% (flour basis) inhibited mold growth and increased the shelf-life of wheat bread by 1–3 days (Lagrange & Payne, 1988). 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 raisin extract did not differ until 4 weeks, however the scores were significantly lower after 6 weeks. The biscuits prepared by the incorporation of antioxidants from DL extract were accepted well during the storage study, which indicates the efficiency of antioxidants in preventing the on set of rancidity.

5. Conclusion

The addition of extracts of the three plant materials, gave an excellent antioxidant effect on the biscuits compared with the effect of BHA. The higher efficiency of the plant extracts could be due to the stability of these natural antioxidant during baking. Results of sensory evaluation reveal that the selected plant extracts at concentrations of 1% and 2% 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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