

Antioxidative Properties of Mint (*Mentha spicata* L.) and its Application in Biscuits

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ABSTRACT

In this study, Mint (*Mentha Spicata* L.) was used as a source of natural antioxidant in the preparation of biscuits with optimized levels of different forms of mint (powder, extract and pure menthol). The biscuits were stored for five months packed in unit pouches of metalized polyester/poly laminated pouch at room temperature and tested periodically for sensory parameters. The stability of biscuit lipids were studied by determining free fatty acid, peroxide value, thiobarbituric acid value and total antioxidant capacity. In sensory evaluation, pure menthol (MNT-M) scored higher ($p < 0.05$) panel score than mint powder (MNT-P) and mint extract (MNT-E) biscuits. The antioxidative activity of mint biscuits was compared with that of biscuit incorporated with Butylated hydroxyl anisole (BHA) initially as well as at the end of five months. The results indicated that mint powder had shown good antioxidant property as natural antioxidant along with other chemical parameters in biscuits, which were quite comparable with BHA biscuits.

Keywords: Mint, antioxidant activity, biscuits, storage stability, sensorial properties.

INTRODUCTION

The role of antioxidants in today's food systems is constantly expanding, given the level of processing and handling that many foods encounter on their way to the consumer. However, the consumer is becoming increasingly concerned about the use of traditional synthetic antioxidants, such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), based on concerns that these compounds may be carcinogenic¹. As a result, there is worldwide interest in finding new and safe antioxidants from natural sources.

The best known natural antioxidants that have proven important in the food industry and

in human health are tocopherols, vitamin C and carotenoids². Herbs and spices are one of the most important targets in the search for natural antioxidants from the safety view point. Many leaf spices are reported to contain biologically active constituents imparting antioxidant, anticarcinogenic and antimicrobial properties to foods^{3,4}. Recent studies have led to the identification of active ingredients and antioxidant components from natural sources, such as rosemary, rice hulls, green tea, sesame seed, canola seed and ginger. Some fruits and vegetables extracts have been reported to be effective antioxidants⁵⁻⁷. 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 storage⁸. Natural aromatic

plants and spices have been widely used in many food products such as meat and meat products, dairy and bakery products⁹. 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 seeds¹. Extracts from *Moringa oleifera*, *Vitis vinifera* and *Embelica officianalis*⁴, green tea¹⁰, *Garcinia* and turmeric powder¹¹ are reported to possess strong antioxidant activity and their application in biscuits was effective in controlling lipid oxidation.

The mint flavor was known in Asia about 2000 years ago¹². It is one of the most important spice extensively used as flavorings in food, cosmetic and pharmaceuticals throughout the world. It is the third largest liked flavor worldwide, liked by all people irrespective of age, gender and ethnic background. Mint belongs to a small genus of aromatic perennial herbs distributed mainly in the temperate regions of the world. Commercially, Mint is also an important essential oil bearing plant with wide usage of spearmint, peppermint and corn mint essence¹³. The most commonly used mints in food are Spearmint (*Mentha spicata* L), Peppermint (*Mentha piperita* L), Horsemint (*Mentha cogifolia*) and Vornmint (*Mentha arvensis* L)¹⁴. *Mentha spicata* and *M piperita* have been investigated for their antioxidant action¹⁵. The chemical composition of the essential oils of *Mentha piperata* has also been widely studied^{13, 16}. The antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties and cultivars is reported¹⁷. The organoleptic properties of various Indian herbal teas including ginger, Tulsi, mint and cardamom is reported¹⁸. A review on evidence based research reports the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.)¹⁹. Our earlier studies have explored the pH, temperature and storage stability of Mint extracts²⁰ and their phenolic extracts from *Mentha spicata* has been studied for its ability to improve the quality and shelf life of fruit juice application in fruit juices²¹.

Although mint has been used for food flavoring and in traditional medicine, there are limited reports on the lipid oxidation inhibiting properties

of mint in baked products. Given the complexity of natural antioxidants, the oxidation process, ingredients used and the baking process, it is essential to study the heat stability of mint extracts during baking.

The present study addresses the utilization of mint as a source of natural antioxidant in biscuit preparation and to investigate the effect of storage of the formulated biscuits on antioxidant stability and sensory attributes.

MATERIALS AND METHODS

Materials

Fresh mint leaves (*Mentha spicata* L.) were washed repeatedly in luke warm water (3-4 times), drained and dehydrated by oven drying at $\leq 60^{\circ}\text{C}$ (overnight). The dried leaves were finely powdered, passed through 60 mesh size and stored in airtight packages, until further use. Shortening (Hindustan Lever Ltd., Mumbai, India), refined flour, sugar powder and other ingredients were procured from local market. BHA was procured from Sd fine chemicals, Mumbai, India. Pure methanol was gifted from PPSFT Department, CSIR-CFTRI, Mysuru. α -tocopherol was procured from Sigma Chemicals, Bengaluru.

METHODS

Preparation of mint extract

To 25 g of mint powder in a stopper flask; ethanol (50-60 ml) was added, shaken in a mechanical shaker at room temperature for 6 h. It was filtered and the residue was treated again with the solvent and filtered. The combined filtrates were stored in amber colored bottle at 4°C . The extract was concentrated by evaporating under vacuum at 50°C in a rotary evaporator. The concentrated extract was stored in the refrigerator until further use.

Selection of Biscuits

In a preliminary study, biscuits prepared with different forms of mint viz., powder, extract and pure menthol were optimized and were studied for sensory studies²². A total of 11 variations were prepared and subjected to sensory evaluation, color and texture measurement. Based on the results, the optimum acceptable levels (on flour basis) for

mint powder (MNT-P), extract (MNT-E) and pure menthol (MNT-M) were 1%, 500 mg and 100 ppm, respectively. These levels were adopted in this study. Control biscuits were prepared without addition of mint/synthetic antioxidant, while the standard variation was prepared with BHA (200 ppm). Biscuits were prepared according to the method of Sai Manohar and Haridas Rao²³. The extracts were blended with fat and emulsion was mixed with sugar. The biscuits after baking (at 160°C for 10 min), were cooled and packed in unit pouches of metalized polyester / polyethylene (LDPE) laminate and stored at room temperature.

Analysis

During the storage period for 5 months, the biscuit variations were evaluated at monthly intervals, for sensory quality and development of lipid oxidation products by means of peroxide value (PV), free fatty acid (FFA), total antioxidant activity (TAOC) and Thiobarbituric acid reactive substances (TBARS) value.

Sensory quality assessment

Sensory evaluation of biscuits (freshly prepared & stored) was carried by a panel of ten judges with over 10 years of experience in the field of baking science and technology.

The biscuit variations presented to the panelists were scored for different quality attributes like color, flavor, texture, taste, mouth feel and overall quality. Since, the incorporation of mint powder and mint extract to biscuits contributed a green color; these two variations were presented to the panelists to prevent bias in sensory evaluation, on separate occasion along with control product^{23,24}.

Total Antioxidant capacity (TAOC)

Ground biscuit samples (5 g) were treated with hexane (25 ml) in a mechanical shaker for 1h at room temperature and filtered. The residue was again treated with hexane (25 ml) in a mechanical shaker for 1/2 h and filtered. The filtrate in the weighed beaker was evaporated on a water bath to obtain sample I. The residue after the filtration was treated with methanol (25ml) in a mechanical shaker for 30 min and filtered. The filtrate in the weighed beaker was evaporated on a water bath to obtain sample II.

10 mg of sample I and II were dissolved in 10 ml of hexane and methanol respectively to obtain sample solution. An aliquot of each sample solution (0.3 ml) was treated with 2.7 ml reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). For the blank, 0.3 ml ethanol was mixed with 2.7 ml of the reagent. The tubes were capped and incubated at 95°C (90 min), cooled to room temperature and the absorbance of the aqueous solution of each was measured at 695 nm against a blank²⁵. α -tocopherol was used as standard. The antioxidant capacity for the samples was expressed as μ mol equivalents of tocopherol /g of biscuit lipid.

Free fatty acids (FFA)

Ground biscuit samples (10 g) were treated with chloroform (50 ml) in a mechanical shaker for 30 min at room temperature. An aliquot (10 ml) of the extract was evaporated at 80°C the residual fat was dissolved in benzene alcohol mixture and titrated against standard alcoholic KOH solution (0.02N) and expressed as % oleic acid²⁶.

Peroxide value (PV) assay

Ground biscuit samples (10g) were treated with chloroform (50 ml) in a mechanical shaker for 30 min at room temperature. To an aliquot (10 ml) of the extract, glacial acetic acid (15 ml) and saturated KI solution (0.5 ml) was added and kept in dark (10-15 min). The flask was removed and 1ml of 1% starch indicator was added and titrated against sodium thiosulphate (0.01N)²⁶.

Thiobarbituric acid assay

Ground biscuit samples (10 g) were homogenized with distilled water (25 ml) and 10% TCA (25 ml) and filtered. To 1ml aliquot, 3 ml of 0.67% TBA solution and 0.05 N H₂SO₄ were added and boiled on water bath for 30 min at 95°C. It was cooled in ice water for 5 min and n-butanol (4 ml) was added and centrifuged for 10 min at 1500 RPM. The organic layer was pipetted out and absorbance measured at 532 nm in a spectrophotometer²⁷.

Statistical analysis

All the above analysis were carried out in 2 replicates in triplicates. The sensory data were subjected to Duncan's new multiple range test²⁸.

RESULTS AND DISCUSSION

There is an increasing interest in the use and measurement of antioxidants in food, pharmaceutical and cosmetic industries. Herbs and spices have traditionally been used to extend the shelf life of foods due to their ability to act as antioxidants in addition to their seasoning properties. Mints are regarded as one of the most important spices throughout the world. In the present study, spearmint and peppermint were used as source of natural antioxidant (AOX). The effect of incorporating different forms of mint in biscuits was evaluated.

The biscuit variations were evaluated for sensory attributes by a trained panel periodically during the storage period (5 months). The mean scores before and after storage, assigned to the five variations are given in Table 1.

In the freshly prepared biscuits, attributes like color, surface character, and crumb color of MNT-M variation received higher scores compared to the biscuits prepared with synthetic antioxidant-BHA (BHA). In terms of texture, taste and mouth feel, the mean scores did not differ

significantly ($p < 0.05$) between the three variations prepared with mint (MNT-P, MNT-M, MNT-E). The control (CNT) and BHA biscuits received significantly higher scores ($P \leq 0.05$) followed by MNT-M, MNT-E and MNT-P, in terms of overall quality. After 5 months of storage, MNT-M received significantly higher scores among the three mint variations; however they were lower than that of BHA. However, before storage, the overall acceptability differed significantly ($P \leq 0.05$) between the variations. Among the 3 mint variations, the scores of MNT-M were significantly higher ($P \leq 0.05$) throughout the storage period. In CNT sample, which did not contain any antioxidant, the mean sensory scores were similar for the first, second and third evaluation, thereafter the scores declined. A similar trend was also seen in the other variations. At the end of storage period, 4 the mean scores at the end of the storage period of all 5 variations were significantly lower ($P \leq 0.05$) than the initial scores. The scores assigned by the panelists at the end of 3rd and 4th month, in MNT-P and MNT-E were comparable, indicating antioxidant stability and acceptability. During storage period, the texture and color measurements of MNT-P was found to be comparable with CNT and BHA indicating their crisp nature²².

Table 1: Sensory quality of biscuits during the storage of biscuits at RT (n=10)

Biscuits		Colour	Surface Character	Crumb Colour	Texture	Taste	Mouth Feel	Over All Quality
CNT	BS	8.1c	8.3c	7.9bc	17.7b	17.7b	7.7b	67.4d
	AS	7.6y	7.7y	7.4y	16.7x	16.8x	7.2y	63.5y
MNT-P	BS	6.0a	6.7a	6.1a	17.0a	17.1a	6.8a	59.7a
	AS	5.8w	6.3w	5.9w	16.2w	16.7x	6.0w	57.0w
MNT-M	BS	7.8c	8.0c	7.5b	16.7a	16.5a	6.5a	63.7b
	AS	7.4y	7.4y	7.0x	16.0w	16.0x	6.3x	60.1x
MNT-E	BS	7.0b	7.4b	6.6a	17.0a	16.9a	6.5a	61.4c
	AS	6.6x	6.6x	6.0w	16.4w	16.4w	6.0w	58.0w
BNT	BS	7.9c	8.1c	7.7b	17.2a	17.5ab	7.8b	66.5d
	AS	7.7y	8.0z	7.5y	16.7x	16.5x	7.4y	63.9y
SEM	BS	0.17	0.15	0.19	0.2	0.17	0.16	0.26
(+/-)Df	AS	0.18	0.16	0.2	0.3	0.25	0.17	0.36

CNT - Control Biscuit; MNT-P - Mint powder Biscuit; MNT-M - Pure menthol Biscuit ;

MNT-E - Mint extract Biscuit; BNT - BHA Biscuit BS - before storage

AS - after storage; Superscripts a, b, c, ... for values in columns for AS and w, x, y, z for BS values differ significantly ($P < 0.05$) at 25 df.

The total antioxidant activity (TAOC) determined in lipids extracted from the biscuits (Table 2) expressed in terms of tocopherol equivalents was considerably higher in MNT-E and MNT-E (fresh) compared to the synthetic AOX. The values obtained for MNT-E (after storage) were similar to those of BHA. This indicates that mint which is a good source of vitamin E, has imparted antioxidant capacity. Although there are a few reports on the antioxidant activity of mint in soda cracker biscuit¹² and in sunflower oil¹⁵ no reports are available on the analysis of TAOC in spearmint and its different forms for comparison. The tocopherols have carry-through properties in baked and fried products. Crackers and pastry prepared with lard treated with 0.01–0.1% tocopherol, either alone or in combination with BHA (0.01%), were appreciably more resistant to rancidity than control samples^{29,30}.

Table 2: Antioxidant activities in biscuit lipids - before & after storage

Biscuit Variations		TAOC* (μmol equivalents of tocopherol /g of biscuit lipid)
CNT	BS	8.6 + 0.008a
	AS	7.3 + 0.006 a
MNT-P	BS	17.7 + 0.023 c
	AS	7.3 + 0.010 a
MNT-M	BS	8.9 + 0.022 a
	AS	8.1 + 0.020 a
MNT-E	BS	17.8+ 0.018 c
	AS	10.1 + 0.017 b
BNT	BS	12.6 + 0.009 b
	AS	10.2+ 0.011 b

Values are mean + SD of 3 replicates. BS - before storage AS - after storage

* TAOC – total antioxidant capacity CNT - Control Biscuit; MNT-P - Mint powder Biscuit; MNT-M - Pure menthol Biscuit; MNT-E - Mint extract Biscuit; BNT - BHA Biscuit

Superscripts a, b, c, ... for values in columns differ significantly ($P < 0.05$)

Data suggest that the members of the genus *Mentha* possess exploitable antioxidant property in vitro. Aqueous extracts of mint are reported to have the lowest antioxidant activity compared to herbs of the species Lamiaceae³¹. The antioxidant efficacy of aqueous extract from *Mentha* species was strongly associated with the phenol content¹⁷.

In an earlier study, addition of extracts of *Moringa oleifera* and *Embellica officianalis* in biscuits were found to have an excellent antioxidant effect compared with the effect of BHA, as they were more effective in controlling lipid oxidation during 6 month storage period. Biscuits treated with natural antioxidants, extracted from raisins and *Moringa oleifera* leaves, received higher panel scores during a storage period of 6 weeks than control, BHA and *Embellica officianalis* extract incorporated biscuits⁴. The findings of the present study are in good agreement with earlier studies on the use of natural antioxidants in baked products. Extracts of *Garcinia* and turmeric powder have been reported to be suitable for use in biscuits as natural antioxidants¹¹.

Free fatty acid value was estimated in all biscuits during storage period to determine the extent of hydrolytic rancidity of the fat used in the biscuit preparation. Free fatty acid value had shown a slow and gradual increase in all the biscuits on storage (Fig. 1a). Initially, the FFA values of antioxidant-incorporated biscuits were lower compared to the control, indicating the potency of antioxidants in inhibiting the formation of FFA. The increase in FFA was higher in control biscuits prepared without the addition of antioxidant, compared to the biscuit variations MNT-P, MNT-E, MNT-M and BHA. However, the FFA values were comparable with each other in these antioxidant-incorporated biscuits till the end of five months.

Peroxide value was estimated in all the biscuit samples to determine the extent of peroxide formation due to oxidation of fat (m. eq. of O_2/kg of fat) during storage period of five months (Fig.1b). It was observed that the peroxide value increased with the storage period reaching a maximum value and followed by a decreasing trend. Initially the PV in all the samples was zero, which remained zero at the end of one-month storage in almost all the

biscuit samples except in biscuits incorporated with MNT-E. There was a sudden increase of PV in MNT-E biscuits from 19.05 at the end of one month storage and the value increased to 32.42 at the end of three months and further started to decrease to 15.12 at the end of 5 months. The PV in these biscuits was highest in comparison to all other biscuits. PV of biscuits incorporated with MNT-P also had a similar trend, as in BHA incorporated biscuits, showing the antioxidant effect of mint powder on the storage quality of biscuits. The PV of control biscuit increased to 21.98 at the end of four months followed by a decrease to 15.91 at the end of 5 months. However, the PV in all the biscuit samples were within the acceptable range and significantly lower in biscuit samples incorporated with MNT-P and BHA. All samples were able to maintain PV less than 20 mEq/kg until the fifth month of storage. As per the BIS standards³², the maximum upper limit for peroxide value is 20 mEq/kg in edible oils. Also, PV range of 10-20 mEq/kg in food product is considered acceptable³³. Green tea extract has shown protective properties against the formation

of primary and secondary oxidation products from fats in biscuits¹⁰.

The TBARS was estimated in all the biscuits every month, during a storage period of five months to determine the extent of oxidation of fat which was measured as malonaldehyde (MDA $\mu\text{g/g}$ of sample) (Fig. 1c). A gradual increase in TBA was observed in all the biscuit variations during the storage period; however, control biscuits had the highest value of 4 μg at the end of 5 month storage. Similar results were also observed in biscuit samples incorporated with Mint extract. The TBA of other biscuit samples, ranged from 3.2 to 3.9 at the end of 5 months storage. The values obtained were within acceptable range in all the biscuit samples and significantly lower ($p < 0.05$) in biscuit samples incorporated with MNT-P, MNT-M and BHA. The results indicate that addition of mint in different forms gave an antioxidant effect on the biscuit, compared with the standard antioxidant –BHA, however, mint powder was more effective in controlling lipid oxidation during storage. The higher efficiency could be due to the stability of antioxidant

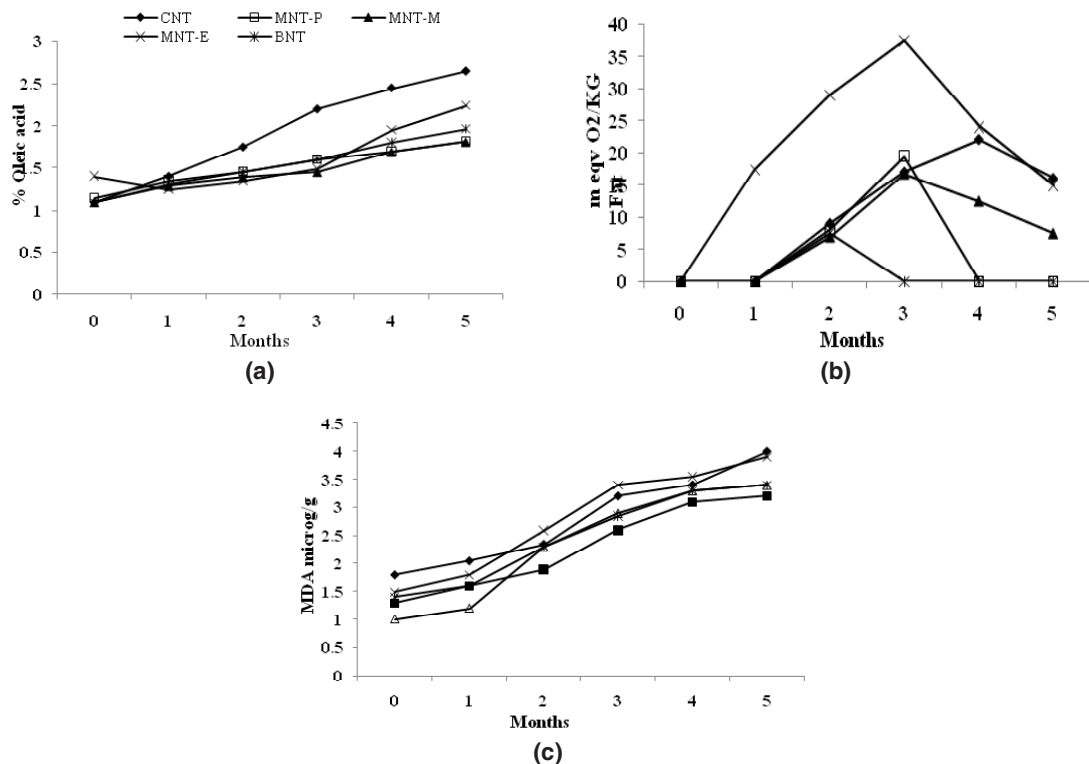


Fig.1: Changes in (1a) Free Fatty Acid value (1b) Peroxide Value and (1c) Thiobarbituric acid value in biscuits during storage period (Room Temperature).

components during baking. TBA value less than 0.576 mg / kg⁻¹ sample are considered not rancid, whereas values of 0.65 – 1.44 mg / kg⁻¹ sample are regarded as rancid but still acceptable and values greater than 1.5 mg / kg⁻¹ sample are said to be rancid and unacceptable³⁴. Reference values for TBARS in baked products are not available.

All the 3 forms of mint were found to exhibit antioxidant activity, with highest activity observed in both fresh and after storage. Among the various forms of mint studied, highest activity in terms of α -tocopherol was seen in menthol powder followed by synthetic antioxidant – BHA. It is essential to consider some aspects before selection and use of natural antioxidants in foods. The form, method and time of incorporation are important. The added antioxidants were not affected by temperature and storage over a period of 5 months.

CONCLUSION

In this study, the overall acceptability of biscuits prepared with natural antioxidant in different forms of mint was acceptable up to a storage period

of 5 months. In terms of both chemical and sensory evaluation, MNT-P biscuits were comparable with that of BHA biscuits compared to MNT –M and MNT-E variations. The study clearly indicated the antioxidant efficiency of different mint forms in preventing the onset of rancidity in biscuits during storage suggesting the retention of bioactive components of mint. With rapid growth and changing eating habits of people, bakery products have gained popularity among masses in India. The bakery products which include bread and biscuit form the major baked foods of total bakery products produced in the country. The study emphasizes the use of mint in baked products as a potential substitute for synthetic antioxidants in food preservation.

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