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Effect of Clove Essential Oil as Antioxidant and Antimicrobial Agent on Cake Shelf Life

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Abstract: The aim of this study was to evaluate efficiency of clove essential oil (CEO) as antioxidant and antimicrobial agent and use in cake preservation as a natural plant preservative due to the harmful effects of synthetic antioxidants. CEO was evaluated their antioxidant effect after adding in cakes (by determining the peroxide value, thiobarbitoric acid and free fatty acid during 28 days of storage at room temperature) and antimicrobial activity (by determining the counts of total bacteria, coliform bacteria, molds and yeasts under the same previous conditions). Also, the effect of CEO on the appearance, color, odor, taste, texture and overall acceptability of the cake samples was sensory evaluated and compared with control sample. The results showed that the CEO was able to retard the oxidation rate and reduction of formed preliminary and secondary oxidation products in cakes compared with synthetic antioxidant, except the sample containing 400 ppm CEO had lower values than control and almost equal with the sample containing BHT. The microbiological study exhibited that CEO was more effective in inhibiting the growth of tested microorganisms with increasing of CEO levels. Also, sensory results indicated that no significant effects were observed between different cake samples in most qualities and they generally were acceptable, except the sample containing 800 ppm CEO was the lowest ($p<0.05$) acceptable sample. It could be concluded that, CEO exhibited high antioxidant activity and effective antimicrobial properties in cakes when compared with the synthetic antioxidant during 28 days of storage. Consequently, this essential oil could be used as natural antioxidant and antimicrobial in foodstuffs, especially those containing lipid and can increase shelf-life of those foods, beside their safe effects on human health.

Key words: Clove · Cake · Antioxidant · Antimicrobial · Sensory evaluation

INTRODUCTION

Cake is one of the most popular bakery items consumed in the world because of nutritional value, different varieties and affordable price. Lipid oxidation and mold growth are major problems in producing cake, which limits shelf-life of this product. The onset rancidity in bakery products causes a great influence on texture, color and organoleptic parameters, also losses the nutritional value [1, 2]. These problems may be prevented by the use of antioxidants and preservatives [3, 4]. The synthetic antioxidants such as butylated hydroxylanisole (BHA) and butylated hydroxyltoluene (BHT) have been used as antioxidants in foodstuff. Use of these synthetic antioxidants, has begun to be limited due to their toxicity, liver damage and carcinogenicity [2, 3]. Therefore development and use of safer antioxidants from natural sources are of interest because of possible negative effects of synthetic food additives on human health [3].

Recently, the interest in therapeutic plants has increased dramatically [5]. The aromatic plants and spices have been widely used in many food products such as meat and their products, dairy and bakery products [1, 6, 7]. Essential oils of herbals and spices are very complex natural mixtures which can contain about 20-60 compounds at quite different concentrations [8]. The composition, structure as well as functional groups of the oils play an important role in determining their antimicrobial activity [9].

The extracts and essential oil of clove are used as a topical application to relieve pain and to promote healing, anti-aging, cardiovascular disease, arthritis, infections

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(skin, flu, bacterial, viral and fungal, hepatitis and parasitic), digestive problems (nausea, vomiting and diarrhea), skin cancer, thyroid dysfunction and also finds use in the fragrance and flavoring industries [1, 10-13].

Aromatic clove oils exhibited antimicrobial activity against eleven pathogenic and spoilage bacteria including *Aeromonas hydrophila, Bacillus cereus, Escherichia coli, Klebsiella pneumonie, Campylobacter jejuni, Listeria monocytogenes, Micrococcus luteus, Pseudomonas aeroginosa, Salmonella enteridis, Staphylococcus aureus* and *Enterococcus faecalis*; and five fungal species: *Aspergillus flavus, Penicillium roqueforti, Rhizopus spp., Mucor plumbeus* and *Eurotium sp.*, as well as against three strains of yeast including *Candida albicans, Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii* at concentrations 50 and 100 μL mL^{-1} and showed a zone of inhibition ranging from 10.45 to 25.12 mm in diameter [14-16]. The other study demonstrated that the clove oils exhibited pronounced and varying degrees of growth inhibition against fungal: *Alternaria alternate, Fusarium oxysporum*, *Aspergillus flavus, Aspergillus strains, Aspergillus acculeatus* and *Apergillus fumigatus* (39 to 86%) and bacterial pathogens: *Pseudomonas syringae, Bacillus subtilis, Escherichia coli, Staphylococcus* sp. and *Aeromicrobium erythreum* (20 to 42%) [13].

Recently, there are many studies about the antioxidant and antimicrobial effects of spices in products of meat, dairy and bakery [1, 6, 7] but, there is an information paucity on regarding the effect of clove essential oil on quality criteria of cake. Hence, the purpose of the study was to evaluate the antioxidant and antimicrobial activities of clove essential oil and compared with BHT in cakes during storage period. In addition, the influence of this essential oil on the sensory properties and shelf life of cakes was studied.

MATERIALS AND METHODS

Materials

Cake Ingredients: Wheat flour (72% extraction), sugar, shortening, baking powder, milk, eggs and vanilla, as well as clove buds *(Eugenia caryophylata thumb)* were purchased from local market, Cairo, Egypt.

Chemicals: Sodium thiosulphate, sodium hydroxide, thiobarbituric acid, butylated hydroxytoluene (BHT), culture media and chemical solvents were purchased from Sigma Chemical Co. (St. Louis, MO).

Methods

Preparation of Clove Essential Oil (CEO): Clove buds were cleaned, ground to a fine powder and passed through a 24-mesh sieve. The essential oil was extracted by steam distillation, using a Clevenger-type apparatus [17]. The obtained essential oil was dried over anhydrous sodium sulphate and stored in refrigerator at 4°C until use.

Preparation of Cakes: Cakes were prepared according to the method described by Lu *et al.* [18] by using the following formula: 1000 g wheat flour (72% extraction) without antioxidants, 850 g sugar, 50 g shortening, 40 g baking powder, 250g eggs, 200 ml milk and 17 g vanilla. BHT or CEO was added to the formula. The ingredients were mixed for 3-4 minutes and placed on aluminum plates then backed in an oven at 160°C for 30 min. Cooking foil was also put in the oven for sterilization. After baking, plates were covered with the sterile cooking foil and transferred to the laminar flow bench. The cakes were exposed to UV light for 10 min to eliminate surface contaminants. They were then cut into 5x5 cm square pieces and packed in polypropylene films to prevent drying before analyses. Cakes were prepared to provide five samples: The first sample without antioxidant agent (control). The second sample was prepared with addition of 200 ppm BHT as synthetic antioxidant. While, the other samples were prepared by adding CEO at three different concentrations 400, 600 and 800 ppm as natural antioxidant.

Preparation of Lipid Extract: The extraction of cakes lipid was carried out according to the method described by Baiano *et al*. [19]. 100 grams of cakes were ground roughly and placed in a closed flask with 200 ml n-hexane. The flask was shaken for 1 hour and then filtered through filter paper (Whatmann No. 1). The solvent was removed from the extracted lipids by rotary at 50°C. The extracted lipids were stored at ambient temperature for subsequent determination.

Chemical Analyses: Oxidation of cakes lipid was periodically determined by the measurement of peroxide value (PV), thiobarbitoric acid (TBA) and free fatty acid (FFA) at 0, 7, 14, 21 and 28 days of storage at room temperature according to the methods of AOCS [20]; AOCS [21] and Bhanger *et al*. [22], respectively. The above analyses were carried out in three replicates and all data were averaged.

Microbiological Analyses: Counts of total bacteria, coliform bacteria, molds and yeasts in the produced cake samples were determined at 0, 7, 14, 21 and 28 days of storage at room temperature according to the method of ISO [23]. All experiments were carried out in triplicates.

Sensory Evaluation: The sensory evaluation of cakes containing CEO at different levels, BHT and control was performed using 20 panelists (the staff of Food Science and Technology Department, Faculty of Agriculture, AL-Azhar University, Cairo). Each panelist was presented with individual cake samples (about 3x3x1.5 cm) which had been baked within the previous 10 h and were placed on a tray in a plastic bag coded with a five-digit random number and served in a randomized order according to the methods described by Chaiya and Pongsawatmanit [24] and Wu1 *et al*. [25]. During the panel test, rinse the panelist's mouth by water to remove any traces of residual food. Each panelist was asked to rate the liking of quality attributes according to appearance, color, odor, taste, texture (hardness and softness) and overall acceptability of each sample using a 9-point hedonic scale (1= dislike extremely and 9 = like extremely).

Statistical Analysis: The data were statistically analyzed by using SPSS (Version 16.0 software Inc., Chicago, USA) of completely randomized design as described by Gomez and Gomez [26]. Treatment means were compared using the least significant differences (LSD) at 0.05 levels of probability and standard error.

RESULTS

Effect of CEO on Chemical Quality of Cakes: The effects of natural and synthetic antioxidants on peroxide value (PV), thiobarbitoric acid (TBA) and free fatty acid (FFA) of cake samples during storage period at room temperature were illustrated in Figures 1, 2 and 3.

The addition of CEO resulted in an evident decrease ($P<0.05$) in PV of cakes when compared with PV of control and sample containing BHT, except the sample containing 400 ppm CEO had lower PV than control and almost equal with the sample containing BHT as shown in Fig. 1. On the other hand, PV increased continuously in all cake samples during storage periods. The increment rate in the value was decreased with increasing of CEO concentration from 400 to 800 ppm.

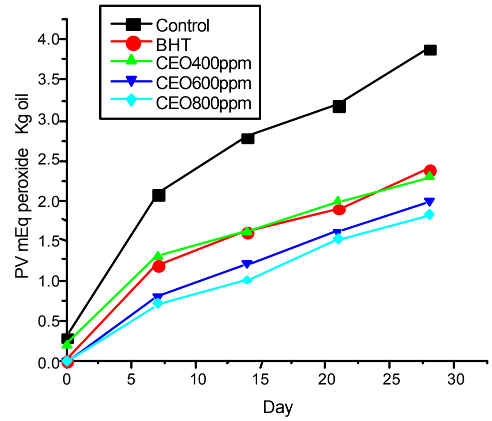

Fig. 1: Effects of BHT and CEO on PV in stored cakes at room temperature for 28 day

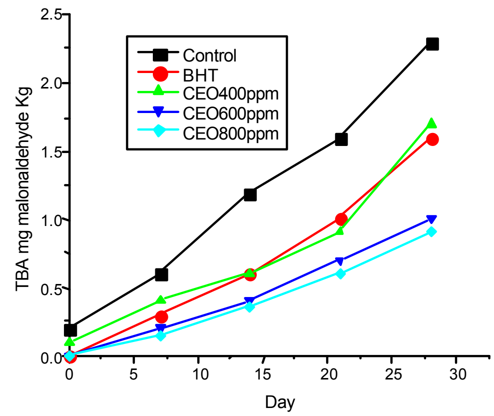

Fig. 2: Effects of BHT and CEO on TBA in stored cakes at room temperature for 28 day

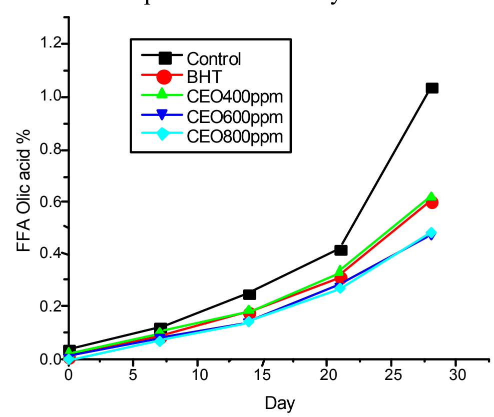

Fig. 3: Effects of BHT and CEO on FFA in stored cakes at room temperature for 28 day

With regard to TBA of cake samples containing CEO at levels 600 and 800 ppm were lower ($P<0.05$) than the control and BHT samples, while the sample containing 400 ppm was almost in equal with BHT sample. In addition, TBA value linearly increased for all cake samples during

Table 1: Microbiological counts* (log CFU /g) of cake samples as affected by addition of different levels of CEO during storage at room temperature for 28 day

			CEO (ppm)		
Storage period (Day)	Control	BHT	400	600	800
		Total bacterial count (TBC)			
0	1.48[a]	1.29[a]	1.31[a]	1.26[a]	1.22[a]
7	2.56[a]	2.07[b]	2.10[b]	1.73[c]	1.61[c]
14	2.78[a]	2.32[b]	2.30[b]	2.16[b]	1.84[c]
21	2.79[a]	2.47[b]	2.38[b]	2.09[c]	1.91[c]
28	2.92[a]	2.63[b]	2.60[b]	2.28[c]	2.19[c]
		Coliform group			
0	ND	ND	ND	ND	ND
7	1.22[a]	1.14[a]	1.09[a]	1.06[a]	1.02[a]
14	1.56[a]	1.30[b]	1.28[b]	1.14[c]	1.09[c]
21	1.81[a]	1.70[a]	1.51[b]	1.36[c]	1.28[c]
28	2.12[a]	1.92[b]	1.88[b]	1.53[c]	1.44[c]
		Molds and yeasts			
0	ND	ND	ND	ND	ND
7	2.26[a]	1.94[b]	1.81[b]	1.56[c]	1.38[c]
14	2.74[a]	2.21[b]	2.09[b]	1.85[c]	1.63[c]
21	2.98[a]	2.48[b]	2.39[b]	2.11[c]	2.01[c]
28	3.42[a]	2.91[b]	2.74[b]	2.47[c]	2.33[c]

* [a, b] and [c] means in the same row with different superscripts are different significantly (P< 0.05). ND: Not detected

Table 2: The mean scores for sensory characteristics* of cakes containing BHT or CEO at different concentrations

	Organoleptic properties					
Cake samples	Appearance	Color	Odor	Taste	Texture	Overall acceptability
Control	8.06[a]	8.44[a]	8.38[a]	8.49[a]	7.93[a]	8.06[a]
BHT	7.84[a]	7.98[a]	7.87[a]	7.78[a]	7.50[a]	7.64[a]
CEO 400 ppm	7.71[a]	7.87[a]	7.81[a]	7.69[a]	7.44[a]	7.66[a]
CEO 600 ppm	7.83[a]	7.79[a]	7.00[b]	6.60[b]	7.58[a]	7.51[a]
CEO 800 ppm	7.69[a]	7.53[a]	6.59[b]	5.74[c]	7.43[a]	6.43[b]

*Sensory scores are based on a 9-point hedonic scale ranging from 1 = dislike extremely to 9 = like extremely. [a, b] and [c] means in the same column with different superscripts are different significantly (p<0.05)

storage period, but it was more evident in control sample than other samples containing CEO or BHT as shown in Fig. 2.

Changes in the FFA values of cake samples were showed in Fig. 3. An increase in FFA value was observed in all the samples during 28 days of storage. The increase was higher (p<0.05) in the control sample than the cake samples containing BHT or CEO at different levels, while non significant difference was observed between two samples containing CEO at 600 and 800 ppm during storage period.

Effect of CEO on Microbiological Quality of Cakes: Data in Table 1 indicated that there are insignificant differences observed among CFU/g values of total bacterial in the tested cake samples at initial time of storage, as well as coliform bacteria group at the seventh day of storage for the same samples. However, coliform bacterial group, molds and yeasts were not detected in all samples at initial time of storage. On the other hand, the antimicrobial effect of CEO was evident at the seventh day of storage (except coliform bacterial group was effected at fourteenth day), where the cake samples containing CEO had a fewer (P<0.05) enumerations compared with the control sample. Also, it is clear that a significant reduction (P<0.05) of counts was occurred among the samples containing CEO. The reduction rate in those values was increased with increasing of CEO level from 400 to 800 ppm.

Effect of CEO on Sensory Quality of Cakes: Cake samples containing BHT or CEO were sensory evaluated and compared to the control sample as shown in Table 2. Data showed that there are non significant differences among cake samples in appearance, color and texture. Also, the same table shows that there were non significant differences among control and samples containing BHT or CEO at level 400 ppm in both odor and taste, but the samples containing 600 and 800 ppm CEO were significantly different ($P<0.05$) as compared with the other samples. With regard to the overall acceptability, the cake sample containing 800 ppm CEO was the lowest acceptable sample, while the other samples were not significantly different as compared with control.

DISCUSSION

In the last few years, an increased attention has been focused on the natural plant extracts and their essential oils, especially those containing phenolic compounds with antioxidant and antimicrobial properties. Clove is one of the important dietary sources of these compounds [7, 10, 14-16].

The addition of natural or synthetic antioxidants to the cake affected the PV, TBA and FFA values by varying degrees during 28 days storage. Some studies [7, 27] indicated that the range of PV from 10 to 20 mEq/kg oil is considered the food product rancid but still acceptable. While more than 20 mEq/kg oil is already rancid and unacceptable to consume. In present study, all samples were considered non-rancid and still acceptable during storage period. However, the control cake exhibited the highest PV throughout the storage period, indicating that the oil in the cakes was oxidized to lipid hydroperoxides. These primary oxidation products were broken down by a free radical mechanism in which the O-O bond was cleaved on either side of the carbon atom bearing the oxygen atom to give the hydroxyl free radical and many secondary products such as alcohols, aldehydes, ketones and malonaldehydes which cause off-flavors [28, 29]. Generally, the higher efficiency of natural antioxidant might be because of the stability of plant oil during baking. These results are in agreement with some previous studies [7, 28], which tested the effect of six Malaysian plants extracts as natural antioxidants and compared it with BHA and BHT in butter cake and biscuit samples. They explored that these extracts at concentration of 1g kg^{-1} oil were more effective in preventing the oxidation than 0.1g kg^{-1} of BHA and BHT after 4 weeks storage.

TBA index measures the formation of secondary oxidation products, mainly malonaldehydes, which may contribute off-flavors to oxidize oil [29]. As is clear from the study, TBA of cake samples increased with storage time owing to the simultaneous increase in PV. During 28 days of storage, TBA value for control sample was higher than other tested samples. Addition of CEO at different levels reduced TBA values compared to the control sample throughout the storage. Some studies [7, 30] indicated that TBA value less than 0.576 mg malonaldehyde / kg sample is considered not rancid, whereas values of 0.65 – 1.44 mg / kg sample are regarded as rancid but still acceptable and values greater than 1.5 mg / kg sample are rancid and unacceptable.

FFA is also a measure of the foods rancidity. It is formed as a result of hydrolysis of triglycerides and may be increase by reaction between oil and moisture [31] or may develop by enzymatic activity due to the microbial growth [32]. After 28 days of storage, acidity of control and cake sample containing BHT was higher than cakes containing different concentrations of CEO. This indicates that the CEO reduced the activity of hydrolytic enzymes, thus cake acidity.

Regarding microbiological quality criteria, the presence of microorganisms is considered to be the factor affecting food safety and organoleptic properties [33]. The current study found a negligible effect of the synthetic or natural antioxidants on the microbial growth in cakes when compared to control sample at initial time of storage. While the antimicrobial effect was evident at the seventh day of storage (except coliform bacterial group was effected at fourteenth day), where that the TBC, coliform bacteria group, mold and yeast counts decreased with increasing level of CEO indicating that the microbial growth was more rapid in the control sample. This result might be attributed to the damage caused by biochemical components from CEO to the membrane permeability of microorganisms [34]. So it was suggested that clove oil could enhance the antimicrobial ability as well as extend the shelf life of the cakes. This result is supported by Yi *et al*. [35], who found that the enhanced antimicrobial activity was related to the effect of polyphenolic compounds, which could alter the integrity of the outer membrane of microorganisms and disrupt cell walls, increasing the permeability and leakage of intracellular components.

Sensory evaluation is an important factor in judging about foodstuffs quality. The different cake samples were evaluated and compared to the control sample to ascertain consumer acceptability for it. No significant differences could be detected among these samples containing CEO and control sample in appearance, color and texture, but the samples containing 600 and 800 ppm CEO were significantly impaired in both odor and taste as compared with the other samples. With regard to the overall acceptability, the cake sample containing 800 ppm CEO was the lowest acceptable sample indicating that increasing the concentration of CEO had negative effect on overall acceptability, while the other samples were not significantly different as compared with control. Generally, it is well known that in complex systems such as cakes, several ingredients interact with each other and affect the sensory properties [36].

It could be concluded that, CEO exhibited high antioxidant activity and effective antimicrobial properties when compared with the synthetic antioxidant during 28 days of storage. The scores of overall acceptability of cakes containing CEO were almost equal to control. However, cake sample containing 800 ppm CEO was less acceptable than the other samples. Consequently, this essential oil could be used as natural antioxidant and antimicrobial in foodstuffs, especially those containing lipid and can increase shelf-life of those foods, beside their safe effects on human health.

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