Evaluation of Antioxidant and Antimicrobial Effects of Chamomile (*Matricaria chamomilla* L.) Essential Oil on Cake Shelf Life

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Received: 30 Oct. 2011 **Accepted:** 13 Oct. 2012

Abstract

Background: Essential oils of medicinal plants such as chamomile are very complex natural mixtures which can contain compounds at quite different concentrations and some of their components have antioxidant and antimicrobial activities in foodstuff.

Objective: The antioxidant and antimicrobial effects of chamomile essential oil in cake preparation were evaluated during 75 days of storage.

Methods: Cake were produced: cakes were treated with chamomile essential oil in three levels (0.05%, 0.1%, 0.15%), cakes were not treated with synthetic and natural antioxidant and antimicrobial agents, and a commercial cake containing synthetic antioxidant (tert butyl hydro quinine= TBHQ) and antimicrobial (potassium sorbate) agents. In all samples peroxide value, thiobarbitoric value, acidity, microbial test (yeast and mould) and sensory test (color, flavor, texture, taste and overall quality) were determined at time intervals 1, 7, 15, 30, 40, 60 and 75 days.

Results: Statistical results showed that, the sample containing chamomile at 0.15%, had good antioxidant and antimicrobial activity in comparison with the control samples (without any synthetic and natural antioxidant and antimicrobial agents) ($p<0.01$). But its activity was less than that of synthetic ones (TBHQ and potassium sorbate as antioxidant and antimicrobial agent) ($p<0.01$). In sensory evaluation, the sample containing chamomile at 0.05% had higher score in flavor, taste and overall acceptability than the samples at 0.15 and 0.1% ($p<0.05$).

Conclusion: Chamomile essential oil as a natural antioxidant and antimicrobial agent can increase shelf-life of food products and due to absence of synthetic agents are safe with no side effect on human health.

Keywords: ***Matricaria chamomilla*, Essential oil, Cake, Antioxidant activity, Antimicrobial activity**

Introduction

Cake manufacturers face a major problem of lipid oxidation and mould growth which limits shelf-life of their product. The use of antioxidant and preservatives can reduce this problem [1]. The antioxidants most frequently used are synthetic phenols such as butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA) and propyl gallate [2]. However the safety of these synthetic antioxidants and preservatives has been questioned due to toxicity, liver damage and carcinogenicity. 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]. Some studies have disclosed the potential sources of natural antioxidants for the bakery products [4]. 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, mace cloves, poppy and sesame [5]. Several studies have demonstrated that a variety of plants such as chamomile, cinnamon possess antioxidant and antimicrobial activities. Chamomile (*Chamomile recutita* L.) is used for a variety of herbal remedies and several classes of biologically activity. Compounds have been identified from the plant including coumarins and sesquiterpenes; there have also been many reports on the identification of flavonoid from this plant. Apigenin is quantitatively the most abundant flavonoid found in chamomile flowers and contributes to the observed pharmacological properties [6]. Chamazulene extracted from chamomile was shown to inhibit Fe^{+2}/ascorbate-induced lipid peroxidation [7]. Water and alcohol extracts of chamomile flowers showed a moderate effect in controlling hydrolytic rancidity, measured by peroxide value and free fatty acids [2].

The composition, structure as well as functional groups of the oils play an important role in determining their antimicrobial activity. Usually compounds with phenolic groups are most effective [8]. Chamomile oil at 3000 ppm exhibited the highest inhibition against *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium moniliforme* [7].

Essential oils of herbals are very complex natural mixtures which can contain about 20-60 compounds at quite different concentrations [9]; some of their components have antioxidant and antimicrobial activities. The aim of this study was to evaluate antioxidant and antimicrobial activity of chamomile essential oil (due to the good antioxidant and antimicrobial effects [2, 7]) on cake shelf life.

Material and Methods

Materials

Chamomile essential oil (*Matricaria chamomilla* L.) was used as source of natural antioxidant and antimicrobial preservatives. It was purchased from the Institute of Medicinal Plants and Natural Products Research in Gorgan, Iran (May 2010) and then the essential oil was extracted by steam distillation, using a Clevenger-type apparatus

[10]. The obtained essential oil was dried over anhydrous sodium sulphate and kept at 4°C until it was used. Oil with no antioxidant was purchased from Behshahr factory, flour with no additive was purchased from Taban Factory, eggs and baking powder were purchased from market. Chemicals (of analytical grade) required: hexane, acetic acid glacial, chloroform, thiobarbitoric acid, tert butyl hydro quinine (TBHQ), sodium hydroxide, ethanol, sodium thiosulfate, saturated potassium iodide, potassium sorbate, pepton water, DG18 (dicholoran 18% glycerol agar) were obtained from authentic companies.

Preparation of cake

Cakes were prepared by the following method. Sugar (20%) and egg (14%) were mixed for 3-4 minutes, after that flour (26%) and baking powder (0.5%) were added and creamed. The essential oil was blended with fat (20%) and mixed with the above cream. After 5 min of mixing water was added to the dough then it was mixed to obtain homogenous dough and placed on aluminum plates, then baked at 20^{o}C for 20 min after baking, plates were allowed to cool, were covered with air-tight sterile foil and stored in ambient temperature.

Cake were prepared to provide five treatments: control treatment 1 received no antioxidant or antimicrobial agent (Con 1= negative control samples), control treatment 2 was prepared with addition of TBHQ (100 ppm) as synthetic antioxidant and sodium sorbate (0.3%) as antimicrobial agent (Con 2= positive control samples), the other variations were prepared by adding chamomile essential oil at 3 different rates of 0.05% (Ch-0.05), 0.1% (Ch-0.1) and 0.15% (Ch-0.15) as natural antioxidant and antimicrobial agent.

Methods

The lipid of ground cakes were extracted using n-hexane by subjected to steam for 30 min [5]. Stability of cake lipids was determined during storage for 75 days [1, 5] at ambient temperature measuring peroxide value, thiobarbitoric acid value, free fatty acids (FFA) and microbial test (yeast and mould counts). The above analyses were carried out in three replicates.

Peroxide value (PV)

Oxidation was periodically assessed by the measurement of peroxide value (PV) at 1st, 7th, 15th, 30th, 45th, 60th and 75th days of storage according to the AOCS method [11].

Thiobarbitoric acid (TBA)

Thiobarbitoric acid (TBA) values of the samples were measured during the same days of storage according to the AOCS method [12].

Free fatty acid (FFA)

Free fatty acid contents were determined during 1st, 7th, 15th, 30th, 45th, 60th and 75th days of storage according to the method described by Bhanger et al., 2008 [13].

Yeast and mould counting

Number of yeast and mould in cakes were counting in Dicholoran Glycerol Agar

(DG18%) medium during 1st, 10th, 30th, 50th, 70th days of storage according to the method of ISO [14].

Sensory studies

Sensory evaluation of cakes was done to determine the acceptability of the cakes prepared by chamomile essential oil addition 10 days after baking. For this study thirteen panelists were recruited from Food Science Faculty of Tarbiat Modares University. The Seven samples were coded differently and served to panelists. Sensory scores for different attributes like color, flavor, texture, taste and overall quality were recorded. The samples were evaluated for sensory attributes on the tenth day after production as excellent= 5, good= 4, suitable=3, not suitable = 2 and ineligible= 1.

Statistical analysis

The experimental data were analyzed statistically for variance by using SPSS 18 program. Data recorded as means ± standard deviation of three replicate measurements. Analyses of variance were performed by ANOVA test and significance of differences between the means was determined by Duncan's multiple range tests.

Results

Chemical analyses

Peroxide values (PVs) were estimated in all cakes to determine the extent of peroxide formation due to oxidation of fat (milliequivalents of O_2/kg of fat) during the storage period of 75 days (Figure 1). Data showed that initially peroxide value in all samples was low and increased during storage. In Cont 1 samples, the rate of hydroperoxides formation was higher than in the other samples treatments at every sampling date. Whereas, the increase in PV in Cont 2 was marginal and was the lowest among all treatments and samples.

In comparison with Cont 1 sample, the cakes containing chamomile essential oil had lower PV but their peroxide values were higher than those of Cont 2. It was interesting to note that cakes prepared by chamomile essential oil at high concentration (0.15%) had the lowest PV during the storage, in fact increased of concentration caused decreased PV. The peroxide values after 75 days were different significantly among the all samples.

Changes occurring in TBA and FFA values of the cake variations during storage are given in Figures 2 and 3. Increase in TBA and FFA values were observed in all cake samples by time. However cont 1 treatment had the highest value (11.9) in FFA test and (0.57) in TBA test after 75 days.

The TBA values of the samples prepared by chamomile essential oil, ranged from 0.03-0.4 and in the Cont 2 ranged from 0-0.08. The values obtained are within acceptable range after 75 days but in the Cont 1 sample ranged from 0.1-0.57.

In the cakes prepared by chamomile essential oil TBA and FFA values increased by decreasing the chamomile concentration.

FFA increased in all samples, it was the lowest in Cont 2 and highest in Cont 1, in cakes containing chamomile essential oil was higher than Cont 1 and lower than Cont 2.

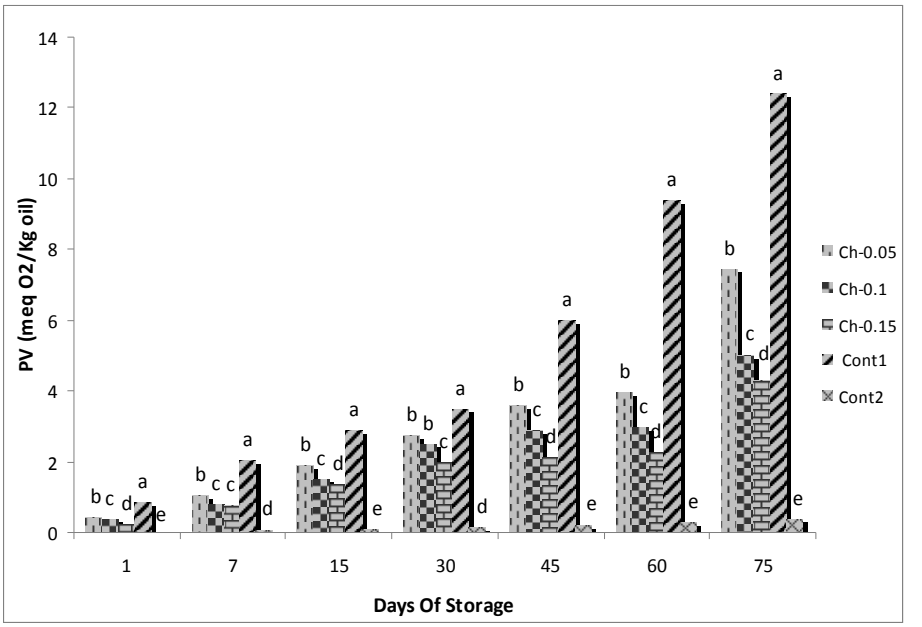

Fig. 1 - PV (meq O_2/kg oil) changes of produced cakes samples during 75 days of storage.
The same letters (a, b, c, d and e) are not significantly different (p<0.01), Adding chamomile essential oil at rates of 0.05% (Ch-0.05), 0.1% (Ch-0.1) and 0.15% (Ch-0.15); Con 1= no antioxidant or antimicrobial agent; Con 2= with addition of TBHQ (100 ppm) as synthetic antioxidant and sodium sorbate (0.3%) as antimicrobial agent

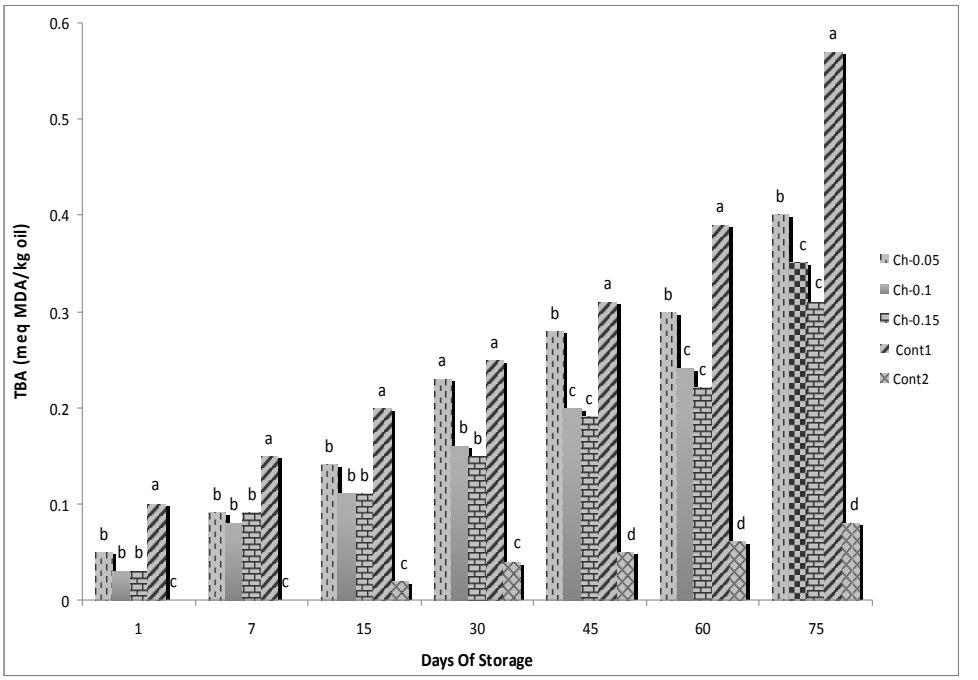

Fig. 2 - TBA (meq MDA/kg oil) changes of produced cakes during 75 days of storage
The same letters (a, b, c, d and e) are not significantly different (p<0.01), Adding chamomile essential oil at rates of 0.05% (Ch-0.05), 0.1% (Ch-0.1) and 0.15% (Ch-0.15); Con 1= no antioxidant or antimicrobial agent; Con 2= with addition of TBHQ (100 ppm) as synthetic antioxidant and sodium sorbate (0.3%) as antimicrobial agent. MDA=malondialdehyde

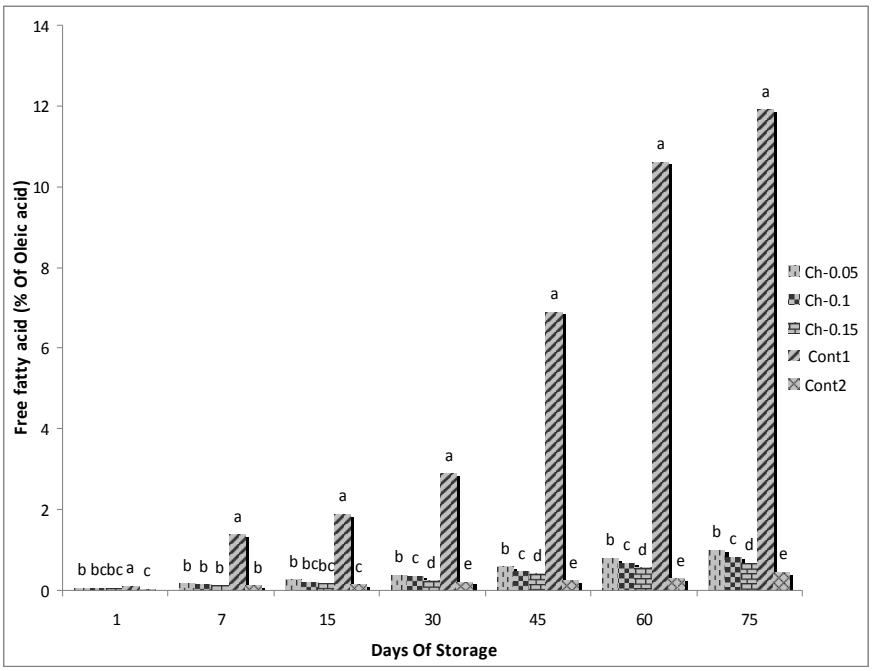

Fig. 3 - FFA (% oleic acid) changes of produced cakes during 75 days of storage
The same letters (a, b, c, d and e) are not significantly different (p<0.01), Adding chamomile essential oil at rates of 0.05% (Ch-0.05), 0.1% (Ch-0.1) and 0.15% (Ch-0.15); Con 1= no antioxidant or antimicrobial agent; Con 2= with addition of TBHQ (100 ppm) as synthetic antioxidant and sodium sorbate (0.3%) as antimicrobial agent

On the whole the changes in TBA and FFA for the different additive treatments followed exactly the same trend by time as did the changes in PVs.

Microbial analysis

As Figure 4 (mean of ratio of mould and yeast number in samples to Cont 1 sample) shows in compare to Cont 1 which is with no antimicrobial, chamomile essential oil retarded mould growth in cakes. Chamomile essential oil in high concentration had more effect on mould, in fact with increasing the concentration of chamomile essential oil inhibitory activity would increase but its affect is less than synthetic ones. After 75 days, growth of moulds was observed in all samples.

Common moulds found in cakes and bakery products are *Rhizopus stolonifer*, *Penicillium expansum*, *Penicillium stoloniferum*, *Aspergillus niger*, *Monilia sitophila* and species of Mucor and Geotrichum. Among these, *Penicillium expansum*, *Penicillium stoloniferum* and Mucor are mycotoxin producers [1]. In this study types of moulds were not identified.

Sensory analyses

Figure 5 gives the mean scores for sensory analysis of cakes on ten days after baking. There was no significant difference in the texture and color of different cake samples as evaluated by the panelists. It was observed that cakes prepared by chamomile essential oil in some attributes and overall quality received lower score than the other samples. Cakes containing 0.15% chamomile essential oil had the lowest score, as shown in Figure 5; you see

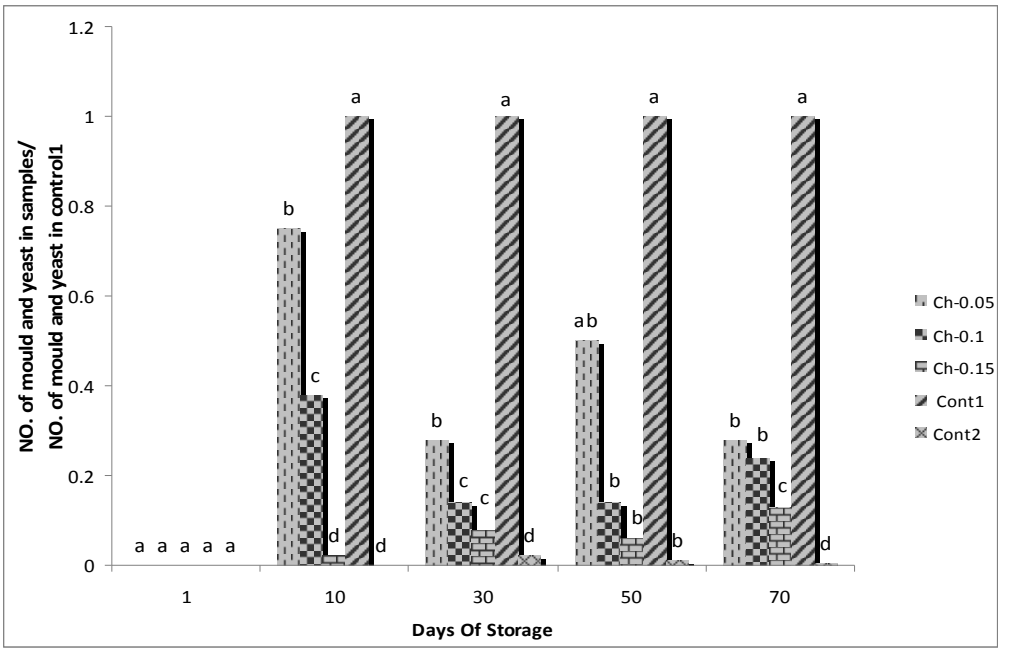

Fig. 4 - Mean of ratios of yeast and mould numbers in samples relative to control (numbers in samples/ numbers in control 1)

The same letters (a, b, c and d) are not significantly different (p<0.01), Adding chamomile essential oil at rates of 0.05% (Ch-0.05), 0.1% (Ch-0.1) and 0.15% (Ch-0.15); Con 1= no antioxidant or antimicrobial agent; Con 2= with addition of TBHQ (100 ppm) as synthetic antioxidant and sodium sorbate (0.3%) as antimicrobial agent

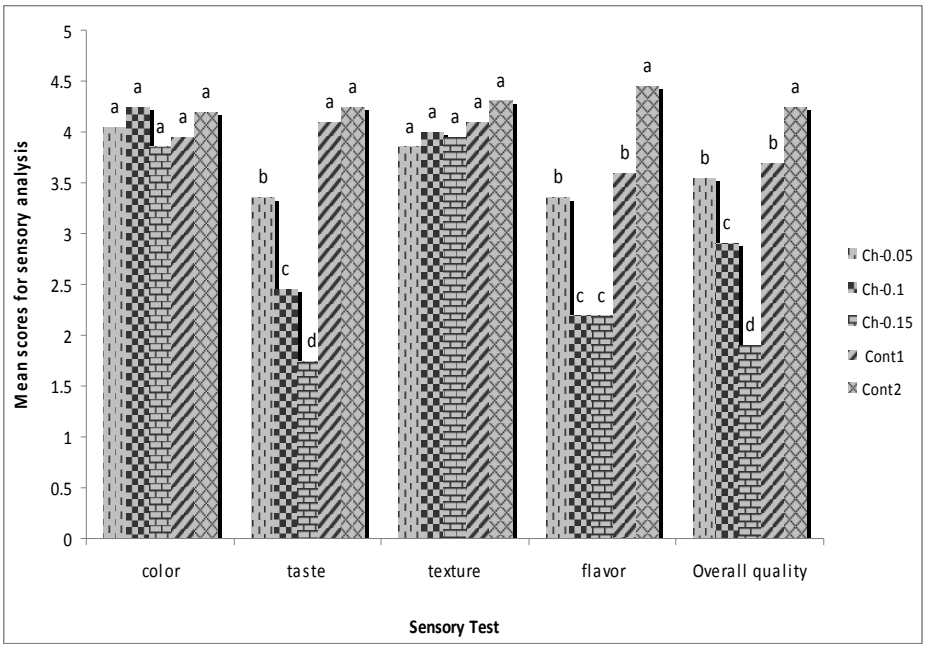

Fig. 5 - Mean scores for sensory analysis of cakes

The same letters (a, b, c and d) are not significantly different (p<0.05), Adding chamomile essential oil at rates of 0.05% (Ch-0.05), 0.1% (Ch-0.1) and 0.15% (Ch-0.15); Con 1= no antioxidant or antimicrobial agent; Con 2= with addition of TBHQ (100 ppm) as synthetic antioxidant and sodium sorbate (0.3%) as antimicrobial agent

sensory scores increased with decreasing the amount of essential oil. The highest scores of overall quality belonged to Cont 2. Sensory qualities of Cont 2 (containing commercial preservatives) were more acceptable than all other treatments.

Discussion

Three different concentrations of chamomile essential oil were used in present study as source of natural antioxidant and antimicrobial agent. The effects of antioxidants on the stability of the added fat were determined by monitoring PV, TBA and FFA periodically under actual storage conditions (ambient temperature). Cakes containing synthetic antioxidant and antimicrobial (TBHQ and potassium sorbate) chemicals possess stronger activity than the natural ones but in comparison to samples with no synthetic antioxidant or antimicrobial agents, chamomile essential oil had significant activity ($p<0.01$) and the effect of chamomile essential oil increased with increasing concentration.

Our results revealed that chamomile essential oil applied to cake was fungicidal and retarded mould growth.

Antioxidant activity of plants is mainly due to the presence of phenolic compounds [2]. Chamomile possesses different phenolics. Some phenolic compounds reportedly have both antioxidant and antimicrobial effects [1, 15].

When essential oils are used in food production systems amounts required are high and as such they are often higher in quality than would normally be organoleptically acceptable [8]. Our results are in agreement with those of Al-Ismail and Aburjai, McKay and Blumberg, Holly and Patal, Pauli and Tolouee et al. [2, 7, 8, 16, 17], because cakes containing 0.15% essential oil had appropriate antioxidant and antimicrobial activity while gained lower score for sensory evaluation.

In order to obtain maximum benefit from use of natural antioxidant and antimicrobial compounds in food products, several points should be taken into consideration, for instance: α_w, temperature, concentration of essential oil and pH should be controlled during preparation and storage. The combined impact of preservatives commonly used in bakery products and other important factors such as water activity (α_w), pH on the prevention of fungal spoilage has been assayed. Potassium sorbate is a suitable preserving agent that inhibits growth of xerophilic fungi in bakery products of pH near 4.5 regardless of α_w level. For products slightly acidic the addition of this preservative must be combined with low water activity levels otherwise other additional controlling factors must be applied [18].

In fact hurdle technology is useful to control oxidation and spoilage due to microorganisms in bakery products of intermediate moisture.

Plant-derived essential oils due to their content of antimicrobial compounds possess adequate potentials as natural agents for food preservation. Their antimicrobial activity is assigned to a number of small terpenoid and phenolic compounds [19].

Conclusion

In conclusion, the above results showed that, the sample containing chamomile at 0.15% had better antioxidant and antimicrobial activities than the samples without any synthetic and natural antioxidant and antimicrobial agents. But its activity was less than that of TBHQ and potassium sorbate as antioxidant and antimicrobial agent (synthetic ones). Results show that essential oils alone cannot provide complete protection against oxidation and microbial contamination in cakes. It is therefore necessary to control the water activity, temperature, pH and packaging conditions to reduce the amount of food preservatives. Natural antioxidants and antimicrobials can increase shelf-life of food products and due to absence of synthetic agents are safe with no side effects on human health.

Acknowledgment

The authors thank the financial support of Tarbiat Modares University Research Council and Center of Excellency for Recycling and Losses of Strategic Agricultural Products.

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