Antioxidant and Antimicrobial Potential of *Echinacea purpurea* Extract and Its Effect on Extension of Cake Shelf Life

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Abstract

Background: Oxidation of lipids causes reduction of different properties of products that contains lipids. Nowadays, there is a growing demand for the natural antioxidants due to the harmful effects of synthetic antioxidants such as BHA, BHT and TBHQ.

Objective: In this study, extract of aerial parts of *Echinacea purpurea* L. was used instead of synthetic antioxidants in cake then, antioxidant and antifungal activity of *Echinacea purpurea* L. extract (EPE) were evaluated in cake.

Methods: The antioxidant activity of EPE and synthetic antioxidant was compared by measuring peroxide and thiobarbituric acid values during 60 days storage at 25 ºC. Antimicrobial properties of EPE were evaluated by counting of molds in Dicholoran Glycerol agar (DG18%) medium and free fatty acid analyses were assessed by IUPAC method. The effect of the extract on the color, texture, taste and total acceptability of the cake samples was carried out by a group of 20 trained panelists using Hedonic test.

Results: Different levels of EPE were able to retard the oxidation rate of cake, the 1000 ppm of it showed the lowest PV and the antioxidant activity of EPE was higher than BHA 200 ppm ($p<0.01$). EPE at 1500 and 2000 ppm showed the best antimicrobial activity ($p<0.01$). Cakes which containing the extract were well acceptable in terms of sensory parameters.

Conclusion: Results showed that EPE was more effective in controlling growing molds and lipid oxidation during 60 days storage at 25 ºC and it can be used instead of synthetic antioxidant and preservatives.

Keywords: ***Echinacea purpurea*** **L., Extract, Antioxidant activity, Antimicrobial activity, Cake**

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 mould growth are major problems in producing cake, they can limit the shelf-life of this product. These problems may be prevented by the use of antioxidants and preservatives [12]. The onset rancidity in bakery products causes a great influence on texture, color, and organoleptic parameters, also losses the nutritional value [3]. The synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) have been used as antioxidants in foodstuff. Use of these synthetic antioxidants, has begun to be limited due to their toxicity and harmful effects on health [23]. This has led to an interest in the find more effective, safer and economical sources of natural antioxidants and preservatives [7].

Recently, use of plants as a source of biologically active substances including antioxidants, antimutagens, and anticarcinogens attracted much attention [3]. The aromatic plants and spices have been widely used in many food products such as meat and meat products, dairy and bakery products [23]. Limited reports are available about antioxidant and antimicrobial effects of extracts and essential oils of natural plants in bakery products. Goli *et al* (2005) had reported that the pistachio hull extract (PHE) was effective in retarding oil oxidation at 60 °C. They reported a positive correlation between extract concentration and antioxidant activity over the range 200-600 ppm. PHE at 600 ppm had antioxidant activity same as BHA and BHT at 200 ppm [8]. It has been shown that pomegranate (*Punica granatum*) peel extract (PPE) at 500 ppm was more effective in retarding oil deterioration than BHA and BHT at 200 ppm [31]. Rajaei et al (2010) showed that phenolic compounds of pistachio green hull have antimutagenicity activity against direct mutagen of 2-nitrofluorene and it may become important as a noticeable source of compounds with health protective potential and antimicrobial activity [21]. Lipid peroxidation in crude soybean oil can be inhibited by the required amount of essential oil of *Bunium persicum* [25]. Lean and Mohamed, 1999 showed that turmeric had antioxidative and antimicrobial activities in butter cake during the 4 weeks storage, and this was better than synthetic ones. It has been reported that rice bran extract has a good antioxidant activity and it can keep cookies against lipid oxidation [7]. Also, Reddy *et al* (2005) reported that drumstick leave and amla extracts have excellent antioxidant activity on biscuit in comparison with BHA during 6 weeks of storage. Increasing shelf life of cakes has great economic importance [6]. Bajaj and Urooj, (2006) studied effect of incorporation of mint as a source of natural antioxidant on sensory parameters of biscuits. They explored that biscuits including mint (*Mentha spicta* L.) in powder form were highly acceptable compared to control and BHA in biscuit. Nanditha *et al* (2009) reported that hexane and chloroform extracts of *Garcinia* and turmeric powder were suitable for use in biscuits as

natural antioxidants and results were comparable with BHA. It has been reported that cinnamon leaf, clove, bay, lemongrass and thyme essential oils have antifungal activity in sponge cake against *Eurotium*, *Aspergillus* and *Penicillium* genus [9]. Rizzello *et al* (2009) reported that the water-soluble extract of *Amaranthus* spp. seeds has an excellent antifungal activity, and large inhibitory against some of the most important bread contaminants such as *Penicillum brevicompactum*, *P.chermesinum* and *Eurotium herbarioum*.

According to the new attraction for natural products such as essential oils and plant extracts, despite their wide use as fragrances, it is important to develop a better understanding of their biological action for new applications as a food preservers and additives in human nutrition [4].

Echinacea purpurea L. is the most common medicinal plant in Europe. A popularly consumption as an ingredient in functional foods, supplements and certain candies seem to be increasing alongside the appearance of clinical evidence for efficiency against common colds [29]. Merali *et al* (2003) reported that extracts of the genus Echinacea have significant antifungal and anti-inflammatory activities. The plant extracts have shown antioxidative, antibacterial, antiviral, and antifungal properties and are used for treating common cold and respiratory and urinary diseases [30]. Cichoric acid (di-caffeoyl tartaric acid), alkamides (alkyl amides), polysaccharides and glycoproteins are considered as the most important compounds of this medicinal plant [29]. Pellati *et al* (2004) have shown that all investigated Echinacea species are a good source of natural antioxidants and could be used to prevent free-radical-induced harmful effects. The antioxidant activity could be ascribed to the phenolic content of the roots, and cichoric acid present in *E. purpurea* was almost as efficient as echinacoside in *E. pallid*. Extracts of the roots and leaves of *E. purpurea* were found to have antioxidant properties in a free radical scavenging assay and in a lipid peroxidation assay [26].

With proliferated knowledge and demand on natural health care, the World Health Organization (WHO), the US Food and Drug Administration (FDA) and European Union Administration have independently announced their management regulations and relevant measures on traditional medicine and medicinal herb [13]. A big advantage of essential oils is the fact that some of them show a very antimutagenic capacity which could well be linked to an anticarcinogenic activity [4]. Natural antioxidants attracted considerable interest in recent years not only for their role in preventing the auto oxidation, but also for their consumer health benefits. Cakes containing *Echinacea purpurea* L. extract with clinical evidence that may provide a health benefit beyond basic nutrition can be used as a functional food.

The objectives of this study are as follows: (i) to evaluate the antioxidant activity of *Echinacea purpurea* L. extract (EPE) as a natural antioxidant in cake, (ii) to investigate its antifungal effects and sensory on cake.

Materials and Methods

Materials

Medicinal Plant

Aerial parts of *Echinacea purpurea* L. which cultivated in Iran were obtained from Institute of Medical Plants, ACECR, Karaj, Iran. 1.0 g of plant powder was taken then, 20 ml methanol (80%) was added and extraction carried out by magnetic stirring at room temperature for 6h [20]. Then, extract was dried by rotary evaporator and stored at 4 °C for other experiments.

Chemicals

All chemicals, with the highest purity available, like BHA, ethanol, thiobarbitoric acid, carbon tetrachloride, sodium hydroxide and culture media (DG 18) were purchased from Merck Chemical Co. (Darmstadt, Germany).

Cake Ingredients

Soybean oil (refined, bleached and deodorized) without any antioxidant was purchased from Parsghoo Oil Factory (Tehran, Iran). Egg and sugar were bought from local supermarket. White flour without any preservative, salt and baking powder were purchased from Golha Co. (Tehran, Iran).

Methods

Determination of Total Phenolic Content

Total phenolic content (TPC) of *Echinacea purpurea* L. extract was determined according to the Folin – Ciocalteu reagent method as described by Bhanger *et al* (2008). Twenty µl of sample was taken in the test tube, then, 1.58 ml distilled water and 100 µl of Folin – Ciocalteu reagent and 300 µl of sodium carbonate were added. After vortexing the reaction mixture, the tube was placed in a dark place for 2 h and the absorbance at 765 nm was measured against a blank. The total phenolic content was expressed as mg gallic acid equivalents (GAE) / g of extract.

Cake Preparation

Cakes were prepared according to the method described by Lean and Mohamed, (1999). Batter for cakes were prepared by creaming soybean oil (500 g), sugar (450 g), salt (2.5 g) and each plant extract or commercial antioxidant for 12 min at medium speed until light and fluffy. Whole eggs (450 g) were then slowly added at low speed to avoid curdling, the mixing bowl was scraped, and this was followed by continuous mixing for another minute. Then, water (150 g) and required amounts of extract were added to the mixture and mixed for a few minute. Sifted flour (500 g) and baking powder (9 g) were gently folded in at low speed for 1 min, before the batter was mixed for another minute at medium speed. The batters were baked at 180 ºC for 20 min. The cakes were cooled, packed in polypropylene film and stored at room temperature for 60 days.

In a preliminary test, sample cakes were prepared by different levels of *Echinacea purpurea* L. extract (250, 500, 1000, 1500, 2000, 2500 and 3000 ppm) and organoleptic evaluation was carried out. The results show that the three levels of 1000, 1500 and 2000 ppm were more acceptable by panelists. For

analyzing antioxidant and antifungal effects of natural extract compared with synthetic antioxidant, cakes were prepared with *Echinacea purpurea* L. extract at concentration of 1000, 1500 and 2000 ppm, BHA at 100 and 200 ppm and control sample without any antioxidant and preservative.

PV, TBA, free fatty acid (FFA) of cakes were determined after 1, 5, 8, 15, 30, 45 and 60 days and mold counting after 10, 30, 45 and 60 days of storage at 25 ºC.

Determination of Antioxidant Activity

Extraction of Lipid

A hundred grams of cakes were ground roughly and was placed in a closed flask; 200 ml of n-hexane was added. The flask was shaken for 1 hour and then filtered. The solvent was removed from the extracted fat by rotary at 50 ºC. The extracted fat was used to various analyses which will be described in the following sections [6].

Determination of Peroxide Value (PV)

Peroxide values of cakes were determined after 1, 5, 8, 15, 30, 45 and 60 days of storage at 25 ºC according to the AOCS method [1]. The results were expressed as mille equivalents of O2 kg^{-1} of oil.

Determination of Thiobarbituric Acid Value

Thiobarbituric acid values (TBA) of the cakes were measured after 1, 5, 8, 15, 30, 45 and 60 days of storage at 25 ºC according to the AOCS method [2].

Determination of Antifungal Activity

Counting of molds was carried out in Dicholoran Glycerol agar (DG18%) medium during 10, 30, 45 and 60 days of storage at 25 ºC according to the method of ISO [10]. The results reported as a mold ratio of sample / control.

Free Fatty Acid Analysis

Free fatty acid analyses during 1, 5, 8, 15, 30, 45 and 60 days of storage at 25 ºC, were assessed by IUPAC standard method [7].

Sensory Analysis

Sensory evaluation of cakes treated with different levels of EPE, BHA and control was carried out by a group of 20 trained panelists using Hedonic test. The panelists were asked to evaluate color, texture, taste and total acceptability of the cake samples on a scale from 5 to 1 indicating decreasing taste. The data from the 20 independent panelists were pooled and the mean values and standard deviations were determined [19].

Statistical Analysis

Data were statistically analyzed using analysis of variance (ANOVA) and differences among the means were determined for significance at $p \leq 0.01$ using least significant differences (LSD) test (by SAS software). The data are presented as mean ± standard deviation of the three determinations.

Results

Total Phenolic Content

The total phenolic content was determined by using Folin-Ciocalteu method. The phenolic content can be used as a critical index for evaluating the antioxidant capacity because phenolic compounds are widely established in the herbs as the secondary metabolites and most plant-derived antioxidants contain large amounts of polyphenols [7, 8, 9]. TPC of EPE was about 60.0 ± 1.0 mg (GAE)/g. Lee and Scagel, (2010) reported that total phenolic content of EPE contained 22.3 ±1.0 mg (GAE)/g. Lee et al., (2009) analyzed TPC of *Echinacea purpurea* extract and explored that it contained 11.0 ± 1.0 mg (GAE)/g. In another study, *Echinacea purpurea* L. dietary supplements from two companies (coded E2 and E3), were evaluated for total phenolic content. TPC of E2 and E3 were 44.64 and 24.78 mg (GAE)/g, respectively [15]. Several factors, such as local, climatic, seasonal and experimental conditions influenced on the composition of any plant-derived and extraction of plant materials is mostly based on the correct choice of solvents, heat and/or agitation that are important to increase the solubility of materials and the rate of the mass transfer [26, 27, 28].

Antioxidant Effect of EPE on Cake

In most cases, the hydroperoxide formation or the production of primary and secondary products of oxidation can be followed by chemical or sensory methods. PV generally used as a factor to indicate the developing oxidation of oil, fat and fatty foods [7]. Figure 1A shows the PV changes of the cakes during 60 days of storage at 25 ºC. As seen from Figure 1A, PVs of samples increased during the storage time. All the samples treated with EPE exhibited a slow rise in peroxide value, but control sample showed a noticeable increase during the storage time. EPE at 1000 ppm had the least PV value in comparison with control and BHA at 100 and 200 ppm ($p < 0.01$). This result shows that EPE at 1000 ppm had the highest antioxidant activity which was higher than BHA at 200 ppm. At higher concentration a prooxidant effect observed.

PV of cake increased by increasing the concentration of EPE from 1000 ppm to 1500 and 2000 ppm showed partially pro-oxidative effect, this result might be due to increasing of phenolic components. Lean and Mohamed, 1999 reported that black pepper leaves appeared to be pro-oxidative, which could be due to the presence of chlorophyll. Light and photosensitizes such as chlorophyll can initiate free lipid radical formation [12].

The addition of natural and synthetic antioxidants to cake also affected the TBA values of them during storage for 60 days (Figure 1B). The values obtained are significantly lower in cakes treated with EPE than control. EPE at 2000 ppm had TBA value almost equal to BHA at 200 ppm. All the levels of antioxidant used decreased the rate of secondary products formation in cakes.

Antifungal Activity of EPE in Cake

As seen from Figure 2, it is clear that the mold ratio (sample / control) of all samples were increased during storage. After 60 days,

there was a significant difference between mold ratio of samples containing EPE and those containing BHA and control. There was no significant difference between samples containing BHA and control after 60 days of storage at 25 ºC. By increasing the EPE concentration from 1000 ppm to 1500 and 2000 ppm, antifungal activity increased, hence conclude that EPE could prevent growth of moulds in cake.

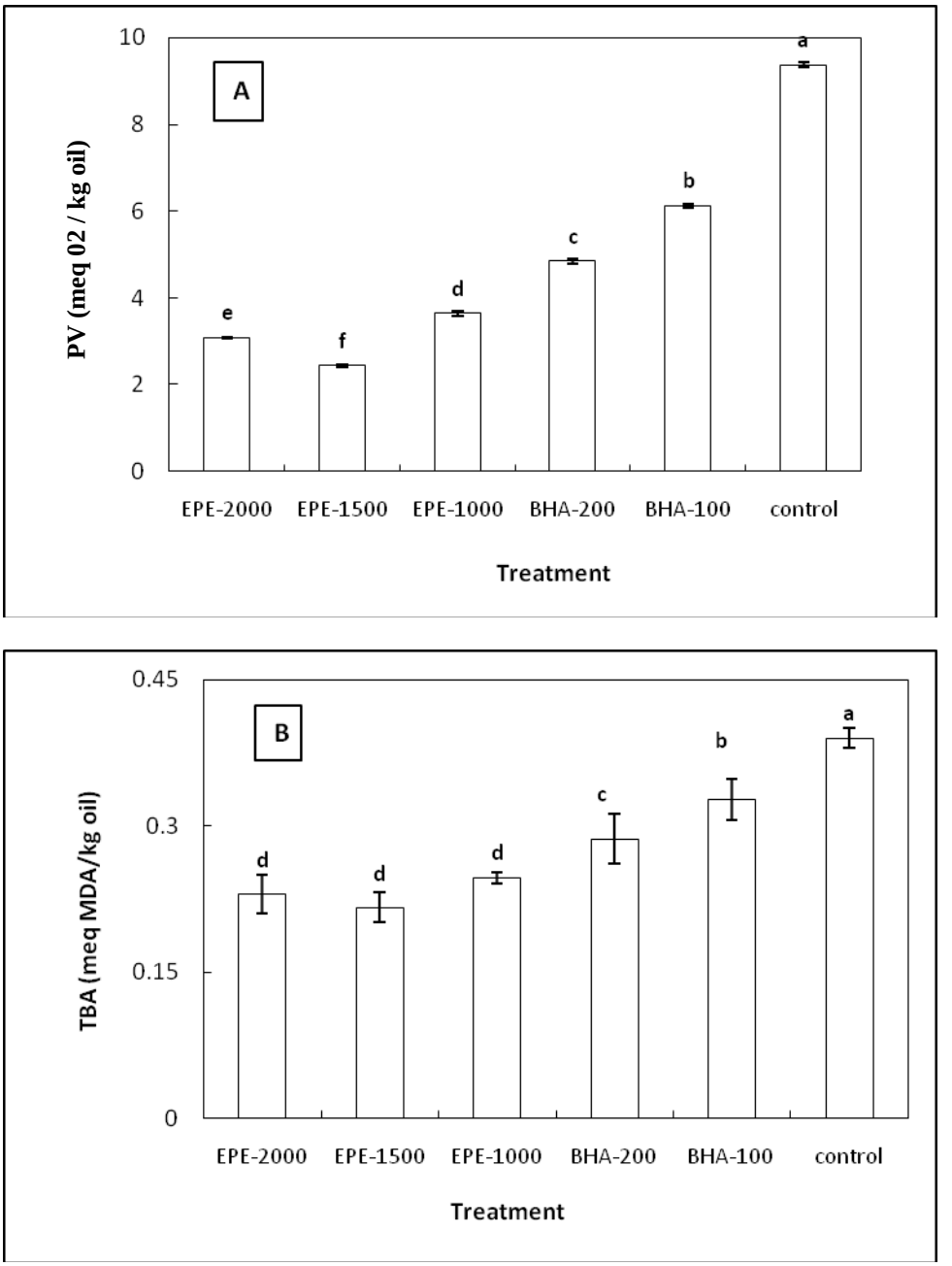

Figure 1- Comparison of PVs (A) and TBA (B) of samples during 60 days of storage at 25 ºC. Values with different letters were significantly different (p < 0.01, LSD test)

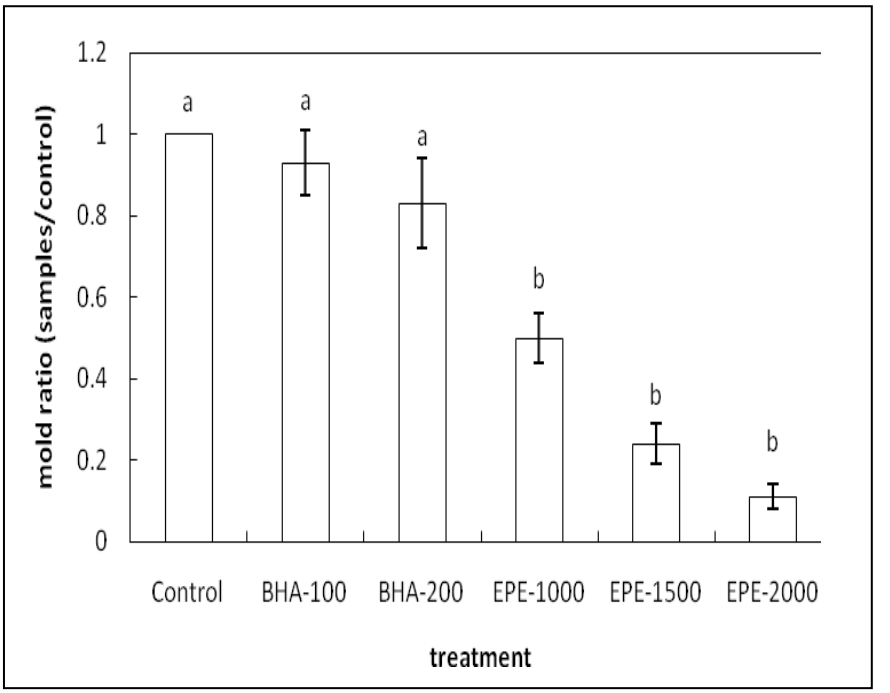

Figure 2- Comparison of mold ration of samples/control after 60 days of storage at 25 ºC. Values with different letters in the same column were significantly different ($p < 0.01$, LSD test).

Free fatty acid contents of cakes are presented in Table 1. As observed from Table 1, control sample had maximum value of acidity 9.32 percentage oleic acid followed by cakes containing BHA at 100 (8.02 percentage oleic acid) and 200 ppm (7.11 percentage oleic acid) after 60 days storage. Cakes containing different concentrations of EPE had lower acidity values. Free fatty acid is formed because of hydrolysis of triglycerides [7] and may develop by enzymatic activity due to microbial growth. The results (Figure 2) showed that EPE could be able to prevent the mould growth and decrease the production of hydrolytic enzymes.

Sensory Assay

Sensory evaluation is an important factor in judging about foodstuffs quality. Samples were evaluated to ascertain consumer acceptability of cakes. There was no different significant difference between cakes containing EPE and control ($p < 0.01$). Cakes containing 1000 and 1500 ppm of EPE had better color, taste, texture and total acceptability in comparison with those prepared with synthetic antioxidant.

Discussion

As shown previously, the EPE had good antioxidant ability for scavenging free radicals [13]. Pellati et al, (2004) reported that some components such as echinacoside, cichoric acid and cynarin, show the highest radical

Table 1- Effect of EPE on FFA content of cake samples*

Sample	Day						
	1	5	8	15	30	45	60
Control	0.09±0.006^{c}	0.10±0.006^{c}	0.14±0.01^{c}	0.23±0.01^{c}	0.92±0.01^{b}	5.86±0.02^{a}	9.32±0.03^{a}
BHA100	0.07±0.01^{c}	0.10±0.01^{c}	0.13±0.005^{c}	0.18±0.01^{d}	0.87±0.01^{c}	3.31±0.01^{b}	8.02±0.03^{b}
BHA200	0.10±0.01^{c}	0.10±0.006^{c}	0.12±0.01^{c}	0.19±0.01^{d}	1.09±0.02^{a}	4.13±0.03^{c}	7.11±0.02^{c}
EPE1000	0.18±0.01^{ab}	0.22±0.02^{a}	0.25±0.02^{a}	0.28±0.02^{a}	0.30±0.02^{de}	0.33±0.02^{d}	0.40±0.05^{d}
EPE1500	0.18±0.01^{ab}	0.21±0.006^{ab}	0.24±0.02^{a}	0.27±0.01^{ab}	0.30±0.01^{de}	0.33±0.03^{d}	0.38±0.02^{d}
EPE2000	0.16±0.02^{b}	0.19±0.02^{b}	0.22±0.01^{b}	0.25±0.02^{b}	0.29±0.01^{e}	0.32±0.02^{d}	0.36±0.02^{d}

* Values with different letters in the same column were significantly different ($p < 0.01$, LSD test)

scavenging activity due to, two adjacent hydroxyl groups on each of their phenolic rings, while chlorogenic, caffeic and caftaric acids with two adjacent hydroxyl groups on one ring showed lower antioxidant activity. The higher the number of hydroxyl groups, the greater the radical scavenging activity. Among components of EPE, cichoric acid is the efficient scavenger of free radicals. Although the alkamide fraction does not exhibit antioxidative activity on its own, it increases the activity of cichoric acid. Improvement of the effect of cichoric acid mixed with alkamides can accordingly be due to surface activity of the alkamides that give the polar cichoric acid better access to inhibit lipid oxidation in the lipophilic droplets of the emulsion [29].

Reddy et al, (2005) were used amla, drumstick leaves and raisins extracts, as natural antioxidants in biscuit. All extracts showed an excellent antioxidant effect on the biscuits in comparison with BHA. PV of drumstick leaves after 6 weeks was 0.8 meq O2/kg oil in comparison with control (3.2 meq O2/kg oil). The higher efficiency of the plant extracts might be because of the stability of these natural antioxidants during baking. In another study, addition of purified extracts of marjoram, mint and basil in biscuits, displayed a good antioxidative effect in comparison with BHA [6]. Lean and Mohamed, (1999) investigated the effect of six Malaysian plants extracts, turmeric, lemon-grass, clove, betal leaves, *Garcinia atriviridis* and black pepper leaves, as natural antioxidants in comparison with BHA and BHT in butter cake samples and PV and TBA of cakes were evaluated after 4 weeks storage. They explored that turmeric, betel leaves, clove and lemongrass at 1g kg^{-1} oil concentration in preventing oxidation were more effective than 0.1g kg^{-1} of BHA and BHT, and black pepper leaves had the pro-oxidant effect. Nanditha *et al*, (2009) reported that hexane and chloroform extracts of *Garcinia* and turmeric powder were suitable for use in biscuits as natural antioxidants and results were comparable with BHA. Among the antioxidants used, biscuits with *curcumin* were found to retain only 11.23 % activity whereas biscuits with chloroform extract of

Garcinia had retained 51 % activity after baking, which was better than TBHQ.

Antimicrobial effects of EPE which obtained by classical and ultrasound solvent extraction were compared. Their results showed that extracts had a noticeably growth inhibition on *Candida albicans* and *Saccharomyces cerevisiae*, while no growth inhibition zones were observed for *Aspergillus niger*. The diameters of inhibition zone observed for all the microorganisms were larger for extracts obtained by classical extraction than those by ultrasound extraction [27]. It has been reported that the *Echinacea purpurea* has been proven to show good immunoregulation, antiinflammation and antioxidant capacity, and with no other side effects during clinical trial stages, and several groups of compounds, including alkamides and caffeic acid derivatives, have important role in EPE activity [11,13,14].

Goynot et al, (2003) investigate antifungal effects of 16 essential oils against common fungi causing spoilage of bakery products, including *E. amstelodami, E. herbariorum, E. repens, E. rubrum, A. niger, A. flavus,* and *P. corylophilum.* They observed that volatile substances from cinnamon leaf, clove, bay, lemongrass and thyme essential oils had excellent antifungal activities against fungi. Lean and Mohamed, (1999) reported turmeric, lemon-grass, *Garcinia atriviridis* and clove were efficient in retarding mould growth in butter cakes because of oxygenated sesquiterpenes and monoterpene hydrocarbons in turmaric and eugenol and phenolic compounds in clove. The volatile components from mustard, cinnamon, garlic and clove essential oils were effective in the control of common bread spoilage fungi [18]. Essential oils of *Carum carvi* exhibited more than 50 % fungi toxicity against two strains of *A. flavus* [11]. Razzaghi- Abyaneh et al, (2009) reported that limonene, a terpenoid hydrocarbon isolated from different plant species especially caraway had antifungal activity against *Aspergillus niger*. It has been reported that the water-soluble extract of *Amaranthus* spp. seeds has an excellent antifungal activity, and large inhibitory against some of the most important bread contaminants such as *Penicillum brevicompactum*, *P. chermesinum* and *Eurotium herbarioum*. The crude water-soluble extract had minimal inhibitory concentration (MIC) of 5 mg of peptides/ml [24]. Suhr and Nielsen, (2003) reported that larger phenolic compounds such as thymol and eugenol had good antifungal activity against rye bread spoilage fungi applied directly, whereas smaller compounds such as citral and allyl isothiocyanate were most efficient when added as volatiles.

Influence of natural antioxidants such as *garcinia* and *turmeric* powder on biscuit was investigated by Naditha *et al* (2009). They indicated that addition of natural antioxidants had no effect on sensory parameters of biscuit and the samples had good acceptability. In another study Bajaj and Urooj, (2006) showed that biscuits including mint (*Mentha spicta* L) in powder form received significantly higher scores in terms of texture, taste and mouth feel in comparison with control and biscuit containing BHA. In this study, cakes

containing EPE had better sensory parameters in comparison with those prepared with synthetic antioxidant.

Conclusion

The chemical, microbial and sensory results indicated that EPE can act as a good replacer of synthetic antioxidant and preservative. The higher efficiency of the plant extract in retarding the rate of lipid oxidation could be due to phenolic content and the stability of this natural antioxidant during baking. Results of antimycotic evaluation showed that selected extract at 1000, 1500 and 2000 ppm had an excellent antifungal effect on the cake. Also, our results showed that EPE had no adverse effect on sensory parameters of cakes. It is noticeable that cakes prepared with EPE have considerably good nutritive and health benefits in comparison with synthetic additives.

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