Evaluation Antioxidant and Antimicrobial Effects of Cinnamon Essential Oil and Echinacea Extract in Kolompe

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Abstract

Background: Applications of natural ingredients not only increase the shelf life of food but also omit the problems of synthetic preservatives compounds.

Objective: *Cinnamon verum* essential oil (CVEO; 0.05, 0.1, 0.15%) and *Echinacea purpurea* extract (EPE; 0.25, 0.5, 0.75%) were added to Kolompe (a traditional cookie in Kerman-Iran) and their antioxidant and antimicrobial effects were compared with the samples of BHA (100 and 200 ppm) and control (without any antioxidant).

Methods: Peroxide and tiobarbituric acid values (PV and TBA) and total count, mold, yeast, *Escherichia coli, Enterobacteriaceae, Bacillus cereus*, positive *Staphylococci –coagulase* were measured for antioxidant and antimicrobial assessment, respectively.

Results: PV and TBA values of control were more than others and antioxidant effect of CVEO-0.05 was similar to BHA-0.01 ($p< 0.01$). The least PV and TBA values were for CVEO-0.1. Application of EPE-0.25 was too led more antioxidant effect (increased by increasing %) than BHA-0.01. All samples lacked aerobic microorganism, yeast and mold except EPE-0.25 (that was the same with control) in 30th day. The number of these microorganisms in EPE-0.5 was lower than control, BHA-0.01 and BHA-0.02. No microorganism was in EPE-0.75 that expressed strong antimicrobial effect of this level extract.

Conclusion: Antioxidant effects for CVEO-0.1, CVEO-0.25, EPE-0.1 and EPE-0.25 were more than BHA. With increasing of concentration, PV and TBA values for pro-oxidant effect increased. Application of CVEO and EPE in Kolompe was strong effect in microbial properties and increase the shelf life of Kolompe.

Keywords: Antimicrobial Effect, Antioxidant activity, *Cinnamomum verum, Echinacea purpurea* L., Kolompe

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Introduction

Almost all foodstuffs consumed by man are likely to be decayed and degraded in different proportions. Therefore, man has to find ways and substances to increase food shelf life. One way to prevent food decaying is adding preservatives such as antioxidant and antimicrobial substances to food stuffs. Nowadays, a large number of synthetic substances including antioxidants such as tert butyl hydro quinine (TBHQ), butylated hydroxyl anisole (BHA), and butylated hydroxyl toluene (BHT) are used in industrial products. As the consumers' knowledge about the harmful effect of synthetic preservatives improves, a promoting tendency is come into existence on using natural substances; especially compounds with vegetable origin to increase the stability of food [1, 2].

Fruits and vegetables, as biologically active sources, contain phytochemicals with antimicrobial, antioxidant, antimutagenic, and anticarcinogenic activities [2-5]. Phytochemical shaving both antioxidant and antimicrobial properties can be used for preserving foodstuffs and increasing their shelf lives [6]. Essential oils and extracts obtained from plants also contain these active compounds. So, they are used in many foodstuffs as natural additives [7]. The main biologic constituents in the extracts and oils that have antioxidant and antimicrobial properties are phenolic compounds [8-10].

Confectionary products have nearly large amounts of oil and moderate moisture; so, they are susceptible to oxidative and microbial degradation and thus, their shelf lives are limited [11]. Considering this, synthetic antioxidants and microbial preservatives like sorbate and propionate are used in industry. However, as it was mentioned, by increasing the consumers' demand for green food products with high safety, quality and nutritional values, it seems that using plants and materials of plant origin such as essential oils and extracts is a suitable way to meet this need [11, 12].

Much research has been performed about using natural substances in preserving confectionary and bakery products. Bassiouny et al. examined the antioxidant effect of fine powders and ether extracts of basil, peppermint, spearmint, and marjoram in cracker biscuit. They found that using each of the powders in 0.5% would bring about antioxidant effect; however, adding a mixture of four powders in 1% caused pro-oxidant effects. Pure ether extracts showed antioxidant effects in biscuits as well, which was higher than that in BHA [4]. Nielsen and Rios used a mixture of spice and herbs in active packaging to prevent bread from fungal contamination. The essential oils of cinnamon, mustard, garlic, and clove showed antifungal effects, among which clove showed the strongest effect. Although oregano oleoresin weakly prevent the growth of most important spoilage fungi of bread, vanilla essential oil had no preventative effect against these fungi [12]. Frutos and Hernandes-Herreroused rosemary extract in concentrations of 2, 4, and 6 g/l mixed with sunflower oil, garlic, and parsley to make a mixture as dressing bread. The peroxide values obtained showed that after

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eight days, rosemary extract caused antioxidant effect on the above-mentioned dressing [1]. Using 5% ginger and cumin for making cookies showed antioxidant effect well; as the total phenolic contents in these cookies were higher than that in the control sample. In addition, higher $DPPH^{\bullet}$ values in cookies with these spice showed antioxidant activities [13].

Cinnamon essential oil is made from bark and leaves of *Cinnamomum verum* obtained by steam distillation. The most important chemical compounds of the essential oil are cinnamaldehyde and eugenol [14]. Mathew and Abraham proved that essential oils from both bark and leaves have antioxidant effects and can be used in preventing oxidative degradation in food products [10]. Cinnamaldehyde, eugenol, cinnamic acid, and cineol are the main compounds in cinnamon that cause the antioxidant activity [15]. Several researches have also reported its antimicrobial effects [6, 16, 17].

The echinacea extract obtained from *Echinacea purpurea* L. has been extensively used as medicine. In different researches, the antioxidant effects of this compound have assessed to be from medium to weak compared with other herbs and aromatics [18]. These effects are related to the phenolic content of the plant [19]. The antimicrobial property of this compound has been also proved. For instance, Stanisavlijevic et al. showed that echinacea extract can prevent the growth of *Candida albicans* and *Saccharomyces cerevisiae*, but has no effect on the growth of *Aspergillus niger* [20].

Kolompe, is an Iranian traditional cookie (in Kerman Province), which made by dough crust and date crumb. If the cookie is used as a snack, it can provide a part of calorie needed for body, since it contains date. Existence of high amounts of oil in the formula of Kolompe makes it susceptible to oxidation. Because of the medium water activity in this material, it is also susceptible to microbial contamination. So far, a comprehensive study has not been performed on the antioxidant activity of natural compounds on preserving Kolompe. The antimicrobial activity of these compounds has not been also evaluated against on microorganisms causing decay in Kolompe. Therefore, we used cinnamon essential oil and echinacea extract as natural antioxidant compounds in Kolompe and compare their effects with BHA antioxidant. The antimicrobial and sensorial properties in these two compounds were also studied.

Material and method

Materials

Cinnamon essential oil and echinacea extract were obtained from the Institute of Medicinal Plants and Natural Products Research, Karaj, Iran. Materials used in preparation of Kolompe consist of sunflower oil without antioxidant from Ferico Co. (Sirjan, Iran), confectionary shortening without antioxidant from Golnaz Co. (Kerman, Iran), flour with 80% extraction from Tak Co. (Kerman, Iran), and date were purchased from local market.

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Chemical materials used included glacial acetic acid, chloroform, hexane, sodium thiosulfate, potassium iodide, starch, sodium hydroxide, and tiobarbituric acid, and culture mediums were plate count agar (PCA), yeast extract glucose chloramphenicol (YGC), violet red bile glucose (VRBG), brilliant green (BG), Buffered brilliant green bile glucose broth (BGBG broth, EE broth), Buffered peptone water (BPW), mannitol egg yolk polymyxin agar (MYP), lauryl sulfate broth (LSB), and baird parker agar (BPA). The media and chemical agents were purchased from Merck (Darmstadt, Germany) and butylated hydroxy anisole (BHA) from Sigma Chemical Co. (MO, USA).

Methods

Preparation of Kolompe

For preparation of Kolompe, 500 g of a mixture containing equal amounts of shortening and sunflower oil were added to 700 g flour. After punching, 300 ml water was added to the mixture and was punched for 15 min until soft dough resulted. The dates, which forms crust after seeding, were manually punched until a soft and uniform mixture was resulted. It should be noted that to avoid the influence of other additives, no spice and herb that is normally used in preparation of Kolompe was used. The Kolompe test samples were prepared in four variations. Control samples were prepared without addition of antioxidant. The other variations were prepared by adding synthetic antioxidant (0.01% and 0.02% BHA according to the lipid weigh), *Cinnamomum verum* essential oil at three different concentrations: 0.05%, 0.1%, and 0.15%, (CVEO-0.05, CVEO-0.1 and CVEO-0.15, respectively) and *Echinacea purpurea* extract with three different concentrations: 0.25%, 0.5%, and 0.75% (EPE-0.25, EPE-0.5 and EPE-0.75, respectively) according to the dough weigh.

Dough and date were formed to 30 and 10 g balls, respectively. After placement of the date balls inside the dough ball, molding was done. The samples were baked at 180 °C for 45 min. The Kolompe samples were cooled, packed in polypropylene film, and stored at room temperature (25± 3 °C) for 60 days. The samples were analyzed at days 1, 5, 8, 15, 30, 45, and 60 for chemical and microbial tests.

Chemical analyzing

Kolompe samples were ground roughly and extracted in n-hexane by Soxhlet extraction for 30 min. and then the solvent was removed by evaporation under reduced pressure and 30 ºC on rotary evaporator Buchi Co. (Switzerland). After extraction, the peroxide value (PV) [21], TBA value [22], and acidity [23] of the oil were determined. The moisture content and pH of the samples were also measured.

Microbial analyzing

In the days mentioned, the total count of microorganisms [24], yeasts and molds [25], *Bacillus cereus* [26], *Enterobacteriaceae* [27], *Escherichia coli* [28], and *Staphylococcus aureus* [29] were assayed.

Sensory studies

Sensorial studies were carried out by semi trained panelists [20] on the basis of color,

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taste, texture, and overall acceptability parameters. Here, the five-point hedonic scale was used within the range of 1 to 5. Maximum score was the best example [30].

Statistical analysis

Statistical analysis of the data was performed using SAS, version 9.1 and significant differences among means from triplicate analyses at $p< 0.01$ were determined by LSD. All results are presented as mean ± standard deviation of the three determinations.

Results

Moisture assay

The amount of moisture was measured in different days. It was shown that the moisture content of all samples decreased in the course of time, such that the moisture level was less than 12% on day 45.

Microbial assay

Studying antibacterial activity of cinnamon essential oil and echinacea extract

The results of identification and enumeration of *E. coli*, *Enterobacteriaceae, B. cereus*, coagulase-positive *Staphylococci* in days 1, 5, 8, 15, 30, 45, and 60 showed that none of the Kolompe samples contained these bacteria. The reason for this finding may be the effect of cooking temperature, as well as the moisture and low amount of water in the Kolompe samples, which was not adequate for bacterial growing.

Studying antimicrobial activity of cinnamon essential oil and echinacea extract on enumeration of aerobic microorganism

The total count of microorganisms in Kolompe samples during 60 days of storage are presented in Table 1. As shown by the results, the colony count was zero in the first day in all samples, but from day 5, the total count increased. From day 8, the total count of microorganism declined, as in day 45, no microorganism was observed in samples except the control and samples containing BHA. This is probably due to circumstances inadequate for microorganism growth, including the moisture decrease, and also the effect of antimicrobial compounds added. Since from day 45, the total count of samples was so low, for better evaluation and comparison of the antimicrobial activities of cinnamon essential oil and echinacea extract, the results of day 30 were used for comparison to obtain more reliable results (Figure 1). The comparison showed that the highest microorganism growth rate was in control, BHA-0.01, BHA-0.02, and EPE-0.25 samples. Adding 0.5% echinacea extract could not completely prevent the microbial growth, though it decreased the total microorganism count. Also, the colony count in samples containing cinnamon and EPE-0.75 was zero.

Studying antifungal activity of cinnamon essential oil and Echinacea extract in Kolompe

On day 1, all samples were free from yeasts

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Table 1- Total count of samples cfu/g on 5, 8, 15, 30 and 45 days[ab]

	Days				
Sample	5	8	15	30	45
Control	60±4 a	52±2 a	30±1 a	2±1 a	10±1 a
BHA-0.01	53±1 a	48±0 a	30±0 a	25±1a	10±0 a
BHA-0.02	51±0 a	50±0 a	28±2 a	27±1a	10±0 a
CVEO-0.05	16±1 d	10±0 d	5±0 d	1±0 c	0±0 b
CVEO-0.10	0±0 e	0±0 e	0±0 e	0±0 c	0±0 b
CVEO-0.15	0±0 e	0±0 e	0±0 e	0±0 c	0±0 b
EPE-0.25	40±3 b	50±2 a	14±2 b	20±1 a	0±0 b
EPE-0.5	22±2 c	27±2 b	9±0 c	6±0 b	0±0 b
EPE-0.75	21±1 c	20±1 e	0±0 e	0±0 c	0±0 b

[a]Values expressed are means ± SD of triplicate measurement

[b]Values with different letters are significantly different at $p < 0.01$

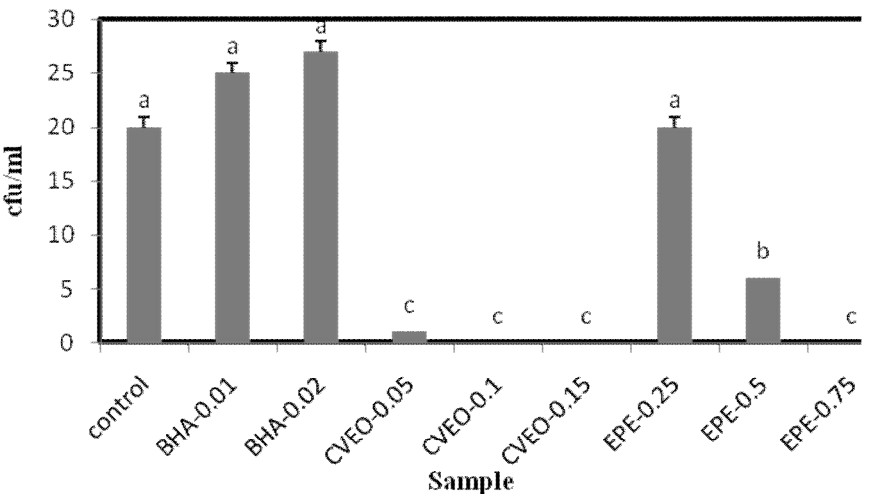


Figure 1- Effect of antimicrobial of *Cinnamomum verum* and *Echinacea purpurea* L. in Kolompe on 30th day

and molds. Fungal growth increased gradually on day 5, owing to the favorable growing conditions, such that the colony count peaked in day 5 (Table 2). Because of the circumstances of inadequate for growth (such as decreased water and moisture content), the number of yeasts and molds decreased too, such that none of them were observed in samples on days 45 and 60. Comparison of the antifungal property in all samples on day 30, it can be concluded that the strongest antifungal property was related to different concentrations of cinnamon, for instance EPE-0.75 was able to prevent fungal growth (Figure 2). Furthermore, the least antifungal activity was observed in EPE-0.25, in which the number of yeast and molds did not significantly differ from control, BHA-0.01 and BHA-0.02 samples.

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Table 2- Total colony of yeast and mold on 5, 8, 15 and 30 days [ab]

	Days			
Sample	5	8	15	30
Control	43±0 [a]	39±4 [a]	15±3 [a]	7±0 [a]
BHA-0.01	40±1 [a]	30±1 [a]	16±1 [a]	8±0 [a]
BHA-0.02	38±1 [a]	19±3 [b]	8±1 [b]	0±1 [a]
CVEO-0.05	20±5 [b]	3±0 [d]	0±0 [c]	0±0 [c]
CVEO-0.10	0±0 [c]	0±0 [d]	0±0 [c]	0±0 [c]
CVEO-0.15	0±0 [c]	0±0 [d]	0±0 [c]	0±0 [c]
EPE-0.25	40±4 [a]	20±5 [b]	9±1 [b]	7±1 [a]
EPE-0.5	39±1 [a]	10±1 [c]	6±2 [b]	4±1 [b]
EPE-0.75	20±5 [b]	11±5 [c]	1±0 [c]	0±0 [c]

Total colony of all samples were zero in days of 1, 45 and 60
[a]Values expressed are means ± SD of triplicate measurement
[b]Values with different letters are significantly different at P< 0.01

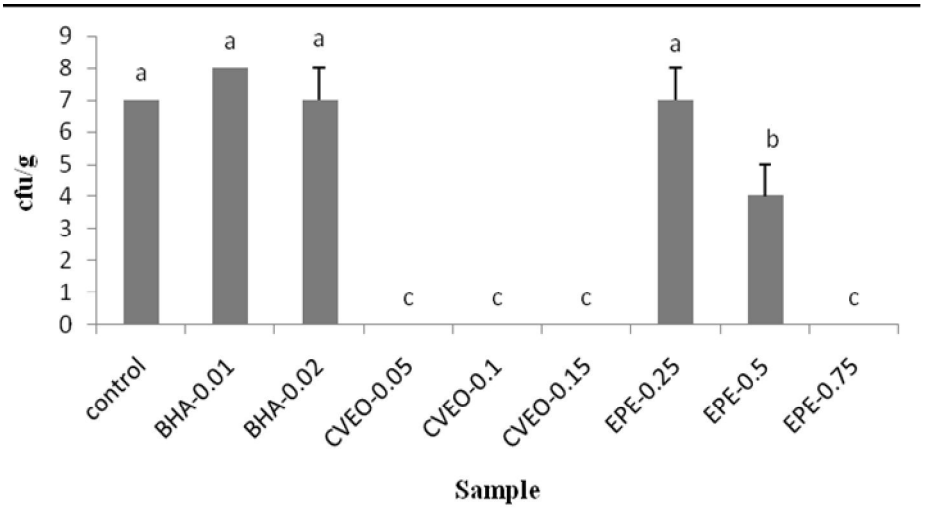


Figure 2- Effect of antifungal of *Cinnamomum verum* and *Echinacea purpurea* L. in Kolompe on 30th day

Antioxidant assay

Measuring peroxide and tiobarbituric acid values

During the 60 days of storage, the highest values of peroxide (meq O_2/kg oil) and tiobarbituric acid (meq MDA/kg oil) were observed in control sample. In other words, this sample had the highest oxidation rate and others samples showed lower rates (Tables 3 and 4). Moreover, higher values were obtained by increasing the storage time. This indicates that the oil in Kolompe was oxidized, and the first and second oxidation products such as peroxides, ketones, aldehydes, and alcohols were produced [31]. For further investigation and comparing the antioxidant activities of the samples, the results of peroxide and tiobarbituric acid value on day 60 were compared.

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Table 3- Peroxide value (meq O_2/kg oil) of samples on keeping days [a]

	Days						
Sample	**1**	**5**	**8**	**15**	**30**	**45**	**60**
Control	0.37±0.04 [a]	1.03±0.09 [a]	1.98±0.03 [a]	2.83±0.05 [a]	3.16±0.01 [a]	3.98±0.06 [a]	4.65±0.09 [a]
BHA-0.01	0.16±0.00 [b]	0.52±0.02 [c]	0.96±0.05 [b]	1.20±0.02 [e]	2.58±0.01 [b]	2.78±0.01 [c]	3.57±0.02 [d]
BHA-0.02	0.13±0.02 [b]	0.21±0.01 [g]	0.84±0.04 [cd]	1.12±0.02 [f]	2.22±0.02 [c]	2.45±0.02 [e]	3.01±0.03 [f]
CVEO-0.05	0.23±0.03 [b]	0.53±0.01 [c]	0.98±0.00 [b]	1.75±0.00 [b]	2.14±0.01 [d]	2.90±0.00 [d]	3.54±0.06 [de]
CVEO-0.1	0.16±0.00 [b]	0.42±0.00 [ed]	0.92±0.00 [bc]	0.98±0.01 [g]	2.22±0.00 [c]	2.28±0.01 [f]	2.84±0.01 [g]
CVEO-0.15	0.16±0.00 [b]	0.63±0.00 [b]	0.81±0.00 [d]	1.22±0.01 [de]	1.75±0.00 [f]	2.48±0.00 [e]	4.28±0.04 [b]
EPE-0.25	0.15±0.05 [b]	0.26±0.03 [fg]	0.67±0.02 [e]	1.09±0.01 [f]	1.70±0.02 [f]	2.78±0.07 [c]	2.96±0.02 [fg]
EPE-0.5	0.18±0.02 [b]	0.45±0.00 [cd]	0.67±0.01 [f]	1.27±0.03 [cd]	2.25±0.04 [c]	2.59±0.07 [d]	3.39±0.03 [e]
EPE-0.75	0.18±0.01 [b]	0.34±0.01 [ef]	0.82±0.08 [d]	1.32±0.04 [c]	1.96±0.04 [f]	2.76±0.04 [c]	3.78±0.07 [c]

[a]Data expressed are means ± SD of triplicate measurements and values with different letters are significantly different at $p< 0.01$

Table 4- Tiobarbituric acid value (meq MDA/kg oil) of samples on keeping days [a]

	Days						
Sample	1	5	8	15	30	45	60
Control	0.37±0.04 [a]	1.03±0.09 [a]	1.98±0.03 [a]	2.83±0.05 [a]	3.16±0.01 [a]	3.98±0.06 [a]	4.65±0.09 [a]
BHA-0.01	0.16±0.00 [b]	0.52±0.02 [c]	0.96±0.05 [b]	1.20±0.02 [e]	2.58±0.01 [b]	2.78±0.01 [c]	3.57±0.02 [d]
BHA-0.02	0.13±0.02 [b]	0.21±0.01 [g]	0.86±0.04 [cd]	1.12±0.02 [f]	2.22±0.02 [c]	2.45±0.02 [e]	3.01±0.03 [f]
CVEO-0.05	0.23±0.03 [b]	0.53±0.01 [c]	0.98±0.00 [b]	1.75±0.00 [b]	2.14±0.01 [d]	2.90±0.00 [b]	3.54±0.06 [de]
CVEO-0.1	0.16±0.00 [b]	0.42±0.00 [ed]	0.92±0.00 [bc]	0.98±0.01 [g]	2.22±0.00 [c]	2.28±0.01 [f]	2.84±0.01 [g]
CVEO-0.15	0.16±0.00 [b]	0.63±0.00 [b]	0.81±0.00 [d]	1.22±0.01 [de]	1.75±0.00 [f]	2.48±0.00 [c]	4.28±0.04 [b]
EPE-0.25	0.15±0.05 [b]	0.26±0.03 [fg]	0.67±0.02 [e]	1.09±0.01 [f]	1.70±0.02 [f]	2.78±0.07 [c]	2.96±0.02 [fg]
EPE-0.5	0.17±0.02 [b]	0.45±0.00 [cd]	0.67±0.01 [f]	1.27±0.03 [cd]	2.25±0.04 [c]	2.59±0.07 [d]	3.39±0.03 [e]
EPE-0.75	0.18±0.01 [b]	0.34±0.01 [ef]	0.82±0.08 [d]	1.32±0.04 [c]	1.96±0.04 [f]	2.76±0.04 [c]	3.78±0.07 [c]

[a]Data expressed are means ± SD of triplicate measurements and values with different letters are significantly different at $p< 0.01$

Studying oxidation process on 60th day

According to Tables 3 and 4, significant differences were observed between treatments of cinnamon essential oil and echinacea extract, and also among cinnamon essential oil, echinacea extract, synthetic antioxidant, and the control ($p< 0.01$). In cinnamon-treated samples, antioxidant properties of CVEO-0.05 was higher than that in BHA-0.01, as its TBA value was significantly lower than BHA, and also its peroxide value was not significantly different from BHA-0.01. Moreover, CVEO-0.1 showed higher antioxidant effect compared with BHA-0.02 and its peroxide value was significantly lower than that in BHA-0.02. With increasing the concentration of essential

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oil from 0.01% to 0.15%, antioxidant effect decreased significantly, and its antioxidant effect was lower than BHA-0.01 (TBA and PV were lower than BHA-0.01).

TBA value in EPE-0.5 and EPE-0.75 were significantly different ($p< 0.01$), and were less than the value obtained in BHA-0.02. Also, their peroxide values were higher than BHA-0.02 and BHA-0.01, respectively. Thus, it is concluded that in the aforementioned concentrations, their antioxidant effect was higher than the synthetic antioxidant. As previously mentioned, the antioxidant property of echinacea is attributed to phenolic compounds, especially chicoric acid [32]. According to Tables 3 and 4, with increasing concentrations of echinacea extract from 0.25% to 0.5%, antioxidant effect decreased (both TBA and PV increased). This was more obvious in EPE-0.75. So, in this sample, TBA value increased significantly, and although its amount was less than that obtained for the control, it was higher than the values obtained for BHA-0.01. This decrease is probably caused by the increasing active compounds such as phenolic compounds existing in the extract and the pro-oxidative effect of the extract [33].

Acidity

The foodstuffs containing lipids have slight and definite amounts of free fatty acids, but the amount may increase because of spoilage and lipid hydrolysis. Lipid hydrolysis is caused not only by the food enzymes but also by microorganism enzymes. Therefore, measuring acidity is considered as an indicator of microbial and chemical spoilage and its level is dependent on the freshness and lipid hydrolysis in sample. In Figure 3, the acidity level did not significant changed during storage. This is probably due to the low activity of microbial and chemical lipase. Relatively low moisture of Kolompe samples may also delay the hydrolysis of triglycerides [23].

pH amounts

The pH amounts of various treatments were not significantly different during the sixty-day maintenance. Although pH level depends on the acidity level, increased acidity in samples did not significantly affect the pH amounts.

Assessing sensorial properties

Kolompe samples were assessed by 20 panelist and results are presented in Table 5. It is necessary to state that the levels mentioned were chosen as acceptable concentrations by panelists, and in this stage, sensorial measurements were performed just to compare cinnamon essential oil and echinacea extract levels with synthetic antioxidant and control samples. The properties tested included color, taste, texture, and overall acceptability.

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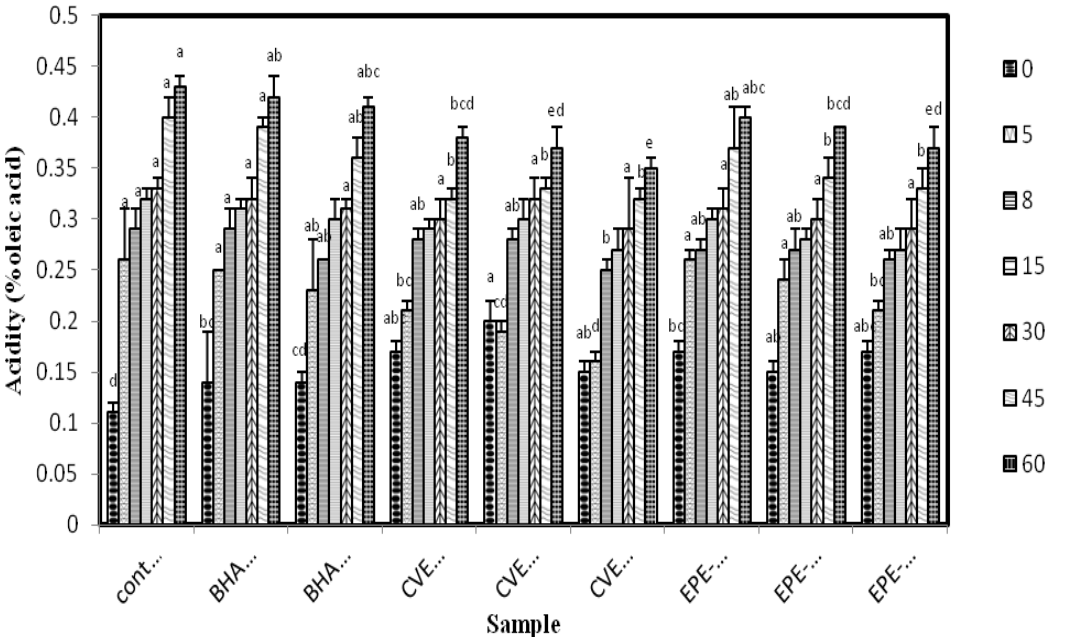


Figure 3- Effect of keeping period on acidity of samples

Table 5- Scores of sensorial analyzing [a]

Sample	Color	Taste	Texture	Overall acceptability
Control	3.40±1.27 [bc]	3.83±1.05 [ab]	3.20±1.00 [a]	3.40±1.04 [a]
BHA-0.01	3.55±1.09 [abc]	3.80±1.05 [ab]	3.50±1.31 [ab]	3.60±1.03 [ab]
BHA-0.02	3.95±1.09 ab	3.95±1.05 [ab]	4.05±0.99 [a]	3.65±1.18 [ab]
CVEO-0.05	3.65±0.67 [abc]	4.15±1.05 [a]	3.60±1.18 [ab]	3.65±0.87 [ab]
CVEO-0.1	4.05±0.82 [a]	3.45±1.14 [ab]	3.65±1.13 [ab]	3.90±0.78 [ab]
CVEO-0.15	3.90±0.96 [ab]	2.55±1.05 [c]	3.55±1.14 [ab]	3.35±0.93 [b]
EPE-0.25	3.65±0.87 [abc]	3.85±0.98 [ab]	3.50±1.00 [ab]	3.45±1.14 [ab]
EPE-0.5	4.10±0.91 [a]	4.25±0.78 [a]	3.55±1.23 [ab]	4.05±0.82 [a]
EPE-0.75	3.10±1.20 [c]	3.55±1.31 [ab]	3.20±0.76 [b]	3.40±0.94 [b]

[a]Data expressed are means ± SD of triplicate measurements and values with different letters are significantly different at $p< 0.01$

With regard to color, the highest score was given to CVEO-0.1 and EPE-0.5. Control, BHA-0.01, BHA-0.02, CVEO-0.05, CVEO-0.15, and EPE-0.25 were not significantly different. The lowest color score was given to EPE-0.75, which is probably because of color change of the sample into green in this step. Other samples were not significantly different.

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CVEO-0.15 obtained the lowest score for taste. Although the highest score was given to CVEO-0.05 and EPE-0.5, they were not significantly different from other samples (except CVEO-0.15). The scores given to texture of different samples were almost the same and they were not significantly different with each other. The highest scores of overall acceptability were given to EPE-0.5. Although the lowest scores were given to control, EPE-0.75, and CVEO-0.15, their scores were not significantly lower than the scores obtained by other samples. Therefore, it can be concluded that the best sample was EPE-0.5 from the assessors' point of view. As the amount of echinacea extract increased to 0.75%, the acceptability of Kolompe decreased, such that this sample, control, and CVEO-0.15 got the lowest acceptability scores. The scores of other samples were statistically similar.

Discussion

For antioxidant and antibacterial effects of cinnamon essential oil and echinacea extract, it can be concluded that the most activity belonged to EPE-0.25 and CVEO-0.1 which were stronger than BHA-0.02. With increasing amount of essential oil and extract, antioxidant activity decreased because of pro-oxidant effect. The antimicrobial activity of EPE-0.5, EPE-0.75 and all of the three concentrations of cinnamon (CVEO-0.05, CVEO-0.1 and CVEO-0.15) showed very well preventing power on aerobic microorganism, yeast and molds. Strong antimicrobial effect in cinnamon essential oil results from the cinnamaldehyde compound. The amount of this compound in essential oil used in the study was 47.25% (the results are not presented). As mentioned earlier, this compound shows strong antimicrobial property against many microorganisms [42]. In addition, presence of phenolic compounds in echinacea extract caused antioxidant and antimicrobial effects. Organoleptic assay showed that EPE-0.5 had the highest and CVEO-0.15 and EPE-0.75 had the lowest acceptability. Antifungal properties of natural extracts may affect the type of substrate. In this respect, Guynot et al. reported that the essential oils of cinnamon, bay, clove, thyme, and lemon grass show good antifungal effect on common fungi causing spoilage of bakery products when they were used in wheat flour-based agar medium. Nevertheless, these essential oils had antifungal effects in sponge-cake just when water activity was kept in low limit [34]. Suhr and Nielsen stated that the antifungal effects of the essential oils were related to the application method. They observed that essential oils with small amounts of effective compounds showed their best antifungal effect when they were used as volatile agents, but essential oils with large phenolic compounds, such as eugenol in cinnamon, have the best effect when it was used directly in medium [35]. Although the antimicrobial properties of synthetic antioxidants has been proved [36], according to the results, their effects cannot be observed in the amounts used [37].

Our results showed good antioxidant and sensorial properties of *Echinacea purpurea* L.

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and *Cinnamomum verum* in Kolompe that were comparable with finding of Balestra et al. they used ginger powder in concentrations of 3, 4, 5, and 6% for bread preparation. Although the highest antioxidant activity was observed for the sample containing the highest dose, this bread had unpleasant sensorial and rheological properties. The breads with 3% ginger powder was better than the control bread not only for the antioxidant effect, but also for sensorial properties [38].

Hix et al. replaced natural compounds of ascorbate, α-tochopherol, mixture of ascorbate and α-tochopherol, sodium phytate, and ferulic acid with BHA in sugar-snap cookies and preserved them at 60ºC. Different experiments showed that among the compounds used, ferulic acid and sodium phytate are the best replacements for BHA, and have suitable antioxidant activity in cookies. Also, the cookies containing phytate were sensorial more acceptable than other samples containing BHA [39]. In another study, the antifungal and antioxidant effects of six plant extracts in butter cake were investigated [11]. The highest antioxidant activity belonged to turmeric and betel leaves. Lemongrass and clove also showed antioxidant property higher than BHA and BHT. Nevertheless, adding pepper leaves caused increased oxidation. The compound mentioned and betel leaves enhance fungal growing, but using turmeric, lemongrass, Asam gelugur, and clove delayed microbial growth in butter cake. In the research done by Reddy et al. the effect of adding plant extraction on keeping biscuits was studied [30]. Adding extractions of amla (*Emblica officinalis*), drumstick leave (*Moringa oleifera*), and raisins (*Vitis vinifera*) in 1%, 1%, and 2%, respectively, after six weeks caused peroxide activity of biscuit samples to be less than that of the control sample (containing 200 ppm BHA). These compounds did not also affect the organoleptic properties of the last product. Bhanger et al. studied peroxide value, iodine value, and free fatty acids of cookies containing methanol extract of rice bran, and found that this compound containing antioxidants, and improves the durability of cookies [23]. Using green tea in concentration of 1% reduced hydroperoxides in biscuits, but did not affect secondary metabolites of oxidation. After storage, the taste of these biscuits was better than those containing BHA and control [40]. Anuradha et al. demonstrated that adding 0.4% natural vanilla extract to biscuits shows stronger effects than 0.2% synthetic vanilla and BHA [41].

Conclusion

In studying antioxidant effects of cinnamon essential oil and echinacea extract, it was defined that these two compounds had the antioxidant effect in all levels. According to the results obtained in this study, we can use cinnamon essential oil and echinacea extract as replacements of synthetic antioxidant. Moreover, they can increase the shelf life of Kolompe, because of the antimicrobial properties and also improve the sensorial quality and product acceptability.

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