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Application of Some Antifungal and Antioxidant Compounds Extracted from Some Herbs to Be Used in Cakes as Biopreservatives

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ABSTRACT

It is highly desirable to employ biopreservatives of the antioxidant and antimicrobial properties to avoid the side effects associated with the use of synthetic preservatives. Cake batters containing essential oil extracts of some such Iranian native herbs as: Lemon balm (*Melissa officinalis*), Camel thorn (*Alhagi maurorum*) and Ajwain (*Trachyspermum copticum*), were prepared. The chemical compositions of the essential oil were determined through GC–MS experiments. The shelf lives of the cakes were estimated by TBA (ThioBarbituric Acid) along with mould count measurements at room temperature during a 6 week storage period. The results revealed that these essential oils were fully effective in retarding mould growth and fat rancidity in the cakes. It was therefore concluded that these essential oils are of the potential to be used in the food industry as promising biopreservatives.

Keywords: Ajwain, Antifungal, Antioxidant, Cake, Camel thorn, Lemon balm.

INTRODUCTION

Cakes as nutritious food products, composed of water, flour, egg and additives are used as accepted alternatives for a main food by individuals, particularly children, all over the world. Cake manufacturers face a major problem of lipid oxidation and mould growth which limits the shelf-life of their products (Gulc, 2003). Because water activity in these products is not high, mould growth on bakery products is a serious problem during storage, (Fustier *et al.,* 1998). The most common spoilage fungi in cakes and bakery products are commonly xerophilic fungi that include *Eurotium*, *Aspergillus* and *Penicillium* species (Suhr and Nielsen, 2004). Moulds present in the cake batter are destroyed during the baking process, however contamination arises again from mould spores derived either from atmosphere or from facility surface during the cooling step (Bennion, 1997). Antimicrobial properties of herbs and spices are recognized since ancient times and have been used in medicine and for food preservation (Oussalah *et al.,* 2006). However there are reports that fungi may show resistance to the natural antimicrobial compounds (Lopez-Malo *et al.,* 2002). Numerous studies have documented that this activity is related to the presence of some such compounds as phenols and phenolics in essential oils of herbs and spices (Aligiannis *et al.,* 2001; Elgayyar *et al.,* 2001). It is also known that the fats present in cakes are oxidized to lipid hydroperoxides, the process

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being termed as autoxidation. These unstable primary oxidation products are subsequently broken down through the free radical mechanisms, producing many such types of secondary products as ketones, alcohols, aldehydes and malonaldehydes which give off-flavors to the product (Kequan and Liangli, 2006). Autoxidation of fats and oils in the processed foods could be prevented by use of oxidation inhibitors known as antioxidants (Huang *et al.,* 2005). Four commercial synthetic antioxidants commonly used in food industries are reportedly toxic to human cells, with Propyl Gallate as the most toxic, followed by BHA (Butylated HydroxyAnisole), BHT (Butylated HydroxyToluene) and α-tocopherol. Dietary BHA is also carcinogenic, causing fatal hemorrhages in the pleural and peritoneal cavities and such organs as testes and pancreas of rats (Hsu and Yen, 2008). Because of the health problems cited, consumers much prefer to use food products that contain preservatives coming from natural source origins as opposed to synthetic additives. Some such essential oils as cinnamon leaf, clove, bay, lemongrass and thyme essential oils have exhibited potential antifungal activities against the common fungi that cause the spoilage of bakery products (Guynot and Ramos, 2003).

The aim followed in this study was therefore to evaluate the antifungal and antioxidant effects of three essential oils extracted from Iranian native herbs namely: Lemon balm, Camel thorn and Ajwain. A comparison with some of the most known synthetic preservatives is also made and presented.

MATERIALS AND METHODS

Chemicals

Ascorbic acid, Butylated HydroxyToluene (BHT), 1,1-diphenyl-2-picrylhydrazy(DPPH), ferrozine,Folin–Ciocalteu's reagent,gallic acid, iron(III)chloride, iron(II) chloride, TriChloroacetic Acid (TCA) were provided from Sigma Chemical Company (Germany). Dibasic potassium phosphate, 2-ThioBarbituric Acid (TBA), sodium carbonate and dibasic sodium phosphate were done so from Merck (Darmstadt, Germany)

Plant Materials

Leaves of Lemon balm, seeds of Camel thorn and Ajwain, in the dried form, were purchased from a local market in Isfahan, Iran.

Isolation of the Essential Oils

Two-g samples of air-dried and finely ground materials were individually subjected to water distillation for 6 h using a Clevenger apparatus. The extracted essential oils were next dried over anhydrous sodium sulfate and, following filtration, stored in the fridge until further subsequent experiments. The percentage yields (%) of the oils, calculated on a moisture-free basis, are given in Table 1.

GC–MS Analysis

Analysis of the essential oils was performed using a Varian GC-3800 MS-Saturn 2200, equipped with a capillary column (30 m-0.25 mm id, 0.25 µm) and an HP 5972 mass selective detector. For GC–MS detection, an electron ionization system with ionization energy of 70eV was employed. Helium was used as the carrier gas at a flow rate of 0.1 m min^{-1}. Injector

Table 1. Essential oils yield.

Essential oil	Essential oil yield (%)
Ajwain	4.5
Camel thorn	4.9
Lemon balm	3.7

and MS transfer line temperatures were set at 220 and 290°C, respectively. Column temperature was initially set at 80°C for 3 minutes, then gradually increased to 180°C at a 3 °C min^{-1} rate, held for 10 minutes at this temperature and finally raised to 280°C at the rate of 10 °C min^{-1}. Diluted samples (1/100 in acetone, v/v) of 1.0 µL were manually injected into the GC-MS apparatus in a splitless mode. The components were identified based on a comparison of their relative retention times and mass spectra *vs.* those of the standards, NIST library data of the GC–MS system.

Being of the Cake

Each samples of cake batter was made in a stainless steel vessel by sugar (500 g), eggs (250 g), with 0.5 g of each essential oil or BHT or potassium sorbate being blended for 3 minutes at a medium speed. Sunflower oil devoid of any antioxidant (260 g), invert sugar (55 g), glucose syrup (33 g), glycerin (2 g), emulsifier (mono and diglycerides) (18 g), water (555 ml) and whole dried milk (22 g) were then added to the mixture in the vessel and mixing continued at high speed for another 3 min. Sifted flour (1,000 g) together with baking powder (18 g) were then gently folded in at low speed for 1 minute. The batter was baked at 165°C with 65% bottom heat for 50 minutes. The cakes were then cooled, packed in polypropylene films and stored at room temperature (27±3°C) for 6 weeks (Bennion, 1997). The storage of cakes was done in two trials, with triplicate samples for each trial. The cakes were analyzed at day 1 and then weekly up to 6 weeks for their ThioBarbituric Acid values (TBAV) and for mould enumeration.

Antifungal Activity Test

Cell counts were performed by plating of serial dilutions of the cake homogenates after 1, 2, and 3 to 6 weeks of storage at room temperature. A 10 g sample of each cake was homogenized into a flask containing 90 ml sterilized saline, giving dilutions of 1:10 or 1:100, 1:1000 or 1:10,000, by need. Each homogenate dilution (1 ml) was inoculated on the plates containing Potato Dextrose Agar (PDA) for mould count. The plates were then incubated at 25°C for 5 days. The number of colonies were finally counted and evaluated in relation with the weight of the cake in test, and then expressed as percent. Moulds were counted in 3 replicates, using three different dilutions of each sample (Doulia *at al.,* 2006).

Antioxidant Activity Test

TBA Method

TBA distillation method was performed to evaluate the level of oxidation as described by Ke *et al.* (1984). Cake samples (10 g) were ground and blended with 90 ml of distilled water plus 2.5 ml of HCl (2:1 in water) in a test tube. A 5 ml mixture of Propyl Gallate and EDTA (1:1 v/v) was then added to each of the tubes. The whole mixtures were transferred into Kjeldahl flasks with a few saddle stones added to each flask to prevent bumping. The Kjeldahl flasks were heated for 10 minutes at the highest heat level obtainable on the Kjeldahl distillation apparatus. When boiling began, a 50 ml distillate was collected from each of the mixtures. The distillates were then gently mixed with 5 ml of each transferred into separate test tubes each containing 5 ml of the TBA reagent. The tubes were immersed into boiling water bath for 35 minutes and then cooled down within 10 minutes to ambient temperature using tap water. A blank distilled water-TBA reagent sample was used as control. An aliquot of each sample was poured into a cuvette and the optical densities assessed through in a UV spectrophotometer (CAM spec m350 UV vis spectrophotometer, England) at a

wavelength of 532 nm. The absorbance was multiplied by the factor 7.8 to calculate the quantity in mg of malonaldehyde per kg of the cake.

Sensory Analysis

A 20-member consumer panel was recruited to act as based on the liking of the cakes and frequency of consumption. The panelists were asked to rate how much they liked the taste of the randomly presented four-digit coded cake samples soon after production, containing Ajwain, Lemon balm, Camel thorn *vs.* control while using a 9-point hedonic scale (1= Dislike extremely and 9= Like extremely). This analysis was conducted at the Laboratory of Department of Agriculture, Food and Nutrition of Isfahan University where the judges, mostly students, teachers and other employees of the campus, who were aware of the experiment being conducted and by having signed the Free and Cleared Consent Form. Statistical analysis was then carried out on the obtained data from the hedonic scale. The samples had been presented along with water being used as mouth cleanser. Data were then analyzed using SAS (Statistic Analysis System, version 9), making use of Analysis of Variance (ANOVA), and applying the Tukey test of 99% confidence ($P< 0.01$) (O'Mahony, 1986).

Statistical Analysis

The tests were conducted under laboratory conditions. Treatments were carried out in triplicate. Statistical analyses were performed eploying SAS version 8.2 (SAS Institute, 1999–2001) to study the effect of some antifungal and antioxidant compounds extracted from herbs and used in cake baking. The analyses of variance and regressions were carried out using the minimum squares method. The data recorded at the laboratory test conditions were statistically analyzed, using two factor randomized design.

RESULTS AND DISCUSSION

The main compounds of essential oils of herbs identified through GC-MS are listed in Table 2. Lemon balm essential oil mainly contained 3-Caren (27.5%) and Thymol (22.16%). Camel thorn essential oil was mainly composed of Squalene (33.72%), Carbamic acid (14.82%) and Thymol (14.12%) while the major components of Ajwain oil were Thymol (60.55%), 1, 8 Cineole (16.33%) and 3-Caren (14.02%). No mould growth was observed in the cake samples up to two weeks past (Figure 1). However, mould growth on negative control and on Lemon balm containing samples was observed during the third week of the experiment. The antifungal activity, up to the sixth week of the experiment was checked because the number of moulds counted was unacceptable.

The Camel thorn containing cakes showed contamination starting from the fourth week while samples with Ajwain oil exhibited good antifungal activity until the beginning of the fifth week as also observed for BHT and Sorbate containing samples (Figure 1). Therefore, it could be concluded that Ajwain bears a maximum antifungal activity among the three essential oils and is able to retard mould growth in the cakes ($P< 0.05$) for a considerable period of time. The results of GC-MS analysis showed that the volatile oils of Ajwain contain ketones, thymol and monoterepene aldehyde (Table 2) which are proven to be responsible for antimicrobial properties against mould growth (Nilsen Rois, 2000). In addition, phenolic compounds present in the essential oils have reportedly antimicrobial effects (Kiran Babu *et al.,* 2007). Camel thorn was the second in inhibitory effect against moulds. As observed and reported by Elgayyar and Draughon (2001 that squalene and thymol are of the potential of antifungal activity, it could be concluded that these two

Table2. Chemical composition of the essential oils extracted from some three Iranian herbs.

				Composition (%)		
	R_t(Min) [a]	Compound [b]	*RI*[c]	Lemon balm	Camel thorn	Ajwain
1	4.234	α-Phellandrene	921	1.7	-	-
2	4.883	α-Pinene	939	0.8	tr [c]	-
3	5.089	Comphene	957	0.8	tr	1.2
4	5.829	Benzen1,2,3,5tetramethyl	970	1.3	-	tr
5	5.849	β-Pinene	982	8.1	8.4	2.8
6	6.116	3-Caren	987	27.5	tr	14.0
7	6.231	β-Myrecene	991	1.1	5.0	tr
8	6.456	1,8 cineole	993	1.1	-	16.3
9	6.815	α-Terpinene	1017	0.4	-	tr
10	6.958	carvacrol	1019	-	2.0	-
11	7.034	ρ-Cymene	1025	1.5	-	5.1
12	7.113	Limonene	1031	tr	-	tr
13	8.976	Linalool	1097	-	-	tr
14	10.374	Menthone	1150	-	tr	-
15	10.617	Isomenthone	1164	-	tr	-
16	12.335	Squalene	1231	-	33.7	
17	12.634	Carvone	1243	1.0	9.1	-
18	13.883	Thymol	1290	22.2	14.1	60.5
19	13.898	Fenchyl acetate	1293	tr	1.3	tr
20	14.382	Carbamic acid	1431	-	14.4	
21	16.085	β-Caryophyllene	1438	tr	0.7	-
22	20.062	Caryophllene oxide	1600	0.9	1.4	-

[a] Retention time (as minutes); [b] Commpound listed in order of elution from an HP 5972 MS column, [c] Trace= 0.1%. [c] Retention indices on HP5972 MS column in reference to n-alkanes.

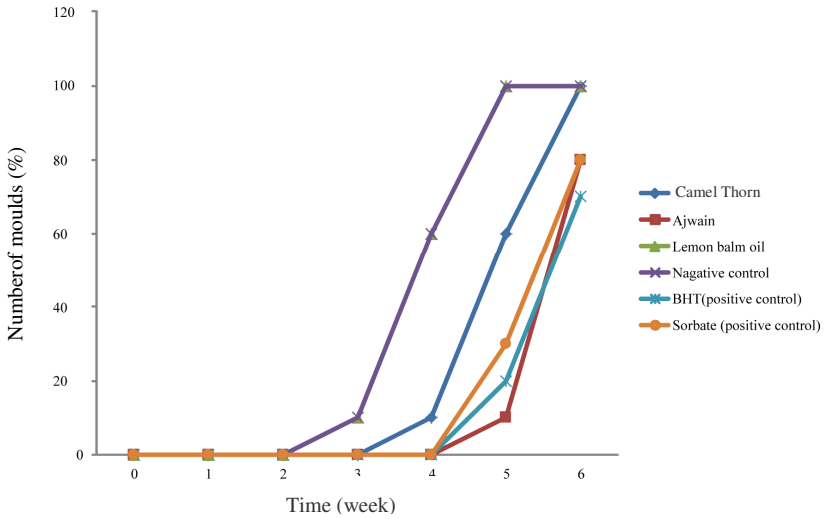

Figure 1. Mould growth on cakes during storage at room temperature. Values are means of three replications.

components present in Camel thorn extract are responsible for the observed inhibitory behavior.

The mean TBAV differences between the experimental and the control cakes were significant (P< 0.05) from the beginning of the experiment up to the end of storage period. According to the literature (Ke *et al.,* 1984), when the level of TBAV in the cakes is less than 0.576 mg Kg^{-1}, they are considered as not rancid, while within the range of 0.65-1.44 mg Kg^{-1}, the sample is even though regarded as rancid but still acceptable. For values greater than 1.5 mg Kg^{-1}, the samples are considered as be rancid and unacceptable. The results in Figure 2 indicate that rancidity was not developed in any sample within the first week. By the second week, the control and BHT containing cakes were rancid but still acceptable, while no change was observed in the samples containing the natural antioxidants. By the end of the fifth week, the cakes containing Lemon balm were turnedrancid although still in an acceptable range. After a passage of six weeks, all the cakes were found rancid except for those containing Ajwain and Camel thorn oils. These results clearly indicate that Ajwain and Camel thorn oils bear the most antioxidant activity and benefit from a much better performance than BHT. Caryophillene oxide present in the essential oils of Camel thorn is perhaps responsible for the strongly observed antioxidant activity. Furthermore, Camel thorn contains such phenolic compounds as Carvacrol that exhibit considerable antioxidant activity (Yanishlieva *et al.,* 1999). More importantly, Ajwain oil was significantly more acceptable than BHT and was the strongest antioxidant to retard lipid oxidation in the cakes within a 6 week period of storage, perhaps due to the presence of Thymol and β-Pinene antioxidants in its chemical composition as also suggested by Yanishlieva *et al*. (1999). The antioxidant activity of phenolics used in different systems has been indicated to be as effective as BHA or BHT (Balasundram and Sundram, 2006; Rababah and Hettlarachy, 2004).

The use of herb essential oils at concentrations required to be effective in cakes as a natural preservative could raise concerns regarding changes in the sensory properties. However, one should consider that the essential oil not only acts as a

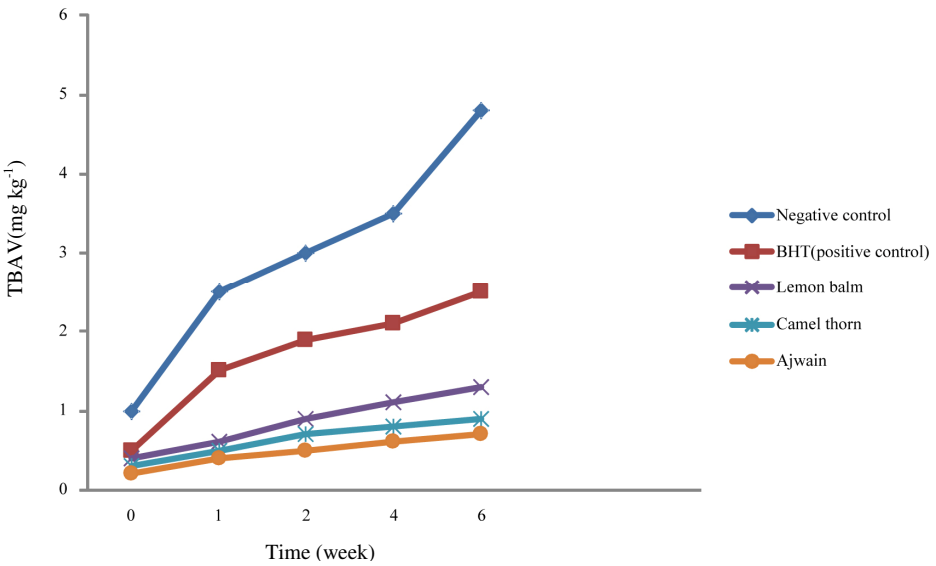

Figure 2. TBA (ThiobarBituric Acid) values of cakes stored at room temperature. Values are means of three replications.

Table 3. Mean of sensory scores of the cakes treated with essential oils

Treatment	Ajwain	Lemon balm	Camel thorn	BHT	LSD
Mean scores	2.30^{a*}	0.35^{d}	1.80^{b}	1.00^{c}	2.23
£f(x)	46	7	36	20	

*Values are means of three replications. Means followed by the same letter in the same row (a, b, c and d) are not significantly different (P> 0.01)

preservative but is also a flavor component. The results of sensory evaluation revealed that the panelists liked the taste of the cakes containing Ajwain essential oils more than they did the taste of other cakes as indicated by its attaining of a higher score (P< 0.01) (Table 3). There were no significant differences observed between the sample containing Ajwain essential oil *vs*. control (P< 0.05).

In conclusion Ajwain essential oil was the most efficient antifungal and antioxidant as regards cake baking in comparison with the other two essential oils and with the synthetic preservatives studied for a test duration of 6 weeks of storage. This was followed by Camel thorn and Lemon balm. Future works are include recommended to the identification and application of the most active components rather than the whole oil. This would further alleviate the organoleptic concerns, whilst retaining antimicrobial activity. On the other hand, the antimicrobial activity of any plant essential oil might be reduced due to its interaction with other ingredients, requiring the addition of the active substances in their greater concentrations. In such cases, further safety studies may also be necessary.

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کاربرد ترکیبات آنتی اکسیدانی و ضد قارچی استخراج شده از بعضی گیاهان دارویی در کیک به عنوان نگهدارنده

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چکیده

با توجه به اینکه اثرات سوء نگهدارنده های شیمیایی و سنتزی، برای مصرف کنندگان مشخص گردیده، در سال های اخیر مطالعات زیادی در زمینه یافتن نگهدارنده های طبیعی صورت گرفته است. در این تحقیق کیک با اسانس های روغنی خارشتر، زنیان و فرنجمشک تهیه شد. ترکیبات شیمیایی اسانس های روغنی به وسیله GC-MS تعیین شد. با استفاده از روش TBA (اسید تیو باربیتوریک) و شمارش کلنی کپک اثر نگهدارنده های طبیعی ذکر شده بر روی حفظ بهتر کیفیت و افزایش مدت ماندگاری کیک در طی دوره انبار داری بررسی گردید. نتایج این تحقیق نشان می دهد که اسانس های روغنی مورد آزمایش در به تاخیر انداختن رشد کپک و اکسیداسیون چربی موثر می باشند. بنابراین می توان از این نگهدارنده های در صنعت غذایی نیز استفاده کرد و اثر ضد قارچی و آنتی اکسیدانی آنها را ناشی از حضور ترکیبات فنولیک دانست.