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USE OF MEDICINAL AND AROMATIC PLANTS FOR INCREASING QUALITY OF SOME BAKERY PRODUCTS

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Abstract. The present study was performed to use medicinal herbs (anise, black cumin, rosemary and sage as natural antioxidants and antimicrobial to increase shelf–life of some bakery products. Essential oils and phenolic compounds of selected herbs were extracted and quantitative determined. Essential oils components and phenolic compounds of herbs were fractionated by Gas Chromatography Mass Spectrum Technique (GC–MS). Essential oils and phenolic compounds of herbs were added at different concentrations (200, 400, 800 and 1000ppm) and BHT (200ppm) to sunflower oil and determined stability by Rancimat method at 100°C±2°C. Also, were screened against four gram–negative bacteria (*Escherichia coli*, *Klebsiella Pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris*) and two gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) at four different concentrations using disc diffusion method. Essential oils and phenolic compounds of selected herbs and BHT were added to cake and determined peroxide value during storage and sensory evaluation. The results indicated that addition of essential oils and phenolic compounds of herbs prolonged the oxidative stability of sunflower oil and can be a good source of antibacterial agents.

Key words: Medicinal plants, essential oils, Phenolic compounds, antioxidant, antiomicrobial.

Introduction

Bakery products are widely consumed and are becoming a major component of the international food market [KOTSIANIS *et al*., 2002]. Cake is one of the most common bakery products consumed by people in the world. Now days, cake manufacture face a major problem of lipid oxidation which limits the shelf life of their products [LEAN & MOHAMED 1999]. Bakery products such as cakes particularly those with high lipid content tend to become rancid after prolonged storage owing to the oxidation of polyunsaturated fatty acids [SMITH *et al*., 2004, RAY & HUSAIN, 2002]. Foods containing higher content of polyunsaturated fatty acids are more prone to oxidation [AARDT *et al*., 2004]. One of the most important changes that occur to food is lipid oxidation. Lipid oxidation lowers the quality and nutritional value of food [SUJA *et al*., 2004].

The susceptibility of lipid to oxidation is one of the major cause oxidative stresses, resulting in the development of rancidity, unpleasant tastes and odours as well as changes in colours [PEZZUTO & PARK, 2002].

In the food industry, the rate of auto–oxidation is reduced by freezing refrigeration, packaging under inert gas in the absence of oxygen and vacuum packaging [DAKER *et al*., 2008].

In cases where these methods are neither economic nor practical from the nutritional and technological points of view, it is highly desirable to control oxidation by the addition of antioxidants, inhibitory substances that do not reduce food quality [GERMZA *et al*., 2006].

Antioxidants also have an important role in preventing a variety of lifestyle–related diseases and ageing because these, too, are closely related to active oxygen and lipid peroxidation [IZZREEN & NORIHAM, 2011]. However, the results of a great deal of research into the antioxidant properties of different substances have not identified any antioxidant that may be active in all food products.

Such a characteristic results from many factors, e.g stability at the time of processing, mixing ability and activity in different lipid systems and the need to fulfill legal requirements [VIUDA–MARTOS *et al*., 2010]. The most widely used synthetic antioxidants in food are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl galate (PG) and Tertiary butyl hydroxyquinone (TBHQ) [KANATT *et al*., 2007].

Although efficient in preventing oxidation, only a few synthetic compounds are currently approved for use in the food industry because of their toxicity and carcinogenic [BOTTERWECK *et al.*, 2000]. To satisfy consumer's preference for natural food additives over synthetic ones, there is increasing importance searching for natural antioxidants from herbs, fruits, vegetable and spices as a less harmful alternative to synthetic antioxidants [DAKER *et al.*, 2008].

Consequently, there has been a growing in interest in searching for and using natural antioxidants for three principal reasons [DASTMLCHI *et al.*, 2007].

a) Numerous clinical and epidemiological studies have demonstrated with reduced risks of developing chronic diseases such as cancer, cardiovascular disorders and diabetes;
b) Safety considerations regarding the potential harmful effects of the chronic consumption of synthetic antioxidants in food and beverages; and
c) the public's perception that natural and dietary antioxidants are safer than synthetic analogues.

The result has been an increased interest on spices, aromatic and medicinal plants as sources of natural antioxidants to replace synthetic antioxidants.

Many sources of antioxidants of a plant origin have been studied in recent years [BASUNY, 2004; BASUNY *et al.*, 200, 2006 & 2011].

Among these, many aromatic plants and spices have been shown to be effective in retarding the process of lipid peroxidation [SULTAN *et al.*, 2009].

One component present in aromatic plants and spices, and which may act as natural antioxidants, is the corresponding essential oil. In general terms, essential oils are composed of>70 components, principally polyphenols, terpenes, monoterpenes and seaquiterpenes [CRAIG, 1999] some of which may represent more than 85 % of the total content.

These oils have been shown to possess antibacterial, antifungal, antiviral in secticidal and antioxidant properties [PRABUSEENIVASUN *et al.*, 2006].

Also, these oils have been used in cancer treatment [SYLVESTRE *et al.*, 2006].

Now day's essential oils and other components such as phenolic compounds are gaining increasing attention because of their relatively safe status, their wide acceptance by consumers, and the possibility of their exportation for potential multi–purpose functional uses [KANTER *et al.*, 2009].

The aim of this study was to determine the essential oils and total phenolic content and the (antioxidant activity and antimicrobial agent) several medicinal herbs widely used in Saudi Arabia: Anise (*Pimpinella anisum*), Black cumin (*Nigella sativa*), Rosemary (*Rosmarinus officinalis*) and Sage (*Salvia officinalis*).

Also mixing these essential oils and phenolic of herbs at various concentrations in some bakery products such as cake and compared with BHT and control treatment without herbs.

Material and methods

– Source of herbs: Anise (*Pimpinella anisum* L.), Black cumin (*Nigella sativa* L.), Rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis L.)* were obtained from local market in Al–Hasa region, Saudi Arabia.

– *Source of sunflower oil:* Refined sunflower oil was obtained from Arma Company (AD–Damam City, Saudi Arabia). Peroxide, acid values of fresh sunflower oil were 0.40meq. Peroxides kg^{-1} and 0.10 mg $KOHg^{-1}$, respectively.

– *Solvent and standard reagents*: All solvents used throughout the whole work were of analytical grade. BHT was purchased from Sigma (St. Louis. MO. USA). Folin–Ciocultreau reagent was obtained from Gerbsaur Chemical Co. (German). Gallic acid (98%) was purchased from Aldrich Chemical Co. (ltd., England).

– *Essential oils*: The essential oils of anise, black cumin, rosemary and sage were extracted by steam distillation according to the method of Kanter [KANTER *et al.*, 2003].

– *Total phenol content*: The total phenol content was determined using Folin–Ciocalteu's [SINGLETON *et al.*, 1999]. Values were expressed as gallic acid equivalent.

– *Identification of essential oils and phenolic components by GC–MS*: Essential oil and phenolic components for selected herbs were identified by using the Gas Chromatography–Mass Spectrum Technique (*Table 1*). The phenolic components and essential oil constituent's percentages were estimated from the measured peak area of the chromatogram according to Prudent [PRUDENT *et al.*, 2002].

Table 1.
Condition of GC–MS

Information	Condition
Instrument	GC5890 Mass spectrophotometer 5989, Hewlett Pachared (HP)
Column	Hp15(30µmx0.25mmi.d.,0.25µm film thickness,
Stationary phase	Polyphenyle methyl silioxane,
Flow rate	0.6 ml Helium min^{-1},
Column temp.	50– 200°C,
Rate temp.	6°C min^{-1},
Injection temp.	200°C,
Detector temp.	220°C,
Recorder	HP

Antioxidant activity. Different concentration of anise, black cumin, rosemary and sage phenolic compounds and essential oils (200, 400, 800 and 1000 ppm) as well as synthetic antioxidant (BHT, 200 ppm) were individually added to sunflower oil to study their antioxidant behavior.

The designation of an induction period, measured by using a Rancimat method 679 9 Metrohom, Switzerland) at 100°C±2°C [MENDEZ *et al.*, 1996].

The antioxidant activity index (AAI) was calculated from the measured induction period (IP) according to the following formula by Foester [FOESTER *et al.*, 2001]. AAI=Induction period of lard with antioxidant/Induction period of pure sunflower

Antimicrobial screening. The antimicrobial screening as described by Burt [BURT *et al.*, 2004]. Microorganisms used were obtained from stock collection of Microbiology Department Faculty of Agriculture Cairo University. Subculture on the nutrient agar slants: four gram–negative bacteria (*Esherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa and Proteus Vulgaris*) and two gram–positive bacteria (*Basillus subtilis and Staphylococcus aureus*). A solution of each essential oils and phenolic compounds (100µl/ disc) was used.

The plates were incubated at 37°C and 25°C for bacteria and fungi respectively, using Rifampicin, Amoxycillin+ Flucloxacillin and Ciprofloxacin (1 mg/ml for each, 100µl /disc) as a control.

The antimicrobial activity was recorded by measured the diameter of inhibition zone after 12–18 and 24–48 hrs. for bacteria. Three replicates were carried out and the average was determined.

Production of cake. The cakes were formulated into ten formations i.e. without additive (control), BHT (200ppm), 200, 400, 800 and 1000 ppm essential oils and phenolic compounds of anise, black cumin, rosemary and sage.

The margarine without antioxidant and sugar were beaten in a mixing bowl on medium speed until light and fluffy (Creamy) for 12 min.

The whole eggs (white and yolk) were added one by one. Addition of eggs had to be careful ensuring that each addition is completely incorporated. The batter was beaten until it is smooth for 5 min.

After that, the antioxidant (natural or synthetic) was added into the batter for 1 min at low speed. With the mixer at low speed, sifted cake flour were fold in carefully without over mixing into the batter for 2 min.

All of these mixtures were put in the pan and backed in an oven at an approximately temperature of 160°C for 60 min. Once backed, the cakes were allowed to cool for 30 min. and then stored in plastic containers at ambient temperature about 15 days for further analysis.

Peroxide value of cake samples.

The peroxide values were determined in triplicate samples by the extraction method of Mielnik [MIELNIK *et al.*, 2006]. Results expressed as meq. /kg oil.

Sensory evaluation of cakes. Cakes produced (different concentrations of essential oils and phenolic compounds of medicinal herbs) were evaluated for their sensory characteristics by ten panelists from the staff of Food Science and Nutrition Dep., Faculty of Agriculture Science and Foods, King Fiasal Univ., Al–Hasa, Saudi Arabia.

The scoring scheme was established as mentioned by Thanaa, (2000) as follows: color of crust, taste, appearance, crunchiness and over acceptability.

Statistical analysis. Convention statistical methods were used to calculate and standard deviations of three simultaneous assays carried out with the different methods.
Analysis of variance (ANOVA) was applied

to the data to determine differences (P ≥ 0.05).

Results and dicussion

Essential oil content. The essential oil content of the anise, black cumin, rosemary and sage herbs are presented in *Table 2*. In the anise a high content of essential oil (2.67%) was obtained.

Prabuseenivasan [PRABUSEENIVASAN *et al.*, 2006] also demonstrated that anise herb have a high essential oil. Rosemary and sage were seen to be a less rich source of essential oils (1.80 and 1.90%), respectively, while black cumin showed the lowest amount of essential oil (0.75%) respectively. Essential oils content could be used important indicator of the antioxidant and antimicrobial properties [BURT, 2004 & KORDALI *et al.*, 2005].

Table 2.

Essential oils content in the studied medicinal herbs

Medicinal herbs	Essential oil content (%)
Anise	2.67±0.20
Black cumin	0.75±0.01
Rosemary	1.80±0.11
Sage	1.92±0.13
Values expressed are means ± standard deviations.	

Phenolic content.

Different phytochemicals have e and various protective and therapeutic effects which are essential to prevent diseases and maintain a state of wellbeing. Anise, black cumin, rosemary and sage were analyzed for their phyto–constituents.

The quantitative estimation of the phytochemical constituents of anise, black, rosemary and sage show that the medicinal herb are rich in phenolic contents according to the data shown in *Table 3*.

In the anise herb high content of total phenols (890.90 μg/gm). Black cumin and rosemary herbs were seen to be a less rich source of total phenols (730.50 and 622.30μg/gm), respectively, while sage showed the lowest amount of total phenolic (460.20 μg/gm).

Many authors [BOTTERWECK *et al.*, 2000 & AARDT *et al.*, 2004] have described the potential antioxidant properties of polyphenols. These compounds act as antioxidants by donation of a hydrogen atom, as an acceptor of free radically, by interrupting chain oxidation reactions or by chelating metals.

Table 3.

Total phenolic compounds in the studied medicinal herbs.

Medicinal herbs	Total phenolic (μg/gm)
Anise	890.90±10.22
Black cumin	730.50±8.50
Rosemary	622.30±7.30
Sage	460.20±4.36
Values expressed are means ± standard deviations.	

Chemical composition of the essential oils. Gas Chromatography–Mass Spectrum Technique was used to determine the chemical composition of four selected medicinal herbs essential oils are listed in *Table 4*.

Table 4.

Chemical components of the studied essential oils of medicinal herbs.

Essential oil components (mg/mg)	Essential oils			
	Anise	Black cumin	Rosemary	Sage
P–Cymene	2.20±0.20	38.25±1.00	0.00±0.00	4.10±1.00
A–Pinene	0.46±0.01	0.10±0.001	36.40±2.71	1.60±0.20
1,8– Cineole	10.11±2.0	0.95±0.10	12.00±1.90	24.70±2.51
Linalool	41.20±4.50	1.60±0.01	2.33±0.20	0.00±0.00
O– Ethylphenel	2.01±0.01	0.00±0.00	0.10±0.001	1.22±0.01
Camphor	0.62±0.1	0.00±0.00	15.60±2.00	2.50±0.19
Isopinocamphone	0.00±0.00	0.22±0.01	0.85±0.10	0.00±0.00
P–Pinene	0.00±0.00	0.00±0.00	0.15±0.001	1.60±0.09
Borneol	1.50±0.10	1.03±0.001	0.00±0.00	0.00±0.00
4–Tepineol	0.88±0.10	0.24±0.001	0.00±0.00	0.00±0.00
A–Terpineal	0.61±0.01	0.00±0.00	0.71±0.001	0.34±0.001
Bornyl acetate	0.00±0.00	0.06±0.001	0.65±0.001	0.00±0.00
Verbenone	0.00±0.00	0.00±0.00	0.41±0.001	0.00±0.00
Camphone	2.01±0.15	0.45±0.01	0.00±0.00	7.60±1.90
Thymoquinone	2.20±0.15	38.25±3.09	0.00±0.00	4.20±1.01
Carvacrol	1.95±0.20	10.38±3.90	1.22±0.01	0.19±0.001
Eugenol	10.90±4.02	2.40±0.81	1.05±0.01	3.50±0.90
Values expressed are means ± standard deviations.				

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In the essential oil of anise, the major constituents were Linalool (41.20%), Eugenol (10.90%) and 1.8 Cineole (10.11%). In black cumin essential oil the major constituents were Thymoquinone (38.25%), P–Cymene (32.02%) and Carvacrol (10.38%). In the essential oil of rosemary the major constituents were α–Pinene (36.40%), Camphor (15.60%) and 1,8–Cineole (12.00%). In sage essential oil the main components were Camphor (25.00%), 1.8–Cineole (24.70%) and Camphene (7.6%).

Chemical composition of the phenolic. Also, Gas Chromatography–Mass Spectrum Technique (GC–MS) was used to identify and determine the components of the phenolic medicinal herbs.

Data in *Table 5* showed that, there were 20 components identified in the phenolic medicinal herbs (anise, black cumin, rosemary and sage).

The major components of anise phenolic were Cinnamate (47.48%) Camphor (16.48%) and Phenyl Propenoic acid (7.92%). Black cumin phenolic the main components were Thymol (39.04%), α–Thyjene (33.50%) and β–Pinene (18.09%).

The principle components of rosemary phenolic were 1,8–Cineole (22.03%), Camphor (16.31%), Borneol (15.50%) and Verbenone (12.06%).

The main components of sage phenolic were α–Thyjene (43.82%), Camphor (18.26%) and α–Terpineol (14.07%).

Table 5.

Chemical components of the studied phenolic compounds of medicinal herbs.

Phenolic compounds (µg/mg)	Phenolic compounds			
	Anise	Black cumin	Rosemary	Sage
Cinnamate	48.48±4.01	1.20±0.10	3.01±0.91	0.00±0.00
Camphor	16.48±2.00	0.00±0.00	16.31±1.98	18.26±2.31
Phenyl Propenoic acid	7.92±1.90	0.00±0.00	4.02±1.02	2.11±0.29
Thymol	0.00±0.00	39.04±3.90	1.22±0.15	0.02±0.001
A–Thyjene	0.00±0.00	33.50±3.01	0.00±0.00	43.82±3.81
B–Pinene	0.06±0.001	18.09±1.85	0.00±0.00	0.00±0.00
1.8–Cineole	0.00±0.00	0.00±0.00	22.03±2.90	0.71±0.15
Broneol	0.92±0.00	4.10±1.00	15.50±1.80	3.50±0.85
Verbenone	0.31±0.10	2.02±0.41	12.06±1.33	0.00±0.00
A–Terpineol	1.09±0.20	0.00±0.00	0.07±0.0001	14.07±1.72
Methyl Cinnamate	0.00±0.00	1.50±0.15	0.00±0.00	0.00±0.00
Gallic acid	2.01±0.35	2.25±0.45	0.00±0.00	0.17±0.01
Elagic acid	3.22±0.90	0.00±0.00	0.13±0.001	2.11±0.30
B–Ocimene	0.00±0.00	0.63±0.10	0.24±0.01	0.00±0.00
A–Terpeneol	0.00±0.00	0.00±0.00	1.21±0.10	0.16±0.10

Values expressed are means ± standard deviations.

Antioxidant activity of the essential oils and phenolic compounds medicinal herbs. The antioxidant activities of herbs have been widely demonstrated, although the mechanism of such activity in not fully understood. Several explanations have been provided, among them the following: the sequestration of free radicals; hydrogen donation; metatic ion chelation; or even acting as substrate for radicals such as superoxide or hydroxyl [VIUDA–MARTOS *et al.*, 2010].

The Rancimat test is a very easy and inexpensive method, which requires small sample volumes and achieves reproducible results.

Although this technique has been questioned, it is commonly used in the food industry and governmental analytical laboratories. *Tables 6* & *7* give the related antioxidant activity of sunflower oil with different concentrations of selected herbs essential oils (200, 400, 800 and 1000ppm) and phenolic compounds (200, 400, 800 and 1000ppm) compared with BHT at 200ppm.

The results illustrated that all the essential oils and phenolic compounds were extracted from medicinal herbs and added at various concentrations to sunflower oil, exhibited antioxidant activity.

However, statistical analysis showed that the essential oils extracted from medicinal herbs had low significant antioxidant effect on sunflower oil stability, compared with phenolic compounds in all herbs (anise, black cumin, rosemary and sage).

Table 6.

Antioxidant activity of essential oils of medicinal herbs by Rancimat methods (hrs)

System	Induction period (hrs)
Sunflower oil (control)	7.30±1.00
Control+BHT (200ppm)	10.50±1.50
Essential oil of anise	
Control+200ppm	10.00±1.26
Control+400ppm	11.30±1.71
Control+800ppm	13.90±2.00
Control+1000ppm	17.20±3.04
Essential oil of black cumin	
Control+200ppm	10.02±1.15
Control+400ppm	11.11±1.60
Control+800ppm	13.50±1.60
Control+1000ppm	17.30±3.13
Essential oil of rosemary	
Control+200ppm	10.60±1.50
Control+400ppm	11.90±1.85
Control+800ppm	15.60±2.05
Control+1000ppm	19.22±3.81
Essential oil of sage	
Control+200ppm	10.00±1.20
Control +400ppm	11.00±1.61
Control+800ppm	13.20±1.86
Control+1000ppm	17.33±3.00
Values expressed are means ± standard deviations.	

Table 7.

Antioxidant activity of phenolic compounds of medicinal herbs by Ramncimat method (hrs)

System	Induction period (h)
Sunflower oil (control)	7.30±1.00
Control+BHT (200ppm)	10.50±1.50
Phenolic compounds of anise	
Control+200ppm	10.60±1.50
Control +400ppm	11.50±1.90
Conrtrol + 800ppm	15.22±2.01
Control + 1000ppm	17.90±2.30
Phenolic compounds of black cumin	
Control + 200ppm	10.70±1.56
Control +400ppm	11.80±1.95
Conrtrol + 800ppm	14.85±1.95
Control + 1000ppm	18.50±2.55
Phenolic compounds of rosemary	
Control + 200ppm	10.77±1.34
Control +400ppm	12.30±2.00
Conrtrol + 800ppm	16.33±2.33
Control + 1000ppm	19.3.00±3.00
Phenolic compounds of sage	
Control + 200ppm	10.50±1.09
Control +400ppm	11.80±1.30
Conrtrol + 800ppm	14.33±1.85
Control + 1000ppm	18.39±2.34
Values expressed are means ± standard deviations.	

The induction periods for all systems were greater than that of the induction period of sunflower oil alone, Generally, the levels of 200 and 400ppm for essential oil and phenolic compounds from all herbs induced antioxidants activity similar to synthetic antioxidant (BHT, 200ppm), respectively. It is worth to mention that the best results in this study were obtained from rosemary (phenolic compounds and essential oils) in retarding sunflower oil oxidative rancidity. Similar results were obtained by [VIUDA–MARTOS *et al.*, 2010] on clove, thyme, rosemary and sage.

Also, Eid, (2006) reported that, the addition of thyme essential oil at 100, 250, 500 and 750ppm to sunflower oil increased its induction period compared with control sample (without antioxidants).

Antimicrobial activity of the essential oils and phenolic compounds of medicinal herbs. The antimicrobial activity of four selected essential oils and phenolic compounds of (anise, black cumin, rosemary and sage) herbs against six bacterial species are summarized in *Tables 8* and *9*.

The results revealed that the selected essential oils and phenolic compounds of medicinal herbs showed antimicrobial activity with varying magnitudes.

The zone of inhibition above 7mm in diameter was taken as positive result.

Generally, most of the tested organisms were sensitive to many of the essential oils and phenolic compounds of all herbs.

Table 8.

Antimicrobial activity of essential oils of medicinal herbs

Treatments	Gram–negative				Gram–positive	
	Escherichia coli	*Klebsiella Pneumonia*	*Pseudomonas Aeruginosa*	*Protrus Vulgaris*	*Bacillus Subtilis*	*Staphylococcus Aureus*
Rifampicin	12.50±1.50	11.90±1,21	10.11±1.03	9.20±1.00	10.00±1.00	11.30±1,51
Amoxicillin+ Flucloxacillin	13.90±2.00	13.80±1.98	12.80±1.60	10.55±1.20	11.22±1.20	12.50±1.88
Ciprafloxacin	14.01±2.02	13.55±1.56	14.10±2.09	11.40±1.16	13.10±1.90	14.21±2.13
Essential oil of anise						
200ppm	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
400ppm	13.80±1.95	0.00±0.00	0.00±0.00	9.41±0.90	0.00±0.00	0.00±0.00
800ppm	16.70±2.50	12.05±1.41	0.00±0.00	10.22±1.00	9.00±0.81	0.00±0.00
1000ppm	18.80±3.41	14.03±1.81	0.00±0.00	12.50±1.45	11.50±1.25	8.22±0.70
Essential oil of black cumin						
200ppm	0.00±0.00	8.02±0.85	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
400ppm	0.00±0.00	10.30±1.00	8.09±0.77	0.00±0.00	8.50±0.79	0.00±0.00
800ppm	16.80±2.40	12.50±1.31	10.12±0.99	10.01±1.00	9.33±0.85	8.04±0.78
1000ppm	18.50±3.30	14.10±2.09	11.50±1.16	12.05±1.40	10.90±1.33	9.50±1.00
Essential oil of rosemary						
200ppm	11.50±1.33	0.00±0.00	0.00±0.00	0.00±0.00	9.50±0.85	9.00±0.95
400ppm	14.50±2.00	10.50±0.98	0.00±0.00	10.55±1.00	10.11±1.00	10.11±1.01
800ppm	18.70±3.11	12.30±1.43	10.08±0.96	12.33±1.81	12.11±1.90	13.50±1.98
1000ppm	20.80±4.02	16.50±2.35	13.05±1.34	14.01±2.00	16.03±2.70	16.90±2.33
Essential oil of sage						
200ppm	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
400ppm	0.00±0.00	0.00±0.00	9.12±0.90	0.00±0.00	0.00±0.00	0.00±0.00
800ppm	12.70±1.20	0.00±0.00	11.05±1.01	10.00±1.00	0.00±0.00	8.50±0.70
1000ppm	14.01±2.00	11.03±1.15	13.12±1.51	12.50±1.56	9.02±0.80	10.50±1.23

Values expressed are means ± standard deviations.

Table 9.

Antimicrobial activity of phenolic compounds of medicinal herbs

Treatments	Gram–negative				Gram–positive	
	Escherichia coli	*Klebsiella Pneumonia*	*Pseudomonas Aeruginosa*	*Protrus Vulgaris*	*Bacillus Subtilis*	*Staphylococcus Aureus*
Rifampicin	12.50±1.92	11.90±1.43	10.11±1.01	9.20±0.82	10.00±0.96	11.30±1.61
Amoxicillin+ Flucloxacillin	13.90±2.03	13,80±1.90	12.80±1.78	10.55±1.31	11.22±1.46	12.50±1.81
Ciprafloxacin	13.01±2.15	13.55±1.75	14.10±1.90	11.40±1.63	13.10±1.91	14.21±2.00
Essential oil of anise						
200ppm	9.50±0.91	0.00±0.00	8.30±0.75	0.00±0.00	0.00±0.00	8.33±0.85
400ppm	10.20±1.00	0.00±0.00	9.44±0.89	0.00±0.00	9.62±0.94	9.90±0.91
800ppm	11.70±1.45	9.00±0.81	12.30±1.89	0.00±0.00	10.81±1.35	10.81±1.34
1000ppm	14.01±2.50	10.22±1.02	13.50±2.15	9.33±0.80	12.06±1.67	12.90±1.89
Essential oil of black cumin						
200ppm	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	7.90±0.66	0.00±0.00
400ppm	0.00±0.00	0.00±0.00	8.50±0.70	0.00±0.00	9.50±0.89	0.00±0.00
800ppm	8.90±0.80	0.00±0.00	10.22±1.15	9.11±0.89	11.41±1.76	12.50±1.78
1000ppm	10.50±1.09	8.50±0.78	12.40±1.89	11.22±1.67	14.00±2.00	15.60±2.41
Essential oil of rosemary						
200ppm	9.30±0.91	0.00±0.00	9.00±0.90	0.00±0.00	9.50±0.90	8.41±0.81
400ppm	11.00±1.20	8.30±0.75	11.31±1.33	10.71±1.33	10.13±1.20	10.22±1.15
800ppm	12.04±1.44	9.71±0.83	13.22±2.25	12.50±1.81	13.50±2.00	12.51±1.60
1000ppm	14.50±2.01	11.82±1.46	16.50±3.00	15.22±2.89	15.80±2.81	16.02±3.20
Essential oil of sage						
200ppm	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
400ppm	0.00±0.00	0.00±0.00	8.22±0.75	0.00±0.00	0.00±0.00	0.00±0.00
800ppm	9.00±0.98	0.00±0.00	10.00±0.95	8.90±0.76	9.50±0.88	0.00±0.00
1000ppm	12.50±1.66	10.00±1.05	12.50±1.93	11.20±1.60	12.50±1.93	10.50±1.20
Values expressed are means ± standard deviations.						

Both gram–positive and gram–negative bacteria were sensitive to the potent essential oils and phenolic compounds. In general, rosemary and sage oils and phenolic compounds showed significant inhibitory effect against six bacterial species. Moderate effects were seen in other oil and phenolic compounds (anise and black cumin). On the other hand, statistical analysis showed that the essential oils extracted from all herbs had low significant antimicrobial effect compared with phenolic compounds in all herbs (anise, black cumin, rosemary and sage).

Plant essential oils and phenolic compounds have been used for many thousands of years in food preservation, pharmaceuticals, alternative medicine and natural therapies [JONES, 1996]. Essential oils and compounds are potential source of novel antimicrobial compounds [MISTSCHER *et. al.*, 1987], especially against bacterial pathogens. In vitro studies in this work showed that the essential oils and phenolic compounds inhibited bacterial growth but their effectiveness varied.

The antimicrobial activity of many herbs has been previously reviewed and classified as strong, medium or weak [ZAIKA, 1988]. In our study rosemary and sage phenolic compounds exhibited strong activity against the selected bacterial strains.

Several studies have shown that clove, sage and rosemary essential oils and phenolic compounds had strong and consistent inhibitory effects against various pathogens. In the present study, showed least black cumin phenolic compounds. Similar results were obtained by [TAKARADA *et al.*, 2002].

Changes in peroxide value during storage of cake

The effect of natural (essential oils and phenolic compounds of herbs) and synthetic antioxidants (BHT) on peroxide value of cake samples over 15 days of storage periods under room temperature was illustrated in *Table 10* & *11*. Peroxide value range of 10–20meq./kg, food product is considered rancid but still acceptable.

But if more than 20meq./kg, it considered food product already rancid and unacceptable to consume [PEARSON, 1970].

All samples were able to maintain peroxide value less than 10 meq./kg until the fifteen day of storage.

Table 10.
Effect of essential oils of medicinal herbs on the peroxide value of cake samples during storage period.

Treatments	Storage period (day)			
	0	5	10	15
Control	0.30±0.01	5.20±0.35	11.20±0.90	16.30±1.31
BHT (200ppm)	0.30±0.01	3.90±0.22	7.00±0.60	9.93±0.81
Essential oil of anise				
200	0.30±0.01	4.00±0.25	7.93±0.66	10.50±0.95
400	0.30±0.01	3.70±0.20	6.67±0.60	9.61±0.91
800	0.30±0.01	2.42±0.15	5.10±0.53	8.22±0.75
1000	0.30±0.01	2.00±0.10	3.35±0.30	6.33±0.60
Essential oil of Black cumin				
200	0.30±0.01	3.71±0.25	6.92±0.60	9.95±0.92
400	0.30±0.01	3.27±0.20	5.81±0.55	8..81±0.76
800	0.30±0.01	2.59±0.16	5.00±0.50	8.00±0.78
1000	0.30±0.01	2.00±0.11	3.90±0.30	6.90±0.62
Essential oil of Rosemary				
200	0.30±0.01	2.98±0.29	6.01±0.60	9.00±0.93
400	0.30±0.01	2.10±0.20	4.37±0.91	7.30±0.68
800	0.30±0.01	1.93±0.15	4.21±0.40	6.90±0.63
1000	0.30±0.01	1.62±0.10	3.11±0.30	5.20±0.51
Essential oil of Sage				
200	0.30±0.01	4.00±0.30	7.00±0.61	10.33±0.93
400	0.30±0.01	3.78±0.25	6.50±0.60	9.60±0.82
800	0.30±0.01	2.61±0.20	5.00±0.51	8.01±0.75
1000	0.30±0.01	1.95±0.10	3.61±0.30	6.55±0.60

Values expressed are means ± standard deviations.

Table 11.
Effect of phenolic compounds of medicinal herbs on the peroxide value of cake samples during storage period.

Treatments	Storage period (day)			
	0	5	10	15
Control	0.30±0.01	5.20±0.30	11.20±0.90	16.30±1.20
BHT (200ppm)	0.30±0.01	3.90±0.30	7.00±0.60	9.93±0.80
Phenolic compounds of Anise				
200	0.30±0.01	3.85±0.20	7.00±0.60	10.22±0.88
400	0.30±0.01	3.50±0.30	6.32±0.55	9.30±0.80
800	0.30±0.01	2.80±0.15	5.00±0.45	8.00±0.75
1000	0.30±0.01	1.92±0.10	3.11±0.30	6.11±0.60
Phenolic compounds of Black cumin				
200	0.30±0.01	3.50±0.30	6.95±0.60	9.85±0.80
400	0.30±0.01	3.00±0.20	6.12±0.60	9.13±0.80
800	0.30±0.01	2.33±0.14	5.09±0.40	8.07±0.78
1000	0.30±0.01	1.80±0.11	3.50±0.30	6.50±0.60
Phenolic compounds of Rosemary				
200	0.30±0.01	2.90±0.15	5.86±0.45	8.80±0.79
400	0.30±0.01	1.98±0.10	4.81±0.40	7.50±0.65
800	0.30±0.01	1.50±0.10	3.12±0.30	6.22±0.60
1000	0.30±0.01	1.00±0.07	2.50±0.20	5.00±0.52
Phenolic compounds of Sage				
200	0.30±0.01	3.80±0.20	7.00±0.60	10.00±0.89
400	0.30±0.01	3.55±0.20	6.50±0.50	9.50±0.85
800	0.30±0.01	2.78±0.16	5.11±0.45	8.20±0.78
1000	0.30±0.01	1.98±0.10	3.00±0.30	6.00±0.60

Values expressed are means ± standard deviations.

Addition of antioxidants (essential oils and phenolic compounds) extracted from herbs (anise, black cumin, rosemary and sage) at various concentrations as well as BHT (200ppm) had significantly lower peroxide value than those of the control, throughout the duration of the study.

However, the peroxide value of rosemary phenolic compounds at 1000ppm was significantly lower than the peroxide value of all systems during storage.

Sensory evaluation of cakes

Mean scores for sensory evaluation of baked cake as affected by different concentrations of essential oil and phenolic compounds of herbs (anise, black cumin, rosemary and sage) are shown in *Tables 12* & *13*.

Results indicated that the addition of these essential oils and phenolic compounds of herbs gave a better rating score in the baked cake samples under study than control samples.

Generally, it could be observed that the addition of medicinal herbs (essential oils and phenolic compounds) to bakery products(cake) exhibited the highest characteristics of sensory evaluation and had higher addition of 200, 400, 800 and 1000ppm, respectively, to obtained good quality productive, suggesting their potential source for alternative dietary oils for manufactured cake.

Bakery products manufacture uses a wide range of ingredients of which the fats and oils offer the highest potential risk of rancidity in the autoxidation of fats; unsaturated fatty acids are oxidation to hydroperoxides whish on subsequent decomposition yield a number of saturated and unsaturated aldehyde and ketones [WHO, 1972].

Table 12.

Sensory evaluation of cake mixed with different concentrations of essential oils of herbs

Treatment	Color	Taste	Odor	Appearance	Overall acceptability
Control	18.50±1.00	18.00±0.90	17.50±0.81	18.30±0,95	18.70±1.00
BHT 200ppm	18.00±0.90	17.50±0.81	18.00±0.92	18.00±0.93	18.00±0.95
Essential oil of anise					
200ppm	17.50±0.85	18.10±0.93	18.80±1.01	18.80±1.09	18.90±1.20
400ppm	18.00±0.88	18.00±0.90	18.00±0.93	17.50±0.81	18.00±0.92
800ppm	18.01±0.91	18.00±0.91	18.50±1.00	18.50±1.00	18.90±1.20
1000ppm	18.50±0.99	18.00±0.88	18.00±0.90	17.50±0.81	17.50±0.85
Essential oil of black cumin					
200ppm	18.00±0.90	18.50±0.99	17.50±0.92	17.70±0.98	17.80±0.95
400ppm	18.50±0.99	18.50±0.99	18.00±0.92	18.30±0,95	18.00±0.93
800ppm	17.51±0.80	18.00±0.90	17.60±0.95	17.00±0.92	17.80±0.95
1000ppm	18.00±0.91	18.00±0.93	18.00±0.90	18.30±0,95	17.80±0.95
Essential oil of rosemary					
200ppm	18.00±0.92	17.00±0.81	16.20±0.70	17.00±0.92	16.70±0.70
400ppm	17.00±0.81	18.00±0.91	18.00±0.92	17.00±0.92	18.00±0.90
800ppm	17.90±0.85	17.00±0.85	16.20±0.70	17.50±0.95	16.50±0.69
1000ppm	18.00±0.93	17.00±0.85	16.20±0.70	18.00±0.90	17.50±0.95
Essential oil of Sage					
200ppm	18.30±0.96	16.50±0.73	16.50±0.75	16.50±0.75	16.50±0.69
400ppm	17.00±0.81	18.00±0.90	17.00±0.85	18.00±0.90	17.50±0.95
800ppm	17.00±0.80	16.00±0.70	16.50±0.75	16.50±0.73	16.50 ±0.69
1000ppm	18.00±0.92	17.90±0.85	17.00±0.85	18.00±0.92	16.20±0.70

Values expressed are means ± standard deviations.

Table 13.

Sensory evaluation of cake mixed with different concentrations of phenolic compounds of herbs

Treatment	Color	Taste	Odor	Appearance	Overall acceptability
Control	18.50±1.00	18.00±0.95	17.50±0.85	18.30±1.00	18.70±1.00
BHT 200ppm	18.00±0.95	17.50±0.81	18.00±0.93	18.00±0.90	18.00±0.95
Phenolic compounds of anise					
200ppm	18.00±0.95	18.00±0.93	17.50±0.84	18.00±0.90	18.00±0.95
400ppm	17.50±0.81	18.50±0.95	17.20±0.83	17.80±0.85	17.50±0.83
800ppm	18.00±0.95	18.00±0.93	17.50±0.84	18.50±0.95	18.00±0.95
1000ppm	18.00±0.93	17.30±0.80	17.50±0.81	17.30±0.80	17.50±0.83
Phenolic compounds of black cumin					
200ppm	17.50±0.81	1700±0.81	17.30±0.80	17.50±0.81	17.50±0.83
400ppm	16.20±0.70	17.50±0.73	17.00±0.80	17.20±0.83	16.50±0.73
800ppm	18.00±0.92	17.20±0.83	17.50±0.73	17.80±0.85	17.50±0.83
1000ppm	17.20±0.83	17.80±0.85	17.50±0.86	17.50±0.81	18.00±0.93
Phenolic compounds of rosemary					
200ppm	17.70±0.86	17.60±0.85	16.50±0.73	18.00±0.90	17.50±0.83
400ppm	18.50±1.00	18.00±0.92	16.20±0.70	17.70±0.86	17.80±0.85
800ppm	17.50±0.80	17.50±0.86	16.50±0.73	18.50±1.00	17.00±0.80
1000ppm	17.20±0.83	17.70±0.86	17.00±0.81	17.50±0.84	16.20±0.70
Phenolic compounds of sage					
200ppm	18.30±0.98	16.30±0.71	17.00±0.80	16.50±0.73	16.50±0.75
400ppm	16.30±0.71	17.50±0.80	17.50±0.86	17.00±0.81	17.50±0.83
800ppm	17.30±0.84	16.50±0.73	17.00±0.80	16.50±0.73	16.50±0.75
1000ppm	18.50±1.00	18.50±1.00	18.50±0.95	17.00±0.80	17.50±0.83

Values expressed are means ± standard deviations.

This study shows that essential oils and phenolic compounds extracted from medicinal herbs are distributed and remained functional as antioxidant and antimicrobial on bakery products.

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