USING NATURAL ADDITIVES FOR INCREASING SHELF LIFE OF PIZZA

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(Manuscript received 1 August 2010)

Abstract

In later years, there is an increasing interest for new natural antimicrobial compounds due to negative effect of artificial food preservatives on human health therefore, in this study the dill, celery and parsley seeds were added individually at levels 1.5 and 2% before baking on surface pizza and its essential oils which were extracted from these seeds were added individually at levels 400 and 600 ppm after baking on pizza surface. The results demonstrated that physicochemical properties of essential oils were in standard limit. Sensory evaluation of pizza showed that pizza color with three seeds additives and pizza taste with dill seeds essential oil added significantly differed taste compared with control sample. GC/MS apparatus estimated 15, 13 and 14 components present in dill, celery and parsley essential oils respectively. Antimicrobial activity of essential oils indicated that essential oils gave greater antimicrobial activity in gram positive bacteria than that in gram negative bacteria, also, essential oils had higher antimolds activity than that of bacteria and yeast. Total count of microorganisms in pizza revealed that adding three essential oils increased shelf life of pizza as compared with adding the three seeds.

INTRODUCTION

Natural antimicrobials, such as spices and spice extracts, for reducing and eliminating pathogen and spoilage microorganisms from fresh produce can be used as an alternative treatment. There is an increasing interest in the possible use of natural compounds to prevent microbial growth in the food items which will result in additive free, fresher and more natural tasting food products. Thus, Consumers all over the world are becoming increasingly conscious of the nutritional value and safety of their food and its ingredients. At the same time, there is an increase preference for natural food and food ingredients which are generally believed to be safer, more healthy and less subject to hazards than food containing artificial food additives (Karapinar *et al.*, 2005).

Recently, there is a growing interest in substances exhibiting antimicrobial and antioxidant properties that are supplied to human and animal organisms as food components or as specific pharmaceutics. It has been well-known that essential oils have antimicrobial and antioxidant effects. Also, common need is available in natural extracts which give a pleasant taste and/or smell combined with a preservative action, aimed to avoid lipid deterioration, oxidation and spoilage by microorganisms (Sacchetti *et al.*, 2005).

Since ancient times, herbs and spices have been added to different types of food to improve the flavor and organoleptic properties. Also, herbal medicines have a great potential in the emerging nutrition industry, because these materials are often considered foods as well as medicines and are used in preventive and curative treatments throughout the world. Especially popular today is the concept of foods that combine nutritional and medicinal benefits, so- called "functional foods" many natural compounds extracted from plants have demonstrated biological activities. Among these various kinds of natural substances, essential oils from aromatic and medicinal plants receive particular attention as potential natural agents for food preservation. In fact, their effectiveness against a wide range of microorganisms has been repeatedly demonstrated (Marino *et al.*, 2001).

Essential oils obtained from plants by steam distillation, pressing or extraction, in addition to their characteristic aroma and flavor, usually contain phenolic compounds with a certain level of antimicrobial and antifungal activity. The activity of essential oils against microorganisms is strongly dependent on the food system in which they are used. It also depends on other variables, for example, plant variety, climatic factors and extraction method. These essential oils have been shown to have useful antimicrobial activity at concentrations suitable for use in food processing (Shelef *et al.*, 1980).

Essential oils (EOs) (also called volatile or ethereal oils) are aromatic oily liquids obtained from plant material. About 300 essential oils are known commercially important-destined chiefly for the flavours and fragrances market. Among the herbs and spices and its oils best known for their antimicrobial properties are celery seeds, dill seeds and parsley seeds (Farrell, 1999).

The present study has been planned to evaluate the efficiency addition of dill, celery and parsley seeds and its essential oils on sensory evaluation and microorganisms content of pizza.

MATERIALS AND METHODS

Materials:

Dill seeds (*Anethum graveolens L.*), celery seeds (*Apium graveolens L.*) and parsley seeds (*Petroselinum sativum M.*) were obtained from Medicinal and Aromatic Plant Research Department, Agriculture Research Center, Giza, Egypt .

Wheat flour (72% extraction) was obtained from Middle and West Delta Mills Company, Cairo, Egypt .

Microorganisms:

Strains used were as follows: Four strains of gram positive bacteria, *Bacillus cereus* (ATCC14579), *Bacillus subtilis* (CGMCC 1.4681), *Listeria monocytogenes* (NRRL, 1951) and *staphylococcus aureus* (ATCC 25923). Two strains of gram negative bacteria, Escherichia coli (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 278530). Four strains of molds, *Aspergillus niger* (ATCC 6880), *Fuzarium oxysporum* (CGMCC 3.12), *penicillium citrnium* (CGMCC 3.27) *Rhizopus nigricans* (CGMCC 3.37). One strain of yeast, *candida albicans* (ATCC 14053). All strains were obtained from Faculty of Pharmacy, Zagazig University, Egypt. The bacterial strains were grown in Mueller Hinton Agar (MHA) plates at 37°C, while the fungi (molds and yeast) were grown in Sabouraud Dextrose Agar (SDA) and potato dextrose agar (PDA) media, respectively, at 20°C.

Methods:

Extraction of essential oils:

The seeds of dill, celery and parsley were individually ground, then mixed with distilled water (seeds: water, 1:5) and submitted to steam distillation about 4hr. via Clevenger- type apparatus. The collected oils were dried by passing over anhydrous sodium sulfate on a filter paper(Whatman No.1) in a glass funnel. The oils were stored in a sealed glass bottle at 4˚c until subsequent tests (AOAC, 2005).

Determination of physicochemical properties of essential oils :

- Specific gravity determined by using a pycnometer (10ml capacity).
- Rferactive index determined by using zeiss refractometer at 20 ˚C.
- Optical rotation determined using polarimeter.
- Acid number, ester number and iodine number were determined according to method of AOAC (2005).

Preparation of pizza:

Pizza was prepared according the method described by Larsen *et al.* (1993). The following ingredients were used for manufacturing pizza dough samples: 1000 g flour, 25 g yeast, 5 g sugar, 1 gm salt, 200 ml corn oil, 50 g egg and sufficient water to make cohesive dough constant consistency. The quantities of water, sugar and yeast were put in a cup to be mixed well and after waiting about 5 min. for the activation of yeast the mixture was transferred to a large mixing bowel. The flour quantities were added followed by salt and oil to be mixed with fork to let all the liquid absorbed by the flour. The dough was Kneaded for 8-10 min. on pastry board until the texture was smooth and uniform. The dough was placed in a bowel and drizzle with corn oil covered with a cloth, let to rise for about an hour. The dough was punch down and after about 45 min. The topping of each pizza consists of 30 gm paste tomato, 25 gm green pepper, 30 gm black olive and 30 gm turkey cheese. pizza dough was put in the oven at 170°C for 30 min. The pizza samples were packed in polypropylene film and stored at 5°C in refrigerator.

Sensory evaluation of pizza:

The characteristics of pizza included, color, taste, odor, texture and general appearance were sensory evaluated according to method of Larsen *et al.* (1993).

Essential oils analysis by Gas chromatography-Mass spectrometry (GC-MS):

The analysis of the essential oils was performed using a Hewlett Packard (Waldbronn, Germany) 5890 II GC, equipped with a 30 m × 0.25 mm i.d., 0.25μm HP-5 MS capillary column and HP 5972 mass selective detector. For GC-MS detection an electron ionization system was used with ionization energy of 70 eV. Helium was the carrier gas, at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 220°C and 290°, respectively. Column temperature was initially at 50°C, then gradually increased to 150°C at a 3°C/min rate, held for 10 min and finally increased to 250°C at 10°C/min. Diluted samples (1/100 in acetone) of 1.0 μL were injected manually and splitless. Identification of the components was based on the comparison of their mass spectra with those of NBS75 K library data of the GC-MS system and literature data. The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on non-polar phases reported in the literature (Adams, 2001).

Antimicrobial disk assay:

Antibacterial and antifungai (molds and yeast) activities of three EO_s were investigated by the disk diffusion method (Xhu *et al.,* 2004). The MHA plates, containing an in column size of 10^6 CFU/ml of bacteria or 2×10^5 CFU/ml yeast cells or molds spores on SDA and PDA plates, respectively, were spread on the solid plates with an L-shaped glass rod. Then, disks (6.0 mm in diameter) impregnated with each extract at a different concentration and placed on the inoculated plates. All of the plates were incubated at 37°C for 24 h. for bacteria and at 28°C for 48 h. for fungi. The sensitivity of the microorganisms species to the EO_s was determined by measuring the sizes of the inhibitory zones (including the diameter of disk) on the agar surface around the disks. All of the experiments were performed in duplicate. The results are reported as the average of two experiments.

Total count of microorganisms in pizza:

Total Microbial count and fungi (mold & yeast) count were determined according to methods of AOAC (2005).

Statistical analysis:

The obtained data were statistically analyzed using Statistical Analysis System SAS (2001).

RESULTS AND DISCUSSION

Physicochemical properties of essential oils:

The physicochemical properties of essential oils extracted from dill, celery and parsley seeds were determined and the obtained results were tabulated as shown in Table (1). The values of specific gravity, refractive index, optical rotation, acid number, ester number and iodine number are within the ranges reported by Food Chemical Codex (1981). Meanwhile, Badei *et al.* (2008) declared that the quality of any essential oil and consequently its value and applications are highly correlated with its chemical constituents which directly affect the physicochemical characteristics of these essential oils.

Sensory evaluation of pizza:

With regard to sensory evaluation of pizza after directly baking the data in Table (2) summarized mean values of color, taste, odor, texture and general appearance. It could be deduced from results that addition of dill, celery and parsley seeds at levels 1.5 and 2% and its essential oils at levels 400 and 600 ppm did not significantly affect on values of sensory evaluation of pizza compared with control pizza with the exception of both color pizza when dill, celery and parsley seeds were added and taste of pizza when essential oil of dill seeds was added whereas significantly different compared with control. In the meantime Anitescu *et al.* (1997) indicated that essential oils have a complex composition, containing several hundred constituents, especially hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, aldehydes, ketones, acids, phenols, oxides, lactones, acetyls, ethers and esters). Both hydrocarbons and oxygenated compounds are responsible for the characteristic odor and flavors.

Chemical components of essential oils:

Table (3) and figures (1), (2) & (3) illustrated the Gas chromatography-Mass spectrometry (GC/MS) profiles of essential oils. The results appeared that myristicin, camphene and β-Pinene were the major components present in dill seeds essential oil. Meanwhile, limonene, camphene and β-Pinene were the major components present in celery seeds essential oil. At the same time, β-Phellandrene, myristicin and limonene were the major components present in parsley seeds essential oil. On the contrary, nerodiol and elemicin were not detected in celery seeds essential oil, while, P-cymene was not detected in parsley seeds essential oil under the condition of the experiment in essential oil.

Antimicrobial activities of essential oils:

Concerning the antimicrobial effect of extracted essential oils the results in Table (4) demonstrated that the essential oils were greater antimicrobial activity in gram positive bacteria than that in gram negative bacteria. These results are in agreement with those obtained by Sue and Young (2000), who observed that gram positive bacteria were more susceptible to inhibition by plant essential oil than gram negative bacteria. This finding could be elucidated by Sue and Young (2000) who reported that gram negative bacteria have a cell wall covered by an outer membrane, phospholipids and some protein, this structure may be prevent either the uptake of the oils or protect the peptidoglycan layer from the oils. The outer lipopolysaccharide (LPS) membrane of gram negative bacteria presents a permeability barrier to hydrophobic substances that can enter and inhibit the growth of gram positive bacteria. In gram positive bacteria, the peptidoglycan layer is on the outside and more available to contact with the oils. Some of the antimicrobial compounds in certain oils may fall into this category, explaining the increased resistance of gram negative bacteria to particular oils since these oil components could not enter the cell as easily.

Regarding antimolds effect of essential oils, the data in Table (4) indicated that three essential oils were higher antimolds activity than that of bacteria and yeast. This result coincide with that mentioned by Shelef (1980) who found that molds are more sensitive than bacteria.

Respecting antiyeast effect of essential oils, the results in Table (4) showed that three essential oils exhibited weak activity against yeast.

Branen *et al.* (1980) mentioned that the mechanism of microorganisms decreasing might be related to the destruction or inactivation of essential enzyme and/or genetic material. Also, inhibition DNA, RNA and protein synthesis. They also added that the monoglycerides depressed the NADH oxidase system by acting on the O_2 side of flavine of NADH dehydrogenase. Maria *et al.* (1996) found that celery and dill essential oils showed antibacterial, antilisteria and antifungal activity. The same author indicated that there is a relationship between the chemical composition of the essential oil and its antimicrobial effect.

Total count of microorganisms in pizza:

With respect to total bacterial count and fungi (molds & yeast) count (CFU/gm) in pizza samples, the results in Table (5) revealed that the antimicroorganisms activity of dill, celery and parsley seeds and its essential oils extracted from these seeds could be ranked as follows dill > parsley > celery. From the same table it could be observed that three essential oils were higher or greater antimicroorganisms than powder seeds specially 600 ppm from essential oils. Therewithal, the pizza samples were found to be free of the microorganisms at zero time. In addition to, pizza samples containing different levels of seeds and its essential oils had a longer shelf life compared with control sample (without additives). This effect is due to that seeds and essential oils contains numerous compounds that have inhibitory properties against the growth of microorganisms. Wei and Wang (2001) mentioned that total phenolic content in dill and parsley seeds were 3.12 and 1.12 mg/g as gallic acid equivalents respectively (on fresh weight basis), while Vinson *et al.* (1998) found that total phenolic content in celery seeds was 1.2 μmol/g as catchin equivalents (on fresh weight basis), and they evinced that microorganisms in foods are influenced by the intrinsic characteristics of foods (i.e., pH, moisture level, oxidation-reduction potential, nutrient content, antimicrobial constituents, biological structures, chemical composition of the food and shape or size of the food) and extrinsic characteristics (i.e., temperature, humidity and gases of the environment. Also, the physical and chemical composition of the microorganisms and their stage of existence (vegetative cell, spore, and phase of growth, moist or wet, etc.)

In conclusion, the aforementioned results declared that physicochemical properties of essential oils were in standard limit. Sensory evaluation of pizza showed that pizza color with three seeds additives and pizza taste with dill seeds essential oil added significantly differed taste compared with control sample. GC/MS apparatus estimated 15, 13 and 14 components present in dill, celery and parsley essential oils respectively. Antimicrobial activity of essential oils indicated that essential oils gave greater antimicrobial activity in gram positive bacteria than that in gram negative bacteria, Also, essential oils had higher antimolds activity than that of bacteria and yeast. Total count of microorganisms in pizza revealed that adding three essential oils increased shelf life of pizza as compared with adding the three seeds. Generally, three seeds and its essential oils have broad inhibitory activities to microorganisms and are promising for incorporation into various food products and an ideal candidate for uses for food preservation.

Table 1. Physicochemical properties of essential oils (EO_s)

Characteristics	Dill seeds EO	Celery seeds EO	Parsley seeds EO
Specific gravity (at 15 °C)	0.901	0.891	1.050
Refractive index (at 20 °C)	1.4835	1.4782	1.4910
Optical rotation	+ 94 °0 /	+ 55 °0/	- 60 °0 /
Acid number	1.03	1.50	1.48
Ester number	35.31	18.0	9.75
Iodine number	112.3	94.8	109.5

Table 2. Sensory evaluation of pizza after directly baking*.

Treatments	Color (10)	Taste (10)	Odor (10)	Texture (10)	General appearance (10)
Pizza (control)	8.95 ±0.26a	9.52 ±0.30a	9.10 ±0.27a	9.00 ±0.08a	9.14 ±0.15a
Pizza +1.5% dill seeds	7.81 ±0.24b	8.85 ±0.34a	9.13 ±0.15a	9.10 ±0.22a	8.93 ±0.19a
Pizza +2% dill seeds	7.12 ±0.19b	8.79 ±0.39a	9.17 ±0.30a	9.16 ±0.21a	8.89 ±0.33a
Pizza +1.5% celery seeds	7.18 ±0.25b	8.90 ±0.28a	9.11 ±0.25a	9.11 ±0.40a	8.49 ±0.18a
Pizza +2% celery seeds	7.12 ±0.33b	8.83 ±0.21a	9.14 ±0.19a	9.13 ±0.37a	8.41 ±0.22a
Pizza +1.5% parsley seeds	7.70 ±0.29b	8.72 ±0.12a	9.18 ±0.24a	9.00 ±0.25a	8.37 ±0.31a
Pizza +2% parsley seeds	7.31 ±0.09b	8.60 ±0.34a	9.21 ±0.31a	9.10 ±0.20a	8.37 ±0.10a
Pizza +400 ppm EO dill seeds	8.35 ±0.20a	8.25 ±0.29b	8.55 ±0.18a	9.14 ±0.10a	9.57 ±0.17a
Pizza +600 ppm dill seeds essential oil	8.19 ±0.40a	8.11 ±0.15b	8.50 ±0.15a	9.17 ±0.19a	9.61 ±0.09a
Pizza +400 ppm celery seeds essential oil	8.17 ±0.16a	8.96 ±0.17a	8.90 ±0.20a	9.22 ±0.26a	9.30 ±0.25a
Pizza +600 ppm celery seeds essential oil	8.12 ±0.35a	8.21 ±0.27a	8.75 ±0.28a	9.00 ±0.38a	9.37 ±0.35a
Pizza +400 ppm parsley seeds essential oil	8.15 ±0.39a	8.70 ±0.16a	9.00 ±0.32a	9.20 ±0.11a	8.76 ±0.30a
Pizza +600 ppm parsley seeds essential oil	8.90 ±0.15a	8.67 ±0.35a	8.89 ±0.18a	9.00 ±0.36a	8.80 ±0.17a
L.S.D. (0.05)	0.20	0.16	N.S	N.S	N.S

* Values are mean ± standard error.

* Values in the same column with different superscript letters (a, b,…) are significantly different.

Table 3. Chemical components of essential oils fraction by GC/MS technique.

Components	Percentage (%)		
	Dill seeds EO	Celery seeds EO	Parsley seeds EO
α-Pinene	9.69	6.07	5.84
Camphene	17.71	15.31	3.98
β-Pinene	13.94	7.28	4.95
β-Phellandrene	8.06	2.00	25.77
Limonene	10.68	26.43	14.27
Myristicin	28.27	2.54	18.85
Camphor	11.59	3.63	7.05
Linalool	7.55	4.38	10.72
P-Cymene	0.85	1.29	—
α-Terpinene	2.13	1.50	1.21
Eugenol	5.46	2.33	1.30
Carvone	2.80	2.40	1.75
Nerodiol	0.91	—	0.65
Apiole	2.18	2.19	1.24
Elemicin	0.79	—	0.51

Table 4. Effect of essential oils (EOs) on growth inhibition zone diameter (mm) of microorganisms.

		Dill seeds EO		Celery seeds EO		Parsley seeds EO	
		400 ppm	600 ppm	400 ppm	600 ppm	400 ppm	600 ppm
Gram (+)	*Bacillus cereus*	11	17	10	11	10	13
	Bacillus subtilis	14	21	8	10	9	14
	Listeria monocytogenes	16	19	11	12	12	16
	Staphylococcus Aureus	15	24	11	14	12	17
Gram (-)	*Escherichia coli*	9	12	8	12	8	10
	Pseudomonas aeruginosa	8	15	9	11	8	12
Mold	*Aspergillus niger*	19	26	12	14	15	18
	Fuzarium oxysporum	20	25	13	14	13	18
	Penicillium citrnium	18	25	11	15	13	15
	Rhizopus nigricans	20	27	12	13	14	17
Yeast	*Candida albicans*	3	5	1	2	2	5

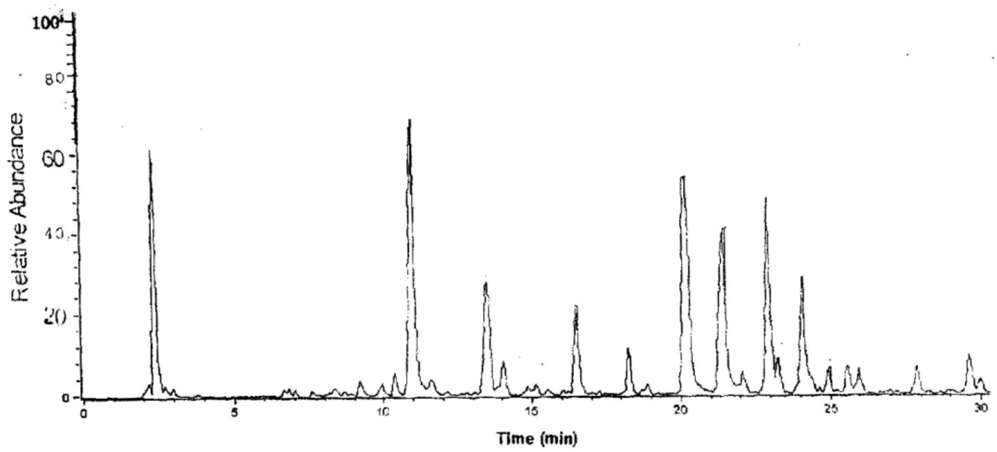

Figure 1. GCMS chromatogram of dill seeds essential oil components.

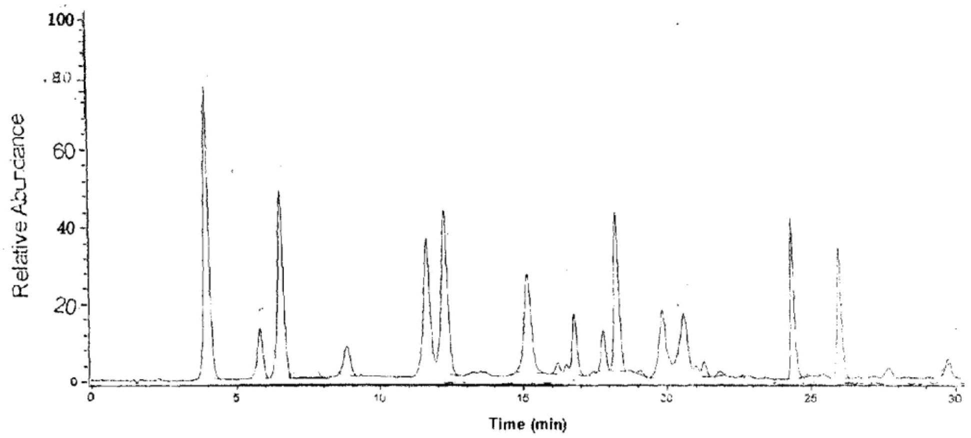

Figure 2. GCMS chromatogram of celery seeds essential oil components.

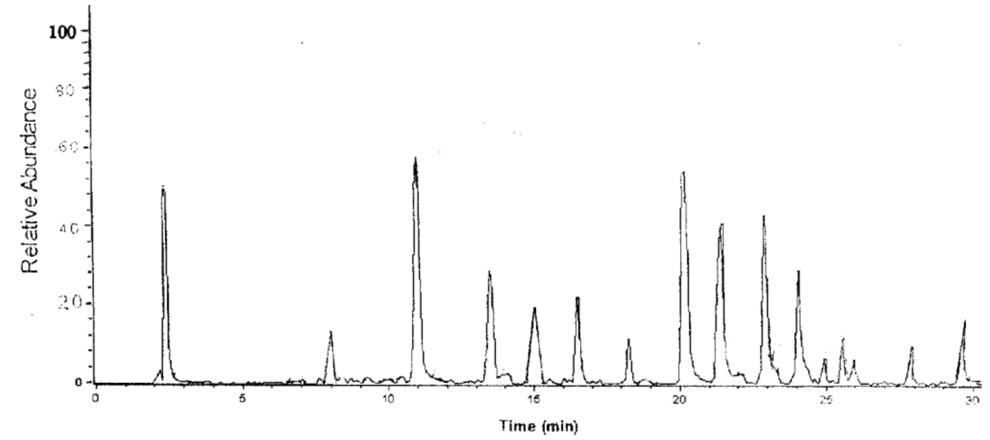

Figure 3. GCMS chromatogram of parsley seeds essential oil components.

Table 5. Effect of seeds and essential oils on total count of microorganisms in pizza (CFU/gm).

Treatments	Storage period (days)							
	Zero time		5 days		10 days		15 days	
	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
Control	ND	ND	3.1×10^2	1.8×10^2	Ex 7 days			
Seeds:								
Dill 1.5 %	ND	ND	1.0×10^2	ND	2.3×10^2	1.0×10	Ex 12 days	
Dill 2 %	ND	ND	8.0×10	ND	1.7×10^2	ND	Ex 13 days	
Celery 1.5%	ND	ND	2.5×10^2	1.0×10^2	Ex 9 days			
Celery 2%	ND	ND	2.0×10^2	ND	2.7×10^2	1.9×10^2	Ex 11 days	
Parsley 1.5%	ND	ND	2.3×10^2	8.0×10	Ex 10 days			
Parsley 2%	ND	ND	1.4×10^2	ND	2.1×10^2	1.0×10^2	Ex 12 days	
Oils:								
Dill 400 ppm	ND	ND	3×10	ND	1.1×10^2	1.0×10	Ex 13 days	
Dill 600 ppm	ND	ND	1×10	ND	7.0×10	ND	Ex 15 days	
Celery 400 ppm	ND	ND	7×10	ND	2.1×10^2	2.0×10^2	Ex 11 days	
Celery 600 ppm	ND	ND	3×10	ND	9.0×10	2×10	Ex 11 days	
Parsley 400 ppm	ND	ND	5×10	ND	1.9×10^2	9.0×10	Ex 11 days	
Parsley 600 ppm	ND	ND	2×10	ND	1.6×10^2	ND	Ex 13 days	

Fungi are molds and yeast.
ND means not detected.
Ex means expired date.

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استخدام إضافات طبيعية لزيادة فترة صلاحية البيتزا

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فى السنوات الأخيرة يوجد اهتمام زائد لاستخدام المركبات الطبيعية كمضادات للميكروبات بسبب التأثير السلبى للمركبات الصناعية التى تستخدم كمواد حافظة للأغذية على صحة الإنسان ولذلك ففى هذه الدراسة تم إضافة بذور الشبت والكرفس والبقدونس كلاً على حده بنسب ١,٥، ٢% من وزن الدقيق قبل عملية الخبيز على سطح البيتزا وكذلك الزيوت الطيارة المستخلصة من هذه البذور بنسب ٤٠٠، ٦٠٠ جزء فى المليون من وزن الدقيق بعد عملية الخبيز على سطح البيتزا. أظهرت نتائج تقدير الخواص الطبيعية والكيميائية للزيوت الطيارة أنها كانت فى حدود المواصفات وأوضحت نتائج التقييم الحسى للون البيتزا المضاف إليها البذور الثلاثة وكذلك طعم البيتزا المضاف إليها زيت بذور الشبت اختلفت معنويًا بالمقارنة بعينة الكنترول. كما تم التعرف على مكونات الزيوت الطيارة باستخدام جهاز التحليل الكروماتوجرافى الغازى/التحليل الطيفى للكتلة وتم التعرف على ١٥ مركب فى زيت الشبت و١٣ مركب فى زيت الكرفس و١٤ مركب فى زيت البقدونس. وأظهرت نتائج تأثير الزيوت الطيارة على بعض سلالات البكتريا والفطر والخميرة أن الزيوت الطيارة كانت أكثر تأثيرًا على البكتريا موجبة لجرام من البكتريا سالبة لجرام وكذلك كانت الزيوت الطيارة أكثر تأثيرًا على الفطر من البكتريا والخميرة. فى حين أظهرت نتائج العد الكلى للبكتريا و(الفطر والخميرة معًا) فى البيتزا أن الزيوت الطيارة كانت أفضل من البذور فى إطالة فترة صلاحية البيتزا.