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Effect of dill seeds as anti-fungal properties for bread

Fatma A. Nada[1], H.A. Amra[2], [2]Mohamed Sh. R. and [2]Noah Badr A.

[1]Bakery and Pasta Res. Dept., Food Tech. Res. Inst., ARC, Giza, Egypt.
[2]Department of Food Toxicology and Contaminants, National Research Centre, Dokki, Giza, Egypt.

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ABSTRACT

The presented study was investigated the effect of dill seeds essential oils on the microbial and fungal activity. The essential oils of dill seeds was used by spraying on pan bread at levels (1; 1.5; 2; 2.5 and 3 ml), also sensory evaluation and total counts were determined. Essential oils was extracted from whole seeds by using hydro-distillation , the dill seeds essential oil compounds were identified by gas chromatography/mass spectphorometr (GC/MS). The results showed that analysis the essential oils composed of 31 constituents. The highest percentage of content were carvon (32.55%), Dillapiole (20.45 %) and D-Limonene (16.06%). The highest total score of bread of sensory characteristics was recorded with spraying of 2ml by essential oil dill seeds (97.47) compared with control (94.08). Meanwhile spraying 3ml by essential oil dill seeds have lowest total score and significantly difference in pan bread (77.89). Also, the essential oils of dill seeds showed antimicrobial and antifungal activity against 4 fungi strains (*Aspergillus flavus* (RCMB 002002) , *Aspergillus niger* (RCMB 002005), *Penicillium expansum* (RCMB 001001 (1)IMI 28169) and *Penicillium italicum* (RCMB 001018 (1)IMI 193019) and 5 bacteria strains (*Staphylococcus aureus* (RCMB 010010), *Bacillus cereus* RCMB 027 (1) and *Bacillus subtilis* RCMB 015 (1) NRRL.B- 534 (gram positive + ve) and *Escherichia coli* (RCMB 010052) ATCC 25955 and *Salmonella typhimurium* (gram negative –ve). Total bacterial counts and yeast and molds showed increased through third till six days in pan bread control sample meanwhile, a little appears for total count bacteria was found in pan bread sprayed with 2ml essential oil of dill seeds during storage for 6 days at room temperature (25±2C°) while such samples still free from molds and yeasts till six days of storage. It can be concluded that the essential oil dill seeds have good antimicrobial and antifungal effects which delayed the microbial growth in the bread which increased shelf life and improved the quality.

***Keywords*:** Dill seeds, essential oils, pan bread, sensory evaluation, antibacterial, antifungal, inhibition zone, total counts, yeast and molds.

Introduction

Bakery products get deteriorated due to physical, chemical and microbiological factors. Major microbiological loss of bakery products is due to mould growth and problems that are associated with it. Shelf life of bakery products is limited by growth of moulds. Blue green *Penicillium* sp. and *Aspergillus* sp. are found along with other genera of mould causing bread spoilage. (Saranraj and Geetha, 2012).

Skandamis *et al.* (2001) reported that to enhance the shelf life of bread, several chemical antimicrobial agents have been employed but they are considered responsible for many carcinogenic and teratogenic attributes and residual toxicity. Due to these reasons, consumers tend to doubtful of chemical additives and thus the demand for natural preservatives has been intensified.

For prevention of spoilage in bakery products natural preservatives are better alternative than artificial preservatives (Anand and Sati, 2013). Dill (*Anethum graveolens)*, is one of the family Umbelliferae plants, an annual herb and it's originates from West Asia and Mediterranean. It is used as a source of essential oil and as a vegetable. Its seeds are used in tea, breads, soup . Hence, there has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils (Al-Snafi, 2014). Tian *et al.,* (2011) reported that the *Anethum graveolens* (dill) is widely used for flavoring foods and beverages. The yield of its essential oil is about 3.5%,

Corresponding Author: Fatma A. Nada, Bakery and Pasta Res. Dept., Food Tech. Res. Inst., ARC, Giza, Egypt. E-mail: fatmanada3@gmail.com

which is relatively high. Thus, the essential oil of A. graveolens is more likely to be developed into a food preservative.

The productivity and quality of essential oil depend on cultivar, climate, sowing and harvest dates, environmental stress, and management practices, (Yili *et al.,* 2006). Tanrueam *et al.,* (2014) reported that carvone is the major component of *Anethum graveolens* which is related to the antibacterial activity.

Essential oils can be a natural alternative for chemical preservatives which is mainly extracted from plant material such as leaves, flowers, buds, seeds, twigs, bark, herbs, fruits and roots. Essential oils had showed antibacterial and antifungal activity against many food spoiling bacteria and fungus (Burt, 2004). Essential oils are rich sources of biologically active compounds. Recently, there has been a profound interest in the antimicrobial properties of the aromatic plants, particularly in their essential oils (Hussein *et al.,* 2015). Dill has been reported to possess antibacterial (Rafii and Shahverdi, 2007).

Tayarani-Najaran, *et al.,* (2016) reported that seeds and leaves of dill plant are the main parts that are being used. The main components of the essential oil obtained from this plant are reported to be carvone, limonene, dihydrocarvone, carvacrol, *p*-cymen, α-phellandrene, and dill apiole.

Kapoor *et al.* (2002) who reported the dill apiole and carvone as the main components, carvone (38.89%), dill apiol (30.81%), limonene (15.93%) and trans-dihydrocarvone (10.99%) were major components. Also, Singh *et al.,* (2005) found that carvone (55.2%), limonene (16.6%), dill apiole (14.4%) and camphor (11.44%), were four major compounds in dill seed oil.

The food industry often uses essential oil instead of dill leaves and seeds due to its characteristic aroma and flavor (Jirovetz *et al.,* 2003). It has been reported that dill has antimicrobial, antihyperlipidemic, diuretic, hypotensive, antispasmodic, antiemetic, laxative effect (Hosseinnzadeh *et al.,* 2002 ; Koppula and Choi, 2011) .

Synthetic compounds are widely used by the food industry to preserve foods, but despite regulation by the health organs, they are reason for concern by consumers principally with respect to their safety, since in some cases they can cause adverse reactions.(Aun *et al.,* 2011).

Much research has already been conducted in the domain of natural antimicrobial preservatives as a result of increasing signs of negative effects due to the intake of chemical preservatives and the changing consumer perception towards food preservatives [(Ring *et al.,* (2001) and Dengate and Ruben, (2002)].

Lucera *et al.* (2012) they highlighted that dipping, spraying, and coating treatment of food with active solutions are currently applied to product prior to packaging as valid options and the use of natural compounds in food sector.

Limonene and carvone, have exhibited strong antifungal activity against *Aspergillus niger, Saccharomyces cerevisiae* and *Candida albicans* (Stravri and Gibbons, 2005; Kaur and Arora, 2010).

The present study aimed to improve the quality of the baked pan bread by using essential oils extracted from dill seeds by the spraying to the crust after baking.

Material and Methods

Materials:

Dried of Dill seeds (*Anethum graeolens* L.), was obtained in the late June, 2017 from Medicinal and Aromatic Research Department Horticultuer Research Institute, Agriculture Research Center, Giza, Egypt. Wheat flour (72% extraction) was obtained from 6 th October for Milling and Marketing Co., 6 th October city, Egypt. Instant active dry yeast, crystal white sugar, sodium chloride, corn oil, obtained from the local market, Cairo, Egypt.

Extraction of Essential Oil (EOs)

The dried Dill seeds (*Anethum graeolens* L.), was submitted to water distillation for 3 hours by hydro-distillation in a Clevenger-type apparatus separately until there was no significant increase in the volume of the oil. The volatile oils were dried over anhydrous sodium sulfate and stored at -18°C in the dark glasses, according to the method described by Asadipour *et al.* (2003).

GC / MS analysis

The analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm i. d. and 0.25 μm film thickness). The carrier gas was helium with the linear velocity of 1ml/min. The injector and detector temperatures were 200º C and 250º C, respectively. Volume injected 1μl of the sample. The MS operating parameters were as follows: ionization potential 70 eV, interface temperature 250º C, and acquisition mass range 50–800.The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature (Rob *et al.,* 2013).

Pan bread Preparation:

The straight dough method for pan bread production was carried out according to the method described by A.A.C.C. (2000) with some modification, recipe of pan bread containing wheat flour (100 g), instant active dry yeast (1.5g) , crystal white sugar (5.0g), sodium chloride (1.0g), corn oil (3.0g), water (60 ml).

Procedure:

The blends of flour with all other ingredients were mixed together for a time obtained from farinograph data. The resulted dough was leave to rest for 30 min (fist proofing), then divided to (125gm), rolled and molded automatically in molding. Each piece was placed in baking pans (10x5x6cm) tightly greased to prevent the loaves from sticking pans and were leave to ferment for 60 min in a cabinet at 30ºC and 85% relative humidity, then baking process was carried out in electrically heated oven at 250ºC for 25-30 min. After baking, loaves were separated from the baking pans and allowed to cool for 1hr at room temperature, then Sprayed 1, 1.5, 2, 2.5 and 3 ml extracted from essential oils on pan bread and then organoleptic evaluation.

Organoleptic evaluation of pan bread:

Fresh samples of pan bread were organoleptically evaluated by taste panellists from the staff in Food Tech. Res. Institute, Agric. Res. Center, Giza, Egypt. They were asked to score the internal characteristics of pan bread for general appearance(15),crust color(15), crumb color (15), distribution of crumb(15),taste (20), flavour (20) and total score (100) according to the method described by A.A.C.C. (2000).

Source of the tested fungi and bacteria:

Pathogenic fungal isolates (*Aspergillus flavus* (RCMB 002002) , *Aspergillus niger* (RCMB 002005)*, Penicillium expansum* (RCMB 001001 (1)IMI 28169) *and Penicillium italicum* (RCMB 001018 (1)IMI 193019) and pathogenic bacteria *Staphylococcus aureus* (RCMB 010010)*, Bacillus cereus* RCMB 027 (1) and *Bacillus subtilis* RCMB 015 (1) NRRL.B- 534 (gram positive + ve) and *Escherichia coli* (RCMB 010052) ATCC 25955 and *Salmonella typhimurium* (gram negative –ve) were kindly obtained from the Regional Center for Mycology and biotechnology,Al-Azhar University, Egypt.

Microbial Evaluation:

Antibacterial test assays:

Antimicrobial susceptibility test was carried out by the wells diffusion method. Bacterial strains were grown and diluted using Mueller-Hinton broth. Bacterial strains were grown to exponential

phase in Mueller-Hinton at 37^0C for 18hr and adjusted to a final density of 10^8 CFU/ml by diluting fresh cultures and comparison with Mc Farland density. The anti-bacterial activity was tested by inoculating 500µl of Mueller- Hinton broth into 25 ml of nutrient agar and allowed to cool under strict aseptic conditions. On solidification of the medium wells were made in petriplates with the help of a sterile metal borer (7mm). The microorganisms used for the antimicrobial assays were *Escherichia coli and Salmonella* (gram negative –ve) and *Staphylococcus aureus*, *Bacillus cereus and Bacillus subtilis* (gram positive + ve). The Petri-dishes containing agar media (nutrient agar NA) was plated with 0.1 ml of *Escherichia coli* and the other Petri-dishes with *Salmonella* (gram negative –ve) cultures and in another 3 plate put 0.1 ml of different microorganisms such as *Staphylococcus aureus*, *Bacillus cereus and Bacillus subtilis*. The inoculated plats with bacteria were made in triplicate. The discs containing the oil dill was placed on the agar using sterile forceps. The control sample was contained Gentamicin (4µg/ml). Plates were incubated at 37 ˚C for *B. cereus* and *E. coli* for about 18-24 hours. After proper incubation, antibacterial activity was determined by measuring the diameter of the zone of the inhibition around the well by using metric scale. The diameter of resultant zone of inhibition was measured in millimetres. Three replicates were carried out for each extract against each of the test organism (Olila, *et al.,* 2001).

Microbiological Analysis of pan bread

All samples of pan bread were analyzed by microbialogical tests including bacteria and yeast & mold, at zero time, 1, 2, 3, 4, 5 and 6 days of storage at room temperature (25±2C˚) ; All analyses were performed by using the standard procedures outlined in the American Public Health Association (APHA 1992).

Statistical analysis

Data were expressed as the means ± SD. Statistical analysis was carried out using one - way analyses of variance, ANOVA (Rao, and Blane, 1985).

Results and Discussion

Essential oil composition (Eo):

The pale yellow in color essential oil of *Anethum graveolens L.* seeds showed 2.47% of Eo yield which was extracted by hydro-distillation. The chemical components of AEo were identified by using GC-MS technique, the percentages of composition are presented in Table (1) The results showed that AEo composed of 31 constituents. The highest percentage of relative content revealed carvon (32.55%), Dillapiole (20.45 %) and D-Limonene (16.06%) as majority of components. Also, our results is in agreement with Radelescu *et al.,* (2010) who found the major component in dill seeds essential oil is carvone (20-60%). Mahmoodi *et al.,* (2012) found that *D*-carvone (36.09%), Limonene (19.89%), dillapiol (16.83%), *E*-dihydrocarvone (7.36%) and *Z*-dihydrocarvone (6.59%) were present as major compounds Analysis in dill seeds essential oil.

Organoleptic evaluation of pan bread

Organoleptic characteristic of produced fresh pan bread (crust color, crumb distribution, crumb color, taste, flavour, general appearance and total score) at different levels of essential oil dill seeds sprayed to the pan bread crust was presented in Table (2). The results showed that there is a significant difference in crust color and taste at 3ml spraying by essential oil dill seeds .Crump distribution , crumb color and general appearance showed that no significant difference in all samples. Concerning to flavour results showed that 2ml spraying by essential oil dill seeds was high significantly compared to control and other samples.

In addition total score decreased significantly in pan bread sprayed with 3ml by essential oil dill seeds (77.89) while total score increased significantly in pan bread sprayed with 2 ml by essential oil dill seeds (97.47) compared with control (94.08).

Table 1: Chemical and Identification of *Anethum graveolens* L. Seeds essential oils by GC/MS.

Components	% Area sum	Components	% Area sum
Methyl 3,5-dimethylbenzoate	1.09	Linolenic acid	0.29
γ-Terpinene	0.35	Eugenol	0.39
β -phellandrene	0.39	Myristicin	1.17
$\acute{\alpha}$ - phellandrene	0.29	Dillapiole	20.45
carveol	2.50	n-Hexadecanoic acid	0.57
o-cymene	0.59	Octadecanoic acid	0.59
D-Limonene	16.06	Phytol	0.46
2-Carene	0.31	Isolongifolol	0.32
Cis-Verbenol	0.28	Elaidic acid	1.97
Pulegone	0.90	Cannabinol	0.61
Myrtenol	0.26	Kaempferol	0.63
Dihydrocarvone	4.98	Methyl myristate	0.26
(R)-Camphor	7.65	Ricinoleic acid	0.38
patchoulane	2.20	Cis-vaccenic acid	0.34
Carvone	32.55	Ethyl Oleate	0.56
3-Thujanone	0.61	Total identified	100

Table 2: Organoleptic evaluation of pan bread sprayed crust by essential oil dill seeds.

Characteristics	Crust color (15)	Distribution of crumb (15)	crumb color (15)	Taste (20)	Flavor (20)	General appearance (15)	Total score (100)
Control	$14.25^{a}\pm 0.5$	$14.38^{a}\pm0.48$	14.59^{a} ±0.11	$18.75^{a}\pm0.50$	$18.11^{b}\pm0.44$	$14.00^{a}\pm0.81$	$94.08^{b}\pm0.92$
Different levels of essential oil dill seeds sprayed to the pan bread crust (ml)							
1	$14.38^{a}\pm0.94$	$14.39^{a}\pm0.05$	$14.60^{a}\pm0.09$	$18.77^{a}\pm0.98$	$18.36^{ab}\pm1.12$	$14.13^{a}\pm0.85$	$94.63^{b}\pm3.68$
1.5	$14.74^{a}\pm0.10$	$14.42^{a}\pm0.10$	$14.59^{a}\pm0.13$	$19.41^{a}\pm0.28$	$18.56^{ab}\pm0.51$	$14.19^{a}\pm0.04$	$95.91^{ab}\pm0.45$
2	$14.85^{a}\pm0.09$	$14.43^{a}\pm0.09$	$14.60^{a}\pm0.12$	$19.71^{a}\pm0.10$	$19.66^{a}\pm0.23$	$14.22^{a}\pm0.09$	$97.47^{a}\pm0.44$
2.5	$14.52^{a}\pm0.36$	$14.41^{a}\pm0.01$	$14.61^{a}\pm0.11$	$19.22^{a}\pm0.92$	$18.39^{ab}\pm0.45$	$14.18^{a}\pm0.14$	$95.33^{ab}\pm1.25$
3	$12.31^{b}\pm0.69$	$14.42^{a}\pm0.10$	$14.58^{a}\pm0.19$	$10.31^{b}\pm1.51$	$12.03^{c}\pm1.78$	$14.23^{a}\pm0.08$	$77.89^{c}\pm1.30$

Means in the same column with different letter are significantly different ($P \leq 0.05$).

Evaluation of Antimicrobial effect of dill seeds essential oils

The antimicrobial activity of dill seeds essential oils at 100 µl/well against selected fungi , *Aspergillus niger* (RCMB 002005), *Aspergillus flavus* (RCMB 002002), *Penicillium expasum* RCMB 001001 (1)IMI 28169 and *Penicillium italicum* RCMB 001018 (1)IMI 193019) , Gram positive bacteria (*Staphylococcus aureus* (RCMB 010010) , *Bacillus cerues* RCMB 027 (1) and *Bacillus subtilis* RCMB 015 (1) NRRL.B- 534) , Gram negative bacteria (*Escherichia coli* (RCMB 010052) ATCC 25955 and *Salmonella typhimurium* RCMB 006 (1) ATCC 14028), are summarized in Table (3).

The results revealed that the dill seeds essential oils showed antimicrobial activity with varying magnitudes compared to positive control. Dill seeds oil was recorded highest action against fungi (*Aspergillus flavus*, *Aspergillus niger*, *Penicillium expasum* and *Penicillium italicum*) the inhibition zone diameter were recorded 60,70, 34 and 33 mm, respectively comparing with control which containing 15,16,17 and 18, respectively Ketoconazol 100µg/ml showed lowest effective in inhibition zone diameter. Also Dill seeds oil was recorded 80, 73 and 38 mm, respectively against selected Staphylococcus aureus, Bacillus cerues, Bacillus subtilis as Gram- positive bacteria comparing with control which containing 24,25 and 26, respectively Gentamycin 4µg/ml . While Dill seeds oil was recorded 78 mm for *Escherichia coli* and 66 mm for *Salmonella typhimurium as Gram-negative bacteria* .whereas, control contained 30 and 17, respectively Gentamycin 4µg/ml. Elgayyar *et al.,* (2001) who reported that the Dill seeds oil was strongly effective against *Staphylococcus aureus, Escherichia* coli, Yersinia enterocolitica, Geotrichum candidum and Rhodotorula glutinis with inhibition zone of 36-69 mm and moderately effective against *Salmonella typhimurium* with inhibitory zone of 26 mm. Dill oil was weakly effective against *Aspergillus niger* with inhibitory zone of 12 mm. Tian *et al.,* (2012) reported that the antifungal activity of dill oil results from its ability to

disrupt the permeability barrier of the plasma membrane and from the mitochondrial dysfunction-induced reactive oxygen species (ROS) accumulation in *A. flavus* , dill oil can be a potential source of eco-friendly antifungal drugs and food preservatives.

Table 3: Antimicrobial activity of Dill seeds essential oil using agar well diffusion method, diameter (mm) of inhibition zone.

Type of strain	Antimicrobial activity of the essential oils on inhibition zone diameter (mm)	
	dill seeds oil	positive control
Fungi		***KETOCONAZOL***
Aspergillus niger (RCMB 002005)	60	15
Aspergillus flavus (RCMB 002002)	70	16
Penicillum expansum RCMB 001001 (1)IMI 28169	34	17
Penicillum italicum RCMB 001018 (1)IMI 193019	33	18
Gram positive bacteria		***GENTAMYCIN***
Staphylococcus aureus (RCMB 010010)	80	24
Bacillus cereus RCMB 027 (1)	73	25
Bacillus subtilis RCMB 015 (1) NRRL.B- 534	38	26
Gram-negative bacteria		***GENTAMYCIN***
Escherichia coli (RCMB 010052) ATCC 25955	78	30
Salmonella typhimurium RCMB 006 (1) ATCC 14028	66	17

Total bacterial counts, yeast and mold cells of spraying (2 ml) dill deeds essential oil on pan bread during storge for 6 days:

The effect of spraying (2 ml) essential oil of dill on pan bread on total bacteria counts, yeast and mold during storage for 6 days at room temperature (25±2C°) was illustrated in Table (4). The data showed that no any detected microbial spoilage in control and pan bread sprayed with (2 ml) essential oil of dill at zero time.

After three days of storage at room temperature, a slight increase in total bacterial count in control, it was recorded (5×10^2 CFU/g), meanwhile, no appears in microbial spoilage and the total bacterial count was found in pan bread samples. After fifth days of storage the control pan bread showed more increase in total bacterial counts was detected (23×10^2 CFU/g) and meanwhile, results showed a slight appears (2×10^2 CFU/g) was found in sprayed pan bread samples. Also total bacterial counts were increased through sixth days in control while little increase was found in pan bread sprayed with (2 ml) essential oil of dill seeds. The decrease in total bacterial counts may be due to the addition of sprayed (2 ml) essential oil of dill which contains high antioxidants which acts as preservative substances and increase shelf life in pan bread. Antimicrobial activity of dill seeds essential oil probably originates from carvone, having in mind literature data about antimicrobial effect of carvone on a large number of bacteria and fungi can be noticed (Singh *et al.,* 2005). Adeniyi *et al*., (2010) reported that the uses of the higher concentration of the preservations guide to the longer shelf life of the product.

Data in Table (4) showed that, mold and yeast were absent after first and second days in all treatments and control. A gradual increase of yeast and molds was found in control sample with increasing storage time up to 6 days which riched 11×10^2 CFU/g in six days of storage, meanwhile pan bread samples sprayed with 2ml essential oil still free from molds and yeasts till six days of storage. This result are in agreement with those reported by Krisch *et al.* (2011) who reported that essential oils represent a natural, effective, and consumer-accepted tool against food spoilage caused by yeast and moulds.

Table 4: Effect of storage on total counts, yeast and molds of pan bread by sprayed (2 ml) dill seeds essential oils.

Pan bread	Time (day)	Total counts $\times 10^2$ CFU/g	Yeast and Molds $\times 10^2$ CFU/g
Control	zero	N.D	N.D
	1	N.D	N.D
	2	N.D	N.D
	3	5	7
	4	16	8
	5	23	9
	6	35	11
Samples	Zero	N.D	N.D
	1	N.D	N.D
	2	N.D	N.D
	3	N.D	N.D
	4	N.D	N.D
	5	2	N.D
	6	4	N.D

N.D: Not detected growth of microorganism

Conclusion

Dill seeds essential oils (EOs) are natural preservative; these oils can be successfully used to enhance the shelf life of pan bread. Essential oils can be help to improve the flavour and aroma of the (bakery products) pan bread along with its shelf life. Also, it has a strong antimicrobial which can be play a significant role in overcoming storage losses and in enhancing food shelf life**.**

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