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Impact of Antimicrobial Properties of Some Essential Oils on Cheese Yoghurt Quality

[1]Sahar H.S. Mohamed, [1]Wafaa M. Zaky, [1]Jihan M. Kassem,
[1]Hayam M. Abbas, [1]M.M.E. Salem and [2]H.A.H. Said-Al Ahl

[1]Department of Dairy, National Research Centre, Dokki, Giza, Egypt
[2]Department of Medicinal and Aromatic Plants, National Research Centre, Dokki, Giza, Egypt

Abstract: Cheese yoghurt samples were prepared by using some essential oils as natural anti-microbial agents. Seven various essential oils were extracted from different plant sources and screened for their anti-microbial effect against five strains of gram positive and negative pathogenic bacteria strains. Obtained results indicated that caraway as well as dill seeds oil had the highest anti-bacterial effect. The minimum inhibitory concentrations (MICs) of the two selected oils were determined. A patch of cheese yoghurt was manufactured from milk which previously contaminated by the five strains of pathogens and was incorporated by the two selected essential oils; then stored at 7°C±2 for 14 days. All fresh and stored contaminated cheese yoghurt samples were periodically assayed for their microbiological examinations after 0, 7 and 14 days of cold storage. Data indicated that gram-positive bacteria were more susceptible than gram-negative. The most sensitive microorganism was *Listeria monocytogenes* which affected by all the seven oils, while the most resistant microorganism: was *E. coli* O157:H7. It was observed that the two essential oils did not affect the growth of both starter cultures (*Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophilus*) in contaminated cheese yoghurt samples compared with control one. Fresh curd weight and whey drainage of uncontaminated cheese yoghurt samples were determined as well as the progress in their titratible acidity and pH values through 14 days of cold storage. It could be notice that, control cheese yoghurt drainage more whey either after 2 hrs or after 24 hrs of hanging and it had less weight. The experimental samples fortified with essential oils seemed softer than control. Thus we recommend that, caraway and dill essential oils could be successfully used as natural and safe anti-pathogens source in production of cheese yoghurt.

Key words: Caraway · Cheese yoghurt · Dill · Essential oils · Pathogens

INTRODUCTION

Food processors, food safety researchers and regulatory agencies have been increasingly concerned with the growing number of food-borne illness outbreaks caused by pathogens like *Staphylococcus aureus*, *Salmonella* sp., *Clostridium perfringens*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus* and entero-pathogenic *Escherichia coli* [1, 2]. These bacteria cause over 90% of all cases of food poisoning. Infections due to bacterial species also remain a serious clinical problem. Emerging resistance of bacterial species is seriously decreasing the number of effective antimicrobials. Because of increasing consumers need and legal authorities; food industries has tended to reduce the use of chemical preservatives in their products to either completely nil or to adopt more natural alternatives for the maintenance or extension of product shelf life [3]. Plants and their essential oils (EOs) are potentially useful sources of antimicrobial compounds. Numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microbes, including food-borne pathogens. Friedman *et al.* [2] Ranèiæ *et al.* [4],

Corresponding Author: Hayam M. Abbas, Department of Dairy, National Research Centre, Dokki, Giza, Egypt.

Mehdizadeh *et al.* [5] and Elaissi *et al.* [6] also mentioned that spices and medicinal plants are widely used as raw materials for pharmaceutical preparations as a supplement for dietetic products, especially for "self medications" in public spices having essential oils which exhibit antimicrobial effects. Weerakkody *et al.* [7] indicated that the clove, cinnamon, oregano, rosemary and dill are considered as the most common spices and herbs with strong antimicrobial activity. They added that their essential oils containing chemical compounds such as carvacrol, cinnamaldhyde, eugenol and camphor which are identified as the major chemical components responsible for exerting antimicrobial activity. Some scientists confirmed the antimicrobial activity of essential oils from oregano, thyme, sage, rosemary, clove, coriander, garlic and onion against both bacteria and molds. The composition, structure, as well as functional groups of the oils play an important role in determining their antimicrobial activity [8, 9, 10].

Most of the antimicrobial activity in EOs is found in the oxygenated terpenoids (e.g., alcohols and phenolic terpenes), while some hydrocarbons also exhibit antimicrobial effects [11, 12]. Interactions between these components may lead to antagonistic, additive or synergistic effects. Some studies have demonstrated that whole EOs usually have higher antibacterial activity than the mixtures of their major components, suggesting that the minor components are critical to the synergistic activity; though antagonistic and additive effects have also been observed [13, 14]. Caraway seeds (*Carum carvi* L.) have an economical importance; it contains several components, carvone and limonene are the main components available in their oil [15]. In recent years the scientific literature reports pharmacological effects of dill such as antibacterial [16, 17], antimycobacterial [18], antioxidant [19, 20], cancer chemopreventive [21]. The well-known properties of dill from the traditional medicine, such as carminative, stomachic, diuretic have been reported by Hosseinzadeh *et al.* [22] and Amin and Sleem [23]. Dill essential oil has hypolipidemic activity and could be a cardioprotective agent [24]. Cheese yoghurt is a traditional fermented milk product. It is a popular food in various parts of the world especially in the Middle East regions where it plays a significant role in the family diet. It has increased in popularity during the last decade. Its perceived nutritional benefits and storage characteristics have led to its increasing economic importance [25].

Therefore, the fundamental goal of this research is studying the antibacterial effect of the extracted essential oils from some natural plant sources. Then; use their essential oil extracts, which had the highest antibacterial effect for preparing cheese yoghurt. Evaluation of some chemical properties as well as microbiological examinations of the resultant product was also aim of this study.

MATERIALS AND METHODS

Materials

Plant Materials: Seven plant essential oils were used to study the activity against the pathogenic bacteria. The tested plants were: dill (*Anethum graveolens*), caraway (*Carum carvi*), coriander (*Coriandrum sativum*) belonging to the family *Apiaceae*, whereas basil (*Ocimum basilicum*) and lemon balm (*Melissa officinalis*) belonging to family *Lamiaceae*. These were obtained from Medicinal and Aromatic Plants Department, National Research Centre, Egypt.

Milk: Fresh full fat buffalo's milk was obtained from Faculty of Agriculture, Cairo University.

Bacterial Strains: Five pathogenic bacterial strains were obtained from Microbiological Resources Center (Cairo MIRCEN): *Listeria monocytogenes* (EMCC 1875); *Staphylococus aureus* (ATCC13565) *Bacillus cereus* (EMCC1080), used as gram- positive bacteria. *Escherichia coli* O157:H7 (ATCC51659) and *Salmonella typhimurium* (ATCC 25566) used as gram- negative. *Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophilus* obtained from Chr. Hansen Denmark were used as starter culture for cheese yoghurt preparation.

Methods

Essential Oils Extraction and (GC/MS) Analysis: Hydro distillation in Clevenger apparatus has been used for essential oil extraction. About 100 g seeds of dill, caraway and coriander or herbs of all tested plants were set with distillated water enough to cover the plant material; the process of hydro distillation tasted 3 hours according to Guenther [26]. The essential oil of each dill or caraway seeds was dehydrated over anhydrous sodium sulphate and stored at -20°C till GC/MS analysis. GC/MS analysis was performed separately with a Hewlett Packard model 5890. Gas chromatograph equipped with 5 series Mass selective detector 9144 (HP). The column used was SE-54 (30 m X 0.25 mm i.d.). The oven temperature was maintained at 60°C for 2 min after injection and then programmed at 5°C min-1 to 270°C. The injector temperature was 270°C and MS conditions were kept at

280°C and 42ev. Compounds were identified by matching of their mass spectra with those recorded in the MS library and further confirmed by comparison of the mass spectra with those of reference compounds or with published data.

Growth Conditions of Pathogenic Bacteria: Stock cultures of *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus, Escherichia coli* O157:H7 and *Salmonella typhimurium* were sub-cultured twice onto Tryptone Soya Agar (TSA) followed by incubation at 37°C. Working cultures were prepared from subcultures and grown overnight in Tryptone Soya Broth medium (TSB, Oxoid, Basingstoke, UK) under optimal conditions for each microorganism. Cocktail bacterial cultures obtained of different strains of each pathogens microorganism were used in application concerning the fate of the inoculated pathogens on cheese yoghurt.

Antimicrobial Assay Using the Disc Diffusion Method: Screening of the seven essential oils (EOs) was studied of each antimicrobial activity on (TSA) at 37°C. Each one of antimicrobial EOs was tested undiluted as well as diluted in Tween 20 at concentrations of 25, 50 and 75ml/100 ml; the amount of undiluted EOs added to each filter paper disc was 10µl, while 20 and 60µl were added from diluted EOs. The concentration of bacteria inoculated in TSA was $2x10^6$ CFU/ml with inoculated volume (0.1 ml). All experiments were performed in duplicate. Sterilized filter paper discs (Whatman No1, 6mm in diameter) were placed on the surface of TSA. The inhibition zone diameter was measured (including the filter paper disc) using Vernier Calipers and expressed in millimeter.

Minimum Inhibitory Concentration (MIC) Assay: Based on the previous screening essential oils; caraway and dill seeds oils were determined for their Minimum Inhibitory Concentrations (MICs) by using agar dilution methods [27]. Series dilutions of each oil, ranging from 0.001 to 0.007 ml/ml were prepared in Nutrient Agar. Plates were dried at room temperature for 30 min prior to spot inoculation with 0.1 ml of culture containing approximately 10^5 CFU/ml of each organism. Inoculated plates were incubated at 37°C for 18 hrs and the MIC was determined. Experiments were carried out in duplicate. Inhibition of bacterial growth in plates containing the tested oil was judged by comparison with growth in blank control plates. The MICs were determined as the lowest concentration of oil inhibiting visible growth of each organism on agar plate [12].

Cheese Yoghurt Preparation: Fresh buffaloes milk was heated, (90°C/20 min.), cooled to 40°C, inoculated with 2% yoghurt starter then contaminated with a cocktail of the five strains of pathogenic bacterial cultures obtained by mixing the same population (≈ 10^6 CFU/ml) of the different strains of each microorganism. The inoculated milk was divided into three equal portions. The first portion served as control (free from EOs), second and third portions were fortified with 0.005 and 0.003 ml/ml of dill and caraway essential oils respectively. All portions incubated at 40°C till complete coagulation. The plastic containers stored at (7°C±2) for 14 days. Another separated uncontaminated samples also fortified with EOs were prepared for chemical analysis. Three replicates were conducted.

Analytical Procedures

Microbiological Examination of Cheese Yoghurt: Microbiological assessments were carried out for all cheese yoghurt samples. The pathogenic bacteria as well as TVC and starter culture was accounted in contaminated samples after 0, 7 and 14 days of cold storage (shelf life of cheese yoghurt according to Egyptian – Standard, ES: 3157-2006 and 2613-2006/2). The pathogenic bacteria were detected using Listeria Selective Agar medium for *Listeria monocytogenes*, Sorbitol MacConky Agar Medium for *Escherichia coli* O157:H7, Baird Parker Agar for *Staphylococcus aureus*, Manitol Egg Yolk Polymxin Agar (MYPA) for *Bacillus cereus* and Salmonella and Shigella Agar Medium for *Salmonella typhimurium*. The total bacterial count was accounted by using Plate Count Agar Medium. The count of starter cultures was carried out on MRS medium for *Lactobacillus delbrueckii* subsp *bulgaricus* and M17 for *Streptococcus thermophilus*.

Chemical Analysis: Whey drainage during hanging and fresh curd weight were estimated of uncontaminated samples. Total solids (TS), total protein (TP) and Fat contents of fresh samples were also determined [28]. Stored cheese yoghurt samples were periodically analyzed after 0, 7 and 14 days of cold storage for their titratible acidity (TA %) [28]. the pH values were measured using a digital laboratory pH meter (HI 93 1400, Hanna instruments) with glass electrode.

RESULTS AND DISCUSSION

Growth Inhibition of Some Pathogens by Eos: The tested seven essential oils showed various degrees of inhibition against the five bacterial strains using the disc diffusion

method as presented in Table 1. Essential oils had an enhanced inhibitory effect in descending order: Caraway seeds oil (inhibited four strains); dill seeds or herb and coriander herbs oils (inhibited three strains); coriander seeds, basil and lemon balm herbs oils (inhibited two strains).The results showed that gram-positive bacteria were more susceptible than gram negative. The most sensitive microorganism was *Listeria monocytogenes* which affected by all the seven oils, followed by *Salmonella typhimurium* which affected by three oils. The most resistant microorganism: in this study; was *E. coli* O157:H7 which affected by dill and coriander herbs oils only. Most studies investigated the action of whole EOs against food spoilage organisms and food borne pathogens reported that, generally, EOs are slightly more active against gram-positive than gram-negative bacteria [12, 29-32]. Gram-negative organisms are less susceptible to the antibacterial action is perhaps to be expected, since they possess an outer membrane surrounding the cell wall [33], which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering [34, 35]. An important characteristic of EOs and their components is their hydrophobicity, which enables them to partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable [36]. Leakage of ions and other cell contents can then occur [37-40].

Effect of Diluted Essential Oils on Pathogenic Bacteria: Essential oils which had the most inhibitory effect against the tested bacterial strains; were selected for determining their inhibitory action diluted in Tween 20 obtaining concentrations of 25, 50 and 75 ml/100 ml using two volumes 20 and 60µl. Table 2 shows the lowest concentrations of two selected caraway and dill seeds oils against the pathogenic bacteria tested by the disc diffusion assay. The results showed that by increasing the volume or concentrations of EOs, the antimicrobial activity was increased. Caraway and dill seeds oils with the minimum concentration of 25 ml/100 ml had an inhibitory action against *Staphylococcus aureus* and *Salmonella typhimurium* using both volume 20 and 60µl. While, caraway and dill seeds oils at concentration of 25 ml/100 ml and volume 20µl had no effect on *Listeria monocytogenes, Escherichia coli* O157:H7 and *Bacillus cereus*. Increasing the volume of caraway and dill seeds oils to 60µl lead to increase the antimicrobial effect on the previous bacteria.

Minimum Inhibitory Concentrations (MICs) Assay: Based on the disc diffusion studies; dill and caraway seeds oils were selected for further studies in cheese yoghurt preparation. Since in disc diffusion experiment the different bacterial strains of *Listeria monocytogenes, Escherichia coli* O157:H7, *Staphylococcus aureus, Bacillus cereus* and *Salmonella typhimurium* responded differently to the action of the essential oils, a cocktail of bacterial cultures was used for the application of essential oils in cheese yoghurt. For practical application of essential oils in cheese yoghurt, the minimum inhibitory concentrations (MICs) of the two selected seeds oils (dill and caraway) were determined. The concentration was ranged from 0.001 to 0.007 ml/ml and the results are illustrated in Table 3. The results revealed that caraway seeds oil with 0.003 ml/ml showed maximum activity values of MICs followed by dill seeds oil with MICs values 0.005 ml/ml against all the tested strains.

Effect of Using Essential oil on the Microbiological Quality of Cheese Yoghurt: Caraway and dill seeds essential oils had the same ability to inhibit the growth of all pathogens in cheese yoghurt samples stored at 7°C±2. The two essential oils were added at concentrations of 0.003 and 0.005 ml/ml caraway & dill, respectively. Caraway and dill were the most inhibitor to *L. monocytogenes* and *S. typhimurium*, they were not detected after 7 or 14 days in samples inoculated with 10^6 CFU/g and stored at 7°C±2 (Table 4). Caraway inhibited *S. aureus* by 1.3-log cycle after 7 days and by 4-log cycle after 14 days, while *E. coli* O157:H7 was inhibited by 1.7-log cycle after 7days and it was not detected at 14 days of storage. The results showed that dill was able to inhibit *E. coli* O157:H7 by1.1-log cycle during 7 days of storage at 7°C±2 and it was not detected at 14 days, while *S. aureus* was inhibited by 0.9 and 1.5-log cycle after 7 or 14 days respectively. Dill and caraway showed bactericidal action at the end of storage period (14 days). Nissen *et al.* [41] mentioned that α-pinene in caraway had a broad-spectrum antibiotic. Tajkarimi *et al.* [42] reported that α-thujene, α-pinene, sabinene are also responsible for the antibacterial and antifungal activity of spices and herbs. While, Bagdassarian *et al.* [43] indicated that solvent extracts of dry seeds from Apiaceae family to which belong: fennel, dill, anise, caraway and coriander are rich in phytochemical contents which possessed high antioxidant and antimicrobial activities. Sokovic *et al.* [3] observed that the antibacterial potential of tested

Table 1: Zone of inhibition growth of pathogenic bacteria by essential oils

	Inhibition zone diameter (mm)[a]				
Essential oils	*L. monocytogenes*	*E. coli* O157:H7	*S. aureus*	*B. cereus*	*S. typhimurium*
Caraway (seeds)	22.0	n.i.	21.7	21.0	13.0
Dill (seeds)	13.0	n.i.	n.i.	15.0	15.0
Coriander (seeds)	13.3	n.i.	n.i.	14.7	n.i.
Dill (herb)	13.0	42.7	n.i.	n.i.	12.7
Coriander (herb)	29.3	29.0	n.i.	n.i.	11.7
Basil (herb)	11.3	n.i.	13.0	n.i.	n.i.
Lemon balm (herb)	15.0	n.i.	18.7	n.i.	n.i.

[a] Obtained by adding 10µl of each undiluted antimicrobial substance. The diameter of the filter paper disc (6 mm).

[n.i] No zone of inhibition observed.

* Three replicates were conducted

Table 2: Zone of inhibition growth of pathogenic bacteria obtained by the lowest concentrations 25, 50 or 75 ml/ 100 ml) of the two selected essential oils

		Inhibition zone diameter (mm)[a]									
		L. monocytogenes		*E. coli* O157:H7		*S. aureus*		*B. cereus*		*S. typhimurium*	
Essential oils	Con. ml/100ml	20	60	20	60	20	60	20	60	20	60
Caraway (seeds)	25	n.i.	23.0	n.i	14.6	10.0	31.6	n.i.	19.0	14.3	30.0
	50	12	27.0	9.6	14.4	13.3	34.3	11.0	26.6	22.3	32.0
	75	21	38.0	13	36.6	24.0	39.6	16.3	30.0	22.6	33.0
Dill (seeds)	25	n.i.	14.0	n.i.	14.7	13.7	34.7	n.i.	16.3	14.0	12.7
	50	n.i.	18.0	n.i.	17.0	21.3	45.0	11.3	14.0	13.6	13.0
	75	10.7	23.3	22.7	18.7	23.7	38.3	20.0	27.7	13.0	22.0

[a] Obtained by adding 20 or 60 µl of each diluted essential oils.

[n.i] No zone of inhibition observed. * Three replicates were conducted

Table 3: The minimum inhibitory concentrations (MICs) of the two selected essential oils on pathogenic bacteria

Essential oils	Con.ml/ml	*L. monocytogenes*	*E. coli* O157:H7	*S. aureus*	*B. cereus*	*S. typhimurium*
Caraway (seeds)	Control[a]	++++	++++	++++	++++	++++
	0.001	+++ [b]	+++	+++	+++	+++
	0.003	- [c]	-	-	-	-
	0.005	-	-	-	-	-
	0.007	-	-	-	-	-
Dill (seeds)	Control	++++	++++	++++	++++	++++
	0.001	+++	+++	++	+++	+++
	0.003	++	++	--	+++	+++
	0.005	-	-	-	-	-
	0.007	-	-	-	-	-

[a] Growth on medium without essential oils.

[b] The present of growth.

[c] The absent of growth.

essential oils' components can be presented as: linalyl acetate < limonene < β-pinene < α-pinene < camphor < linalool < 1, 8-cineole < menthol < thymol < carvacrol. In control samples of cheese yoghurt, a decrease of the inoculated population was observed during storage at 7°C±2 (Table 4). Generally, the susceptibility of bacteria to the antimicrobial effect of EOs also appeared to increase with a decrease in the pH of the food, the storage temperature and the amount of oxygen within the packaging [44, 45]. At low pH the hydrophobicity of EOs increases, enabling it to more easily dissolve in the lipids of the cell membrane of target bacteria [46].

The effect of the two essential oils on population of starter cultures in cheese yoghurt samples made by contamination with pathogenic bacteria (stored at 7°C±2 during 14 days) was illustrated in Table 5. The results

Table 4: The effect of adding the two selected essential oils in cheese yoghurt samples stored at 7°C±2 for 14 days on the bacterial pathogens count (CFU/g)

Microorganisms	Storage period (days)	Control[a]	Caraway *	Dill **
L. monocytogenes	Zero	6.0	6.0	6.0
	7	5.0	- [b]	-
	14	3.4	-	-
E. coli O157:H7	Zero	6.0	6.0	7.1
	7	5.0	4.3	6.0
	14	4.3	-	-
S. aureus	Zero	6.3	7.3	7.9
	7	5.0	6.0	7.0
	14	4.3	3.3	6.4
B. cereus	Zero	6.0	6.0	6.0
	7	7.6	-	5.0
	14	5.0	-	-
S. typhimurium	Zero	6.0	6.0	6.0
	7	5.0	-	-
	14	3.4	-	-

[a] Control without added essential oils. [b] Absent of pathogenic bacteria.

* Caraway oil conc. was 0.003 ml/ml ** Dill oil conc. was 0.005 ml/ml.

Table 5: The viable cell counts (log_{10} CFU/g) of starter culture and total bacterial counts in cheese yoghurt contaminated with pathogenic bacteria and stored at7°C±2 for 14 days

Essential oils	Storage period (Days)	*S. thermophilus*	*L. bulgaricus*	TVC[b]
Control [a]	Zero	7.7	6.3	7.7
	7	7.9	7.8	7.9
	14	7.1	6.5	9.3
Dill (seeds)	Zero	7.5	7.4	7.1
	7	8.9	8.6	8.5
	14	8.5	8.6	9.3
Caraway (seeds)	Zero	7.2	6.5	7.8
	7	7.9	7.5	8.2
	14	7.3	7.2	9

[a] Control made without essential oils and within pathogenic bacteria

[b]TVC: Total bacterial viable counts

* Three replicates were conducted

Table 6: Chemical compounds (%) identified in essential oil of *Carum carvi* and *Anethum graveolens* seeds

Compounds (%)	*Carum carvi* (Caraway)	*Anethum graveolens* (Dill)
α-pinene	8.29	-
α-phellandrene	1.20	-
Limonene	15.55	20.64
Carvone	74.40	64.59
Dihydrocarvone	-	1.95
Dillapiol	-	12.04
Identified compounds	99.44	99.22
Unidentified compounds	00.56	00.78

Table 7: The amount of whey drainage (ml) and fresh curd weight (kg) resultant from 4 kg fresh milk

Items	Control	E_1	E_2
Whey after 2 hrs (ml)	1310	1220	1250
Whey after 24 hrs (ml)	1100	950	1000
Total whey Drainage (ml)	2400	2170	2250
Curd weight after complete drainage (Kg)	1.250	1.350	1.400

E_1: cheese yoghurt fortified with dill oil, E_2: cheese yoghurt fortified with caraway oil

* Three replicates were conducted

revealed that essential oils did not affect the growth of both starter cultures (*Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophilus*) in contaminated cheese yoghurt compared with control. The lactic acid bacteria are gram positive fermentative bacteria which are resistant to essential oils [11]. A small difference in lactic acid bacteria count was observed between the control and experimental samples. This is in agreement with Vinderola *et al*. [47], who reported that lactic acid starter and probiotic bacteria growth was not affected by vanilla, banana and strawberry added to pure cultures. Total bacterial viable counts (TVC), data in Table 5 indicated that in all cases, the respective counts increased gradually up to the end of storage. These results are in agreement with those reported by Khaleel [48] and El-Nawawy *et al*. [49].

Chemical Compounds Identified in Essential Oils of Caraway and Dill Seeds: Data presented in Table 6 reflected the main constituents of the two selected essential oils isolated from caraway and dill seeds. This table reveals that carvone is the major component in the two oils where it represented 74.40% and 64.59% in caraway and dill oil respectively. Limonene came in the second order where dill had 20.64% followed by caraway which had 15.55%. Carvon is aliphatic compound which plays an anti-oxidant role and has an important therapeutic action [11]. Alpha-pinene was represented about 8.29% in caraway seeds oil followed by α-phellandrene, while they were absent in dill seed oil. Caraway is an annual and herbaceous plant which is rich in carvon [50]. Koppula *et al*. [51] mentioned that the main component of caraway is carvon (40-60%) followed by limonene and carveol. They added that the aqueous extracts of caraway had an adaptogenic and nootropic activities as well as they had an ability to inhibit lipid peroxidation in liver & brain. Al-Asmail *et al*. [52] reported that water-extract of dill seeds, showed higher anti-oxidant activity which is mainly due to the presence of phenolic compounds. In 2010 Stankeviciws [53] reported that the highest content of phenolic compounds was found in dill extract when it compared with celery, onion and parsley as well as it had the highest radical scavenging. However, Sedlakova *et al.* [50], Gwari *et al*. [54] and Meshkatasadat *et al.* [15] determined the main compounds & aroma profile of caraway EOs from species, while Samojlik *et al.* [55] studied the influence of caraway EOs on pharmacokinetic of paracetamol.

Properties of Cheese Yoghurt Samples

Whey Drainage and Curd Weight: Data in Table 7 reflected amount of whey drainage and the yield of fresh cheese yoghurt-curd of both control (C) and experimental cheese yoghurt samples fortified with dill seeds oil (E_1) and that fortified with caraway seeds oil (E_2). It could be notice that control cheese yoghurt curds drainage more whey either after 2 hrs or after 24 hrs of hanging and they had less weight (1.250 kg). However, experimental cheese yoghurt (E_1) drainage less whey and had 1.350 kg against 1.400 kg for E_2- cheese yoghurt sample. Foda *et al*. [56] reported that Herby-White- cheese fortified with spearmint essential oil contained high moisture and had softer structure than control. They explained this observation that the presence of EOs in cheese matrix enhanced the enzymatic activity and so, produced softer structure. They also added that herbs increased the binding water and herby cheese had less casein aggregation. Mocanu *et al*. [57] added that synersis is influenced by the product composition, especially titratible acidity. The synersis is more intense for sample with higher titratible acidity.

Main Chemical Composition of Fresh Cheese Yoghurt: Table 8 shows the main gross composition of fresh cheese yoghurt samples which fortified with dill and caraway essential oils. It is obvious that TS content of control was higher (28.15%) than E_1 (21.68%) or E_2 (24.57%). Subsequently fat and protein contents of control were also higher than the experimental cheese yoghurt samples These data confirmed the data presented in Table 7, which showed that samples contained essential oils drainage less amount of whey and higher weight than control one.

Values of pH and Titratible Acidity of Cheese Yoghurt Samples During Storage Period: Data presented in Table 9 revealed that pH values and titratible acidity (%) of both control and experimental samples seemed to be nearly the same at zero time. No clear differences were observed during storage period where control samples possessed slightly increasing acidity (less pH) followed by E_1 then E_2. It was observed that TA% increased gradually during storage period. The trend of the changes in pH values of all treatments was opposite to that of acidity. Al-Otaibi and El-Demerdash [58] observed that thyme, marjoram and sage had a stimulatory effect on the starter culture and total viable counts so, titratible acidity was increased in labneh samples fortified with EO. Mortiz *et al*. [59]

Table 8: Proximate analysis of fresh cheese yoghurt samples

Components (%)	Control	E_1	E_2
TS	28.15	21.68	24.57
Fat	13.00	10.50	11.00
Fat/DM	46.18	48.43	44.77
Protein	9.64	6.99	7.88
Protein/DM	34.25	32.24	32.07

E_1: cheese yoghurt fortified with dill oil, E_2: cheese yoghurt fortified with caraway oil

* Three replicates were conducted

Table 9: Values of pH and titratible acidity (%) of cheese yoghurt samples during storage period.

	Control		E_1		E_2	
Storage period (days)	pH	TA	pH	TA	pH	TA
Zero	4.33	1.63	4.36	1.60	4.34	1.61
7	4.19	1.73	4.15	1.69	4.10	1.68
14	4.10	1.78	4.08	1.73	4.06	1.72

E_1: cheese yoghurt fortified with Dill oil, E_2: cheese yoghurt fortified with Caraway oil

TA: Titratible acidity (%). * Three replicates were conducted

mentioned that cinnamon-EO had antimicrobial activity against *L. rhamnosus* (as starter culture), which interfering with lactic acid production in the fermented milk, however Shori and Baba [60] reported that herbal water extracts does not affect the viability of lactic acid bacteria in bio-yogurts. Salem *et al*. [61] concluded that dry leaves of *Moringa oleifera* had stimulatory effect on the starter culture. It can be concluded that the effect of the EOs on the viability of starter culture depended upon the type of microorganism and essential oil as well as their concentrations [11].

CONCLUSION

It could be concluded that caraway as well as dill seeds oils had the highest anti-bacterial effect against the five tested pathogenic bacteria among all the tested essential oils. The most sensitive microorganism was *Listeria monocytogenes* which affected by all the seven oils, while the most resistant microorganism was *E. coli* O157:H7. It was observed that essential oils did not affect the growth of starter cultures in cheese yoghurt samples. Control cheese yoghurt samples drainage more whey either after 2 hrs or after 24 hrs of hanging and it possessed less weight than the experimental samples fortified with essential oils. The experimental samples had low total solids as well as fat and total protein contents. No clear differences were observed in titratible acidity or pH values of control or experimental samples during 14 days of cold storage. Subsequently; it could be recommended that caraway and dill oils can be used as natural and safe anti-microbial substances in cheese yoghurt.

REFERENCES

1. Wilson, C.L. and G.G. Droby, 2000. Microbial Food Contamination; CRC Press: Boca Raton, FL, USA, pp: 149-171.
2. Friedman, M., R.P. Henika and E. R. Mandrell, 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica*. Journal of Food Protection, 65: 1545-1560.
3. Soković, M., J. Glamoèlija, P. D. Marin, D. Brkić and L.J.L.D. van Griensven, 2010. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an *in vitro* model. Molecules, 15: 7532-7546
4. Rancic, A., M. Sokovic, J. Vukojevic, A. Simic, P. Marin, S. Duletic-Lauševic and D. Đokovic, 2005. Chemical composition and antimicrobial activities of essential oils of *Myrrhis odorata* (L.) Scop, *Hypericum perforatum* L. and *Helichrysum arenarium* (L.) Moench. Journal of Essential Oil Research, 17: 341-345.

5. Mehdizadeh, T., H. Tajik, S.M.R. Rohani and A.R. Oromiehie, 2012. Antibacterial, antioxidant and optical properties of edible starch-chitosan composite film containing *Thymus kotschyanus* essential oil. Veterinary Research Forum., 3(3): 167-173.
6. Elaissi, A., K. Salah, S. Mabrouk, S. Larbi, K. Chemli and F. Harzallah-Skhiri, 2011. Antibacterial activity and chemical composition of 20 *Eucalyptus* species' essential oils. Journal of Food Chemistry, 129: 1427-1434.
7. Weerakkody, N., N. Caffin, L. Lambert, M. Turnera and G. Dykes, 2010. Synergistic antimicrobial activity of galangal (*Alpinia galanga*), rosemary (*Rosmarinus officinalis*) and lemon iron bark (*Eucalyptus staigerana*) extracts. Journal of Science Food Agriculture, 91: 461-468.
8. Omidbeygi, M., M. Barzegar, Z. Hamidi and H. Naghdibadi, 2007. Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste. Food Control, 18: 1518-1523.
9. Yesil, C.O., K.E.E. Hames, E. Bedir, S.F. Vardar, T. Ozek and K.H.C. Baser, 2007. Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. Food Chemistry, 100: 553-559.
10. Vimal, M., P.P. Vijaya, P. Mumtaj and M.S. Seema Farhath, 2013. Antibacterial activity of selected compounds of essential oils from indigenous plants. Journal of Chemical and Pharmaceutical Research, 5(1): 248-253.
11. Burt, S., 2004. Essential oils: Their antimicrobial properties and potential applications in foods: A review. International Journal of Food Microbiology, 94: 223-253.
12. Delaquis, P.J., K. Stanich, B. Girard and G. Mazza, 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. International Journal of Food Microbiology, 74: 101-109.
13. Mourey, A. and N. Canillac, 2002. Anti-*Listeria monocytogenes* activity of essential oils components of conifers. Food Control, 13: 289-292.
14. Bassolé, I.H.N. and H.R. Juliani, 2012. Essential oils in combination and their antimicrobial properties. Molecules, 17: 3989-4006.
15. Meshkatalsadat, M.H., S. Salahvarzi, R. Aminiradpoor and A. Abdollahi, 2012. Identification of essential oil constituents of caraway (*Carum Carvi*) using ultrasonic assist with headspace solid phase microextraction (Ua-Hs-Spme). Digest Journal of Nanomaterials and Biostructures, 7(2): 637-640.
16. Singh, G., I.P.S. Kapoor, S.K. Pandey, U.K. Singh and R.K. Singh, 2001. Studies on essential oils: part 10, Antibacterial activity of volatile oils of some spices, Phytother. Research, 16: 680-682.
17. Lopez, P., C. Sanchez, R. Batlle and C. Nerin, 2005. Solid and vapor-phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains, Journal of Agricultural Food Chemistry, 53(17): 6939-6946.
18. Stavri, M. and S. Gibbons, 2005. The antimycobacterial constituents of dill (*Anethum graveolens*). Phytother. Research, 19 (11): 938-941.
19. Satyanarayana, S., K. Sushruta, G.S. Sarma, N. Srinivas and G.V. Subba Raju, 2004. Antioxidant activity of the aqueous extracts of spicy food additives-evaluation and comparison with ascorbic acid in *in-vitro* systems Journal of Herb. Pharmacother., 4(2): 1-10.
20. Singh, G., S. Maurya, M.P. De Lampasona and C. Catalan, 2006. Chemical constituents, antimicrobial investigations and antioxidative potentials of *Anethum graveolens* L. essential oil and acetone extract: Journal of Food Science, 70(4): 208-215.
21. Zheng, G., P.M. Kenney and L.K.T. Lam, 1992. Anethofuran, carvone and limonene: Potential cancer chemopreventive agents from dill weed oil and caraway oil. Planta Medica, 58: 339-341.
22. Hosseinzadeh, H., G.R. Karemi and M. Ameri, 2002. Effects of Anethum graveolens L. seed extracts on experimental gastric irritation models in mice, Journal of *BMC* Pharmacology, 2(1): 21-30.
23. Amin, W.M. and A.A. Sleem, 2007. Chemical and biological study of aerial parts of dill (*Anethum graveolens* L.), Egyptian Journal of Biomedical Sciences, 23(1): 73-90.
24. Hajhashemi, V. and N. Abbasi, 2008. Hypolipidemic activity of *Anethum graveolens* in rats, Phytother. Research, 22(3): 372-375.
25. Shamsia, S.M. and M.S. El-Ghannam, 2012. Manufacture of Labneh from cow's milk using ultrafiltration retentate with or without addition of permeate concentrate. Journal of Animal Production Advances, 2(3): 166-173.

26. Guenther, G., 1961. The Essential Oils VIII. Robert E. D. Nastrand Comp. Inc. Toronto, New York, London.
27. Prudnet, D., F. Perineau, J.M. Bessiere, G.M. Michel and J.C. Baccou, 1995. Analysis of the essential oil of wild oregano from Martinique (*Coleus aromaticus* Benth.) – Evaluation of its bacteriostatic and fungistatic properties. Journal of Essential Oil Research, 7: 165-173.
28. AOAC, 2007. Association of Official Analytical Chemists. Official Methods of Analysis. 20th Ed., Gaithersburg, M D, USA.
29. Marino, M., C. Bersani and G. Comi, 2001. Impedance measurements to study the antimicrobial activity of essential oils from *Lamiacea* and *Compositate*. International Journal of Food Microbiology, 67: 187-195.
30. Cimanga, K., K. Kambu, L. Tona, S. Apers, T. De Bruyne, N. Hermans, J. Totte, L. Pieters and A.J. Vlietinck, 2002. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. Journal of Ethnopharmacology, 79: 213-220.
31. Pintore, G., M. Usai, P. Bradesi, C. Juliano, G. Boatto, F. Tomi, M. Chessa, R. Cerri and J. Casanova, 2002. Chemical composition and antimicrobial activity of *Rosmarinus officianalis* L. oils from Sardinia and Corsica. Flavour and Fragrance Journal, 17: 15-19.
32. Harpaz, S., L. Glatman, V. Drabkin and A. Gelman, 2003. Effects of herbal essential oils used to extend the shelf life of freshwater-reared Asian sea bass fish *(Lates calcarifer*). Journal of F ood Protection, 66(3): 410-417.
33. Ratledge, C. and S. G. Wilkinson, 1988. An Overview of Microbial Lipids. In: C. Ratledge and S.G. Wilkinson, (Eds), Microbial Lipids, Vol.1. Academic Press, London, pp: 3-22.
34. Vaara, M., 1992. Agents that increase the permeability of the outer membrane. Microbiological Reviews, 56(3): 395-411.
35. Wilkinson, J.M., M. Hipwell, T. Ryan and H.M.A. Cavanagh, 2003. Bioactivity of *Backhousia citriodora*: Antibacterial and antifungal activity. Journal of Agricultural and Food Chemistry, 51: 76-81.
36. Sikkema, J., J.A.M. De Bont and B. Poolman, 1994. Interactions of cyclic hydrocarbons with biological membranes. Journal of Biological Chemistry, 269(11): 8022-8028.
37. Cox, S.D., C.M. Mann, J.L. Markham, H.C. Bell, J.E. Gustafson, J.R. Warmington and S.G. Wyllie, 2000. The mode of antimicrobial action of essential oil of *Melaleuca alternifola* (tea tree oil). Journal of Applied Microbiology, 88: 170-175.
38. Lambert, R.J.W., P.N. Skandamis, P.J. Coote and G.J.E. Nychas, 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of Applied Microbiology, 91: 453-462.
39. Carson, C.F., B.J. Mee and T.V. Riley, 2002. Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on Staphylococcus aureus determined by time-kill, lysis, leakage and salt tolerance assays and electron microscopy. Antimicrobial Agents and Chemotherapy, 46(6): 1914-1920.
40. Ultee, A., M.H.J. Bennink and R. Moezelaar, 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen Bacillus cereus. Applied and Environmental Microbiology, 68(4): 1561-1568.
41. Nissen, L., A. Zatta, I. Stefanini, S. Grandi, B. Sgorbati and B. Biavati, 2010. Characterization and antimicrobial activity of essential oils of industrial hemp varieties (*Cannabis sativa* L.). Fitoterapia, 81: 413-419.
42. Tajkarimi, M.M., S.A. Ibrahim and D.O. Cliver, 2010. Antimicrobial herb and spice compounds in food. Food Control, 21: 1199-1218.
43. Bagdassarian, V.L.C., Bagdassarian, K.S. and Maria Stefanova Atanassova, 2013. Phenolic profile, antioxidant and antimicrobial activities from the *Apiaceae* family (dry seeds). Mintage Journal of Pharmaceutical and Medical Sciences, 2: 26-31.
44. Skandamis, P.N. and G.J.E. Nychas, 2000. Development and evaluation of a model predicting the survival of *Escherichia coli* O157:H7 NCTC 12900 in homemade eggplant salad at various temperatures, pHs and oregano essential oil concentrations. Applied and Environmental Microbiology, 66(4): 1646-1653.
45. Tsigarida, E., P. Skandamis and G.J.E. Nychas, 2000. Behaviour of Listeria monocytogenes and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5°C. Journal of Applied Microbiology, 89: 901-909.

46. Juven, B.J., J. Kanner, F. Schved and H. Weisslowicz, 1994. Factors that interact with antibacterial action of thyme essential oil and its active constituents. Journal of Applied Bacteriology, 76: 626-631.
47. Vinderola, C.G., S. Costa, G.A. Regenhardt and J.A. Reinheimer, 2002. Influence of compounds associated with fermented dairy products on the growth of lactic acid starter and probiotic bacteria. International of Dairy Journal, 12: 579-589.
48. Khaleel, E.M., 2000. Studies on milk and milk products. M.Sc. Thesis. Fac. of Agric., Zagazig University, Egypt.
49. El-Nawawy, M.A., Y.M. El-Kenany and E.A. Abd El-Ghaffar, 1998. Effect of some herb plants on the use of yoghurt culture. Annals of agriculture Sci. 7th Conf. Agric. Dev. Res. Fac. Agric. Ain Shams University, Cairo, Egypt. 15-17 December.
50. Sedlakova, J., B. Kocourkova, L. Lojkova and V. Kuban, 2003. Determination of essential oil content in Caraway (*Carum carvi* L.) species by means of supercritical fluid extraction. Plant Soil Environ, 49(6): 277-282.
51. Koppula, S., S.R. Kopalli and S. Sreemantula, 2009. Adaptogenic and nootropic activities of aqueous extracts of *Carum carvi Linn* (Caraway) fruit: an experimental study in wistar rats. Australian Journal of Medical Herbalism, 21(3): 72-78.
52. AL-Asmail, K.M. and T. Aburjai, 2004. Antioxidant activity of water and alcohol extracts of chamomile flowers, anise seeds and dill seeds. Journal of the Science of Food and Agriculture, 84: 173-178.
53. Stankevicius, M., I. Akuneca, I. Jākobsone and A. Maruška, 2010. Analysis of Phenolic Compounds and Radical Scavenging Activities of Spice Plants Extracts. Maisto Chemija IR Technologija, T.44, 2.
54. Gwari, G., U. Bhandari, H.C. Andola, H. Lohani and N. Chauhan, 2012. Aroma profile of seeds of Carum carvi Linn. Cultivated in higher hills of Uharakhand Himalaya. Indian Journal of Natural Products and Resources, 3(3): 411-413.
55. Samojlik, I., K.D. Svajcer, B. Bozin and M. Mikov, 2012. Herb-drug interactions: the influence of essential oil of Caraway (*Carum carvi* L.) on the pharmacokinetics of paracetamol. Pharmacology and Toxicology, 13(Suppl.1:A27).
56. Foda, Mervat I., M.A. El-Sayed, Marwa M. El-Moghazy, Amal A. Hassan and Nagwa M. Rasmy, 2008. Effect of spearmint essential oil on ripening of white cheese. The 3rd International Conference on Food Science and Nutrition, Nov.3-5, NRC, Cairo, Egypt, pp: 252-266.
57. Mocanu, D., G. Rotaru, E. Botez, D. Andronoiu and O. Nistor, 2010. Probiotic yogurt with medicinal plants extract: Physical – chemical, microbiological and rheological characteristics. Journal of Agroalimentary Processes and Technologies, 16(4): 469-476.
58. Al-Otaibi, M. and H. El-Demerdash, 2008. Improvement of the quality and shelf life of concentrated yoghurt (labneh) by the addition of some essential oils. African Journal of Microbiology Research, 2: 156-161.
59. Moritz, C.M.F., V.L.M. Rall, M.J. Saeki and A.F. Junior, 2012. Inhibitory Effect of Essential Oils against *Lactobacillus rhamnosus* and Starter Culture in Fermented Milk During its Shelf-life Period. Brazilian Journal of Microbiology: 1147-1156.
60. Shori, Amal B. and A.S. Baba, 2012. Viability of lactic acid bacteria and sensory evaluation in *Cinnamomum verum* and *Allium sativum*-bio-yoghurts made from camel and cow milk. Journal of the Association of Arab Universities for Basic and Applied Sciences, 11: 50-55.
61. Salem, A.S., W.M. Salama, A.M. Hassanein and H.M.A. ElGhandour, 2013. Enhancement of nutritional and biological values of labneh by adding dry leaves of Moringa oleifera as innovative dairy products. World Applied Sciences Journal, 22(11): 1594-1602.