Iraqi Journal of Science, 2022, Vol. 63, No. 7, pp: 2839-2849
DOI: 10.24996/ijs.2022.63.7.8

ISSN: 0067-2904

Microbiological Effect of Lemongrass *Cymbopogon Citratus* and Spearmint *Mentha Spicata* Essential Oils as Preservatives and Flavor Additives in Yogurt

Ibrahim Jabber Abed[1] Alyaa Razooqi Hussein[1]Ghusoon Ali Abdulhasan[1*]Anwar Neamah Dubaish[2]

[1]*Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.*
[2]*The State company for diary Products, Abu-Graib, Baghdad, Iraq.*

Received: 10/8/2021 Accepted: 21/9/2021 Published: 30/7/2022

Abstract

This study was designed to evaluate the antimicrobial effect of *Cymbopogon citratus* and *Mentha spicata* essential oils, separately and mixed, against the microorganisms in yogurt, as well as study the possibility of these essential oils (EO) as natural preservatives and flavors additives/enhancers in yogurt product. Yogurt samples were treated with lemongrass and spearmint EOin different concentrations (250, 500, 1000 ppm: 6250µg/50 ml yogurt, 12500 µg/50 ml yogurt and 25000 µg/50 ml yogurt respectively). The control and treated samples were preserved both at room (25°C) and refrigerator (5°C) temperatures. In control, the contamination was observed through 2 weeks at 25°C and for about one month at 5°C. The samples treated with lemongrass and spearmint EOs (500pmm), the contamination showed up late, after 45 and 30 days at 25°C respectively. While at 5°C, the contamination appeared after 90 and 60 days respectively. The effect of lemongrass and spearmint EO, separately or mixed (synergistic effect), on the growth of fungi that was isolated from spoiled samples, was studied in different concentrations (125, 250, 500 ppm). Microbiological examination was done in the control and treated yogurt samples. There was a significant difference ($P \leq 0.05$) between microbial spoilage (coliform, yeast and fungi) count during different periods of incubation which decreased in the samples treated with essential oils as compared with the control. The results of the percentage of growth inhibition revealed that lemongrass EO, around 80-100%, is the best in inhibiting the molds and yeasts causing yogurt damage as compared to the use of spearmint EO which was 27-60%, and the synergistic effect of about 35-39%. The results of the toxicity assay of the maximum effect of EO *in vivo* proved their validity for consumption when added both as preservative and flavor. The concentrations used for the dosage ranged from 250 ppm to 5000 ppm.

Key words: Yogurt, Lemongrass, Spearmint, Essential oil, Preservative.

تأثير الزيوت الطيارة لنباتي حشيشة الليمون والنعناع كإضافات حافظة ومنكهة في الزبادي

إبراهيم جابرعبد[1] ، علياء رزوقي حسين[1]، غصون علي عبدالحسن[1] ، انوار نعمه دبيش[2]

[1]قسم علوم الحياة ,كليةالعلوم ,جامعةبغداد ,بغداد ,العراق.

[2]ابو غريب , بغداد, العراق. ,الشركة العامة لمنتوجات الالبان

*Email: ghusoon.ali@sc.uobaghdad.edu.iq

الخلاصه

صممت هذه الدراسة لتقييم التأثير المضاد للميكروبات للزيوت العطرية.لنباتي *Cymbopogon citratus* و *Mentha spicata* ، تم اضافة الزيوت الطيارة لعشبة الليمون والنعناع الى عينات الزبادي بتراكيز مختلفة (250 ، 500 ، 1000 جزء في المليون: 6250 ميكروغرام / 50 مل زبادي، 12500 ميكروغرام / 50 مل زبادي و25000 ميكروغرام / 50 ملزبادي علىالتوالي). تم حفظ عينات التحكم والعينات المعالجة في درجة حرارة الغرفة (25 درجة مئوية) ودرجة حرارة الثلاجة (5 درجة مئوية). في السيطرة ، لوحظ التلوث خلال أسبوعين عند 25 درجة مئوية وحوالي شهر واحد عند 5 درجات مئوية. في العينات المعالجة ، أظهر عشب الليمون والنعناع (500جزء في المليون) التلوث متأخرًا بعد 45 و 30 يومًا عند 25 درجة مئوية على التوالي. بينما عند 5 درجات مئوية ظهر التلوث بعد 90 و 60 يومًا على التوالي. تمت دراسة تأثير الزيوت الطيارة لعشبة الليمون والنعناع اما لوحدها أو مختلطة (تأثير تآزري) على نمو الفطريات المعزولة من عينات الزبادي بتركيزات مختلفة (125 ، 250 ، 500 جزء في المليون). تم إجراء الفحص الميكروبيولوجي في عينات لبن الزبادي المعالج والسيطرة. توجد فروق معنوية ($P\leq 0.05$) بين عدد التلف الجرثومي (القولونيات،الخميرة والفطريات) خلال فترة الحضن بدرجات حرارة مختلفة والتي انخفضت في العينات المعالجة بالزيوت الطيارة مقارنة بمعاملة السيطرة. أظهرت نتائج النسبة المئوية لتثبيط النمو أن الزيت الطيارلعشب الليمون(80–100٪)هو الأفضل في تثبيط العفن والخمائر المسببة لتلف الزبادي مقارنة باستخدام الزيت الطيارللنعناع (27–60٪)والتأثير التآزري للتثبيط حوالي (35. –39٪). أثبتت نتائج اختبار السمية لأكبر تأثيرمن الزيوت الطيارةفي الجسم الحي صلاحيتها للاستهلاك عند إضافتها كمواد حافظة ونكهة. تراوحت التراكيز المستخدمة للجرعة من 250– 5000 جزء في المليون.

Introduction

Yogurt is a healthy and nutritive dairy product. It is worth for controlling the growth of bacteria and in treating intestinal diseases like constipation, diarrhea and dysentery [1]. Yogurt is made of milk cultured with live bacteria. The lactic acid resulting from the growth of bacteria coagulates the milk proteins, making yogurt slightly sour in flavor and thick [2]. The bacterial cultures used for synthesis of yogurt are *Streptococcusthermophilus* and *Lactobacillus bulgaricus*. The acidity of yogurt acts as a barrier to bacterial growth, as does the high temperature achieved during the yogurt-making process [3]. Yogurt products must be preserved because they are vulnerable to degradation for longer times by spoilage organisms, mostly yeasts and molds. Several researchers have used different preservation treatments to extend the shelf life of yogurt such as pasteurization, addition of chemical and natural preservatives, effective packaging, high pressure technology, irradiation or using a combination of two or more of these treatments [4].

Preservatives are used to get better yoghurt consistency which prolongs the shelf life of foods and drinks by preventing microbial growth. Preservatives are chemicals which prevent the fermentation and spoilage of foods and drinks without causing any harmful effect to the people who consume them [5]. Recently preservation methods have a great role in food industry. Food additives is one of the preservation methods in dairy products. According to Codex Alimentarius Commission food additives are substances that are added to food for maintaining or improving nutritional value. In late 1988, the European Community approved using of additives in food for human consumption [6]. The first chemical preservative approved by the US Food and Drug Administration (FDA) is sodium benzoate (E211) which is affected by pH. Therefore, the effectiveness of benzoate decreases with the increasing the pH of a medium [7]. Sodium benzoate, potassium sorbate and natamycin are mainly used to control mold, inhibit growth of yeast and some bacteria [8]. As a result of the increased consumption of preservatives and their negative effects on health, extensive search is being sought for alternative food preservatives which are natural, safe and less toxic, especially preservatives derived from herbs and plant products [9, 10]. The use of herbal products as

antimicrobial and antioxidant food preservatives has gained popularity among researchers in the past few decades because of their desirable characteristics, including availability and fewer adverse effects as compared to the other obtainable antimicrobial agents [11]. The natural preservative antioxidants used in exchange for synthetic ones have been found to get better yogurt quality without a change in its nutritional value [12]. In particular, some essential oils derived from plants like lemongrass, are potential food preservatives [13]. Many of them are easy to obtain, have low toxicity in mammalian and quickly degrade in water and soil, making them environment friendly and, hence, resulting in prompting their use in food preservation and processing. Among essential oils used as potential food preservatives is lemongrass EO that is derived from *Cymbopogon citrates* [14, 15]. *C. citratus* extract and essential oil have been used due to their widely reported therapeutic and pharmacological activities in traditional therapy for coughs, consumption, elephantiasis, malaria, ophthalmia, pneumonia and vascular disorders. Researchers have found that lemongrass holds antidepressant, antioxidant, antiseptic, astringent, bactericidal, fungicidal, nervine and sedative properties [16]. It has also been applied in food flavoring, cosmetics, and food and industrial preservation [5]. On the other hand, essential oils derived from Lamiaceae family (e.g. oregano, thyme, basil, mint, spearmint (*Mentha spicata)*, pennyroyal (*Mentha pulegium)*, rosemary, siderites and sage) are recognized as effective inhibitors of some important yeasts that cause spoilage, and therefore, can be used as natural food preservatives [17]. In Iraq, due to increased consumption of dairy products, including yogurt, there has been an attempt to manufacture dairy products that contain natural antioxidants as preservatives, thus improving human health at the same time. Therefore, the present study was designed to evaluate the antimicrobial effect of *C. citratus* and *Mentha spicata* essential oils separately and in combination (synergistic effect) against the spoilage resulting from yeast and fungi in yogurt. As well as study the possibility of these essential oils as natural preservatives and flavor additives in yogurt product.

Materials and Methods

Yogurt Preparation

Powderd milk Nido that consists of fat (28.2g), high quality protein (23.6g), carbohydrates (38g), calcium (860 mg), iron (10 mg), zinc (4.5 mg) and vitamin A (1800 IU),was used in preparing yogurt.The milk was dissolved in sterile distilled water and then boiled.The milk suspension was cooled to 45℃, and ,then in sterile condition, the starters (*Lactobacillus bulgaricus* and *Streptococcus theromophilus*,SACCO, Italy) were added to the cooled milk and mixed.The mixture was then filled in a sterile container (50 ml), before being incubated at 40°C for 3-5hrs. Fresh yoghurt was kept at 4°C for physical, chemical and microbiological analysis.

Lemongrass and Spearmint Essential Oils Extraction

The essential oils of both lemongrass and spearmint were extracted from air dried leaves (250g), using cleavenger hydrodistillation method. The distilled water was boiled with plant material for 3hrs., and then the extracted oils were kept at 4ºC [18].

Addition of EOs to Yogurt Prepared from Milk Samples

After adding the starter in previous step, oils of lemongrass and spearmint were added to the milk container in order to obtain concentrations of oil (125, 250 and 500 ppm).In this experiment the oils were added at zero time and one hour after the beginning of fermentation process.The control was left without oil. The containers were incubated at 37º C for 18hrs. Control and treatment yogurt were kept in refrigerator (5ºC) and at room temperature (25℃) for monitoring the signs of microbial spoilage, with or without essential oils.

Microbiological Examination

1ml of milk sample was pulled-up and added to 9ml of peptone water as a diluent solution. This represented the first dilution 10^{-1}. After that 1ml of samples were taken and placed in

sterile dishes and the appropriate culture media were poured after it cooled down to 45°C. These media are MacConkey agar for coliform bacteria and yeast extract agar for yeast count, and malt extract agar for mold rot [19].

Isolation of Fungi(Mold and Yeast) from YogurtSamples

Isolates were sampled according to FDA criteria [19] and yeasts were identified to species level by physiological and morphological standard methods as recommended by Kurtzman and Fell [20]. Fungal isolates were identified by colony, cell morphology and microscopical observation of conidiospore formation [21].

Any sample that appeared microbially spoiled was examined. 5gm of yogurt samples were mixed with 45 ml of sterile distilled water. 0.1ml of the mixture was cultured by pouring plate method on sabaroud dextrose agar and malt extract agar. The inoculated plates were incubated at 25ºC for 7 days and examined under the light microscope. The isolated fungi were cultured on potato dextrose agar and incubated at 28°C for weeks [22].

Determination of EOs Antifungal Properties

An agar dilution technique was implemented to reveal a different inhibitory effect on the concentrations of lemongrass and spearmint EOs on the radial growth of isolated fungi. Essential oils were prepared in different concentrations (125,250,500 ppm), using DMSO and then powered in potato dextrose agar and inoculated with cutting the fungal disc from seven days old culture by cork porer and was then put on a solid center medium and later incubated. Three replicates were used within each treatment and the control was performed without EOs. Colony diameters were measured and the inhibition percentage was calculated based on a formula [23]:

Growth inhibition% = [(growth in control – growth in sample)/growth in control] × 100

Toxicity Study

Toxicity study for the most effective EO was carried out by using 48 male and female balb/c weighing 20-25g, aged two months. The animals were distributed into six groups, containing eight animals per group. To determine the LD50, one group for control (group C) was given 0.1ml of physiological solution by mouth using oral syringe, while other groups (G1, G2, G3, G4 and G5) were treatment orally with different concentrations of EOs (125, 250,500, 1000, 2000 and 5000 ppm). The animals were monitored for 14 days to record the data of deaths [24].

Statistical Analysis

To analyse the resulting data, SPSS (version 20) was used. Z- test was used to compare two proportions for fungi growth inhibition resulting from exposure to three concentrations of lemongrass, spearmint and mixture of them. LSD was used for microbial spoilage count differences and toxicity study. Differences at $P \leq 0.05$ were assumed statistically significant [25].

Results and Discussion

The results of yogurt preparation in laboratory showed that all inoculated samples with starter produced yogurt from milk. Two different species of bacteria can ferment lactose in milk, namely *Lactobacillus bulgaricus and Streptococcus thermophilus*. Enzymes in the bacteria convert the lactose into lactic acid. As the acid accumulates in the solution, milk thickens and takes on a “sour” taste. More milk protein coagulates when the pH is lowered [3]. Moreover, the results of this study showed that the samples inoculated with EOs of lemongrass and spearmint in concentrations (125, 250 and 500 ppm: 6250µg/50 ml yogurt, 12500 µg/50 ml yogurt and 25000 µg/50 ml yogurt respectively) at zero time and after one hour of addingthe starter,converted milk to yogurt with flavor.The control also gave a positive result.

The EOs are also used as flavoring materials because they contain aromatic components as they are a source of good smell and health-beneficial compounds [26]. The desired taste of

lemongrass resulted from their components such as neral, citronellal, linalool, geranial, limonene, 6-methyl hept-5-en-3-one, caryophyllene and beta-myrcene [27].

The results of the chemical tests showed that the proportion of fat and solids of non-toxicity remained constant in all models.The only variable was the percentage of acidity. The percentage of fat or fat content was 3.6% and the percentage of non-soluble solids was 13%.

One of the most important microbiological tests conducted on the milk product, is the examination of coliform bacteria,yeast and molds (Figure 1). The name was called the direct number on the milk samples taken from milk directly without dilution and dilution 10^{-1}(Table 1). There were significant differences ($P \leq 0.05$) between microbial spoilage (coliform, yeast and fungi) count during different periods of incubation which decreased in samples treated with EOs as compared with controls from one side, as well as between samples with different concentrations.

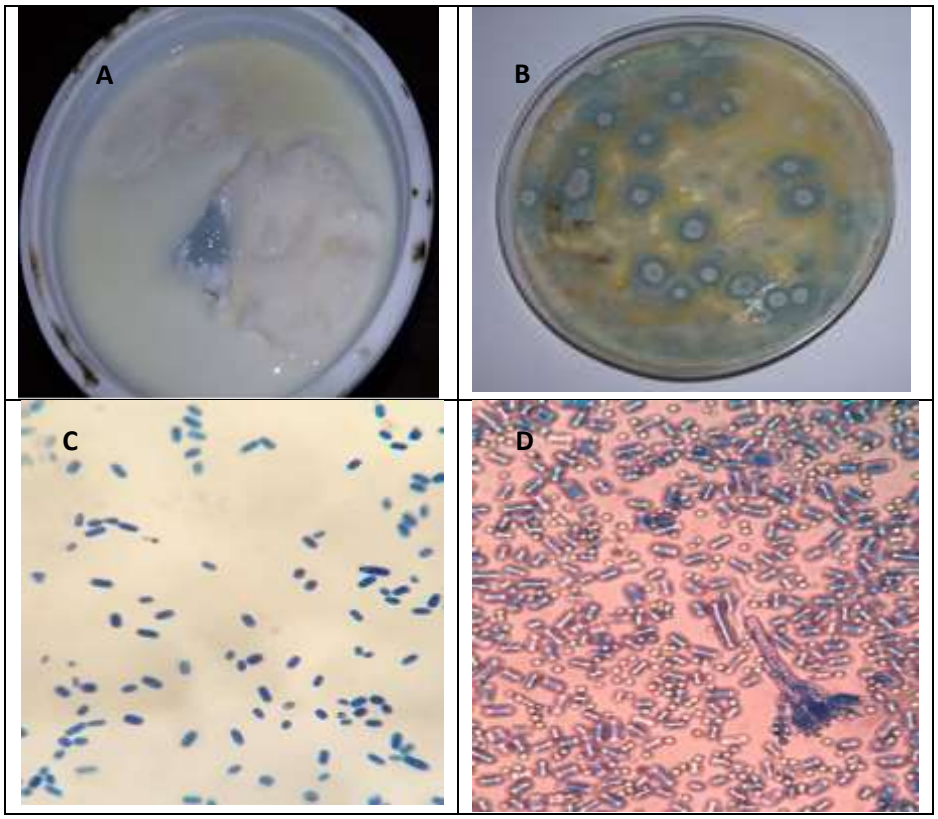

Figure 1- Identification of mold and yeast in yogurt. A: growth of mold and yeast on the surface of yogurt. B: colonies of mold on the surface of malt extract agar. C: yeast cells under microscope (40X). D: mixture of yeast and *Penicillium* mold under microscope.

Table 1 - The microbial number in control and samples treated with lemongrass and mint EOs.

Duration of treatment	Sample	Dilution	Lemongrass Essential Oil		Spearmint Essential Oil	
			CFU Coliform	CFU Fungi and Yeast	CFU Coliform	CFU Fungi and Yeast
1 week	control	direct	10	3	11	4
		10^{-1}	3	1	2	1
	125ppm	direct	7	1	9	1
		10^{-1}	4	0	7	0
	250ppm	direct	2	0	4	0
		10^{-1}	0	0	0	0
	500ppm	direct	0	0	0	0

		10^{-1}	0	0	0	0
2 week	control	direct	65	25	67	30
		10^{-1}	5	5	5	5
	125ppm	direct	24	18	32	23
		10^{-1}	5	3	4	5
	250ppm	direct	18	0	23	0
		10^{-1}	2	0	3	0
	500ppm	direct	0	0	0	0
		10^{-1}	0	0	0	0
3 week	control	direct	120	37	120	39
		10^{-1}	22	6	22	8
	125ppm	direct	38	42	45	57
		10^{-1}	6	10	8	14
	250ppm	direct	28	0	35	0
		10^{-1}	2	0	4	0
	500ppm	direct	11	0	15	0
		10^{-1}	1	0	2	0

After the ending of fermintation process, the yogurt samples that were treated with lemongrass and spearmint essential oil concentrations (125, 250 and 500 ppm), were divided into two groups. One group was left at 25°C, while the other group was preserved at 5°C, with control. The result of control sample appeared spoiled after two weeks at room temperature (25°C), while samples treated with EOs concentrations did not show any microbial spoilage signs during this period. Moreover, the microbial spoilage of the first yogurt sample with concentration 125, 250 and 500 ppm of lemongrass oil was observed after 3, 4 and 6 weeks of preservation respectively at 25°C. Whereas the spearmint oil showed spoilage after 18 days, 3and 4 weeks for the same concentrations and temperature. At 5°C, the samples treated with lemongrass and spearmint (500pmm) showed the contamination late, after about three and two weeks respectively. The other concentrations of both essential oils stayed more periodically uncontaminated at 5°C as compared to 25°C (data not shown).

The microbial spoilage was delayed in the samples treated with EOs in both preserved temperatures. It may be due to the preservative effect resulting from antimicrobial activity of EOs. The duration of spoilage was also different between the tempratures used for incubation that may belong to this temperature suitable for growth of fungi (molds and yeast) [28].

A study done on yoghurt by Abd-El Fattah*et al* .[29] reported that the effective lemongrass EO dose for fungi and mycotoxin production inhibition was 0.1%. Hence, the rise of EO dose to 0.3 % resulted in completely fending off the fungi growth and toxin creation. He also had reported that 0.1% concentration could saveyogurt for more than 28 days at refridgerator temprature. Abu-Seif *et al* .[30] recorded that EO from leaves and stems of *C. citratus* at 0.3 percent lead to full inhibition of fungi (*A. flavus, A. parasiticus and A. ochraceus*) and their toxin production. Lemongrass and cinnamon oils significantly inhibited the growth of mycelia and conidia germination of fungi (*Colletotrichum musae, Fusarium incarnatum and F. verticillioides*) isolated from the banana crown rot disease [31].

The effectiveness of EOs is due to the nature of their basic chemical texture. Major components are often responsible for antifungal activity. The lemongrass EO is largely affluent with linalool acetate (41.29%) and geraniol (32.15%). Morever, the inhibitory effect of the EO is not only due to its main components, but also because of the synergistic effect of its main and/or major compounds [32]. The important characteristic of EO is their hydrophobicity which leads to the fragmentation of lipids in the cell membranes of fungi. It affects its composition and causes leakage of ions and other cellular contents [33].In addition, the EO from peppermint, zataria and basil used for preparing probiotic yogurt formula,

improved antioxidant efficacy of the product. Other essential oils such as clove, rosemary and cinnamon had potential role in increasing the shelf life of the concentrated yogurt [34].

The results also revealed that saving yogurt at refrigerater temperature could enhance the inhibitory action of EO. These results were nearly similar with the results obtained by Eissa *et al.* [35] who proved that 5^{o}C temprature could promote some spices extracts inhibitory effect. These results are asserted by those of Muramatsu*et al.*[36] whoreported that lemongrass and clove demonstrated a powerful anti-fungal effect for 30 days. The fact that lemongrass EO acts as a preservative can be attributed to its containment of aldehydes and volatile materials that have dynamic effect on the inhibition of growth of microorganisms.The safety of food products and their shelf life depends on a number of factors including the type and quantity of the volatile oil added to the food product.

Isolation of Fungi from Yogurt and Determination of Antifungal Effect of EO: The results of microscopic examination in the current study show that the predominant microorganisms are fungi (molds and yeasts) that are responsible for spoilage of yogurt samples, even if preserved in refrigerator at 5°C. The growth of yeasts in yogurts was related to their ability to grow at refrigerator temperature, and their fermentation of lactose and sucrose as well as hydrolyze milk casein. It was found that almost all yeast isolates could grow in the existence of preservatives like sorbate and benzoate (100 µg/ml) [37]. After the isolation of mold and yeast, the results of identification showed four mold and two yeast isolates that included two isolates of *Penicillium*, *Aspergillus niger*, *Mucor*, yeast and *Saccharomyces cerevisiae.*

Many dangerous fungi, such as *Aspergillus* sp.,*Mucor* sp. and *Penicillium* sp., were isolated from yogurt [38]. Lee *et al*. [39] isolated *Mucor circinelloides*strain from a yogurt container. Consumers of this fungus became ill with nausea, vomiting and diarrhea. The growth of fungi on food is a major concern, especially toxin-producing fungi. Yogurt is exposed to spoilage with fungi, either through the lack of hygiene or insufficient storage conditions. Therefore, fungal contamination can occur during the process of transportation or when packing the product, in addition to its ability to remain in the food due to its adaptability in different conditions.

The results of our study showed that all the tested EOs concentrations have antifungal activity against all tested microorganisms. The effect of lemongrass oil was the strongest in comparison to the effect of spearmint separately ($P \leq 0.05$) or when it was mixed with lemongrass oil ($P \leq 0.05$), as shown in Figure 2. Also the effect of lemongrass oil alone differed according to the type of tested mold or yeast. The maximum effect of lemongrass was at 500 ppm which lead to 100% inhibition in growth of all fungi isolated from yougert, except *Mucor* (80.9%). Also, the spearmint showed the maximum inhibion effect against isolated fungi at 500 ppm (27-60%). Furthurmore, the mixed EO showed the maximum effect for all fungi at 500 ppm (35-39%), except *A. niger* at 125 ppm (57.27%). The sensory evaluation of yogurt treated with lemongrass and spearmint EOs in this study observed the acceptability of this yogurt in different concentrations.

Our results were in agreement with the worldwide published results. Soares *et al*. [28] reported that the *Cymbopogon citratus* EO manifested high antibacterial and anti-yeast characteristics. Among them are resistant strains of *S. aureus*, *S. epidermidis*, *E.coli*, *K. pneumonia,* and NCAC non-*Candida albicans Candida* spp *C. parapsilosis* and *C. tropicalis*. It can be concluded that it meets several features that make it a good candidate for the biopreservative of food.

During the study done by Abd-El Fattah *et al.* [29], yogurt samples at 5°C and 28ºC temperatures stored for 28 days with different concentrations of the lemongrass EO, illustrated different acidity and total bacterial cells count than the control samples. Also, the inhibitory effect of EO against yeast depended on EO concentration. 0.1% concentration of

EO preserved the yogurt during this period. All the sensory tested preferred the yogurt with EO than without it. They suggested the possibility of using EO for decontamination of yoghurt from toxigenic fungi and mycotoxins formed by them.

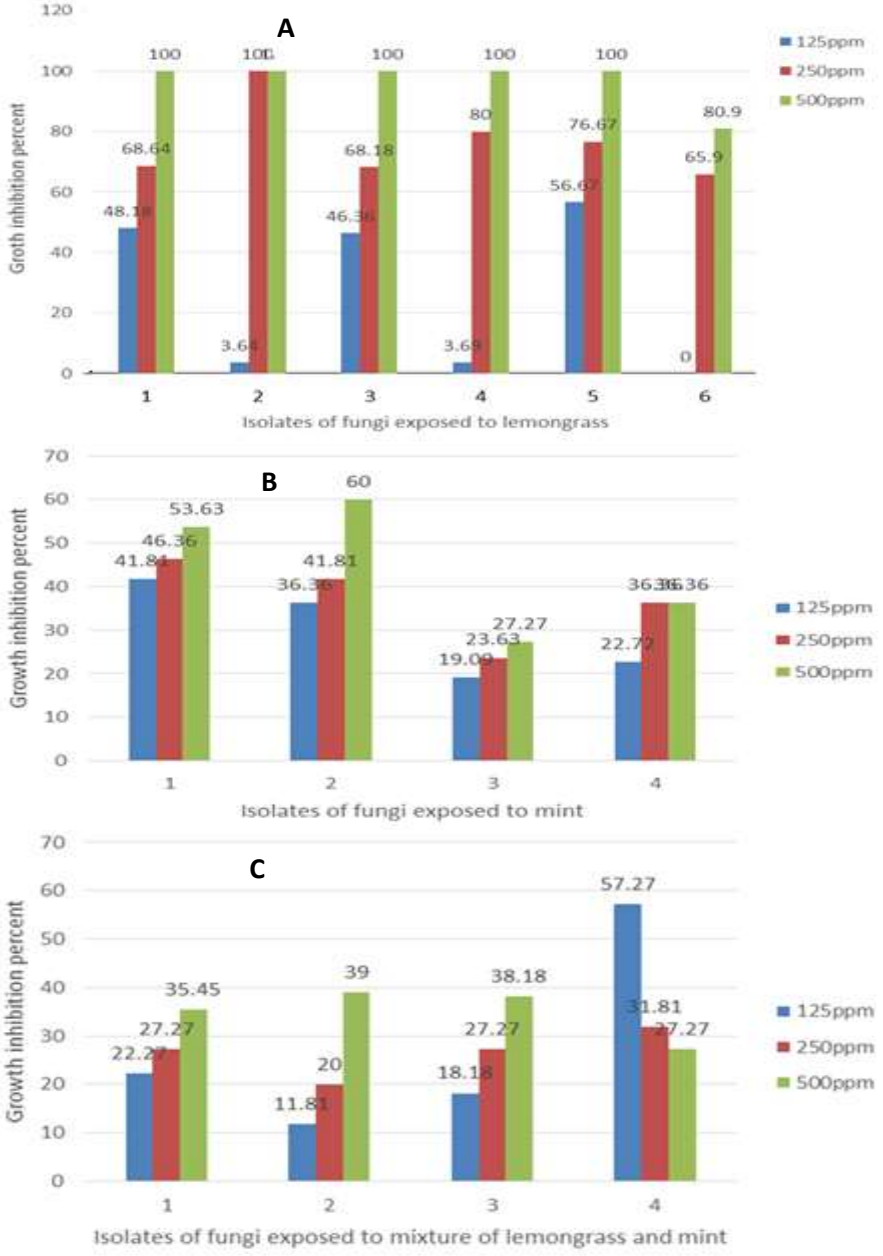

Figure 2 - The effect of essential oils (lemongrass A, spearmint B and a mixture of lemongrass and spearmint C) on fungi isolated from yogurt (1. *Penicillium* sp., 2. *Penicillium* sp., 3. Yeast, 4. *Aspergillus niger*, 5. *Saccharomyces cerevisiae* and 6. sp.*Mucor*. Note: the 5 and 6 isolates were lost during study and were, therefore, not tested for spearmint and mixed EO experiments.

Recently Othman *et al.* [40] reported that lemongrass EO at 4 µl/ ml had 100% inhibitory effect against fungi like *Penicillium* sp. and 20% for *A. flavus*. The differences in the results were attributed to the differences in resistance of the studied fungal isolates.

The aqueous leaves extract of *Mentha spicata L*. were prepared in different concentrations of 1%, 2%, 3%, 4%, 5%, v/v at 100°C for 15 mins, producing thick yogurt for 14 days at 5°C. The pH value decreased with increasing storage period.The total phenolic content was increased with increased concentration and sensory evaluation observed that all thick yogurts,

in different concentrations of *M. spicata* extract, were acceptable by the panelist [41]. Mint essential oil was used for inactivation of *Listeria monocytogenes* and *L. innocua* by more than 1 log cfu/ml [42]. The EO of *M. spicata* and *M. aquatica* was reported to have high inhibition activity against *Staphylococcus aureus*, *Lactobacillus reuteri*, *Bifidobacterium animalis* and *Clostridium perfringens* grown in industrial liquid kashk (yogurt). The concentrations 1500 and 2500 ppm were the most preferred which reduced the viable count of *S. aureus* below 5 log CFU. The population of *C. perfringens*, *L. reuteri* and *B. animalis* decreased less than 1 log CFU after 4 days as well as the EO of *M. aquatic* had less effect on the lactic bacteria [39]. In another study, the *M. spicata* and *M. pulegium* EO had affected the growth of yeast in Doogh (yogurt) in different concentrations which decreased the log number of microorganisms when the concentration was increased, making the EO of *Mentha* the best alternative preservative for chemical hazardous compounds [43].

Toxicity Study:

According to the results of antifungal effect of EO, the most effective EO (lemongrass) was chosen for toxicity determination in animals. The results of this examination showed no significant differences ($P>0.05$) for various concentrations used in the dosage, which started with a concentration of 250 ppm to 5000 ppm. They were monitored for two weeks, as no deaths occurred in the experimental animals under study, noting that the concentrations used for flavoring and preservation of the product were 500 ppm and that this concentration was safe, in addition to the concentration of 5000 ppm, which was ten times more than it was safe, as no deaths occurred. From the results obtained by Costa *etal.* [44] who found that the treated groups with single acute oral dose (3500 mg/kg) and 21 repeated oral doses (1, 10 or 100 mg/kg) of lemongrass EO resulted in no significant changes in pathology, histology of many body organs, urine assay or biochemistry. Analysis were shown in mice treated with lemongrass EO as compared to the control. Furthermore, the blood cholesterol decreased after oral treatment with EO and the the comet assay also showed no genotoxic effect. Later, Sinha *et al.* [45] studied the cytotoxic and genotoxic in human lymphocytes for four EOs (palmarosa, citronella, lemongrass and vetiver) as well as citral and geraniol, using MTT test and trypan blue dye exclusion. The results showed that these EOs and citral, at higher concentration, induced cytotoxicity and genotoxicity but at low concentration were considered safe for human consuming.

In conclusion, lemongrass EO could be recommended for use as a preservative for prolonged shelf life of yogurt due to its components which inhibit the growth of microorganisms and has an acceptable taste.

References

[1] A. ERTÜRK and E. DEMİRKOL, "The Effect of Some Medicinal Plant Extracts on Biochemical, Physicochemical, and Antimicrobial Activity of Exract Added Yogurt," *Harran Üniv Vet Fak Derg*, vol. 3, no. 2, pp. 78-83, 2014.

[2] D. C. o. California. (2014). *Yogurt Nutrition* Available: http://col.st/a23tL

[3] B. A. Nummer. (2002). *Fermenting Yogurt at Home*. Available: http://col.st/1AtTl2u

[4] S. A. Hussain, F. C. Garg, and D. Pal, "Effect of different preservative treatments on the shelf-life of sorghum malt based fermented milk beverage," *Journal of food science and technology*, vol. 51, no. 8, pp. 1582-1587, 2014.

[5] C. E. Ekpenyong and E. E. Akpan, "Use of Cymbopogon citratus essential oil in food preservation: Recent advances and future perspectives," *Critical reviews in food science and nutrition*, vol. 57, no. 12, pp. 2541-2559, 2017.

[6] F. Zamani Mazdeh *et al.*, "Simultaneous determination of preservatives in dairy products by HPLC and chemometric analysis," *International journal of analytical chemistry*, vol. 2017, 2017.

[7] L. J. Ogbadu, "PRESERVATIVES| Permitted Preservatives–Benzoic Acid," 2014.

[8] Z. Esfandiari, M. Badiey, P. Mahmoodian, R. Sarhangpour, E. Yazdani, and M. Mirlohi, "Simultaneous determination of sodium benzoate, potassium sorbate and natamycin content in

Iranian yoghurt drink (Doogh) and the associated risk of their intake through Doogh consumption," *Iranian Journal of Public Health*, vol. 42, no. 8, p. 915, 2013.

[9] A. L. Harvey, "Natural products in drug discovery," *Drug discovery today*, vol. 13, no. 19-20, pp. 894-901, 2008.

[10] G. A. Abdulhasan, "The biological effect of Rosmarinus officinelis L. essential oil on biofilm formation and some fimbrial genes (fimH-1 and mrkD) of Klebseilla pneumoniae," *Iraqi Journal of Science*, vol. 56, no. 3C, pp. 2553-2560, 2015.

[11] M. D. Soković, J. M. Glamočlija, and A. D. Ćirić, "Natural products from plants and fungi as fungicides," *Fungicides-showcases of integrated plant disease management from around the world*, pp. 185-232, 2013.

[12] C. Caleja, L. Barros, A. L. Antonio, M. Carocho, M. B. P. Oliveira, and I. C. Ferreira, "Fortification of yogurts with different antioxidant preservatives: A comparative study between natural and synthetic additives," *Food chemistry*, vol. 210, pp. 262-268, 2016.

[13] M. O. Soares, A. F. Vinha, C. Sousa, A. Castro and P. C. Pires. *Food Preservative Potential of Lemongrass (Cymbopogon citratus) Essential Oil*, In: João Silva Dias, editor. Prime Archives in Agricultural Research. Hyderabad, India: Vide Leaf, 2020.

[14] M. Vazirian *et al.*, "Antimicrobial activity of lemongrass (Cymbopogon citratus (DC) Stapf.) essential oil against food-borne pathogens added to cream-filled cakes and pastries," *Journal of Essential Oil Research*, vol. 24, no. 6, pp. 579-582, 2012.

[15] B. Balakrishnan, S. Paramasivam, and A. Arulkumar, "Evaluation of the lemongrass plant (Cymbopogon citratus) extracted in different solvents for antioxidant and antibacterial activity against human pathogens," *Asian Pacific Journal of Tropical Disease*, vol. 4, pp. S134-S139, 2014.

[16] M. McGuffin , C. Hobbs and R. Upton , *American herbal products association botanical safety handbook*. Boca Raton: CRC press, 1997.

[17] G. Karim, M. A. Meshgi, R. K. Ababil, and S. Bokaie, "Antimicrobial effect of Mentha spicata and Mentha pulegium essential oils in two storage temperatures on the survival of Debaryomyces hansenii in Iranian doogh," *Applied Food Biotechnology*, vol. 3, no. 2, pp. 99-104, 2016.

[18] H. Ali, I. Abed, R. Augul, and H. Fadhil, "INSECTICIDAL ACTIVITY OF EUCALYPTUS SP. VOLATILE OIL AGAINST BACKSWIMMER INSECT ANISOPS SARDEA," *The Iraqi Journal of Agricultural Science*, vol. 51, no. 1, pp. 470-482, 2020.

[19] B. A. Manual, "Gaithersburg," *MD: AOAC International*, 1995.

[20] C. Kurtzman, J. W. Fell, and T. Boekhout, *The yeasts: a taxonomic study*. Elsevier, 2011.

[21] C. J. Alexopoulos, C. W. Mims, and M. Blackwell, *Introductory mycology* (no. Ed. 4). John Wiley and Sons, 1996.

[22] S. R. Moreira , R. F. Schwan, E. P. de Carvalho and A. E. Wheals. " Isolation and Identification of Yeasts and Filamentous Fungi from Yoghurt in Brazil".*Brazilian Journal of Microbiology* ,vol.32,pp.117-122 ,2001.

[23] A. Dalili, S. Bakhtiari, H. Barari, and M. Aldaghi, "Effect of some fungicides against the growth inhibition of Sclerotinia sclerotiorum mycelial compatibility groups," *Journal of plant protection research*, vol. 55, no. 4, 2015.

[24] C. Bhrger, D.R.Fischer, D. A.Cordenunzzi, A.P. Batschauer de Borba, V.C. Filho, and Soares dos Santos, AR. "Acute and subacute toxicity of the hydroalcoholic extract from Wedelia paludosa (Acmela brasilinsis) (Asteraceae) in mice,"*J. Pharm. Sci*., vol 8, no.2), pp. 370-373, 2005

[25] B.Rosner, Fundamentals of Biostatistics; Brooks/cole/cengage learning. Inc., Boston, USA, 2010.

[26] V. A. Worwood, *The Complete Book of Essential Oils and Aromatherapy,Revised and Expanded: Over 800 Natural, Nontoxic, and Fragrant Recipes to Create Health, Beauty, and Safe Home and Work Environments*. New World Library, 2016.

[27] H. S. Laswai *et al.*, "Use of locally available flavouring materials in suppressing the beany taste in soymilk," *African Journal of Food, Agriculture, Nutrition and Development*, vol. 9, no. 7, 2009.

[28] M. O. Soares *et al.*, "Evaluation of antioxidant and antimicrobial properties of the Angolan Cymbopogon citratus essential oil with a view to its utilization as food biopreservative," 2013.

[29] S. Abd-El Fattah, A. Yahia Hassan, H. Bayoum, and H. Eissa, "The use of lemongrass extracts as antimicrobial and food additive potential in yoghurt," *J. Am. Sci*, vol. 6, pp. 582-594, 2010.

[30] F. A. Abu-Seif, M. Abdel-Fattah, A. Sreia, and M. M. Ramadan, "Antifungal properties of some medicinal plants against undesirable and mycotoxin-producing fungi," *Journal of Food and Dairy Sciences,* vol. 34, no. 3, pp. 1745-1756, 2009.

[31] N. Kamsu *et al.*, "Antifungal activities of essential oils of cinnamon (Cinnamomum zeylanicum) and lemongrass (Cymbopogon citratus) on crown rot pathogens of banana," *Indian Phytopathology,* vol. 72, no. 1, pp. 131-137, 2019.

[32] D. Sivakumar and S. Bautista-Baños, "A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage," *Crop Protection,* vol. 64, pp. 27-37, 2014.

[33] C. Carson, K. Hammer, and T. Riley, "Melaleuca alternifolia (tea tree) oil: a review of antimicrobial and other medicinal properties," *Clinical microbiology reviews,* vol. 19, no. 1, p. 50, 2006.

[34] C. Elama, M. Alayoubi, M. Jazzar, and F. Al-Rimawi, "Effect of different essential oils on the shelf life of concentrated yogurt," 2019.

[35] H. A. Eissa, S. M. Abd-Elfattah, and F. A. Abu-Seif, "Anti-microbial, anti-browning and anti-mycotoxigenic activities of some essential oil extracts in apple juice," *Polish journal of food and nutrition sciences,* vol. 58, no. 4, 2008.

[36] K. Muramatsu, M. Ogata, M. Sugawara, and K. Kiuchi, "Antifungal activities of essential oils of spice and sucrose ester of Lauric Acid against Saccharophilic Fungus Wallemia sebi," *JOURNAL OF ANTIBACTERIAL AND ANTIFUNGAL AGENTS JAPAN,* vol. 26, pp. 3-10, 1998.

[37] V. Suriyarachchi and G. Fleet, "Occurrence and growth of yeasts in yogurts," *Applied and Environmental Microbiology,* vol. 42, no. 4, p. 574, 1981.

[38] N. De, T. Goodluck, and M. Bobai, "Microbiological quality assessment of bottled yogurt of different brands sold in Central Market, Kaduna Metropolis, Kaduna, Nigeria," *Int. J. Curr. Microbiol. Appl. Sci,* vol. 3, no. 2, pp. 20-27, 2014.

[39] S. C. Lee *et al.*, "Analysis of a food-borne fungal pathogen outbreak: virulence and genome of a Mucor circinelloides isolate from yogurt," *MBio,* vol. 5, no. 4, 2014.

[40] M. Othman, H. Saada, and Y. Matsuda, "Antifungal activity of some plant extracts and essential oils against fungi-infested organic archaeological artefacts," *Archaeometry,* vol. 62, no. 1, pp. 187-199, 2020.

[41] A. Farhan, H. N. Al-Zobaidy, and M. F. Al-Quraishi, "FORTIFICATION OF THICK YOGURT WITH MINT (MENTHA SPICATA L.) LEAVES EXTRACT."

[42] G. A. Evrendilek and V. Balasubramaniam, "Inactivation of Listeria monocytogenes and Listeria innocua in yogurt drink applying combination of high pressure processing and mint essential oils," *Food Control,* vol. 22, no. 8, pp. 1435-1441, 2011.

[43] L. Golestan, L. Seyedyousefi, H. Kaboosi, and H. Safari, "Effect of Mentha spicata L. and M entha aquatica L. essential oils on the microbiological properties of fermented dairy product, kashk," *International journal of food science & technology,* vol. 51, no. 3, pp. 581-587, 2016.

[44] C. A. Costa, L. T. Bidinotto, R. K. Takahira, D. M. Salvadori, L. F. Barbisan, and M. Costa, "Cholesterol reduction and lack of genotoxic or toxic effects in mice after repeated 21-day oral intake of lemongrass (Cymbopogon citratus) essential oil," *Food and Chemical Toxicology,* vol. 49, no. 9, pp. 2268-2272, 2011.

[45] S. Sinha, M. Jothiramajayam, M. Ghosh, and A. Mukherjee, "Evaluation of toxicity of essential oils palmarosa, citronella, lemongrass and vetiver in human lymphocytes," *Food and Chemical Toxicology,* vol. 68, pp. 71-77, 2014.