The Use of lemongrass extracts as Antimicrobial and food additive potential in yoghurt

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ABSTRACT: The following study was conducted to investigate the antifungal and food additive potential of medicinal plants. herbal decoction and essential oil (EO) extracts of *Cymbopogon flexuosus* (lemongrass) leaves and stems were tested for their inhibitory action against spoilage organisms and mycotoxins formation in two separated experiments. In the first experiment, yeast- extract sucrose medium (YES) was used as a basal medium to examine the mold growth and mycotoxin production by three pathogenic fungi: *Aspergillus flavus (A. flavus), Aspergillus parasiticus (A. parasiticus) and Aspergillus ochraceus (A. ochraceus).* The YES medium was supplemented with four different concentrations of Lemongrass oil, inoculated with 1-mL of a spore suspension containing 10^5-10^6 conidia of each test mold and then incubated at 28º C for 14 days. After incubation period, cultures were analyzed for mycelial dry weight and mycotoxin accumulation. In the second experiment, yoghurt medium was used as a basal medium and the same system of study was applied in two different degrees of temperature (5ºC and 28ºC) for 4 weeks. Evaluation of the Lemongrass oil activity in yoghurt samples focused on the microbial stability of yoghurt, sensory evaluation as well as mold growth and mycotoxin formation. In the 1st experiment, the level of 0.1% of the EO extract was effective in inhibition both mold growth and mycotoxin production for all tested molds, and 0.3 % extract completely prevented the growth and toxin production. whereas, 1% of the decoction extract was effective. So, the EO extract was the suitable agent in the second experiment. It is of interest to note that while reduction in mold growth due to increasing extract concentrations was observed, the most striking effect was the reduction of mycotoxin production. The obtained data from the second experiment showed that the EO extract (0.1% concentration) inhibited viable yeasts and preserved yoghurt for over 28 days at 5ºC**.** Also, the inhibitory action of the EO extract against yeasts was concentration dependent. The maximum inhibitory effect of was found when the extract level increased above 0.1%. Incubation temperature had an important role in growth and mycotoxin production in yoghurt medium. During cold storage for 28 days at 5°C, the different concentrations of the EO extract added to the yoghurt samples displayed different titratable acidity and total bacterial cells and pH than the control yoghurt ($p < 0.05$). Overall sensory acceptability of yoghurt supplemented with the EO extract was higher than that of the control yoghurt prepared without the EO extract. Total sensory evaluation of experimental yoghurt used as a control was up to 4.3 scores lower compared to yoghurt samples treated with the EO extract. The results indicate that the addition of the appropriate the EO concentrations (0.1%, w/v) improved the physicochemical properties as well as sensory characteristics of yoghurt, could be used for decontamination of dairy products such as yoghurt from mycotoxigenic fungi and mycotoxins formation, beside its beneficial properties as a functional food.

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INTRODUCTION

Yoghurt in addition to its high nutritional value, it possesses antagonistic and therapeutic values. The valuable sensory characteristics of yoghurt are due to its content of carbonyls, mainly acetaldehyde, acetone, diacetyl and ethanol, produced by yoghurt bacteria. Yoghurt provides higher levels of protein, carbohydrate, calcium and certain B vitamins than milk **(Gurr, 1987; Deeth** and **Tamime, 1981).** The shelf life of yoghurt is short, i.e., 1 day under ambient condition (25–30ºC) and around 5 days at 7ºC, which hinders its commercialization **(Salji, 1987)**. Yoghurt defects due to microbial contamination are widely reported in literature; the most frequent contaminants are yeasts and moulds **(Spolaor *et al,* 1988; Foschino** and **Ottogalli, 1988;** and **Abdel-Fattah** and **Abdel-Salam 2004)** usually causing the swelling of packs, the presence of superficial coloured spots and abnormal tastes **(Ottogalli, 1991).** The low pH of yoghurt offers a selective environment for the growth of acid tolerant yeasts and molds **(Banaquio *et al.,* 1981, Spillmann** and **Geiges, 1983).** Therefore, it is

not surprising that various investigators have found that yeasts are the primary spoilage microorganisms for yoghurt and that fruits, flavors, and coloring agents are frequent contamination sources **(Main, 1984; Weber** and **Broich, 1986).** The spoilage of yoghurt by yeasts has been generally characterized by yeasty offflavors, loss of textural quality due to gas production, and swelling and occasional rupturing of the product containers **(Davis, 1974).** As a result, there is an apparent need for an effective preservation method to control acid-tolerant spoilage yeasts and molds in yoghurt.

Micotoxigenic Fungi and Pathogenic bacteria can grow at refrigeration temperature to numbers, which can result in an infection. For this reason dairy products should be kept well covered to prevent contamination, should ideally be consumed within two days of opening, or used in cooked foods after that two-day period **(Potter** and **Hotchkiss, 1995).** Mycotoxins may be found in milk products, originating from three possible sources: raw milk (such as aflatoxin M1 which present as a consequence of aflatoxin B1 metabolism by the animal); growth of a toxigenic fungal strain on product and mycotoxins synthesis, and production of these toxins in dried milk used to make milk product **(Jose *et al.,* 1988).**

Hence, it is highly desirable to prevent mould growth or to prevent mycotoxins formation in contaminated food. Several chemicals have been used to detoxify mycotoxins but these chemicals can not be added to foods to prevent mycotoxins formation because of their hazardous effect on human health. In recent years, studies on the natural antifungal agents, herbs and spices, have been reported by numerous investigators **(Afroditi, 1995; Hiroshi** and **Jun Sato, 2002;** and **Abd-EL Fattah and Abdel-Salam, 2004).** They found that some herbs and spices had antifungal effect against some kinds of mycotoxinic fungi, such as *A. flavus*, *A. ochraceous and A. parasiticus*, in synthetic medium. Among herbs and spices used, were sage, thyme, rosemary, mint, and Lemongrass.

Cymbopogon flexuosus (Lemongrass) is an economically important plant that has been used for centuries, as a medicine because of its wide-ranging therapeutic properties included relief of rheumatic and other pain and healing effect on ulcers **(Fenwick *et al.,* 1990).** Flavonoids extracted from Lemongrass are of considerable interest as natural plant components with antioxidant and antifungal activity **(Pratt** and **Hudson,1990; Nieto *et al.,* 1993;** and **Abu-Seif, *et al.,* 2009).** Of the flavonoids present in Lemongrass, licochacone A and licochacone B which have equal antioxidant activity of vitamin E, and glabrene which is 3 times as active when compared with vitamin E **(Okuda *et al.,* 1989).**

One objective of the present study was to investigate the inhibitory action of Lemongrass oil against spoilage organisms and mycotoxins formation in yoghurt under laboratory conditions. The use of Lemongrass herb in this study was due that Lemongrass is naturally occurring material, widely cultivated, cheep, had a medical functions and safe. These properties and the antifungal activity, if possible, make lemongrass oil may be potential multi-functional food additives. The physic-chemical properties, color characteristics, total phenol content, microorganisms, sensory evaluation and the effect of storage time at 5ºC for 2 months of yoghurt were also studied.

2- MATERIALS AND METHODS

2.1- Experimental design.

Depending on our previous results **(Abdel-Fattah, 2002; Abdel-Fattah** and **Abdel-Salam, 2004** and **Abu-Seif, *et al.,* 2009),** concerning the antimicrobial effects of herbal extracts, this study was achieved. Two separated experiments were carried out during this study. The first experiment was to examine the best of the tow different extracts of Lemongrass, essential oil (EO) extract and decoction extract as antifungal agents. In the second experiment, the best extract selected from the first study was used on yoghurt medium, to test its antifungal effects and to study the physico-chemical properties, color characteristics, total phenol content, as well as microbial stability and sensory evaluation of yoghurt during storage time at 5ºC for 4 weeks.

2.2- Organisms.

a- Fungal strains: *Aspergillus flavus* (*A. flavus*), aflatoxigenic local strain; *Aspergillus parasiticus* (*A.parasiticus*) NRRL 2999 and *Aspergillus ochraceus* (*A.ochraceus*) NRRL 3174, were obtained as lyophilized preparation from the Mycotoxin lab. , National Research Center, Dokki, Giza , Egypt.

b- Bacterial strains: Starter culture of Yoghurt Streptococcus salivarius subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus (1:1) was obtained from HACCP certified and ISO22000: 2005 Dairy Company, and used as a source of the starter culture.

2.3- Milk used for making of lab. Yoghurt:

Raw buffalo's milk used for making yoghurt, the milk was obtained from a dairy farm at Agriculture

faculty, Cairo University Governorate. Starter cultures used for making plain yoghurt Old plain yoghurt obtained from HACCP certified & ISO22000: 2005 Dairy Company was used as a source of the starter culture.

2.4- ***Plant material:***

Lemongrass powder (leaves and stems) was purchased from an Egyptian local market (Harraz Co., Cairo, Egypt).

2.5- Mycotoxins standards:

All standards of Mycotoxins (Aflatoxins, B1, B1, G1, G2, and ochratoxin A) were purchased from sigma company, USA.

2.6- **Analytical methods:**

2.6.1- Extract preparations of Plant material:

Lemongrass powder was purchased from a local market and the samples were extracted as follow:

a- Preparation of herbal decoction: Decoctions of lemongrass leaves were prepared by boiling the powder material at solid : liquid ratio 1:10 with distilled water for 5 minutes. The vessel containing the decoction and herb was then covered and removed from the heat and allowed to cool for 5 minutes. The herbal material and liquid was then strained through cheese cloth and the resulting decoction placed into 100 mL reagent bottles which had been kept for use as the test decoction **(Abdel-Fattah** and **Abdel- Salam, 2004).**

b- Preparation of herbal Oil: Briefly, 250 g fresh plant material (leaves and stems) of lemongrass plant was put in a round bottom flask and 1000 mL distilled water was added before subjecting to hydro-distillation **(Bankole** and **Joda, 2004)** for 6 hours. The oil was recovered and dried over anhydrous sodium sulphate.

2.6.2- Spore suspension of fungal strains:

These culture strains were grown on (PDA) slants for 10 days at 25 °C Untill will sporulated . the spores were washed from the slants with a sterile 0.01% solution of tween 80 as a spore despersal agent. The final spore preparations were resuspended in the appropriate volume of sterile saline to yield a direct microscopic count of approximately 10^5-10^6 spores /mL, of each tested fungus.

2.6.3- Preparation of yoghurt containing Lemongrass oil:

Raw buffalo's milk was subjected to a heat treatment at 72°C for 2 seconds to kill microorganisms followed by cooling to 40 – 45°C. Oil extract of Lemongrass was added to milk before processing with different concentrations. As starter culture yoghurt (*L.bulgaricus* and *S. thermophilus*) was added (1.5%) to the milk, followed by mixing, and packed in sterilized glass capped cups 100 mL capacity, followed by incubation at 40°C for 3 hours till gel forms (pH 4.5). Freshly yoghurt was cooled and stored at refrigeration at 5°C till examination to slow down the physical, chemical and microbiological analysis.

2.6.4- Antifungal assay:

2.6.4.1- With yeast extract sucrose medium (YES): Yeast extract- sucrose (YES) broth (2% yeast extract, 15% sucrose) was used in the 1st experiment as a basal medium for mould growth and mycotoxin production in stationary cultures **(Davis *et al.*,1966).** Each medium (50 mL) in 250 mL Erlenmeyer flasks was sterilized at 121ºC for 15 min. For each organism used, the appropriate amounts of lemongrass essential oil and lemongrass decoctions were added into strile YES to obtain the concentrations of 0.05, 0.1, 0.3, 0.5 and 1.0 %. YES without any herbal extract added served as control. Each flask with or without appropriate extract was inoculated with 0.1 mL spore suspension, then cultures were incubated at 28°C in the dark for 14 days. Each concentration of tested extracts was tested twice, sometimes three times.

2.6.4.2- With yoghurt medium: The same antifungal assay on YES, was applied on yoghurt medium except that extract used was the EO extract, added to milk before processing and the culture media were incubated at two different temperature, 5 and 28 ºC for 35 days. Yoghurt medium were inoculated with mycotoxiginic fungi and stored for 35 days at 5 and 28 ºC. Semi quantitative assay of the tested oil extract was conducted according to **Harboure (1973).**

2.6.4.3- Evaluation of antifungal properties of lemongrass extracts.

a- *With YES broth media*: Contents of flasks, with and without (serve as the control) lemongrass extracts, were analyzed in triplicates for dry weight of mycelium and mycotoxin accumulation. Dry weight of mycelium was determined according the method of **Davis *et al.*, (1966).** Aflatoxins were extracted according to the modified procedure of **Eleghede, (1978).** After extraction, aflatoxins (B_1+G_1) were determined according to the **AOAC methods, (1980).** Ochratoxin A (OA) was extracted and determined

according to the method of **Scott *et al.*, (1971).** Percent reduction or accumulation over control, in growth or mycotxin production was calculated by the following equation:

Percent reduction = $100 - \{(A_1 / A_0) * 100\}$

Accumulation over control = $\{(A_1 / A_0) * 100\} - 100$

Were: A_1 = The amount obtained by treatment.

A_0 = The amount obtained by control.

b- *With yoghurt medium:* Fungal growth of all tested molds were visually assessed using a semi-quantitative scale, Viz.(0) no growth; (1) very little growth covered the surface of the plate; (2) 25 % of the plate surface covered; (3) 50% of the plate surface covered; (4) 75 % of the plate surface covered and (5) 100% of the plate surface covered. Yoghurt medium were examined for the presence of aflatoxin production as described by the **AOAC methods, (1980).** For ochratoxin A, cultures were extracted, and OA levels were determined as described by **Valenta** and **Michael, (1996).**

2.6.5- Microbiological stability of yoghurt:

The microbial stability of yoghurt containing the EO extract of lemongrass during storage at refrigerator (5ºC) for 28 days were investigated. The populations of total bacteria, yeast and molds were determined by the method of **Sadler, *et al.*, (1992).** The counts of total bacteria (TPC), yeast and molds (Y&M) calculated per one gram of all yoghurt slices using plate count agar and malt extract agar (Merck KGaA, Darmstadt, Germany). The number of colonies (TPC or Y&M) that appeared on the plates was counted and expressed as Colony Forming Unit (CFU/g).

2.6.6- Determination of pH:

The pH of fruit sample was measured using a combination pH electrode with a digital pH mater (HANNA, HI 902 meter, Germany) standardized with stirring as described in **(AOAC, 2000).**

2.6.7- Determination of total soluble solids (TSS):

The percent total soluble solids, expressed as oBrix, were determined with a refractometer (ATAGO, Japan).

2.6.8- Determination of Titratable acidity (TA):

Titratable acidity were determined as described by **(Tung Sung Chung, *et al.*, 1995)** by using approximately 10 g portion of yoghurt sample blended with 100 mL distilled water for 30 sec in blender and was titrated to pH 8.0 with a 0.1N NaOH solution. The end point was determined with a pH-meter. Titratable acids in the sample were calculated as percent of lactic acid or malic acid.

2.6.9- Viscosity measurements:

The viscosity measurements were carried out using a HAAKE viscometers (HAAKE, Mess-Technik Gmbhu. Co., Germany) with thermostatic bath to control the working temperature within the temperature of 25°C. Results of viscosity were expressed in centipoise (cP) according to the method of **Ibarz *et al.*, (1994).**

2.6.10- Total phenol determination:

Total phenol content of the untreated and treated samples was measured by the method of **Amerine** and **Ough (1980),** the absorbance was measured at 765nm using Spectrophotometer, UVD-3500, Labomed, USA and the results were expressed as milligram of garlic acid as standard equivalent per gram.

2.6.11- Color characteristics determinations:

Color is one of the more important quality parameters in processed products. Undoubtedly, possible color changes would influence the Organolyptic properties of samples and would limit their potential applications.

Hunter a*, b* and L* parameters were measured with a color difference meter using a spectrocolorimeter (Tristimulus Color Machine) with the CIE lab color scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode. The instrument was standardized each time with white tile of Hunter Lab Color Standard (LX No.16379): X= 72.26, Y= 81.94 and Z= 88.14 (L*= 92.46; a*= -0.86; b*= -0.16) **(Sapers and Douglas, 1987).** Color difference, Delta E, was calculated from a*, b* and L* parameters, using Hunter-Scotfield's equation **(Hunter., 1975)** as follows.

Delta E = (delta a^2+ delta b^2 + delta L^2) 1/2 -----(1)
where: a - a_o, b - b_o and L - L_o; subscript "o" indicates color of control or untreated sample.

The Hue-Angle (H)*, Chroma (C)* and Browning Index (B_I) was calculated according to the method of **Palou *et al.*, (1999)** as follows:

H* = tan-1 [b*/a*]…….…… (2)
C* = square root of [a^2* + b^2*]....……...…. (3)
BI = [100 (x-0.31)] 10.72…….……. (4)

Where: X = (a*+ 1.75L*) / (5.645L*+ a* - 3.012b*)

2.6.12- Sensory Evaluation:

Sensory evaluation of the studied was carried out included 20 experienced panelists. The attributes such as: flavour intensity, body, texture and color were organolptically assessed at stated by **(Crandall, *et al.*, 1990).** The all tested samples subjected to sensory evaluation after 28 days in yoghurt samples.

2.7- **Statistical Analysis:**

Analyses for experiments were performed in duplicated, and results were averaged. A Duncan Multiple Range Test was carried out by means of the "shortest significant ranges SSR" **(Larmond, 1974)** to determine the differences between the treatments using HDSS statistical analysis program.

3- RESULTS AND DISCUSSION

3.1- Antifungal effect of the two different extracts of lemongrass on YES borth medium:

Data presented in Table (1) clearly indicate that mould growth by all tested strains, were suppressed by lemongrass oil extract or decoctionextract. The inhibitory effect of these extracts was proportional with their concentrations. Slightly effect on fungal growth was observed when low concentration (0.05% and 0.1%, respectively) of the EO extract and decoction extract were applied, whereas high concentrations of these extracts inhibited fungal growth and, consequently, mycotoxin formation. The maximum inhibitory effect of these extracts were recorded at the level 1 % and 0.3% for decoction extract and EO extract, respectively (Table, 1).

In regard to Table (2), *A. ochraceus* was more sensitive one for the two lemongrass extracts than the two other molds. The EO extract was more effective agent on mycelial growth than decoction extract. The inhibitory effect of the two different extracts on mycelial growth according to the mold type, may be rankled as follow: *A.ochraceus* > *A.flavus* > *A. parasiticus*, for the EO extract. However, for decoction extract were: *A.ochraceus* > *A. parasiticus* > *A.flavus.* These differences in the inhibitory effect may be mainly due to interfering some factors: the mold type, incubation temperature and type of extract and subsequently the differences in the chemical composition for each extract. In this respect, many publications indicated that the compositions and concentrations of compounds within the distinct types of herbal extract preparations would differ and play an important role in its antifungal activity action **(Buchanan** and **Shepard, 1981; Lienert *et al.*,** and **Nass-Reinhold *et al.*, 1998;** and **Abdel-Fattah** and **Abdel-Salsm, 2004).** Also, **El GendY** and **Marth, 1980,** reported that temperature is one factor affects mold growth and mycotoxin production by toxigenic aspergilli and penecillus and in the presence of lactic acid bacteria. **Abdel-Fattah, (2002),** found that mold type and incubation temperature were important factors affecting mold growth and aflatoxin production by *A. flavus* and *A. parasiticus* in media contained solvent extracts of licorice.

Data represented in Table (2) clearly indicate that increasing levels of the extract, in YES broth media, resulted in detection of decreasing levels of mycotoxin production. At the lowest level (0.05% extract), reduction in mycotoxin production was slightly decreased in the media supplemented with decoction extract compared to those supplemented with oil extract. Increasing extract concentrations caused a linear depression in mycotoxin formation by the all tested molds, but the maximum inhibitory effect was differ, and this may be referred that organism was more variable in its reaction to the extract. This trend indicates that extract inhibited mycotoxin formation by inhibiting the mould growth. In a similar study, **Masood** and **Ranjan (1991)** reported that extracts of *Argemone mexicana* and *cyperus rotudns* inhibited aflatoxin production by inhibiting the growth of *A. flavus.* Also, **Mahmoud *et al.*, (1994),** found that extracts of lupinus and xanthium punens inhibited aflatoxin production by inhibiting the growth of *A. flavus.*

Reduction of fungal growth and mycotoxin production by the EO extract in our study was due to interference by active principles of these extracts. Such interference may be at the biosynthetic levels. In this respect, **Kumar** and **Prasad (1992)** suggested that growth and aflatoxin production by *A. flavus* are proportionate processes. However, **Bhatnagar** and **McCromick (1987)** reported that the growth and aflatoxin production by *A. parasiticus* are independent phenomena. On the other hand, **Abu-Seif, *et al.*, (2009)** reported that oil extract of lemongrass leaves and stems, completely inhibited mycelial growth and mycotoxin production of *A. flavus, A. parasiticus and A. ochraceus* at level (0.3 %) of YES broth medium.

Data represented in Table (1) showed that there were a considerable differences in mold or mycotoxin inhibition in YES medium supplemented with the EO extract or the decoction extract, in trend to EO extract. Therefore, the EO extract of Lemongrass leaves and stems was selected, as the best, to examine its antimicrobial effect on yoghurt medium.

Table (1): Mold growth (mg/50 mL media) and percent reduction of the tested molds as affected by the two different extracts of lemongrass on YES broth media for 14 days at 28° C.

Extract level, %	Mold growth and percent reduction for different toxigenic strains					
	A. flavus		*A. parasiticus*		*A. ochraceus*	
	Mould growth	percent reduction	Mould growth	percent reduction	Mould growth	percent reduction
Control, 0.0%	410±13.4^{F}	0.0	305±14.8^{F}	0.0	840±10.5^{F}	0.0
Decoction extract						
0.05						
0.1	325±16.5^{E}	20.73	251.5±19.5^{E}	17.54	599.6±16.3^{E}	28.62
0.3	304±8.3^{E}	25.85	251.5±8.3^{E}	17.54	538±22.4^{D}	35.95
0.5	259±13.0^{D}	36.83	217.5±14.7^{D}	28.70	315±16.5^{C}	62.50
1.0	192±21.5^{C}	53.17	176.5±9.5^{C}	42.13	110±8.7^{B}	86.90
EO extract	175±13.5^{C}	57.32	170.0 ±7.3^{C}	44.26	80±7.2^{B}	90.48
0.05						
0.1	180±11.6^{C}	56.10	65±0.9^{B}	78.69	113±11.4^{B}	86.55
0.3	112±5.8^{B}	72.68	0.0^{A}	100	0.0^{A}	100
0.5	0.0^{A}	100	0.0^{A}	100	0.0^{A}	100
1.0	0.0^{A}	100	0.0^{A}	100	0.0^{A}	100
	0.0^{A}	100	0.0^{A}	100	0.0^{A}	100
LSD at ($p \leq 0.05$)	28.8	-	21.2	-	36.5	-

Each value represents the mean ± SE of three replicates.

Table (2): Mycotoxin production and percent reduction of the tested molds as affected by the two different extracts of lemongrass on YES broth media for 10 days at 28° C.

Extract level, %	Mycotoxin production (µg per 50 mL YES) and percent reduction for different toxigenic strains.					
	A. flavus		*A. parasiticus*		*A. ochraceus*	
	Mycotoxin production	percent reduction	Mycotoxin production	percent reduction	Mycotoxin production	percent reduction
Control, 0.0%	265	0.0	345	0.0	270	0.0
Decoction extract						
0.05	180	32.07	295	14.49	150	44.44
0.1	135	49.06	210	39.13	103	61.85
0.3	55	79.24	73	78.84	45	83.33
0.5	56	79.24	31	91.01	0.0	100
1.0	0.0	100	0.0	100	0.0	100
EO extract						
0.05	125	52.83	113	67.25	28	89.63
0.1	105	60.38	93	73.04	0.0	100
0.3	0.0	100	0.0	100	0.0	100
0.5	0.0	100	0.0	100	0.0	100
1.0	0.0	100	0.0	100	0.0	100

Each value represents the mean of three replicates.

3.2- Antifungal effect of lemongrass oil on yoghurt medium:

Results obtained from Table (3) showed that increasing levels of EO extract added to yoghurt either incubated at 5 or 28 ºC for 28 days, an inhibitory effect was noted on the growth of the all tested molds. At the lowest concentrations of extract (0.05%), the mold growth by the tested molds, were comparatively no changed over control. However,

increasing concentration level up to 0.1% completely prevented the mold growth, either when the media incubated at 5or 28 ºC. These results also indicate that growth was influenced by both mold type and incubation temperature. Also, these results revealed that 28 ºC was the optimum incubation temperature for growth and consequently, mycotoxin formation occurred in this study. The mold growth was higher by *A. flavus* and *A. ochraceus* than *A. parasiticus.* Also, growth was influenced by mold type and temperature degree of incubation. Temperature is one factor which affects mold growth and mycotoxin production. Other publications supported our results **(EL-Gendy** and **Marth, 1980; Mashaly** and **El-Deeb, 1982,** and **Abdel-Fattah, 2002, Abdel-Fattah and Abdel-Salam, 2004).**

When mycotoxin production was determined in the media incubated at 5 and 28ºC, the effect of EO extract was even more striking (Tables 4 and 5). Increasing concentration of extract resulted in detection of decreasing levels of mycotoxin production. At the lowest level of extract (0.05%), a great reduction mycotoxin production were found, and the reduction was especially pronounced with aflatoxins and ochratoxin A. No toxin was detected when the extract level increased to 0.1%.

Regarding results represented in Tables 3, 4 and 5, The lack of mycotoxin production on yoghurt raises questions concerning the reasons for this phenomenon. The possibility that ingredients contained in yoghurt, but not in YES, might be inhibitory to growth and toxin production was considered.

It is possible that antifungal activity of the used lemongrass essential oil in this study is due to an unidentified component of the antioxidants extracted (perhaps phenols, flavonoids, flavones,..etc) . In this respect, **Rosenthal *et al.,* 1997,** reported that phenols play an important role as antifungal agents. They found that ferulic acid, p-coumaric acid and other plant cell wall phenols, had antifungal actions in microorganisms isolated from dairy products**. Also, Abu-seif *et al.,* 2009,** found that phenolic compounds extracted from Lemongrass leaves had antifungal effects on *A. flavus and A. parasiticus.* **Hiroshi** and **Sato (2002),** reported that lemongrass extract with 80% methanol was found to have high fungicidal effect against Arthrinium sacchari M001, and its active compound was identified as glabridin. Strong antioxidant activity has been observed in flavonols such as quercetin, flvonones such as luteolin, and chalcones such as butin **(Hudson** and **Lwis, 1983).**

Table (3): Effect of various concentrations lemongrass oil extract on molds incubated for 28 days at 5 and 28° C.

Extract ,%	*A. flavus*		*A. parasitics*		*A. ochraceus*	
	5° C	28° C	5° C	28° C	5° C	28° C
Control, 0.0%	3	5	3	5	2	4
EO extract						
0.05	2	4	3	4	1	3
0.1	0	0	0	0	0	0
0.3	0	0	0	0	0	0
0.5	0	0	0	0	0	0
1.0	0	0	0	0	0	0

(0) no growth; (1) very little growth ; (2) 25 % of the plate surface covered with mycelia ; (3) 50% of the plate surface covered with mycelia; (4) 75 % of the plate surface covered with mycelia and (5) 100% of the plate surface covered with mycelia.

Table (4): Effect of various concentrations lemongrass oil extract on mycotoxin production in yoghurt medium for 28 days at 5 and 28ºC.

Extract level	Mycotoxin production (µg per 50 mL YES)					
	At 5°C			At 28°C		
	Total Aflatoxins		Ochratxin A	Total Aflatoxins		Ochratoxin A
	A. Flavus	*A. parasiticus*		*A. Flavus*	*A. parasiticus*	
Control, 0.0%						
EO extract	35.3	84.0	116.0	108.6	142.5	185.0
0.05						
0.1	0.0	0.0	0.0	65	56.5	0.0
0.3	0.0	0.0	0.0	43	40.15	0.0
0.5	0.0	0.0	0.0	0.0	0.0	0.0
1.0%	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0

- Each value represents the mean of three replicates.
- ND: No toxin detected.

Table (5): percent reduction in mycotoxin production over control by mold with oil extract of lemongrass in yoghurt media for 35 days at 5 and 28° C.

Extract level	Percent reduction					
	At 5°C			At 28°C		
	Total Aflatoxins		Ochratoxin A	Total Aflatoxins		Ochratoxin A
	A. Flavus	*A. parasiticus*		*A. Flavus*	*A. parasiticus*	
Control, 0.0% **Oil extract**	0.0	0.0	0.0	0.0	0.0	0.0
0.05	100	100	100	40.14	60.35	100
0.1	100	100	100	60.40	71.82	100
0.3	100	100	100	100	100	100
0.5	100	100	100	100	100	100
1.0%	100	100	100	100	100	100

Each value represents the mean of three replicates.

3.2- The effect of essential oil of lemongrass on the log (CFU/gm) of growth yeast and molds (Y&M) and bacteria in yoghurt during storage at refrigerator for 4 weeks.

The microbial stability of yoghurt supplemented with extracts of lemongrass during storage at refrigerator (5°C) for 4 weeks were investigated. Tropical spices (lemongrass) may prove useful in preservation of yoghurt by hurdle technology **(Ejechi, *et al.*, 1998).** Total microbial count of different yoghurt treatments with 0.0, 0.05, 0.1, and 0.3 % of lemongrass EO extract, and of untreated yoghurt were followed up through 4 weeks at 5°C. The effect of treating yoghurts with the studied various volatile or essential oil extract and stored at 5°C for 4 weeks on inhibiting the microbial counts are seen in Table (6). It can be observed that the yoghurt treated with low concentration of lemongrass extract have the highest inhibition of yeast and molds (Y&M) and bacteria (B) followed by those treated with high concentration of lemongrass extract after 4 weeks at 5°C. Untreated yoghurt was 6.75 log (CFU/gm) of B compared to 0.00 log (CFU/gm) in case of those Y&M. Whereas, the yoghurt treated with 0.5% lemongrass was 0.00 log (CFU/gm) of Y&M and 6.10 log (CFU/gm) of B.

The results from Table 6 showed that the yoghurt treated with high concentration of lemongrass has also the highest reduction of Y&M and B followed by low concentration of lemongrass, but untreated samples were the lowest reduction of Y&M and B for 4 weeks stored at 5°C.

These results are partially confirmed by those of **Kanako *et al.*, (1998).** They found that Limon grass and clove exhibiting strong anti-fungal activity for 30 days. Whereas no colonies were seen for 30 days and fungal growth was inhibited for more than 30 days. On the other hand, **Sebti** and **Tantaoui, (1994)** showed that cinnamon powder although very efficient at inhibiting the fungi, imported a dark color to the papers and therefore is not recommended. While, cinnamon water extract did not inhibit fungal growth up to concentration of 80 g/kg (8%). Also, results from Table (6) showed that refrigeration temperature 4 oC of yoghurt could enhance the inhibitory effect of volatile or essential oil lemongrass extract. These results nearly in consistent with results given by **Eissa *et al.*, (2003a, b), Eissa *et al.*, (2008)** and **Ting and Deibel, (1992)** who appeared that refrigeration temperature (5°C) could enhance the inhibitory effect of some spices extracts but not others. When 0.5 or 1.0 % cloves were tested, the organism died more rapidly in tryptic soy broth at 24°C than at 5°C. Whereas, > 5 log reduction in CFU was observed after 7 days of incubation at 5°C and after 3 h incubation at 24°C in tryptic soy broth.

In general, the refrigeration of yoghurt effects increased the inhibition of bacteria, yeast and mold counts. Also, the results showed that the yoghurt treated with different concentrations of lemongrass extract were observed no browning and lowest microbial count (T and M and B) during storage at 5°C for 5 weeks. Lemongrass extract as preservatives may be due to it contain aldehydes and volatile compounds that have efficient on inhibition of browning and inhibition of growth microorganisms. **Nakatani, (1994)** proposed inhibitory mechanism that the anti-fungal action of the aldehydes that was due to the ability to form charge transfer complexes with electron donors and reactivity with SH group in cystein or glutathione moieties.

Zaika, (1988) reported that food product safety and shelf life depend in some part on the type, quantity, and character of volatile oil spices extracts added to the products.Then, our results showed that refrigerating at 5°C and 0.05% volatile or essential oil extracts treatments caused a marked reduction in yeast and bacteria populations with acceptable taste and extension shelf life of yoghurt up to 5 weeks.

Table 6. Effect of essential oil extracts on the number of colonies (TPC or Y&M) that appeared on the plates and expressed as the log (CFU/gm) in yoghurt during storage at refrigerator (5 °C) for 4 weeks.

Extract level,%	Zero time		1 week		2 weeks		4 weeks	
	*M&Y	TPC	M&Y	TPC	M&Y	TPC	M&Y	TPC
0.0	0	6.1	0	6.50	0	6.50	0	6.75
0.05	0	5.95	0	6.20	0	6.30	0	.506
0.1	0	5.90	0	6.00	0	6.10	0	5.95
0.3	0	5.90	0	5.90	0	6.00	0	6.10

- Each value represents the mean of three replicates
- (CFU/gm).means Colony Forming Unit per gream yoghurt.

3.3- Effect of lemongrass essential oil on physico-chemical content in yoghurt during storage time:

Quality evaluation of yoghurt products.

The following discussion of the chemical characteristics for fresh, products and lemongrass extract pre-treated yoghurt is based on the data given in table (7). The pH of fresh and treated yoghurt ranged from 4.04 to 4.49 showing a increase in pH values. The increase in pH was directly related to increase lemongrass extract concentrations in yoghurt. TSS (g/Kg) of yoghurt products after 28 days storage was lower than the fresh yoghurts. Whereas, the increase of TSS was obvious with increasing of lemongrass extract concentration. This increase of TSS was attributed to the greater degree of tissue breakdown, releasing more components that contribute to soluble solids **(Tung-Sun, *et al.,* 1995).** The TSS / acid ratio is the major analytical measurement for quality in fresh and treated yoghurt. The TSS / acid ratio of fresh, and treated yoghurt was increased by increasing of lemongrass extract concentration. TSS / acid ratio was shown to be correlated with sweetness but not so closely with flavour **(Guyer, *et al.,* 1993).**

Titratable acidity of yoghurt products was lower than fresh yoghurt (Table 7), which may due to enzymatic desertification and degradation of pectin resulting in an increased of total acid.

The viscosity (cP) was selected as a measure of yoghurt quality. However, the viscosity between fresh and treated yoghurt samples were decreased from 2.92 cP to 2.64 by increasing the lemongrass extract concentration in yoghurt than fresh sample (2.86 cP), respectively as seen in Table (7). Total phenol content were decreased also by increasing of lemongrass extract concentration in all yoghurt samples, as seen in table (7).

Table (7): Effect of Concentrations on physico-chemical properties in yoghurt at zero time and after 21 days.

Extract level,%	TSS		pH		% acidity		TSS/acidity		Viscosity (cP)		Total Phenols	
	at zero time	after 28 days	at zero time	after 28 days	at zero time	after 28 days	at zero time	after 28 days	at zero time	after 28 days	at zero time	after 28 days
0.0	7.50	6.00	4.04	4.39	0.94	0.94	7.8	6.38	2.86	2.60	1342.85	1342.85
0.05	7.00	6.00	4.00	4.30	0.96	0.92	7.4	6.51	2.92	2.80	1345.97	1345.97
0.1	7.50	7.00	4.08	4.39	0.97	0.94	7.7	7.44	2.75	2.60	1338.17	1338.17
0.3	8.00	7.00	4.11	4.49	0.98	0.95	8.17	7.36	2.64	2.52	1317.87	1317.87

3.4- Effect of lemongrass essential oil concentrations and storage on Color characteristics of yoghurt:

a- Color parameters during storage time of yoghurt:

Tristimulus Reflectance Colorimetry (TRC) measuring the reflectance L*, a* and b* values) was used to follow the extent of browning in yoghurt and change of color in foods **(Sapers** and **Douglas., 1987).** The results in Table (8) showed change of color in yoghurt during storage time up to 28 days at 5°C. These results illustrated the changes in color of yoghurt in terms of redness a*, yellowness b* and lightness L* during 28 days of storage at 5°C. In addition to determination of the lightness L*, redness a* and yellowness b*, for experimented yoghurt. Hue angle (H*) as well as the chromaticity (C*) were determined. Hue is the aspect of color that we describe by words such as green, blue, yellow or red. The chroma refers to reflection at given wavelength and indicates how much a color differs from gray **(Eissa** and **Moharam, 2001)**. The equations No. 1, 2, 3 and 4 are showed the DE, B_1, H* and C*.

The H* values were closely stable in all samples with increasing of storage time up to 28 days at 5°C. The chromaticity (C*) increased by the increasing of stoarge time in yoghurt up to 28 days. Thereafter, no relation was

noticed. It can be observed that the a*-value of the fresh yoghurt was -2.22 compared to -2.27after 7 days, -2.39 after 14 days and increased -2.18 after 28 days, as seen in Table (8) .

Regarding the lightness L* and the yellowness b*. It is clear that the lightness L* as well as the yellowness b* were decreased as a result of increasing the time of storage up to 28 days. The effect of storage time on increasing the a*-value from -2.22 at zero time days to -2.18 after 28 days was noticed. The change in color may be referred to chemical changes occurred during storage **(Kumar *et al.*, 2006).**

The analysis of variance identified the significant (p<0:05) effect of stoarge time on Hunter values of yoghurt. Although the a-value showed a definite increased trend throughout stoarge, the L-value decreased and the b-value increased as the yoghurt storage aged, as seen in Table (8).

It can be concluded that the stoarge of yoghurt slightly inhibited the changes in color yoghurt. The total color differences (DE) increased by the increasing of storage time in yoghurt up to 28 days as presented in Table 1, total colour differences of yoghurt were small, which almost correspond to the sensory difference threshold **(Rohm** and **Jaros, 1996b).** However, greatly different values of DE were found for yoghurts at 7, 14 and 28 days of storage and at different concentrations lemongrass extract treated samples. The almost identical colour values found in yoghurt could be attributed to their similar structure. The browning index (B_I) increased by the increasing of stoarge time in yoghurt up to 28 days, especially in high concentration treated yoghurt (0.3%). Thereafter, no relation was noticed.

b- Non-enzymatic browning of yoghurt samples:

Non-enzymatic browning in yoghurt is only one component that determines overall color and might not be a problem at low levels. The effects of heat treatment of milk and yoghurt products in the inhibition of the browning reaction are listed in Tables (8). It is obvious that the yoghurt product treated and untreated yoghurt samples inhibits the development of A 420 nm and red colour a*. For example, the A420 nm and a*-value of fresh yoghurt was 64.34 compared to 64.11, 64.36 and 61.56 in case of the different concentrations of lemongrass extract yoghurt samples, respectively. **Crandall *et al.*, (1986)** concluded that two measures of browning were used, color a* or L* and absorbance at 420nm where the higher numbers indicate increased absorbance due to the formation of brown pigments. Browning is also indicated by a decrease in the color L* toward black and an increase in the color a* toward brown or red.

Table (8): Effect of on colour characteristics in yoghurt during storage at 5 oC for 35 days.

	L*	a*	b*	Delta E	C*	H*	B_I	OD 420nm
At zero time								
0.0	92.22	-2.22	11.38	11.65	11.59	78.96	20.15	64.34
0.05	91.89	-2.14	11.04	11.31	11.25	79.03	19.60	64.11
0.1	92.19	-2.25	11.38	11.65	11.60	78.82	20.11	64.26
0.3	91.33	-2.27	11.91	12.22	12.12	79.21	21.46	61.56
Storage after 7 days								
0.0	93.68	-2.27	11.75	12.04	11.97	79.07	20.54	65.83
0.05	93.41	-2.24	11.08	11.35	11.30	78.57	19.20	66.3
0.1	93.97	-2.09	10.21	10.52	10.42	78.43	17.44	68.88
0.3	93.51	-2.38	10.32	10.63	10.59	77.01	17.34	68.34
Storage after 14 days								
0.0	92.4	-2.39	11.94	12.19	12.18	78.68	21.08	64.3
0.05	92.33	-2.09	11.12	11.34	11.31	79.36	19.75	64.77
0.1	92.09	-2.53	13.01	13.27	13.25	79	23.33	61.71
0.3	92.97	-2.23	12.12	12.35	12.32	79.57	21.57	63.56
Storage after 28 days								
0.0	93.2	-2.18	10.47	10.74	10.69	78.24	18.02	67.83
0.05	92.6	-2.25	11.92	12.17	12.13	79.31	21.20	64.47
0.1	91.69	-2.04	11.25	11.52	11.43	79.72	20.26	63.63
0.3	92.4	-2.59	13.75	14.03	13.99	79.33	24.82	60.63

3.5- Sensory evaluation of Yoghurt:

The results of sensory evaluation of the products based on colour, odour, taste, texture and appearance are shown in Table (9). Sensory evaluation of the yoghurt samples was carried out during 4 weeks of samples at 5°C by

20 experienced panels using 10 points scales. Difference in sensory properties of yoghurt samples due to the effect of different concentrations of lemongrass extract was determined by analysis of variance (ANOVA). Sensory attributes are of great importance to measure consumer attitudes and their influence on food choice and acceptability. The colour of yoghurt is the first quality attribute used to judge acceptability of yoghurt products.

The change in color may be referred to chemical changes occurred during storage of yoghurt samples **(Young-Hee,** and **Song Sun, 2009).** The mean value of flavor or odour scores for all treatments of yoghurt stored was 8.13, 7.75, 7.00 and 7.38 at 0.0, 0.05, 0.1 and 0.3 % concentration of lemongrass extract, respectively. The texture of the tested samples was affected by refrigeration. The over all mean score for the texture of yoghurt was only 8.13, 7.63, 8.13 and 8.25 at 0.0, 0.05, 0.1 and 0.3 % concentration of lemongrass EO extract, respectively (Table 9). These levels of score indicate the importance of the lemongrass extract in keeping a texture for the tested yoghurt. However, there were significant differences between the individual ripening. Samples pre-treated for 0.3% lemongrass EO extract showed the highest score (8.25) in texture stored at 5°C.

In general, the lemongrass extract pretreated yoghurt samples received higher sensory scores than untreated sample. For example, color values were 8.0 – 8.63 the EO extract pretreated yoghurt samples but were 8.13 – 8.75 in untreated samples, these differences were nonsignificant ($P<0.05$) for all samples. Samples treated with 0.05 and 0.1 % EO extract generally had better score for all sensory characteristics at all samples. On the contrary, the sample treated with 0.3% EO extract had lower score for all sensory characteristics at all samples especially in taste characteristics. However, concentration of lemongrass extract had a positive influence on acceptability of colour and flavor of yoghurt samples. Increased concentration of lemongrass extract also showed that the same odour, colour texture and acceptability characteristics in hoghurt samples. However, all sensory scores were in the acceptable range, which greater than 5 scores.

It is clear that the the lemongrass EO extract pretreated yoghurt gave higher mean panel scores (8.0- 8.5) than the untreated yoghurt sample, which were the most preferred in all the studied characteristics. The lemongrass EO extract pretreated yoghurt samples with different concentrations had a non-significant difference ($P<0.05$) between these samples.

Table (9): effect of lemongrass concentrations on sensory evaluation of youghurt stored at 5°C for 4 weeks.

Extract level,%	Appearance	Texture	Colour	Taste	Odour
0.0	8.13^{A}	8.13 ^{A}	8.75 ^{A}	8.50 ^{A}	8.13 ^{A}
0.05	8.0 ^{A}	7.63 ^{A}	8.50 ^{A}	7.13 ^{B}	7.75 ^{A}
0.1	8.50 ^{A}	8.13 ^{A}	8.50 ^{A}	7.13 ^{B}	7.00 ^{A}
0.3	8.63 ^{A}	8.25 ^{A}	8.38 ^{A}	6.88^{B}	7.38 ^{A}
LSD	NS	NS	NS	S	NS

LSD = least significant difference at 0.05 level.

4- CONCLUSION

While the primary function of Lemongrass may not be preservative in nature, it has preservative properties, which may useful in built-in safety systems in food. In addition, Lemongrass herb is cheep, safe, and had a medical functions. Our results show that water extract from Lemongrass, at level 0.1% was effective agent to inactivate mold growth and mycotoxin formation and increasing level to 0.3% completely prevented growth and mycotoxin production , for the all tested molds, in YES broth medium. However, in yoghurt medium its inhibitory effect was different according to mold type, supplemented concentration and constituents of the used medium. Its evident from our data that, if possible, a sufficient amount of lemongrass oil to prevent mold growth needs to be used if one wishes to prevent mycotoxin production. It can be concluded from the results of this work that of lemongrass volatile oil extract treatments with refrigeration at 5oC may serve as alternative to conventional chemical preservatives in the preservation of yoghurt by hurdle technology.

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