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Utilization of Essential Oils from Citronella and Geranium as Natural Preservative in Mayonnaise

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Abstract: *Background:* Essential oils are one of the natural products that are used in different aspects of life due to biological activities. The current investigated was aimed to study the effect of essential oils of citronella (*Cymbopogon nardus*), geranium (*Pelargonium graveolens*) and there mixture as a source of natural antioxidant and antimicrobial in formulating a new mayonnaise product during storage to improve its nutraceutical value and shelf-life. *Methodology:* The essential oils were added to oil of new mayonnaise formula at the rates of 50, 100 and 150 µl/100 gm from *Cymbopogon nardus* and *Pelargonium graveolens* and 100 µl/100 gm from there mixtures. The effect of essential oils on oxidative stability, sensory characteristics and microbial evaluation of mayonnaise was studied during 4 months. *Results:* The results of essential oils analyzed using GC/MS technique indicated that the major components of citronella oil were citronella (48.73%) and geraniol (33.39%), meanwhile geraniol (40.49%) the major of geranium oil. The essential oils of citronella significantly cytotoxic at high concentrations whereas geranium oil did not show any cytotoxicity. The oils were investigated antimicrobial activity against 7 bacteria strains and 3 fungi strains. Citronella oil had a stronger antibacterial and antifungal potential against selected microorganisms at low concentration was more effective than geranium oil. Sensory evaluation of mayonnaise samples prepared by add essential oils showed no significantly (P >0.05) effect on texture properties. But, odour and appearance indicated that the addition of (citronella 100, geranium 150 and mixture 100 µl/100gm) gave a better rating score in the mayonnaise samples. At the end of storage the peroxide and acid value of mayonnaise formed with essential oils significantly lower than control. Also, Decreasing in microbial loadings of all mayonnaises samples tested were noticed by increasing the essential oils concentration in mayonnaise samples. Mayonnaise prepared with citronella, geranium at concentration 100 and 150 µl/100 gm respectively exhibited lowest total count bacteria and fungi. *Conclusion:* So, the addition of essential oils prolonged the oxidative stability of mayonnaise and they can be used as antibacterial agents.

Keywords: Essential Oil, GC Mas, Cytotoxicity, Antioxidant, Antimicrobial Activity, Mayonnaise and Chemical Composition

1. Introduction

Foodborne Diseases (FBD) is caused by eating food that was contaminated by an infectious agent or a toxin produced by it. [1] According to the World Health Organization (WHO), 30% of people in industrialized countries suffer from FBD and in 2000 only at least two million people worldwide died of diarrhea caused or not by *Salmonella* [2, 3]. Essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). Steam distillation is most commonly used for commercial production of essential oils [4]. The greatest use of EOs (Essential Oils) in the European Union (EU) is in food (as flavourings), perfumes (fragrances and aftershaves) and pharmaceuticals for their functional properties [5]. The essential oil has antifungal, physicochemical and insect-repelling activity, antioxidant activities [6]. The plant used as sources of antioxidants to enhance health and food preservation. These effects have been attributed to antioxidant components such as plant phenolic, including flavonoids and phenylpropanoids among others [7].

The *Pelargonium* (Geraniaceae family) and *Vitex*

(Verbenaceae family) genera are two important sources of foods, medicines and cosmetics in the world. They are also sources of distilled volatile oils [8]. These genera have been found to possess significant pharmacological and biological activities, including antioxidant, anti-neuroinflammatory, antiinfluenza, anticancer, antimicrobial and antifungal activity [9, 10].

The genus *Cymbopogon* belongs to the family Poaceae and possesses more than 100 species in tropical countries. Of those species, approximately 56 are aromatic. A few of them should be given special attention for their wide use in folk medicine and high content of essential oils with quite varied purposes, such as therapeutic and cosmetic [11]. Within this genus are *Cymbopogon nardus* (L.) Rendle (citronella), native to Ceylon, known for the repellent power of its essential oil rich in citronellal. 6 The use of essential oils from species of the genus *Cymbopogon*, such as *C. citratus* and *C. nardus*, for the control of foodborne pathogenic bacteria is an interesting alternative, since these plants have a high essential oil yield [12].

According to McClements [13] mayonnaise is semisolid, oil-in-water (O/W) emulsion that contains egg yolk, salt, vinegar, thickening agents, and flavoring materials, consequently, The emulsion is formed by slowly blending oil with a pre-mix that consists of egg yolk, vinegar, and mustard because mixing the oil and aqueous phase at once will result in formation of a water-in-oil emulsion. It is probably one of the oldest and most widely used sauces in the world today. Traditional mayonnaise is an oil-in-water emulsion containing 70–80% fat [14].

Lipid oxidation is a considerable problem in lipid bearing foods, especially in food products containing lipids with highly polyunsaturated fatty acids (PUFA). Lipid oxidation of foods containing these lipids takes place almost instantly unless careful precautions are taken. Particular problems arise when the highly unsaturated oils are emulsified into various food systems [15]. There is at present increasing interest both in the industry and in scientific research for spices and aromatic herbs because of their strong antioxidant and antimicrobial properties, which exceed many currently used natural and synthetic antioxidants. The application of plant EOs for control of food-borne pathogens and food spoilage bacteria requires the evaluation of efficacy within food products or in model systems that closely simulate food composition.

Therefore, this study was carried out to reveal the chemical composition, antioxidant potential and antimicrobial activity of the essential oil of geranium and citronella as well as to investigate the effects of different concentrations of their essential oils on physico-chemical, microbial population, shelf life, and organoleptic properties of mayonnaise.

2. Materials and Methods

2.1. Plant Material

Dried of *Pelargonium graveolens* and *Cymbopogon nardus*, collected in the late July, 2015 Medicinal and Aromatic Research Department Horticultuer Research Institute Center, Giza, Egypt.

2.2. Extraction of Essential Oil (EOs)

The powdered dried leaves of *Pelargonium graveolens* and *Cymbopogon nardus* was submitted to water distillation for 3 hours by hydro-distillation in a Clevenger-type apparatus separately until there was no significant increase in the volume of the oil collected for 3 h. The volatile oils were dried over anhydrous sodium sulfate and stored at (-18°C) in the dark for analysis and further antibacterial studies [16].

2.3. Production of Mayonnaise

The production of mayonnaise was carried out as described by Leuschner and Boughtflower [17], and comprised three successive stages: Blending of all ingredients except oil, addition of oil under controlled conditions during continuous blending. The ingredients in the mayonnaise were corn oil 66%, full egg 13.5%, water 7.1%, vinegar 8.0%, sugar 2.1%, salt 1.1%, xanthan 1.2%, 0.1%, all of food grade. The essential oils were added to oil of mayonnaise at the rates of 50, 100 and 150 µl/100gm from*Pelargonium graveolens* and *Cymbopogon nardus* and 100 µl/100gm from their mixtures. The mayonnaise samples were transferred to 5 glass bottles (100 ml) screw caps and stored 120 days at 4°C temperature until analysis. The Proximate analysis of mayonnaise were determined according to AOAC. [18] official methods and found content the moisture (21.81-21.96), protein (3.18-3.28), fat (72.40-72.50), and ash content (0.32-0.36) with no significant difference between all samples.

2.4. Acid Value, Peroxide Value, pH and Water Activity (a_w) Measurements

According to AOAC [18], lipids were extracted from mayonnaise samples, acid and peroxide values of extracted lipids were determined.

pH values of mayonnaise samples were measured at a homogenized sample was measured directly, using a pH meter (Janway 3515 pH meter UK)., and water activity (a_w) was measured at 25°C using Decagon Aqualab Meter Series 3TE (Pullman, WA, USA). Three replicate readings (three different samples) were taken for each pH and a_w measurements of mayonnaises.

2.5. Free Radical Scavenging Activity

Free radical scavenging activity of the citronella grass and geranium essential oils was measured by DPPH assay Gulluce *et al.* [19]. The oil sample was dissolved in methanol to give concentration from 0.2 – 0.8 µl/ml for citronella grass oil and geranium oil. Then 1 mL of the essential oil solution and added into 2 mL methanolic solution of 100 µM DPPH. For control reaction, essential oil was replaced with 100% methanol. The mixture was incubated for 2 hours in dark at ambient temperature. Finally, the absorbance was measured at 517 nm using an ultraviolet-visible spectrophotometer (Agilent 8453 UV-Vis Spectroscopy System, Germany). Antioxidant activity was calculated using the following equation.

Scavenging activity (%) = 1- (absorbance of essential oil / absorbance of control) × 100

Where is the absorbance of the control reaction (containing all reagents with methanol), and is the absorbance of the essential oil sample in the DPPH solution. The % DPPH radical inhibition was plotted against the sample concentrations and regression curve was established for calculation of the IC_{50} value.

2.6. Gas Chromatograph Analysis

The essential oil was analyzed using a gas chromatograph Hewlett Packard (HP) 6890 series equipped with flame ionization detector and capillary column HP-5(30m x.25mm. 25m film thickness). The oven temperature increased from 70 to 20°C at a rate of 8°C / min. The injector and detector and hydrogen was used as the carrier gas. The identification of the compounds was done by matching their retention times with those of authentic samples injected under the same conditions.

2.7. Cytotoxicity Essential Oils

Measuring of cytotoxicity by sulforhodamine B (SRB) assay: Potential cytotoxicity of essential oils was tested using the method of Skehan *et al.* [20] using Human Embryonic Kidney (HEK 293) normal cell line

2.8. Microbial Evaluation

2.8.1. Antimicrobial Activity Assay

Antibacterial activity of the essential oils was investigated against 7 bacterial strains, *Escherichia coli* (ATCC 35218), *Salmonella typi* (ATCC 13076), and *Pseudomonas aeruginosa* (NCIM 5029), *Streptococcus pneumonia* (ATCC 49619), *Staphylococcus aureus* (ATCC 13565), *Bacillus cerues* (ATCC 11778) *and Bacillus subtilis* (RCMB 010010),by the agar well diffusion methods Donaldson *et al.*, [21] at concentration 10, 20, 30, 40 and 50µl/well of the test compound. The antifungal activity was measured by disc volatility method of Sharma and Tripathi [22]. The following fungi strains were used: *Aspergillus flavus Candida albicans* (RCMB05036) *Geotrichum candidum* (RCMB 05097).

2.8.2. Microbiological Analysis of Mayonnies

All samples were analyzed for aerobic plate count, *E. coli*, *Salmonella*, *S. aureus*. Psychrophilic bacteria and yeast & mold; All analyses were performed by using the standard procedures outlined in the American Public Health Association (APHA 1992) [23].

2.9. Sensory Evaluation

Sensory evaluation was conducted on the mayonnaise samples after one-day storage at room temperature according to Worrasinchai *et al.* [24]. Sensory characteristics: appearance, colour, odour, texture, taste, and overall acceptability were evaluated by 10 trained panel on 9-point hedonic scale, 1 = the least, the lowest; 9 = the most, the highest.

2.10. Statistical Analysis

The collected data of biological examination were statistically analyzed by the least significant differences (L.S.D) at the 5% level of probability procedure according to Snedecor and Cochran. [25]

3. Results and Discussion

3.1. Chemical Analysis of Essential Oils

The light yellow essential oil of *Cymbopogon nardus* and *Pelargonium graveolens* were obtained in yields of 1.29% and 1.49%, respectively, based on dried extracted material. The percentage composition of the studied oil samples are listed in Table 1. Twelve and fourteen compounds in citronella and geranium oils, respectively. In citronella oil two compounds accounting for (82.12%), there compounds were citronella (48.73%) and geraniol (33.39%), respectively. Koffi *et al.* [26] found that the eighteen compounds were identified in the essential oil of *C. nardus* representing 95.9% of detected constituents including citronellal (35.5%), geraniol (27.9%) and citronellol (10.7%) as major components. Also, our results is in agreement with Koba *et al.* [27] who found the citronellal is the major compound of *C. nardus* essential oil, which gives the characteristic with higher percentage of citronellal (35%).

The constituent of Geranium essential oil showed nearly14 compounds the major compound was geraniol (40.49%), citronellal (14.40%), linalool (9.38%) and citronellol (7.28%), respectively. This data agree well with Rana *et al.* [28] they reported that the geranium essential oil contented thirty compounds and the main components identified were citronellol, geraniol and linalool. The composition of EOs from a particular species of plant can different between harvesting seasons and between geographical sources, (Faleiro, and Satarkar) [29].

Table 1. *Chemical and Identification of citronella and geranium essential oils by GC/MS.*

Components	Citronella	Geranium
α-Pinene	1.98	1.63
Myrcene	1.13	1.61
p-cymen	1.40	1.95
Limonene	1.04	5.99
Linalool	2.09	9.38
Citronellal	48.74	14.40
Geraniol	33.39	40.49
Citronellol	ND	7.28
Citronellyl formate	2.52	ND
Eugenol	1.23	ND
β- Caryophyllene	2.97	3.79
Geranyl acetat	ND	9.69
Unknown	1.01	0.97
Unknown	2.50	0.63
Unknown	ND	1.44
Unknown	ND	0.75

ND: Not detected

3.2. Evaluation of DPPH Free-Radical Scavenging Activity of Essential Oils

In order to determine the effect of concentration on radical scavenging power by DPPH method, five different working solutions were used (0.2 to 0.8 mg/mL). According to the results (Figur. 1) obtained, citronella oil was found the strongest DPPH free-radical scavenging activity at all concentration, the activity of citronella oil was found (73.16%) at 0.8 µl/ml. Meanwhile, geranium oil was found (29.26%) at the same concentration. Džamić *et al.* [30] presented, *P. graveolens* oil was found to possess slightly lower antioxidant activity. Meanwhile, Scherer *et al.* [31] found the antioxidant activity citronella highest antioxidant activity has been evaluated by DPPH (2, 2-diphenyl-1-pycriyl hydrazyl) method.

The IC_{50} values of DPPH free radical scavenging citronella and geranium oil were found to be 0.468 and 1.378 µl/ml, respectively, (Figure. 1). The higher antioxidant capacity of citronella oil as compared to geranium oil may be attributed to the present of eugenol but no found in geranium oil according presented in Table (1). Eugenol being a phenolic compound reported that is in literature to be an antioxidant by donating a hydrogen atom to the free radicals. Politeo *et al.* [32].

Therefore, Citronella and Geranium essential oils are possible sources of antioxidant compounds since these extracts were relatively non-toxic Sacchetti *et al.* and Lalli *et al.* [33, 34]. The antioxidant activity of essential oils is a biological property of great interest because they may preserve foods from the toxic effects of oxidants.

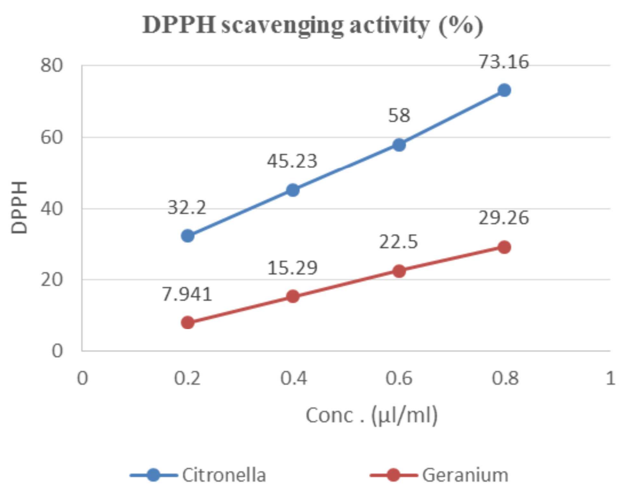

Figure 1. *DPPH free radical scavenging citronella and geranium oils.*

3.3. Evaluation of Cytotoxicity Against HEK 293 Cell Line

The results of the cytotoxicity assays revealed a reduction in viability of normal human Embryonic Kidney (HEK 293) Cell treated with the citronella and geranium essential oils (Table 2). The result of the test showed a decrease in cell viability when used geranium oil. Meanwhile, treatment with citronella oil showed a decrease in cell viability to 76.54 at the concentration 50µl/well. The essential oils of citronella were found to be significantly cytotoxic at high concentrations whereas geranium oil did not show any cytotoxicity as revealed by the MTT assay. The low toxic effects of the citronella oil may be due to the presence of citronolla as the major component whereas considerable less toxic potentials of geranium oils could be attributed to the presence of geraniol. The inhibitory activity against normal Cell line Human Embryonic Kidney (HEK 293) was detected under these experimental conditions with IC_{50} =>50 µl/Well and >100 µg/Well, respectively.

Table 2. *Cytotoxic activity of citronella and geranium EOs with different concentrations against normal cell line Human Embryonic Kidney (HEK 293).*

Sample conc. (µl/well)	Viability %	
	Citronella	Geranium
50	76.54	81.37
25	88.93	92.04
12.5	94.62	98.85
6.25	98.76	100
3.125	100	100
1.56	100	100
0	100.00	100.00
IC_{50}	*>50 µl/Well*	*>100 µg/Well*

Our results agree with Sinha *et al.* [35] they reported that citral (as major component of citronella oil) showed the potential maximum cytotoxic which reduced cell viability to 75.69%. Also, our study supports the cytotoxic properties of citronella and geranium oils assume significance and importance, since these oils are widely used flavoring of many food preparations, cosmetics and also of Medicine [36].

3.4. Evaluation of Antimicrobial and Antifungal Effect of Essential Oils

The antibacterial activity of citronella and geranium essential oils at different concentration (10 to 50 µl/well) against selected Gram negative bacteria (*Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa*) and Gram positive bacteria (*Streptococcus pneumoniae Bacillus cerues Staphylococcus aureus Bacillus subtilis*) are summarized in Table (3). The results revealed that the medicinal herbs essential oils showed antimicrobial activity with varying magnitudes. Citronella oil was recorded highest action (complete inhibition) against *B. cereus and Staphylococcus aureus* (100 mm) at 20µl/well. *Streptococcus pneumonia and Bacillus subtilis as* Gram positive bacteria were the most sensetive strains at concentration 50ul/well with values 20 and 77 mm, respectively. While, the highest inhibition zones were (100mm) obtained of citronella oil at 20 µl/well was completely inhibited the growth of Gram negative strains *(Escherichia coli* and *Salmonella typhi*). While, *Pseudomonas aeruginosa* was more resistant to citronella oil as it recoreded inhibition zone about (66.6 mm) at 50ul/well.

Table 3. *Antibacterial activity of the essential oils from citronella and geranium using agar well diffusion method, diameter (mm) of inhibition zone.*

Type of strain	Antibacterial activity of the essential oils diameter (mm)									
	Citronella (µL/welL)					Geranium (µL/welL)				
	10	20	30	40	50	10	20	30	40	50
	Gram-negative									
Escherichia coli (ATCC 35218)	77.7	100	100	100	100	16.6	42.2	55.5	66.6	85.5
Salmonella typhi (ATCC 13076)	88.8	100	100	100	100	87	93	100	100	100
Pseudomonas aeruginosa (NCIM 5029)	9	12.5	28.8	47	66.6	3.2	6	18	30	53
	Gram-positive									
Streptococcus pneumonia (ATCC 49619)	-	-	8	14	20	5	9	18	22	33
Bacillus cerues (ATCC 11778)	92	100	100	100	100	82	100	100	100	100
Staphylococcus aureus (ATCC 13565)	96	100	100	100	100	20	22	27	33	45
Bacillus subtilis (RCMB 010010)	20	34	44	60	77	11	23.4	32.8	42.9	55.1
	Fungi									
Aspergillus flavus	28	33	79	100	100	10	18	23	31	40
Candida albicans (RCMB05036)	30	49	60	76	85	4.3	18	35	40	68
Geotrichum candidum (RCMB 05097)	18	29	40	60	50	6	15	25	34	42

The zone of inhibition above 6 mm in diameter was taken as positive result.

Inhibition zones formed by geranium essential oil against *Salmonella typhi* showed high inhibition at 30 µl/well concentration. Meanwhile, at these concentration inhibition zones recorded 55.5 and 18 mm against *Escherichia coli,* and *Pseudomonas aeruginosa,* respectively. Geranium oil observed highest action against *Bacillus cerues* from Gram positive bacteria selected, In contrast the lowest action was recorded for *Streptococcus pneumonia* at all concentration. Generally, the citronella oil had an excellent antimicrobial potential form against selected microorganisms was more effective than geranium oil. Furthermore, the citronella oil was found to be more effective than rose geranium oil *Aggarwal et al.* [37] Citronella and geranium oils as such were not effective against Gram negative bacteria but their components viz. citronellol and d-citronellol of citronella geranium oil showed antibacterial activity against gram-negative bacteria, but much less than Gram positive bacteria.

The essential oil of citronella was employed most effective on all strain of fungi than geranium oil. They found that the growth inhibition of citronella essential oil agains*t A.Flavus* started at the lowest oil concentration of 10 µl/well and reached to a maximum of 100 mm at the concentration of 40 µl/well. While, geranium oil at concentration 20 µl/well caused slightly growth inhibition of *A.Flavus* on agar plates. On the other hand, antifungal activities of citronella oil with more slightly inhibition with *Geotrichum candidum than Candida albicans.* Furthermore, with the increase of citronella oil concentration, the antifungal activity gradually strengthened compared with geranium oil with the prolongation of treatment time, the fungicidal rate was also improved. Sindhu *et al.*[38] sugested that the fungistatic and fungicidal effects of the essential oil may be due to the effect of the individual constituent in the oil, and the interactive effects of other minor components may also be responsible for the antifungal effect. Nogueira *et al* [39] reported that the high effective antifungal activity of essential oils might be related to their lipophilic characters which can penetrate the plasma membrane.

Rasooli and Owlia [40] elected that citronella oil penetrated across not only the cell wall, but also the cell membrane. And the citronella oil changed the cellular permeability and caused the leakage of cytoplasm by interacting with the cytoplasm membrane of fungus.

3.5. Sensory Evaluation of Mayonnaise Prepared with Essential Oils

The results of statistical analysis of sensory evaluation of prepared mayonnaise using different concentrations of with citronella (50, 100, and 150 µl/100gm), geranium oils (50,100 and 150 µl/100gm) and mixture from Cit: Ger 100 µl/100gm (1:1) are presented in Table 4. Mayonnaise samples prepared by add different concentrations from essential oils showed no significantly ($P > 0.05$) affected on texture properties. On the other hand, the colour attribute of mayonnaise samples prepared by add were less significantly with increased the concentration of EOs than control sample. Meanwhile, samples prepared with added (citronella 100 µl, geranium 150µl, and mixture 100 µl) recorded higher significant score in taste (9.8, 9.4 and 9.5) and overall acceptability (9.8, 9.5 and 9.6) than other samples. Results of odour and appearance indicated that the addition of (citronella 100, geranium 150 and mixture 100 µl/100gm) gave a better rating score in the mayonnaise samples under study than control samples. These data agree with Govaris *et al.* [41] showed that the addition of oregano essential oil at 0.6 or 0.9% in minced sheep meat was organoleptically accepted. Kishk and Elsheshetawy, [42] found that addition of ginger to mayonnaise significantly ($p < 0.05$) enhanced the sensory attributes depending on its concentration. The panelists preferred the 1.0% and 1.25% ginger in mayonnaise more than the control. Generally, it could be observed that the addition of medicinal herbs essential oils to mayonnaise exhibited the highest characteristics of sensory evaluation.

Table 4. *Sensory evaluation of mayonnaise containing citronella and geranium essential oils at different concentrations.*

	Appearance	Colour	Odour	Texture	Taste	Overall acceptability
Control	$9.7^{a}\pm0.33$	$9.8^{a}\pm0.41$	$8.9^{b}\pm0.23$	$9.2^{a}\pm0.22$	$9.6^{ab}\pm0.22$	$9.4^{b}\pm0.28$
Citronella (50µ)	$9.3^{b}\pm0.42$	$9.6^{a}\pm0.35$	$9.1^{b}\pm0.26$	$9.1^{a}\pm0.27$	$8.7^{c}\pm0.18$	$8.9^{c}\pm0.31$
Citronella (100µ)	$9.7^{a}\pm0.31$	$9.4^{b}\pm0.37$	$9.5^{a}\pm0.34$	$9.3^{a}\pm0.23$	$9.8^{a}\pm0.23$	$9.8^{a}\pm0.35$
Citronella (150µ)	$9.3^{b}\pm0.29$	$9.1^{bc}\pm0.31$	$8.5^{c}\pm0.33$	$9.2^{a}\pm0.29$	$7.8^{e}\pm0.31$	$8.3^{d}\pm0.36$
Geranium (50µ)	$9.2^{b}\pm0.30$	$9.6^{a}\pm0.28$	$8.8^{bc}\pm0.31$	$9.2^{a}\pm0.27$	$8.2^{d}\pm0.21$	$8.2^{d}\pm0.39$
Geranium (100µ)	$9.2^{b}\pm0.36$	$9.5^{ab}\pm0.42$	$9.1^{b}\pm0.26$	$9.1^{a}\pm0.19$	$8.6^{c}\pm0.17$	$8.9^{c}\pm0.26$
Geranium (150µ)	$9.6^{a}\pm0.42$	$9.4^{b}\pm0.39$	$9.3^{a}\pm0.29$	$9.2^{a}\pm0.24$	$9.4^{b}\pm0.21$	$9.5^{ab}\pm0.33$
Citr.: Ger. 1:1 (100µ)	$9.7^{a}\pm0.32$	$9.6^{a}\pm0.32$	$9.4^{a}\pm0.35$	$9.2^{a}\pm0.28$	$9.5^{b}\pm0.19$	$9.6^{a}\pm0.37$
LSD	0.35	0.37	0.28	0.26	0.21	0.32

* Values given represent mean ± S.D. of triplicates.

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

3.6. pH of Mayonnaise Prepared with Essential Oils

The pH of mayonnaise showed a dramatic effect on the structure of the emulsion. The pH values of mayonnaise samples recorded a storage period of over 4 months have been presented in Figure 2. The defrances of pH values of freshly prepared mayonnaise samples were no significant and ranged 3.74 to 3.78 which indicated that the mayonnaise must be acidic in nature. These data agree with Pons *et al.* [43] found that mayonnaises had pH 3.6–3.9 values. But, Rasmy *et al.* [44] found the initial pH values of the control and samples treated with BHA and sage essential oil at different concentrations were 4.43 for all samples.

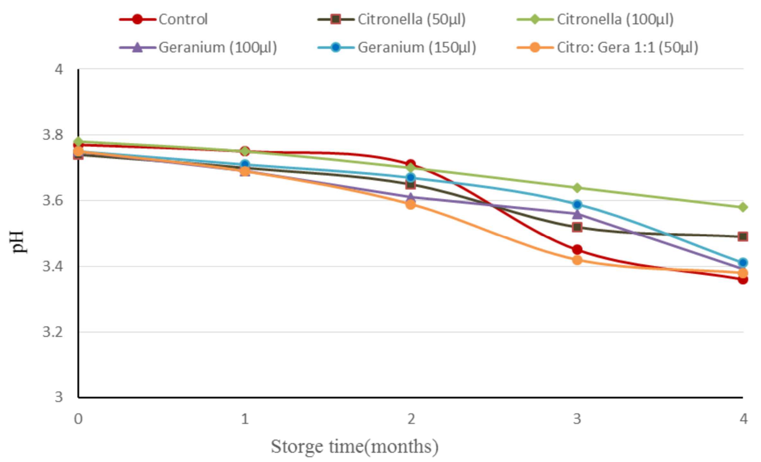

Figure 2. *pH values of mayonnaise containing citronella and geranium essential oils at different concentrations during storage.*

During storage the pH values decreased continuously in all mayonnaise samples. At the end of storage the pH decreased by 0.4 value at control mayonnaise, meanwhile, the mayonnaise formed with citronella oil (100 µl) decreased by 0.2 value. As a result of activity of lactic acid bacteria. Generally, pH value of mayonnaise formed with citronella oil (100 µl) and geranium oil (150 µl) was slightly changed on pH value than other samples. It was noticed that increasing of the citronella and geranium oils concentration lead to reducing bacterial activity and retarding the decreasing of pH values as a result of its antibacterial effect. These obtained data are agree with El-Bostany *et al.* [45] and Kishk and El sheshetawy [42] found that the pH values decreased continuously in mayonnaise samples during the storage period.

3.7. Water Activity of Mayonnaise Prepared with Essential Oils

Water activity predicts safety and stability with respect to microbial growth, chemical and biochemical reaction rates, and physical properties. Therefore, by measuring and controlling the water activity, it is possible to predict microorganisms will be potential sources of spoilage and infection; maintain the chemical stability of products; and optimize the physical properties of products such as moisture migration, texture, and shelf life. Data present in Figure. 3 indicated that water activity of control mayonnaise was gradually increased from (0.898 to 0.988) during storage for 4 months. However, a slightly changed increased in water activity of experimental mayonnaise compared with control. The lowest changed in water activity showed in mayonnaise prepared with 100 µl citronella oil followed by Geranium 150 µl. At the end of storage water activity of control mayonnaise is higher than other treatment mayonnaise with different concentration of essential oils this result to early microbial spoilage of control mayonnaise and allows growth of bacteria, yeast and other molds. According to Troller and Christian [46] water activity, temperature and pH are the most important factors that control rates of deteriorative changes and microbial growth in foods.

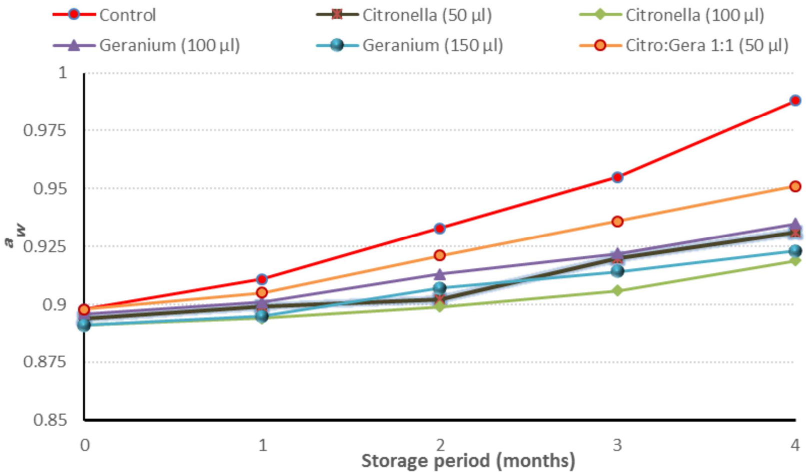

Figure 3. *Water activity a_w of mayonnaise containing citronella and geranium essential oils at different concentrations at storage period.*

3.8. Peroxide Value of Mayonnaise Prepared with Essential Oils

Mayonnaise is susceptible to spoilage through the auto-oxidation of unsaturated and polyunsaturated fatty acids in oil. Lipid peroxidation, in food emulsions leads to the production of off-flavors and off-odors, thereby shortening the shelf life of these products[Halliwell. [47] Changes in the PV of freshly mayonnaise and during storage are illustrated in Figure. 4. The peroxide values in different mayonnaise samples was the same being 0.46 and not affected by adding different concentrations of essential oil of citronella and geranium at zero time. Data revealed that PV values for all investigated samples were significantly (P ≤ 0.05) increased at storage period increased reaching to their highest values after 4 months.

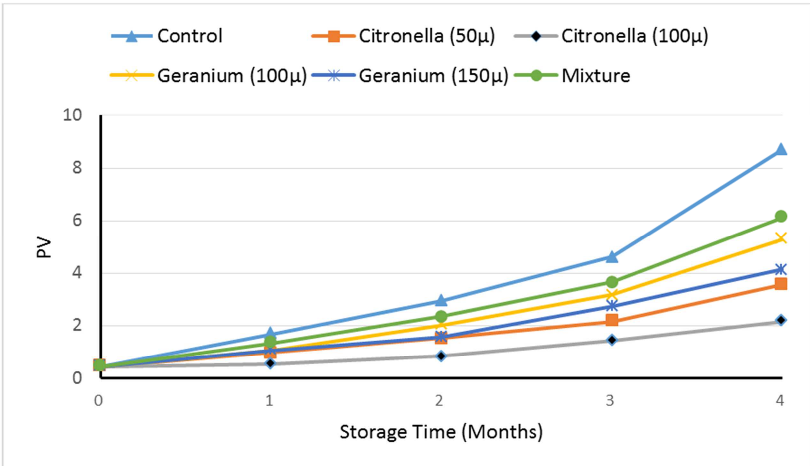

Figure 4. *Peroxide value of mayonnaise containing citronella and geranium essential oils at different concentrations at storage period.*

At the end of storage the PV value of control mayonnaise was significantly higher than other samples formed with essential oils. Meanwhile, mayonnaise formed with citronella (100 µl) was lower PV value 2.17meq peroxide/kg oil. It was clear that treatment of mayonnaise samples either with citronella (50 and 100 µl) and geranium oils (100 and 150 µl) resulted in inhibition of oil oxidation in mayonnaise by 58.99, 75.00, 39.05 and 52.07%, respectively, compared to the control ones, at the end of the storage period. Meanwhile, the uses of 100 µl from a mixture of citronella and geranium oils (1:1) resulted in inhibition of oil oxidation in mayonnaise by 29.38%.

During storage mayonnaise may be affected by a number of factors causing deterioration (physical destabilization, chemical oxidation, hydrolysis and microbiological), which interact. The antioxidant activity of natural antioxidants is higher than those reported by other researches. Shahidi *et al.* [48] they reported that the antioxidant effect of essential oil from aromatic plants is due to the presence of hydroxyl groups in their phenolic compounds. Previous studies by Pokorny *et al.* [49] showed that sage extract has a strong antioxidant effect in sunflower oil.

3.9. Acid Value of Mayonnaise Prepared with Essential Oils

According to Kishk [50] free fatty acids may be produced by the oxidation of double bonds of unsaturated fatty acid esters. In advanced stages of oxidation, free fatty acids with low molecular weight were developed through the accumulation of acidic cleavage products and subsequently increased the acid value. This oxidation could have occurred with the aid of oxidative enzymes and the presence of a proportion of atmospheric oxygen in the headspace and incorporated into the mayonnaise. The acid value of mayonnaise was used as a measure of lipid hydrolysis that leads to the formation of free fatty acids. Data present in Figure. 5 indicated that acid values of mayonnaise increased gradually with significant difference (P< 0.05) during storage period reaching their maximum after 4 months. Control sample had acid value significantly (P< 0.05) higher than other samples prepared using different concentrations of citronella oil and geranium oil. The increase in acid value could be mainly attributed to the activity of acid tolerant microorganisms such as lactic acid bacteria which present in the aqueous phase in mayonnaise Pourkomailian, and Karas *et al.* [51, 52.] Also, Stefanow [53] reported that these increases were probably due to the activity of hydrolytic and oxidative enzymes present in eggs. On the other side, it could be noticed that increasing the essential oils concentration in mayonnaise samples significantly (P< 0.05) inhibits the progress in acid value. Output data from the regression analysis of acid values versus the storage period appeared the relation between essential oils concentrations and acid values. [54]

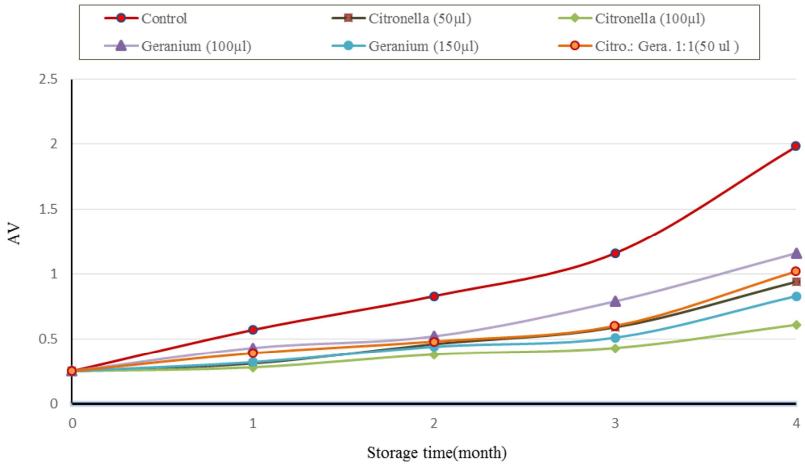

Figure 5. *Acid value of mayonnaise containing citronella and geranium essential oils at different concentrations at storage period.*

3.10. Microbial Evaluation of Mayonnaise Prepared with Essential Oils

The quality of mayonnaise prepared with the different concentration of citronella and geranium essential oils was evaluated microbiologically. Total bacterial count, yeast & mold and Psychrophilic bacteria on control mayonnaise and it's prepared with citronella and geranium oils after storage for 4 months storage at 4°C are shown in Table 5. Data indicates that control mayonnaise and mayonnaise treated with essential oils at zero time were contained lower total bacterial counts (TBC) with value range $0.5x10^2$ to $0.6x10^2$. The current results reveal that the (TBC) of control mayonnaise increased through storage 4 month were $0.5X10^2$, $0.9X10^2$, $1.5X10^2$, $4.3X10^3$ and $6.8X10^4$ respectively. Data revealed that total count bacterial values for all investigated samples were increased at through storage period. On the other side, it could be noticed that increasing the essential oils concentration in mayonnaise samples decreased of total count bacteria. Mayonnaise prepared with citronella: geranium at concentration 100 µl/100 gm was lowest total count bacteria than experimental mayonnaise. Pourkomailian [51] and El-Bostany *et al.* [45] found mayonnaise with full fat (FF) formulation gave the higher possibility that microorganisms this was possibly due to the higher oil content in the oil phase. Karas *et al.* [52] reported the increase in the total bacterial count at the end of the 20 weeks of storage period may be due to the growth of acid tolerant microorganisms such as lactic acid bacteria. On the contrary, Worrasinchai *et al.* [24] reported that after 64 days storage, the total bacterial count of mayonnaise samples decreased.

Data presented in Table 5 indicates that molds and yeasts were not detected in the different mayonnaise samples during the first 2 months. However, they occurred later on and their growth rate increased during the storage period. The growth rate of mayonnaise prepared with the the different concentration of citronella and geranium essential oils was lower than that recorded in control mayonnaise. On the other hand, mayonnaise made from a higher concentration of essential oil was less contaminated than lower concentration of essential oils. From a microbiological safety point of view, it is generally recommended that mayonnaise made with essential oil reduce the risk from microorganisms. The result indicates that all mayonnaise samples were free from *Salmonella, E. coli, and Staph arues.* Meanwhile, Psychrophilic bacteria was detected after 3 months in control mayonnaise and after 4 months on mayonnaise prepared with essential oils. Our results confirmed by Abou-Zeid [55] who found that preventing salmonelosis transmission by home mayonnaise the use of vinegar as acidulants in order achieve a pH between 3.6 - 4.0 and storage in a worm place is recommended.

4. Conclusions

Generally, it could be observed that the addition of medicinal herbs (citronella and geranium) essential oils to mayonnaise exhibited the highest characteristics of sensory evaluation to obtained good quality productive, suggesting their potential source for antioxidant and antimicrobial for manufactured mayonnaise.

Table 5. *Effect of storage on Microbiological characteristics of mayonnaise with different conc. Citronella oil and Geranium oil.*

Sample	Total bacterial count (cfu/gm)					Yeast and mold (cfu/gm)					Psychrophilic bacteria (cfu/gm)				
	Months														
	Zero	1	2	3	4	Zero	1	2	3	4	Zero	1	2	3	4
Control	0.5X10^{2}	0.9X10^{2}	1.5X10^{2}	4.3X10^{3}	6.8X10^{4}	ND	ND	1.6X10^{2}	3.5X10^{3}	8.4X10^{4}	ND	ND	ND	1.2X10^{1}	2.6x10^{2}
Citronella (50µ)	0.5X10^{2}	0.8X10^{2}	1.4X10^{2}	2.7X10^{2}	4.2X10^{3}	ND	ND	ND	1.3X10^{2}	4.5X10^{2}	ND	ND	ND	ND	0.9x10^{1}
Citronella (100µ)	0.5X10^{2}	0.7X10^{2}	1.1X10^{2}	2.0X10^{2}	3.5X10^{3}	ND	ND	ND	0.9 X10^{2}	3.7X10^{2}	ND	ND	ND	ND	0.3x10^{1}
Geranium (100µ)	0.6X10^{2}	0.9X10^{2}	1.3X10^{2}	2.9X10^{2}	4.2X10^{3}	ND	ND	ND	2.4X10^{2}	5.1X10^{2}	ND	ND	ND	ND	1.6x10^{1}
Geranium (150µ)	0.5X10^{2}	0.7X10^{2}	1.2X10^{2}	2.3X10^{2}	3.4X10^{3}	ND	ND	ND	1.6 X10^{2}	4.6X10^{2}	ND	ND	ND	ND	1.2x10^{1}
Citr.: Ger. 1:1 (100µ)	0.5X10^{2}	0.7X10^{2}	1.1X10^{2}	2.2X10^{2}	3.2X10^{3}	ND	ND	ND	2.1X10^{2}	4.2X10^{1}	ND	ND	ND	ND	1.4x10^{1}

N.D: Not detect

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