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ENHANCING BEEF BURGER PROPERTIES USING LEMONGRASS OIL NANOEMULSION

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Received: 26 February 2024; **Accepted:** 24 March 2024

ABSTRACT

Lemongrass (*Cymbopogon citratus)* essential oil (LEO) and concentrations (1 and 1.5 %) of its nanoemulsion (LGNE) were included in beef burger formula to investigate their effect on shelf life, microbial, chemical indices, and technological properties. The product was frozen at -18±3ºC and examined at intervals (0, 48hrs, 4 weeks, then at a month interval for up to 4 months). GC analysis of LEO revealed that citric acid, verbenol, β-pinene, á-Myrcene and ethyl acetate formulate preponderance of the oil. FTIR, PDI, TEM and cytotoxicity were used for nanoemulsion characterization. The sensory attributes study disclosed that the raw oil drastically impacted the sensory criteria "colour and odour"; though samples with LGNE showed better acceptability. Samples with LEO showed the lowest TBC ($P<0.05$). Both LEO and LGNE showed great antibacterial effect against TCC. TYMC exhibited a numeral decrease in the count ($P>0.05$) except for the second month of storage ($P<0.05$). LGNE showed significant antioxidant efficacy nevertheless LEO samples showed higher TBARs values. TVBN was significant lower in LGNE samples especially in the last 2 months of storage. LGNE controlled the increase in samples pH compared to the control ($P<0.05$). WHC and cooking yield % showed improvement in the treatment's samples. As well, diameter loss showed numeral decrease in treatments ($P>0.05$). In conclusion, LGNE generally improves the sensory and cooking properties of burger, over the LGO or the control samples.

Keywords*:* Lemongrass Oil, Nanoemulsion, Beef Burger, Shelf Life, Chemical, Microbial, Sensory, WHC, Cooking Yield, pH

Abbreviations: FTIR= Fourier-transform infrared spectroscopy, PDI= polydispersity index, TEM= Transmission Electron Microscope, TBC= Total bacterial count, TCC=Total coliform count, TYMC= Total yeast and mould count, TBARs= Thiobarbituric acid reactive substance, TVBN= Total volatile base nitrogen, pH= power of Hydrogen, WHC= water holding capacity.

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INTRODUCTION

Burger is a famous formed comminuted meat product made from minced meat consumed by millions of people. burger earns its popularity among other meat products because of its nutritional value, affordability, and sensory acceptance. For the previous reasons, this product rules the global fast-food market, as well as restaurants and retail establishments. However, it has limited stability, mainly due to microbial growth and lipid oxidation (Davis and Lin, 2005; Gahruie *et al.,* 2017; Mizi *et al.,* 2019; Ruiz-Capillas *et al.,* 2021; Mujović *et al.,* 2023).

The deteriorative effects, such as off-tastes, off-odors, and color change, can result in the production of toxic compounds that render meat unsuitable for human consumption (Papuc *et al.,* 2017; Villalobos-Delgado *et al.,* 2019).

Consumer's demand for fresh, healthy, and nutritious food products, aligned with food short shelf-life, resulting in a huge amount of food waste. Thus, turned food waste reduction into a fundamental challenge. Quality of the ingredients, structure, composition, processing, manufacturing conditions, and associated packaging system are factors determine food stability and consequent shelf-life relies (Nunes *et al.,* 2023).

There are various chemical preservatives available in the market that can be used to prevent food degradation. However, the use of chemical preservatives has been linked to potential health risks (Mwale, 2023). Preserving food and ensuring its safety from harmful microbes, while also maintaining consumer acceptability and prolonging shelf life without posing health hazards, have prompted industries to explore alternatives to chemical additives by incorporating natural substitutes. In response, the industry has turned to essential oils as a potential solution to these challenges (Pateiro *et al.,* 2021; Faheem *et al.,* 2022).

Researches and reports on the benefits of incorporating essential oils or plant extracts that have been deemed generally recognized as safe (GRAS) have been conducted on a variety of meats, including lamb, beef, and pig (Nieto *et al.,* 2010).

Lemongrass got its name from the distinctive citrus aroma that the green leaves have when crushed. Pleasant lemon scent of this plant has long been used in the food industry, as well as in perfumery and other cosmetics (Kumar *et al.,* 2010; Ranade and Thiagarajan, 2015). Lemongrass oil may easily substitute synthetic antioxidants that people are concerned about using due to their potential health risks (Olorunsanya *et al.,* 2010).

However, EO exhibits many undesirable physicochemical properties that hinder its widespread use. They are highly volatile, sensitive to light, produce off-flavors, change the color of food, affect the texture of food and have low bioavailability (Joye *et al.,* 2015; Chivandi *et al.,* 2016; Zhang *et al.,* 2021). In this sense, several nanotechnological approaches are currently being applied to overcome these disadvantages without compromising the beneficial properties (Sun *et al.,* 2021; Mohammad *et al.,* 2022).

Nanoproducts can be classified into nanoparticles, nanoencapsulation, nanoemulsions...etc. Nanocoating (nanoemulsions/ nanoencapsulations) is primarily used in food preservation as it has the advantage of encapsulation of bioactive compounds, antimicrobial activity and nutrient delivery (Hegde *et al.,* 2022).

Nanoemulsions are represented by an oily system dispersed in an aqueous system or aqueous system dispersed in an oily one. This nanofabricates offer better dispersion within the final mixture, optical clear nanoproduct, reduces the amount of surfactant required, improve shelf life of the food as it readily available also it modulates product texture (Nema *et al.,* 2022).

Due to diminutive size of the nanomaterials, these structures possess distinctive, groundbreaking, and highly appealing functionalities (Avramescu *et al.,* 2020).

This article delves into the preservative and technological properties improvement effects of lemongrass essential oil and its nanoemulsion on frozen beef burger allover storage period (-18ºC.

MATERIALS AND METHOD

Lemongrass *(Cymbopogon citratus)* essential oil (LEO) was purchased from National Research Center, Giza, Egypt; Polyethylene glycol sorbitan monooleate (Tween* 80) and (Tween* 20) El-Nasr pharmaceutical company" was purchased from El- gomheria incorporation; and Deionized water was obtained from the central laboratory of veterinary medicine, Assiut University. Those chemicals were of analytical grade (AR) and classified as generally recognized as safe (GRAS).

Components analysis for lemongrass essential oil (LEO)

Volatile components were analyzed by Gas chromatography– mass spectrometry (GC / MS) (Thermo Scientific TRACE 1300 Series Gas Chromatograph, USA) at the Department of Chemistry - Faculty of Science - Assiut University according to Abd El-Kareem *et al.* (2020). All volatile components were identified by comparing the recorded mass spectra to standard NIST11.L database mass spectra.

Preparation of LGNE. (Lemongrass oil nanoemulsion) (Ghosh *et al.*, 2013)

Oil-in-water NE was prepared by dissolving 20 v/v % Tween 80 in deionized water at room temperature (5 ml tween 80 into 20 ml deionized water). The mixture was shaken for 10 minutes using a magnetic stirrer to obtain a homogeneous solution. Then, 1 ml of LEO (3.8 %) was then slowly added with rate of 1 drop/ 10 seconds using a syringe. Acetic acid (20 µl) was added and mixed with a direct-driven stirrer (hot plate stirrer, DAIHAN Scientific Co., Ltd, Korea) for 1 hour. The resulting emulsion was sonicated using a 25 kHz ultrasonic homogenizer (USH650, Maximum power: 650 W) for 20 minutes and kept refrigerated (4 °C ±0.2) till use.

Characterization of the prepared NE

Measurement of particle size and polydispersity index (PDI)

The particle size and PDI of the nanoemulsions were measured at 25 ± 0.2°C using a Zeta-sizer (3000 HS, Malvern Instruments, Malvern, UK) at Faculty of Pharmacy, Al-Azhar University -Assiut branch. According to Baboota *et al.* (2007).

Fourier-transform Infrared Spectroscopy (FTIR)

Spectral analysis was carried out in the Analytical Chemistry Laboratory "accredited" Department of Chemistry, Faculty of Science, Assiut University, Egypt. FTIR was measured with a Fourier transform infrared (FTIR) spectrometer (Thermo Scientific Nicolet IS 10, USA) with the Smart OMNI Sampler Accessory. According to Gurpreet and Singh (2018).

Morphological study of NEs

High resolution Transmission electron microscopical scanning (HRTEM) was performed at the Electron Microscopy unit of Assiut University according to Shakeel *et al.* (2009).

Cytotoxicity assay of lemon grass nanoemulsion

It was performed in Nawah Scientific Inc., (Mokatam, Cairo, Egypt) using The Green monkey kidney cell culture (Vero) as described by Skehan *et al.* (1990). Cell viability was assessed by Sulforhodamine B assay (SRB) executed according to (Allam *et al.,* 2018). The absorbance was measured at 540 nm using a BMG LABTECH®-FLUOstar Omega microplate reader (Ortenberg, Germany).

Experimental Design:

A three trial-based experiment was designed to investigate the effect of raw Lemongrass

essential oil (LEO) and different concentrations of its nano emulsion on experimental manufactured beef burger. Burger samples [control with no treatment (C), treated with 1.0 % of lemon grass nano emulsion (1 N), treated with 1.5 % of lemon grass nano emulsion (1.5 N), and treated with 0.5% raw lemon grass oil (0.5 O)] were prepared as three batches from each treatment and the products were kept frozen at -18 ± 3 ºC and examined at intervals (0, 48hrs, 4 weeks then at a month interval) till appearance of deterioration signs.

Burger manufacturing:

Twenty kilograms of beef burger were manufactured in the experimental meat processing unit; at the "Training Center of Quality of Meat, Poultry, Fish and Their Products", Teaching Veterinary Hospital, Faculty of Veterinary Medicine, Assiut university. Legal requirements were according to (ES, 2005) as follows: Meat (chuck piece) was representing 75% with intrinsic fat; soya bean texture was representing 12 %; and seasonings {(bell pepper, hot pepper, onion, tomato, garlic and salts (common salts, phosphates and monosodium glutamate)} was representing 13 % of the final product.

The imported frozen beef chuck was purchased from a local imported meat distributer during the 1st half of its shelf life (6 months). Other materials including food quality grade soya bean texture and other seasoning were purchased from a local apothecary.

Soyabean was soaked in twice its weight water and kept in the refrigerator for the second day before use and was with other seasonings, coarsely ground in a Sirman mincer (Sirman Meat Grinder, model TC 42 Montana Y12, Italy) through an 8 mm plate; then all were thoroughly mixed in meat mixer (Sirman meat mixer, model IP 80 XP BA, Italy). The mixture was then partitioned into 4 equal groups [Group 1: control with no treatment (C), Group 2: treated with 1.0 % of lemon grass nano emulsion (1 N), Group 3: treated with 1.5 % of lemon grass nano emulsion (1.5 N) and Group 4: treated with 0.5% raw lemon grass oil (0.5 O)] N.B: each treatment was made in triplicate.

All prepared mixtures were then shaped into 70 to 75 grams patties using an automatic patty former (Minerva group refrigerated hamburger forming machine, model C/E653 R, Italy). Samples of the freshly prepared burger were taken and analyzed (zero time). The remaining patties were kept in a freezer at -18 ± 3 º C.

Sensory evaluation:

Three samples from each of the beef burger patties were sensory evaluated by an odd number of members from the Department of Food Hygiene, Safety and Technology, Faculty of Veterinary Medicine, Assiut University. The samples color was examined before cooking. The burger patties were cooked in a preheated grill for a total of 5 minutes, 2.5 minutes for each side (reaching 70ºC core temperature) before being coded and evaluated for texture, odor, taste, and overall acceptability after cooking using 5-point hedonic descriptive scales according to Minim (2006) as follows in Table A:

Score	Color	Odor	Texture	Taste	General acceptability
1	Dark yellow or strongly disliked	Strongly disliked	Very poor	Very poor	Strongly disliked
2	Slightly dark or moderately disliked	Moderately disliked	Poor	Poor	Moderately disliked
3	Moderate or indifferent	Indifferent	Fair	Fair	Indifferent
4	Very light or moderately liked	Moderately liked	Good	Good	Moderately liked
5	No yellow color or strongly liked	Strongly liked	Very good	Very good	Strongly liked

Microbiological evaluation of burger samples

Preparation of samples for serial dilution was applied according to (ISO, 2017) under aseptic conditions, using ten grams of sample with ninety ml of a sterile 0.1% peptone water in sterile polyethylene bag and stomached (Seward laboratory mixer Stomacher 400, type BA7021) for 2 minutes to obtain a dilution 1/10; then ten folds serial dilutions were prepared using test tubes each contain 9 ml of sterile diluent (0.1% peptone water).

Total bacterial count (ISO, 2022)

From each of the prepared dilutions, 100 µl was evenly distributed over a dry surface of plate count agar (HI-MEDIA, M091) plates media using clean flame-sterile glass loop. Inoculated plates were incubated at 30°C for (24- 48) hours.

Coliform count and (ISO., 2004)

Violet red bile glucose agar (VRBG) agar (Feng *et al.,* 2002) "7 g peptone, 3 g yeast extract, 5 g sodium chloride, 1.5 g bile salts mixture, 10 g lactose, 0.03 neutral red indicator, 0.002 crystal violet and 15 g agar / 1-liter distilled water; final pH (at 25°C): 7.4±0.2; sterilized by boiling" plates were prepared and were inoculated each with 100 µl from the prepared serial dilutions. Inoculated plates were incubated at 37ºC for 24 hours and characteristic colonies (pink to red or purple with or without precipitation haloes) were counted.

Total yeast and mold count (Hungerford *et al.,* 1998).

Plates of Sabouraud Dextrose agar medium (HI-MEDIA, MH063) containing 0.05 mg of chloramphenicol per ml were inoculated each with 100 µl from the prepared serial dilutions. Inoculated plates were incubated at 25ºC for 5 days before being counted. The yeast and mold count per gram of the sample was then calculated and recorded.

***E. coli* counting (Sahibzada *et al.,* 2018):**

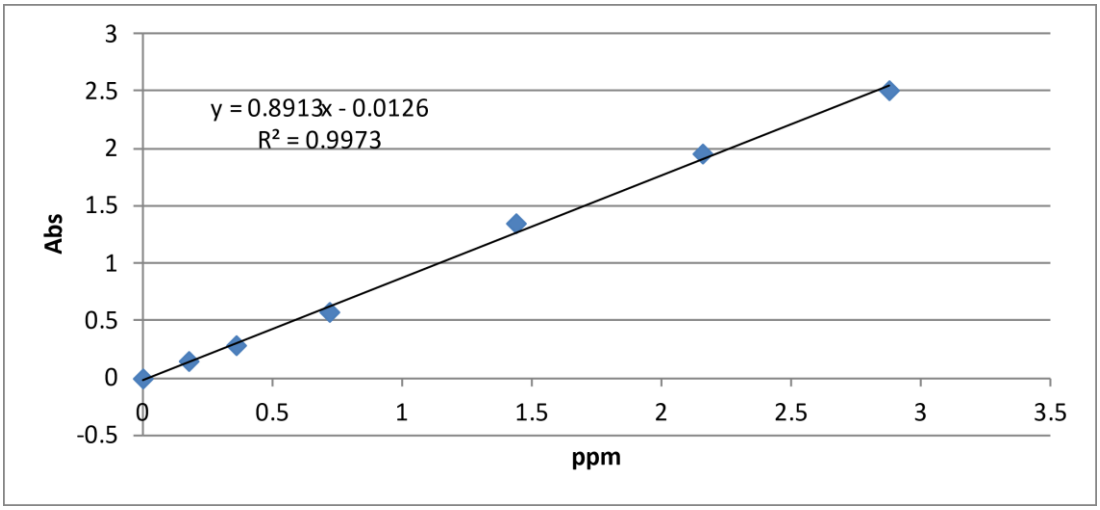
From each dilution 100 µl was used to inoculate EMB plates (HI-MEDIA, M317). The plates were then incubated at 37°C for 24 hours. Characteristic *E. coli* colonies (nucleated colonies with or without metallic sheen) were identified and the count per gram was calculated and recorded.

Chemical quality indicators:

Determination of Thiobarbituric acid-Reactive substances (TBARs):

The sample oxidation was measured according to Ismail *et al.* (2008). The absorbance of the sample was measured at 531 nm using a spectrophotometer (Thermo Scientific Evolution 300UV-Vis) against the 2 blanks. The concentration of in the sample was determined by using the standard curve equation and expressed as mg of malondialdehyde (MDA) / kg of burger, while accounting the dilution factor of 6, as following:

$$\text{TBARS } (mgMDA/kg) = \left(\frac{\text{Spectrophotometer Reading}+0.0126}{0.8912}\right)X6$$



TBARs standard curve

Determination of Total volatile base nitrogen (TVBN) according to **(Pearson, 1976):**
Ten grams of homogenized diluted sample were used for the distillation. The resulting distillate was titrated with 0.05M (0.1N) sulfuric acid to the end point (light yellow). A blank was prepared using the same procedures excluding the sample. The amount of 0.1N sulfuric acid consumed in the titration was used to calculate TVBN according to the following formula: TVBN = (titration value - blank) × 14

Determination of hydrogen ion concentration "pH" according to **Yalcin *et al.* (2018)**
Hydrogen ion concentration was measured directly in the sample after thawing using pH meter with electrode for semisolid samples (testo 205 - One-hand pH/temperature measuring instrument). The pH meter was calibrated with buffer solutions of pH 4 and 7. The probe of the pH meter was then inserted directly into the thawed burger sample to measure its pH value.

Technological characters
Water holding capacity according to **Al-Sultan *et al.* (2022):**
To estimate the water holding capacity (WHC), the pressing method of Honikel and Hamm (1994) was used applying weight of 2 kg for 4 min. Retained weight (RW)% is estimated using the following formula: $RW\ \% = \frac{W1-W2}{W1} x\ 100$ and WHC were calculated as: WHC (Percent of Water Retained) = 100 - RW% ($\frac{W1-W2}{W1} x\ 100$)

Cooking loss according to **Bakhsh *et al.* (2021)**
Samples were weighted while raw and after grilling. Cooking loss was calculated using the

following equation:
$$\text{Cooking loss (\%)} = \frac{(Raw\ weight - Grilled\ weight)}{Raw\ weight} \times 100$$

Cooking yield according to **Akwetey and Knipe (2012)**
To estimate the cooking yield "the weight retained in the sample after grilling", the following equation was used:

$$\text{Cooking yield (\%)} = \frac{Grilled\ weight}{Raw\ weight} \times 100$$

Diameter loss according to **Modi *et al.* (2004):**
The diameter of each sample was measured before and after grilling using a caliper, each at 2 randomly chosen points and the average was calculated. Diameter loss percentage was recorded according to the following equation:

$$\text{Diameter loss (\%)} = \frac{(Raw\ dimater - Grilled\ diameter)}{Raw\ diameter} \times 100$$

Statistical analysis:

The statistical program Graph Pad Prism 9 (version 9.5.1) was used for data analysis. One way ANOVA statistics applied, and results were expressed as mean ± standard error (SE). Differences between means were assessed by Tukey's method ($P<0.05$). Figures were designed by Excel software 2019. FTIR results were analyzed and plotted into graphs using Originlab® origin application 2022.

RESULTS

Table 1: GC/MS analysis of lemongrass (*Cymbopogon citratus*) essential oil (LEO).

Compound name	Formula	Relative peak area %
Citral (Citric acid)	**$C_{10}H_{16}O$**	**28.97**
Citric acid and Verbenol	**$C_{10}H_{16}O$**	**24.95**
β-pinene, **á-Myrcene**	$C_{10}H_{16}$	19.22
Ethyl Acetate	$C_4H_8O_2$	10.32
á-Linalool	$C_{10}H_{18}O$	3.52
Anthranilic acid	$C_{17}H_{23}NO_2$	
Linalyl butyrate	$C_{14}H_{24}O_2$	
2,6,6-Trimethyl-2-cyclohexene-1-carboxaldehyde (Organic oxide) and Verbenol	$C_{10}H_{16}O$	2.35
1,3,4-Trimethyl-3-cyclohexenyl-1-carboxaldehyde (organic oxide) and Verbenol	$C_{10}H_{16}O$	2.20
1,3,6-Octatriene, 3,7-dimethyl-, hept-2-ene, 2,6,6-trimethyl-, (ñ)- Ocimene	$C_{10}H_{16}$	1.13
Ethanol	$C_{20}H_{40}O_2$	1.05
Octadecynoic acid	$C_{18}H_{32}O_2$	
13-Heptadecyn-1-ol	$C_{17}H_{32}O$	
6-Octenal, 3,7-dimethyl-(á-Citronellal)	$C_{10}H_{18}O$	0.9
Propanoic acid, ethyl acetate	$C_5H_{10}O_2$	0.6
8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	0.31
Z,Z,Z-4,6,9-Nonadecatriene	$C_{19}H_{34}$	
8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_2$	
9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester,(Z,Z,Z)-	$C_{21}H_{36}O_4$	
5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	$C_{21}H_{34}O_2$	
3-Cyclohexene-1-carboxaldehyde, 1,3,4-trimethyl	$C_{10}H_{16}O$	0.29
1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl	$C_{10}H_{16}O$	
Bicyclo[3.1.1]heptane-2-carboxaldehyd e, 6,6-dimethyl	$C_{10}H_{16}O$	
2-Isopropenyl-5-methylhex-4-enal	$C_{10}H_{16}O$	
Cyclopentaneacetaldehyde, 2-formyl-3-methyl-à-methylene	$C_{10}H_{14}O_2$	

Table 1: GC/MS analysis of lemongrass (Cymbopogon citratus) essential oil (LEO)-Continued-

Compound name	Formula	Relative peak area %
trans-2-Caren-4-ol	$C_{10}H_{16}O$	0.28
(1,3-Dimethyl-2-methylene-cyclopenty l)-methanol	$C_9H_{16}O$	
2,3-Dehydro-1,8-cineole	$C_{10}H_{16}O$	
Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl	$C_{10}H_{16}O$	
1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethyl)-	$C_{10}H_{16}O$	
7-Oxo-2-oxa-7-thiatricyclo[4.4.0.0(3,8) decan-4-ol	$C_8H_{12}O_3S$	0.2
1-(Cyclopropyl-nitro-methyl)-cyclopen tanol	$C_9H_{15}NO_3$	
13-Heptadecyn-1-ol	$C_{17}H_{32}O$	
9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester,(Z,Z,Z)-	$C_{21}H_{36}O_4$	
Cyclohexanone, 2-(1-methyl-2-nitroethyl)-	$C_9H_{15}NO_3$	
Cyclopropanemethanol, 2-methyl-2-(4-methyl-3-pentenyl)-	$C_{11}H_{20}O$	0.15
9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	
Bicyclo[2.2.1]heptan-2-ol, 2-allyl-1,7,7-trimethyl	$C_{13}H_{22}O$	
Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	
2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)-	$C_{15}H_{26}O$	
Ingol 12-acetate	$C_{22}H_{32}O_7$	0.14
9-Hexadecenoic acid	$C_{16}H_{30}O_2$	
2,6-Octadiene-1,8-diol, 2,6-dimethyl-	$C_{16}H_{30}O_2$	
2-Butenoic acid, 2-methyl-, 2-(acetyloxy)-1,1a,2,3,4,6,7,10,11,11adecahydro-7,10-dihydroxy-1,1,3,6,9-pe ntamethyl-4a,7a-epoxy-5H-cyclopenta[a]cyclopropa[f]cycloundecen-11-yl ester	$C_{27}H_{38}O_8$	
Octadecanal, 2-bromo-	$C_{18}H_{35}BrO$	
Others (<0.14)		3.32
Total		99.9

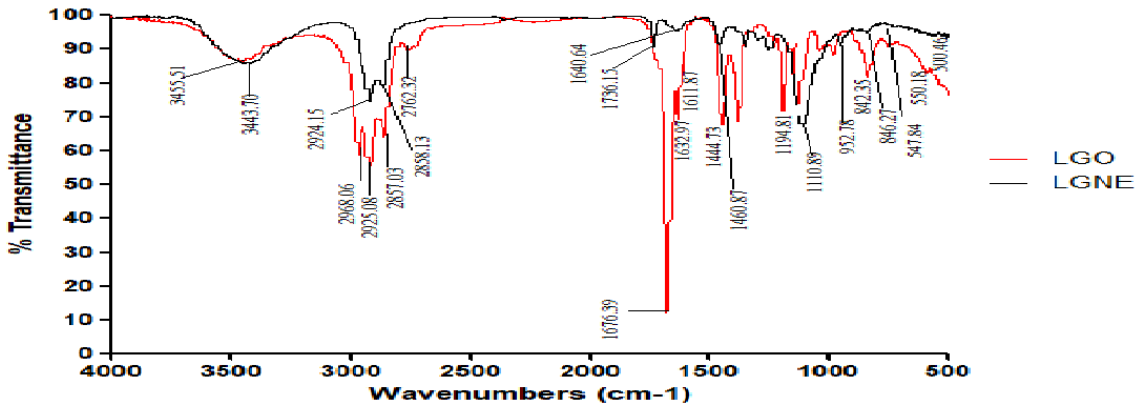


Figure 1: FTIR of Lemongrass essential oil (LEO) and its nanoemulsion (LGNE).

Table 2: Particle size and PDI of nano-fabricate lemongrass essential oil

Nanomaterial	Average droplet size (nm) ± St. Dev.	Polydispersity index (PDI)
LGNE	486.7 ± 108.3	0.221

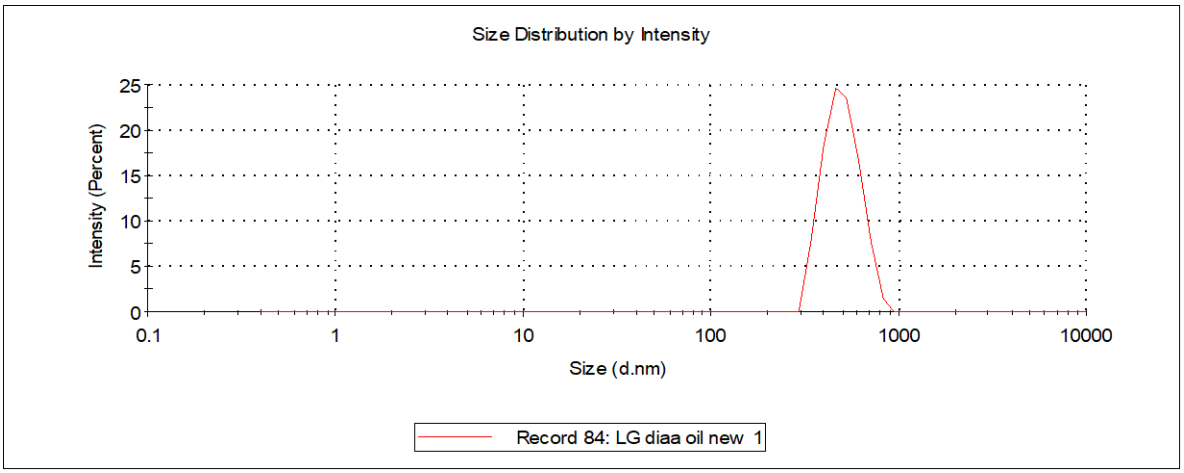


Figure 2: Size distribution by intensity of LGNE

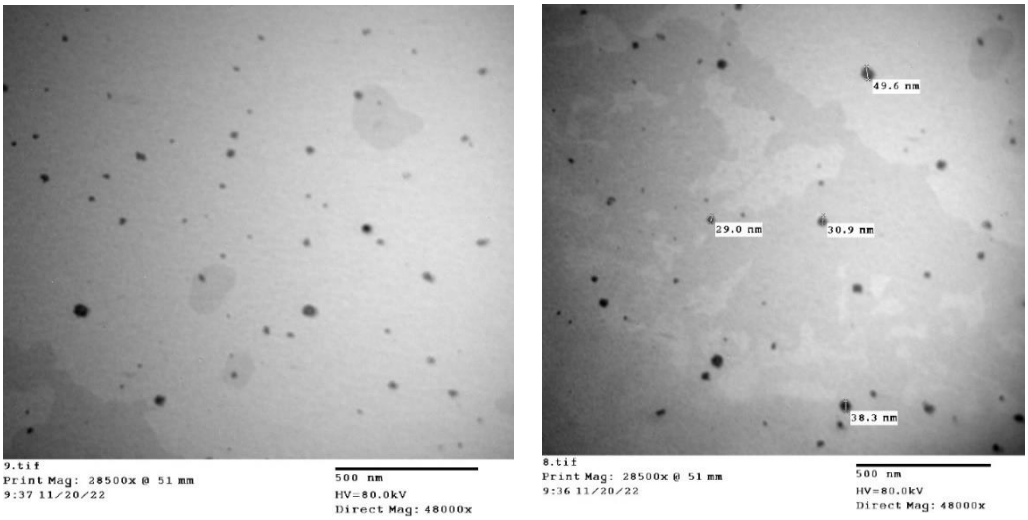


Figure 3: HRTEM of LGNE with spherical shape and mean nano-size ± standard error (30.94±4.12)

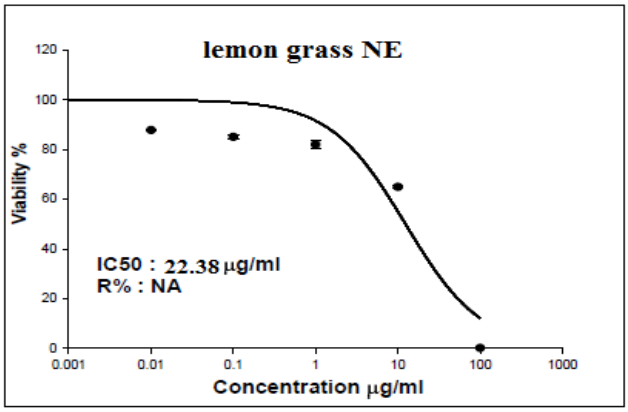


Figure 4: Cytotoxicity of lemon grass nanoemulsions showed half-maximal inhibitory concentration (IC_{50}) equal 22.38 µg/ml

Table 3: Sensory scores of control and treated burger with lemon grass or its nanoemulsions during frozen storage.

Sensory attributes	Time	Control	1 N	1.5 N	0.5 O	P value
Color	0 time	4.5±0.5[a]	4.5±0.28[a]	3.5±0.64[a]	1.5±0.28[b]	0.0016
	48 Hrs	4.25±0.47[a]	4.25±0.47[a]	4.5±0.28[a]	1.75±0.25[b]	0.0009
	1st month	3.83±0.30[a]	4.0±0.51[a]	4.0±0.25[a]	2.333±0.21[b]	0.0061
	2nd month	3.0±0.57	3.667±0.33	3.0±0.57	2.333±0.33	0.3300
	3rd month	3.333±0.66	3.0±0.57	3.0±0.57	1.667±0.33	0.2272
	4th month	3.0±0.57	3.0±0.57	3.333±0.33	1.667±0.33	0.1375
Texture	0 time	3.5±0.28	4.0±0.57	4.0±0.70	4.0±0.40	0.8732
	48 Hrs	4.5±0.28	4.5±0.28	4.5±0.28	3.75±0.47	0.4053
	1st month	4.16±0.30	3.5±0.42	4.16±0.30	3.83±0.30	0.3302
	2nd month	4.33±0.33	3.67±0.509	3.66±0.88	4.33±0.33	0.7569
	3rd month	3.66± 0.33	3.66±0.66	3.66±0.33	4.33±0.33	0.6495
	4th month	3. 0±0.57	3.0±0.57	3.0±0.57	3.667±0.33	0.7569
Odor	0 time	4.25±0.25[a]	4.5±0.28[a]	3.75±0.47[a]	1.75±0.47[b]	0.0012
	48 Hrs	4.0±0.40[a]	4.25±0.47[a]	4.0±0.7[a]	1.25±0.25[b]	0.0028
	1st month	3.333±0.42[a]	4.16±0.30[a]	2.83±0.47[a]	1.5±0.22[b]	0.0005
	2nd month	3.66±0.33[a]	3.33±0.33[a]	3.33±3.33[a]	2.0±0.0[b]	0.0151
	3rd month	3.0±0.57[ab]	3.66±0.33[a]	3.66±0.33[a]	2.0±0.0[b]	0.0402
	4th month	2.33±0.33	3.0±0.0	3.33±0.57	2.0±0.0	0.1598
Taste	0 time	5.0±0.0[a]	3.25±0.62[b]	3.25±0.47[b]	x	0.0363
	48 Hrs	4.0±0.57	4.0±0.57	3.5±0.86	x	0.8411
	1st month	4.16±0.30	3.66±0.55	3.0±0.51	x	0.2487
	2nd month	4.33±0.33	4.33±0.66	3.0±0.57	x	0.2160
	3rd month	3.66±0.33	4.0±0.57	2.66±0.66	x	0.2729
	4th month	3.33±0.33	3.0±0.57	2.66±0.66	x	0.7023
General acceptability	0 time	4.75±0.25	3.25±0.62	3.25±0.47	x	0.0855
	48 Hrs	4.0±0.57	4.25±0.47	3.75±0.94	x	0.8809
	1st month	4.0±0.36	3.66±0.55	3.0±0.68	x	0.4455
	2nd month	4.0±0.0	3.66±0.57	3.0±0.57	x	0.2519
	3rd month	3.66±0.33	3.0±0.0	2.66±0.66	x	0.3170
	4th month	3.33±0.33	2.66±0.33	3.0±0.57	x	0.5787

1 N: treated with 1 % LGNE; 1.5 N: treated with 1.5% LGNE; 0.5 O: treated with 0.5 % LEO
a-b: In the same raw, means with different superscripts differ significantly (P< 0.05)
X: samples had too strong flavor rendering them inedible.

Table 4: Efficacy of LEO and its NE on TBC (log_{10} CFU/g) in treated burger samples

Time	Control	1 N	1.5 N	0.5 O	P. value
Zero time		6.001 ± 0.2706			-
After 48 Hrs	5.79 ±0.02[a]	5.74 ± 0.07[a]	5.70±0.11[a]	5.13± 0.02[b]	0.0121
1st month	5.66±0.13[a]	5.42± 0.08[a]	5.28±0.01[ab]	4.85±0.15[b]	0.0062
2nd month	5.44±0.14[a]	5.35±0.09[a]	5.29± 0.01[a]	4.36±0.18[b]	0.0009
3rd month	5.38± 0.12[a]	5.11± 0.09[a]	5.05±0.14[a]	4.19±0.19[b]	0.0022
4th month	5.33± 0.13[a]	4.76± 0.22[a]	4.76±0.06[a]	3.82±0.04[b]	0.0004

1 N: treated with 1 % LGNE; 1.5 N: treated with 1.5% LGNE; 0.5 O: treated with 0.5 % LEO
a-b: In the same raw, means with different superscripts differ significantly (P< 0.05)
Underlined values exceeded TBC permissible limit stipulated by **ES (2005)** "10^5"**.**

Table 5: Efficacy of LEO and its NE on TCC (log_{10} CFU/g) in treated burger samples

Time	Control	1 N	1.5 N	0.5 O	P. value
Zero time	4.25±0.1407				-
After 48 Hrs	4.90±0.12^{a}	4.73±0.21^{a}	4.71±0.07^{a}	4.25±0.04^{b}	0.0182
1st month	4.68±0.34	4.55 ±0.07	4.20±0.2	4.15± 0.15	0.3146
2nd month	4.54±0.16^{a}	3.92±0.46^{a}	3.66 ±0.33^{a}	2.99± 00^{b}	0.0375
3rd month	4.35± 0.06^{a}	3.49 ± 0.11^{b}	3.36±0.22^{b}	2.99±0.001^{b}	0.0005
4th month	3.99±0.2^{a}	3.24±0.15^{b}	3.25±0.13^{b}	2.99±0.001^{b}	0.0068

1 N: treated with 1 % LGNE; 1.5 N: treated with 1.5% LGNE; 0.5 O: treated with 0.5 % LEO
a-b: In the same raw, means with different superscripts differ significantly (P< 0.05)
All values exceeded TCC permissible limit stipulated by **ES (2005)** "10^{2}".

Table 6: Efficacy of LEO and its NE on TYMC (log_{10} CFU/g) in treated burger samples

Time	Control	1 N	1.5 N	0.5 O	P. value
Zero time	3.000±0.00				-
After 48 Hrs	3.67±0.1	3.77±0.04	3.33±0.2	3.301± 0.0	0.0463
1st month	2.24± 0.1	2.1±0.07	2.14±0.08	1.95±0.13	0.3991
2nd month	4.09±0.12^{a}	3.72±0.12^{ab}	3.49±0.11^{b}	3.53±0.11^{b}	0.0263
3rd month	3.49±0.11	3.55±0.07	3.31±0.15	3.1±0.1	0.0930
4th month	3.25±0.13	3.2±0.1	2.99±0.001	2.99±0.001	0.1333

1 N: treated with 1 % LGNE; 1.5 N: treated with 1.5% LGNE; 0.5 O: treated with 0.5 % LEO
a-b: In the same raw, means with different superscripts differ significantly (P< 0.05)

Table 7: Efficacy of LEO and its NE on TBARs (mg MDA/kg) in treated burger samples

Time	Control	1 N	1.5 N	0.5 O	P. value
Zero time	0.825±0.0067				-
After 48 Hrs	0.88±0.043^{ac}	0.84± 0.029^{ac}	0.81± 0.057^{bc}	1.25± 0.171^{a}	0.0335
1st month	1.18± 0.04^{ac}	0.99±0.05^{bc}	0.84 ±0.04^{b}	1.23± 0.04^{a}	0.001
2nd month	1.25 ± 0.03^{a}	0.99±0.03^{b}	0.89± 0.03^{b}	1.23±0.04^{a}	0.0003
3rd month	1.29± 0.02^{a}	1.02± 0.01^{b}	0.93±0.04^{b}	1.27±0.04^{a}	0.0001
4th month	1.29±0.01^{a}	1.03±0.02^{b}	0.95±0.05^{b}	1.22±0.02^{a}	0.0004

1 N: treated with 1 % LGNE; 1.5 N: treated with 1.5% LGNE; 0.5 O: treated with 0.5 % LEO
a-b: In the same raw, means with different superscripts differ significantly (P< 0.05)

Table 8: Efficacy of LEO and its NE on TVBN (mg N/100g) in treated burger samples.

Time	Control	1 N	1.5 N	0.5 O	P. value
Zero time	17.27± 1.68				-
After 48 Hrs	18.20±0.80	16.33± 0.46	16.33± 0.46	15.87± 0.46	0.0797
1st month	19.60± 0.80	17.73± 0.46	16.80±0.80	19.13± 1.23	0.1699
2nd month	20.53± 0.46	18.67±0.46	17.27± 0.46	21.00±2.13	0.1566
3rd month	21.47± 0.46^{a}	20.07± 0.46^{ac}	17.73±0.46^{b}	19.13±0.46^{bc}	0.0030
4th month	23.80±0.80^{a}	21.47±0.46^{ab}	20.07±21.47^{b}	21.47±21.47^{ab}	0.0004

1 N: treated with 1 % LGNE; 1.5 N: treated with 1.5% LGNE; 0.5 O: treated with 0.5 % LEO
a-b: In the same raw, means with different superscripts differ significantly (P< 0.05)

Table 9: Efficacy of LEO and its NE on pH of treated burger samples

Time	Control	1 N	1.5 N	0.5 O	P. value
Zero time	6.03 ± 0.0116				-
After 48 Hrs	6.06±0.005	6.03± 0.02	6.02± 0.008	6.06± 0.008	0.0867
1st month	6.10± 0.006^{b}	6.07± 0.006^{c}	6.06±0.008^{c}	6.14± 0.005^{a}	0.0003
2nd month	6.20± 0.011^{a}	6.14±0.015^{b}	6.16± 0.008^{bc}	6.21±0.003^{ac}	0.0007
3rd month	6.21± 0.003^{a}	6.16± 0.008^{b}	6.22±0.00^{a}	6.23±0.005^{a}	0.0001
4th month	6.28±0.015^{bc}	6.24±0.005^{b}	6.3±0.0^{ac}	6.35±0.0^{a}	0.0008

1 N: treated with 1 % LGNE; 1.5 N: treated with 1.5% LGNE; 0.5 O: treated with 0.5 % LEO
a-b: In the same raw, means with different superscripts differ significantly (P< 0.05)

Table 10: Efficacy of LEO and its NE on WHC (%) of treated burger samples.

Time	Control	1 N	1.5 N	0.5 O	P. value
Zero time	50.34±4.59				-
After 48 Hrs	56.16± 3.69^{b}	58.42± 1.76^{ab}	60.25± 0.65^{ab}	68.03± 1.17^{a}	0.0217
1st month	50.76± 2.52^{a}	60.90± 1.10^{b}	56.67±4.04^{ab}	59.65± 0.87^{b}	0.0099
2nd month	58.39± 2.60	61.63±0.84	58.90± 0.42	59.97±2.61	0.6526
3rd month	64.01± 1.31	65.37± 0.64	65.81±2.13	63.84±1.47	0.7349
4th month	65.15±1.63	63.30±0.79	63.24±1.69	60.19±1.55	0.1987

1 N: treated with 1 % LGNE; 1.5 N: treated with 1.5% LGNE; 0.5 O: treated with 0.5 % LEO
a-b: In the same raw, means with different superscripts differ significantly (P< 0.05)

Table 11: Efficacy of LEO and its NE on cooking loss (%) in treated burger samples.

Time	Control	1 N	1.5 N	0.5 O	P. value
Zero time	26.03±0.9796				-
After 48 Hrs	28.34± 2.18	29.10± 1.52	25.16± 4.64	24.58± 1.32	0.5946
1st month	29.76± 0.88^{a}	29.68± 0.58^{a}	20.18±1.93^{b}	24.42± 0.68^{b}	0.0010
2nd month	28.61± 2.24	28.55±1.7	24.78± 1.03	23.68±1.47	0.1502
3rd month	30.28± 1.6	27.14± 1.92	21.92±2.91	22.98±0.94	0.0581
4th month	30.03±1.82^{a}	30.45±1.16^{a}	26.44±0.89^{ab}	22.57±1.8^{b}	0.0178

1 N: treated with 1 % LGNE; 1.5 N: treated with 1.5% LGNE; 0.5 O: treated with 0.5 % LEO
a-b: In the same raw, means with different superscripts differ significantly (P< 0.05)

Table 12: Efficacy of LEO and its NE on cooking yield (%) in treated burger samples.

Time	Control	1 N	1.5 N	0.5 O	P. value
Zero time	73.97±0.97				-
After 48 Hrs	71.66± 2.18	70.90± 1.52	74.84± 4.64	75.42± 1.32	0.5946
1st month	70.24± 0.88^{b}	70.32± 0.58^{b}	79.82±1.93^{a}	75.58± 0.68^{a}	0.0010
2nd month	71.39± 2.24	71.45±1.7	75.22± 1.03	76.32±1.47	0.1502
3rd month	69.72± 1.6	72.86± 1.92	78.08±2.91	77.02±0.94	0.0581
4th month	69.97±1.82^{b}	69.55±1.16^{b}	73.56±0.89^{ab}	77.43±1.8^{a}	0.0178

1 N: treated with 1 % LGNE; 1.5 N: treated with 1.5% LGNE; 0.5 O: treated with 0.5 % LEO
a-b: In the same raw, means with different superscripts differ significantly (P< 0.05)

Table 13: Efficacy of LEO and its NE on diameter loss (%) in treated burger samples.

Time	Control	1 N	1.5 N	0.5 O	P. value
Zero time	22.33± 0.8819				-
After 48 Hrs	26.00±0.57^{ab}	27.67± 0.35^{a}	25.33± 0.66^{b}	22.67± 0.33^{c}	0.0007
1st month	26.67± 0.88	26.67± 0.88	24.00± 0.57	23.67± 0.88	0.0507
2nd month	25.33± 0.33	27.00± 0.57	24.67± 0.88	24.33± 0.88	0.1075
3rd month	26.33± 0.33	26.67± 0.66	24.33± 0.66	23.67± 0.88	0.8783
4th month	25.67±0.88^{ab}	26.67± 0.33^{a}	25.00± 0.57^{ab}	23.00± 0.57^{b}	0.0178

1 N: treated with 1 % LGNE; 1.5 N: treated with 1.5% LGNE; 0.5 O: treated with 0.5 % LEO
a-b: In the same raw, means with different superscripts differ significantly (P< 0.05)

DISCUSSION

Meat products, especially beef burgers, are one of the major nutrient food resources in human diet worldwide. However, it is an ideal environment for bacteria to multiply, leading to food spoilage with significant economic losses to the industry producing packaged foods (Surendhiran *et al.,* 2020).

The essential oil of lemongrass (LEO) has good antimicrobial effects against a variety of microorganisms; however, being has reduced stability and compatibility. Essential oil (EO) is dispersed in the burger mixture in the form of nanoemulsions, allowing proper distribution in the main phase with higher bioavailability, stability, and anti-aggregation

properties. The size of the encapsulated LEO emulsion droplets also directly affects the antimicrobial activity (Mendes *et al.,* 2020)

Nanoemulsions are thermodynamically suitable for fusion with lipid membranes. This effect is enhanced by the electrostatic attraction between the cationic charges of the nanoemulsion and the anionic charges in the pathogen and leads to cell lysis and death of the bacteria. This does not create drug-resistant strains and therefore considered promising antibacterial agents (Guerra-Rosas *et al.,* 2017).

Chemical composition of lemongrass essential oil using gas chromatography mass spectrometry (GC/ MS).

In this study, Lemongrass essential oil was subjected to GC/MS analyses to identify their composition (Table 1). The result showed presence of forty-five compounds with considerable amount (> 0.14%). The main components were Citral "citric acid" (28.97 %), Citric acid and Verbenol (24.95 %), β-pinene - á-Myrcene (19.22 %) and Ethyl Acetate (10.32 %).

The most important component is citral by which the essential oil quality can be determined. In order to meet the criteria for a high-quality essential oil, literature suggests a minimum citral content of 75% (Barbosa *et al.,* 2008).

The obtained essential oil was found to contain approximately 28.97 % of citral and 24.95 % of citral and verbenol with a total of 53.92 % of citral and verbenol. This amount was inconsistent with that found by Fatunmibi *et al.* (2023) neither Viuda-Martos *et al.* (2010); citral representing 53.48 % and 37.44 % of the total constituent of *C. citratus* essential oil, respectively.

Fourier-transform Infrared Spectroscopy (FTIR)

It is used to identify functional groups and their binders and molecular fingerprints. This is based on the fact that each molecule and chemical structure produces a distinct spectrum, enabling precise identification (Dutta, 2017).

Figure 1 shows the result of IR of crude lemongrass oil compared to the nano fabricate one. The IR spectra of oil revealed a peak at 3455 cm^{-1}, indicating the presence of OH. Peaks at 2857, 2925 cm^{-1}, and 2968 cm^{-1} indicated C–H stretching, while a peak at 1676 cm^{-1} indicated the presence of C=O stretching group.

Citral is the primary constituent found in *C. citratus* oil. Upon analyzing the IR spectrum various functional groups were identified. Strong vibration was observed at 2925 cm^{-1} corresponds to the asymmetric stretching of -CH_3, indicating the presence of an alkyl saturated aliphatic group. Additionally, a symmetric stretching of -CH_2 was found at 2857 cm^{-1}. The band observed at 1676 cm^{-1} indicates the presence of conjugated double bonds, suggesting the presence of an aldehyde group which indicates citral presence. Finally, at the peak of 1444 cm^{-1}, bending of the -CH group was observed. The FTIR result of lemongrass oil agreed with that obtained by Ogede and Abdulrahman (2022).

The IR result of LGNE showed similar peaks with slight shift towards lower wave number. Such shift can be attributed to the increase in molecular mass. The mass of a molecule is inversely proportional to the vibration frequency. The greater the molecular mass, the lower the frequency of vibration and the lower the wave number (Kaur *et al.,* 2020).

Measurement of particle size and polydispersity index (PDI)

The physical stability and appearance of the final emulsion are directly influenced by the droplet sizes and polydispersity index, making them critical physical parameters to consider (Acosta, 2009).

Table 2 showed the particle size and PDI of the nano-fabricate. The average droplet size (nm) ± St. Dev. was 486.7 ± 108.3 and PDI was 0.221. Furthermore, Figure 2 showed size distribution which designates uniform droplets with narrow size distribution. The low PDI value (<0.5) indicates better stability and uniformity of the dispersion medium (Ali & Hussein, 2017; Singh *et al.,* 2023). As well,

the droplet size was falling within the normal range of nanoemulsions (20 to 500 nm) as mentioned by Gupta, (2020)

High-definition transmission electron microscopy (HRTEM) of LGNE

The utilization of electron microscopy has been identified as a suitable method for examining nanoemulsions and nanomaterials present in food products (Blasco and Pico, 2011; Klang *et al.,* 2012). As shown in figure 3 droplets size range from 29 to 49.6 nm in diameter. The droplets had a dark appearance, spherical in shape, widely separated from each other, and contained an amorphous core.

Cytotoxicity of fabricated lemongrass nanoemulsion

The IC_{50} refers to the compound ability to elicit changes in cellular behavior and vital processes, ultimately leading to cell death or a significant decrease in cell survival (Niles and Riss, 2015).

Figure 4 shows the cytotoxic effects of lemongrass nanoemulsion determined by Sulforhodamine B assay (SRB). The IC_{50} of fabricated lemongrass nanoemulsion was 22.38 µg/ml, being lower than that obtained by Youssef *et al.* (2022) which found rosemarry microemulsion with/without chitosan have IC_{50} > 100 µg/mL.

In this context, the prepared nanomaterial exhibits certain cytotoxicity to the cells, this indicates careful use of the nanoemulsion and should require further investigation to ensure the safety of the fabricated nanoproduct.

1. Sensory evaluation

During storage, sensory changes in the color, odor, taste and texture of meat occur due to bacterial growth and chemical changes such as oxidation, proteolysis with the production of volatile compounds. These undesirable changes reduce the shelf life and acceptability of meat products (Malekmohammadi *et al.,* 2023).

In the current study, samples color was examined before cooking whereas texture, odor, taste, and overall acceptability were evaluated after cooking. Burger samples containing 0.5 % LEO (0.5 O) exhibited an unmissable obvious yellow color. Data in Table 3 showed significant color difference ($P<0.05$) between samples of 0.5 O treatment and samples of other treatments and control at 0 time, 48 Hrs. and 1st month. On the 2nd month, 1 N samples showed better scores compared to 0.5 O ($P<0.05$). The color of all samples began to fade with no significant differences between samples of all treatment as well as the control since the third month. It was noted that, samples treated with the nanoemulsion showed no color difference ($P>0.05$) with control samples throughout the experiment time. This was a repercussion to the white milky color of the fabricated nanoemulsion in contrary to raw LEO which has dark yellow color.

Concerning texture, samples showed fair to good texture with no significant differences ($P>0.05$) between treatments and control over the period of examination. Scores decreased gradually over the examination period.

Regarding odor, samples of 0.5 O treatment showed a strong lemon odor that scored lower ($P<0.05$) than samples of other treatments and control; over the period of the first 2 months of examination. By the third month, 0.5 O samples showed significantly lower scores compared to nanoemulsion samples (1N and 1.5N). Since the second month, control samples scores showed consistent degradation in contrast to the nanoemulsion treatments, which displayed a more moderate level of degradation.

As for flavor, results showed degrading values over the storage period. The (0.5 O) burger samples were inedible due to the strong flavor of the essential oil. Moreover, there was significant difference among the samples of the control and the two other treatments containing lemongrass nanoemulsion “1N and 1.5 N”. This was consistent with Hassoun and Çoban (2017) who alluded that essential oil can interact with certain food ingredients and, when used at concentrations close to or above 1% (v/w), may produce strong odors and aromas, resulting in aftertaste (persistence) and bitterness. On the other hand, the obtained

results disagreed with Hussein *et al.* (2015) who mentioned that addition of 2 % of lemongrass essential oil on manufactured burger resulted in values similar to the control samples.

General acceptability or overall acceptability was evaluated as a single item. The values decreased over time of storage; and there was no significant difference among samples score of the nanoemulsion treatments and the control. Regarding (0.5 O) samples, general acceptability could not be evaluated as they were inedible.

2. Microbiology

2.1.Total Bacterial count

Table 4 showed that all treatments had lower mean values of total bacterial count compared to control samples. Lowest mean values were seen in samples treated with 0.5 % LEO (0.5O) that differ significantly ($P<0.05$) with control samples over the whole period of examination. Also, a significant difference between samples of 1 N and 0.5 O treatments was noted in the first month of examination. However, addition of lemongrass nanoemulsion to the samples caused numeral reduction in the total bacterial count ($P>0.05$) compared to controls. This may be attributable to the low concentration of the active antimicrobial agents incorporated into the nanoemulsion and subsequently into the burger mixture.

The very potent antibacterial effectiveness lemongrass essential oil exhibited; being in the same trend as Boudechicha *et al.* (2023) who mentioned that lemongrass antibacterial activity is very high even for standard antibiotics. The findings were also consistent with those reported by Zaki *et al.* (2018) that total bacterial count values in chilled camel burger samples treated with 0.5 % lemongrass essential oil, were lower than values of control samples and Hosny *et al.* (2020) who found lemongrass oil addition (0.5 %) at beef kofta remarkably decreased the total bacterial count throughout 10 days storage at 4 °C; and Morshdy *et al.* (2021) who found that dipping rabbit meat in 0.5 % lemongrass reduced total bacterial count than control samples over the 12 days of chilled storage.

2.2. Total coliform count

Table 5 showed that addition of raw essential oil results in the lowest coliforms mean values (i.e. the highest antibacterial effect). Nanoemulsion was observed to have an inhibitory effect that increased with its concentration. After 48 hours a significant difference was noted between control samples and samples of the 0.5 O treatment. Surprisingly, there were no differences between different treatment samples in the first month. However, in the subsequent months (2^{nd}, $3^{rd,}$ and 4^{th} months), samples of all treatments showed a significant lower count than the controls ($P<0.05$).

The effective antibacterial effect of lemongrass essential oil was attributed to three key components: geranial (trans citral isomer), neral (cis citral isomer), and myrcene (Onawunmi *et al.,* 1984). The potent effect of citral has been contributed to its ability to change the membrane integrity, intracellular ATP, pH and membrane potential (Adukwu *et al.,* 2016; De Silva *et al.,* 2017). α-Pinene is another major constituent in the composition of oil, which also has antibacterial activity against Gram-negative and Gram-positive bacteria (Youssef *et al.,* 2022).

The obtained results agreed with the results of Salem *et al.* (2010) who mentioned that control samples exhibited the highest coliform counts when compared to other treatments of minced meat containing varying concentrations of lemongrass preserved at a temperature of 4 °C for a storage period of 6 days; Kamona and Alzobaay (2021) who attained lower mean coliform count from chilled fish balls treated with lemongrass (5 µl/g) compared to control samples; and Mozafari *et al.* (2023) who found adding rosemary essential oil or its nanoemulsion to burger samples lowers the coliform count significantly than control samples.

2.3. Total yeast and mold count

Table 6 showed the efficacy of treatments (LEO and its NE) on total yeast and mold count (TYMC) declared a significant

difference was present between control and both treatments (1.5 N and 0.5 O) in the second month.

There was a numeral decrease in the mean count values of treatments compared to control samples, where control samples showed the highest mean values, while samples treated with the raw oil showed the lowest values over most of the storage period. The samples treated with the nanoemulsion showed lower counts than the control samples; along higher antifungal effect when a higher concentration of the nanoemulsion was used. The antifungal efficacy of lemongrass is ascribed to the existence of citral and its isomers (Leite *et al.,* 2014). The antifungal characteristics of citral were linked to the destruction of cell membranes and the subsequent release of cellular components. Additionally, the inhibitory potential of lemongrass essential oil may arise from the combined impact of various minor or major compounds (Nguefack *et al.,* 2012; Majewska *et al.,* 2019).

The obtained results agreed with Ibrahim and Salem (2013) who found that addition of lemongrass extract, decreased mean values of total mold and yeast count compared to control samples of chilled chicken patties; and Kamona and Alzobaay (2021) mentioned that total yeast and mold population gradually declined during the chilled storage of fish balls in lemongrass extract treated samples while in control samples increased.

It is worth pointing out that the sudden drop in the TYMC of control and treatment samples at the 1st month count could be attributed to the sudden effect of freezing during the first period of storage. Mean results of control and treatments followed the same pattern, where increased initially then decreased at the first month and again increased to finally decrease over the last 2 months of examination.

2.4. The *E. coli* count

Characteristic *E. coli* colonies (nucleated colonies with or without metallic sheen) could not be identified on all inoculated EMB plates for all control and treatments samples. Hosny *et al.* (2020) pointed out that addition of 0.5% lemongrass oil to beef Kofta during 10-days storage period at 4 °C demonstrated significant effect on *E. coli* count.

3. Chemical indices

3.1. Thiobarbituric acid reactive substances value "TBARs"

This method allows for the measurement of malondialdehyde, a compound that forms a pink chromophore with thiobarbituric corrosiveness when lipid hydroperoxides disintegrate through oxidation. In an acidic environment, thiobarbituric acid and malondialdehyde combine to create a vibrant compound that absorbs light at 531 nm. Thiobarbituric acid **(**TBA) can react with a wide variety of mixtures to generate a chromophore (Adetuyi *et al.,* 2024).

As detailed in Table 7, the mean TBARs values of samples treated with 0.5 % LEO were relatively high. This could be explained by the effect of the intense yellow color of lemongrass oil present in the samples that obscured the antioxidant effect of the oil (false increase in spectrophotometer reading). The samples treated with 1.5 % nanoemulsion (1.5 N) showed the lowest TBARS mean values compared to the control ($p<0.05$) and other treatments over the whole period of storage. The higher nanoemulsion concentration (1.5 %) showed more reduction in the TBARs values than lower concentration (1 %) ($P>0.05$).

These results disagreed with that obtained by Hosny *et al.* (2020) who mentioned that malondialdehyde "TBARs" values (mg MDN/kg) decreased in 0.5 % lemongrass treated beef kofta samples during a 10-day storage period at 4 °C compared to control samples; Morshdy *et al.* (2021) recorded that TBA mean values in control rabbit meat samples were higher than values of samples dipped in 0.5% LEO; and Zaki (2022) reported that TBARs mean values in burger treated with 0.5 % lemongrass extract showed lower values than control samples. The disagreement with other reviews might be attributable to the use of different methodology in determination of TBARs, use

the leaves of the plant (not the essential oil) or different extract methods.

In the 3rd and 4th month of examinations, there were significant differences between means of control samples and samples of nanoemulsion treatments. It is assumed that the nanoemulsion had good antioxidant effect originated from phenolic compounds of lemongrass oil. Baschieri *et al.* (2017) explained that citral is the main terpene component of lemongrass essential oil and has excellent antioxidant effects. In addition, high bioactive content including tannins, phenols, and flavonoids such as ethyl acetate, ethanol and N- hexane have antioxidant effects due to their ability to scavenge free radicals (Anagnostopoulou *et al.,* 2006; Falah *et al.,* 2015; Wuryatmo *et al.,* 2021).

3.2. Total volatile basic nitrogen "TVBN"

Table 8 showed that TVBN mean values (Mg N/100g) of all beef burger samples had increased during the 4 months of frozen storage. The lower rate of increase was for 1.5 N treatment samples; showed lower mean TVBN values compared to control and other treatments, with a significant difference against the control samples in the last 2 months of storage.

The current result concurs with the result achieved by Hussein *et al.* (2015) who pointed out that control beef burger samples showed higher TVN mean values than correspondent values found in 2% lemongrass formula during 3 months of frozen storage. Zaki *et al.* (2018) who noticed that TVBN mean values of refrigerated camel burger control samples were higher than those recorded in the samples treated with 0.5 % lemongrass essential oil; Hosny *et al.* (2020) indicated that TVBN mean values in beef kofta samples treated with 0.5% lemongrass oil were lower compared to mean values of control during a 10-days storage period at 4 °C; and Morshdy *et al.* (2021) proclaimed higher TVBN mean values in control rabbit meat samples chilled for 12 days, compared to the samples dipped in 0.5% lemongrass essential oil.

It is of value to mention that, nanoemulsions and oil treated samples showed lower TVBN mean values than control samples, consistent with the bacterial results discussed earlier. Sarnes *et al.* (2020) stipulates that concentration of added essential oil may affect TVBN value. TVBN is formed through bacterial activity and the breakdown of proteins by autolytic enzymes. The resulting protein degradation is volatile products such as ammonia, H_2S, phenol, mercaptans, indole, cresol, and skatole, dimethylamine and trimethylamine (Riquixo, 1998; Suranaya Pandit *et al.,* 2007).

3.3. Hydrogen ion concentration "pH"

The initial pH of all samples was 6.03 (Table. 9); 1 N and 0.5 O treatments assumed the lowest and the highest pH values at the end of storage period, respectively. The pH values of all beef burger samples increased during storage time. However, 1 N samples showed the lowest incremental pH values compared to control samples and other treatments.

Addition of 0.5 % essential oil to burger samples showed less potent effect than nanoemulsion (Table. 9). This may be attributed to the freezing effect on the essential oil, (Gómez-Estaca *et al.,* 2010) reported that low water activity (freezing condition), high protein, and fat content were obstacles to the essential oil.

The present data declared significant difference between pH mean values of both nanoemulsion treatments (1 N and 1.5 N) and control samples; revealing that addition of Lemongrass nanoemulsion to burger samples was controlling the increase in the pH compared to the control samples in the first 2 month of storage.

Hosny *et al.* (2020) pointed out that addition of 0.5% lemongrass oil to beef Kofta during 10-days storage period at 4 °C demonstrated strong lowering effect on incremental pH values compared to control samples and Morshdy *et al.* (2021) demonstrated that pH of samples were increasing and control chilled rabbit meat mean values were higher than the mean values of samples treated with 0.5% LEO. On the other hand, Zaki (2022)

reported that chicken burger samples displayed decreasing values and samples treated with 0.5 % lemongrass extract showed lower pH mean values than control samples and Mozafari *et al.* (2023) found pH mean values followed a decreasing pattern over the storage period; and pH mean values recorded in control samples were slightly decreased by addition of 0.1 % rosemary essential oil and its nanoemulsions (0.5 %); that was in partial agreement with the current findings.

The fluctuations in pH levels during the storage process are influenced by various factors including storage temperature, protein degradation, and enzyme activity (Biscalchin-Grÿschek *et al.,* 2003). However, increase of pH over the storage period is contributed to accumulated alkaline compounds (Trimethylamine, ammonia, etc.) produced by microbial and enzymatic activities (Ahmad *et al.,* 2012; Utami *et al.,* 2018).

4. Technological characters

4.1. Water holding capacity (WHC) %

Juiciness contributes to eating quality and plays a key role in meat and plant-based products texture, therefore water holding capacity (WHC) is one of the most important eating quality properties being affect meat juiciness (Hussein *et al.,* 2015; Zhou *et al.,* 2022).

WHC % mean values of all burger samples in zero time were 50.34 % (Table. 10), 0.5 O and control treatments samples showed the lowest and the highest mean values (60.19 and 65.15 %), respectively by the 4th month of frozen storage. Results by 48 Hrs of storage revealed significant difference between means of control samples and 0.5 O samples. By the 1st month there was significant difference between both 0.5 O samples and 1 N samples with the control.

The current pattern concur with that obtained by Zaki *et al.* (2018) who found that WHC of all camel burger samples exhibited a notable increase as the duration of cold storage progressed; and camel burger formulated with lemongrass oil consistently displayed higher value compared to the control samples throughout the entire cold storage period. However, the obtained results partially disagreed with those acquired by Hussein *et al.* (2015) who disclosed that WHC in beef burger exhibited a gradual decrease and the highest values regarding WHC were recorded in the samples of lemongrass treated group.

4.2. Cooking loss and cooking yield %

Cooking loss is assumed to be the percentage of liquids lost (which may include water, protein, fat, and minerals) while cooking yield are the weight retained after cooking (Vu *et al.,* 2022). Cooking loss and yield are a major factors which has great impact on appearance and customer acceptability of meat and its products (Noori *et al.,* 2018).

Tables 11 and 12 are showing that cooking loss and yield were significantly affected by added lemongrass nanoemulsion ($P < 0.05$), this was revealed by the presence of a significant difference between means of control samples and samples of 1.5 N treatment, however there was no significant difference between control and samples of 1 N treatment. In addition, there was a significant difference between control samples and samples of 0.5 O treatment by the first month examination. At the end of storage period, the highest mean value of cooking loss was seen in control samples and the lowest was recorded in 0.5 O samples with significant difference between either of control or 1 N samples and 0.5 O samples. In sum, compared to control samples, addition of 0.5 % raw essential oil obviously decreased cooking loss (Table 11) and increased mean values of cooking yield % (Table 12) over the period of storage, also lower cooking loss. As well, higher cooking yield were obtained from samples treated with the nanoemulsion (1 N and 1.5 N).

Hussein *et al.* (2015) found that cooking yield decreased, and cooking loss values increased during frozen storage period of beef burger; and the highest cooking yield, and lowest cooking loss values were of the lemongrass group, that in part agreed with present results. Also, Zaki *et al.* (2018) noticed that the

lowest cooking loss, and highest cooking yield values was found in camel burgers formulated with lemongrass oil compared to control and other treatments stored at refrigerated temperature for 12 days. On the other hand, Awad (2019) revealed in a study on beef burger that cooking yield declined during frozen storage and samples contain 1 % of dried lemongrass leaves showed lower values compared to control samples.

4.3. Diameter loss %

Diameter loss can be attributed to the evaporation and release of liquid from the patties during cooking. As well, the diameter of meat patties is affected by the meat raw materials. The higher fat content of meat patties the greater diameter shrinkage (Oroszvári *et al.,* 2005; Vu *et al.,* 2022).

Table 13 shows the diameter loss results in control and treated samples. Initial diameter loss mean value was 22.33 %. The highest and lowest diameter loss % at the end of frozen storage period was found in samples treated with 1 % LGNE (1N) and samples treated with 0.5 % LEO (0.5 O), respectively. Significant differences by 48 Hrs examination were found between means of control samples and samples of 0.5O treatment; as well as, between samples of the 3 different treatments (0.5 O, 1N, and 1.5N). By the 4th month of storage, there was a difference ($P<0.05$) between 1 N and 0.5 O samples.

Despite values of diameter loss % were oscillating, the values of 0.5 O samples were undeniably lower than values in control and other treatments.

The obtained result were consentient with Zaki *et al.* (2018) who found the lowest reductions in diameter (%) was in refrigerated camel burgers formulated with lemongrass oil; and mean percentage of diameter reduction recorded in samples treated with 0.5 % lemongrass were lower than values of control samples. However, Awad (2019) mentioned that diameter reduction (%) of frozen beef burger contain 1 % of dried lemongrass leaves was higher than control samples. This disagreement might be attributed to use of leaves instead of essential oil.

CONCLUSION

This obtained result discourages using lemongrass in its raw essential oil form due to its negative effect on sensory attributes however recommend the possibility of using lemongrass nanoemulsion at a concentration of 1.5% in burger to discourage the growth of spoilage bacteria, extend the shelf-life and improve the cooking properties.

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تعزيز خصائص البرجر البقري بإستخدام مستحلب زيت عشبة الليمون النانوي

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تمت إضافة زيت عشبة الليمون وتركيزات من مستحلبه النانوي (١و ١,٥ %) على عجينة البرجر لتقييم تأثيرها على مدة الصلاحية، والمؤشرات الميكروبية والكيميائية للجودة، وكذلك خصائص الطهي. تم حفظ المنتج بعد تجهيزه عند درجة حرارة - ١٨ م وفحصه مباشرة بعد التحضير، بعد ٤٨ ساعة، ثم كل شهر لفترة أربعة أشهر. تم تحليل زيت عشبة الليمون باستخدام جهاز كروماتوجرافيا الغاز، ووجد أن حامض الستريك، والفيريبينول، وبيتا-بينين، وإل-ميرسين، وأسيتات الإيثيل تشكل الجزء الأغلب في تركيب الزيت. كذلك تم تحديد خصائص المستحلب النانوي باستخدام تحليل التحويل الفوريه للطيف بالأشعة تحت الحمراء، مجهرية النفاذ الإلكتروني ومؤشر التشتت المتعدد ومدى السمية الخلوية. تم الفحص الحسي للعينات حيث وجد أن زيت عشبة الليمون قد أثر بشكل ملحوظ على اللون والرائحة مما جعل البرجر غير مقبول وفي مقابل ذلك، أظهرت العينات المضاف عليها المستحلب النانوي قبولا أفضل. أوضحت نتائج العدد الكلي للبكتريا الهوائية ان العينات المعاملة بزيت عشبة الليمون كانت الأقل في العد بين باقي العينات مع وجود فارق معنوي بين هذه النتائج ونتائج العينة الضابطة. كما أوضحت النتائج وجود تأثير مضاد للبكتريا القولونية خاصة في الشهرين الأخيرين من التخزين. كذلك كان هناك انخفاض في العد الكلي للخمائر والفطريات، في العينات المضاف اليها زيت عشبة الليمون ومستحلبه النانوي مقارنة بالعينة الضابطة وقد لوحظ فرق معنوي بين العينة الضابطة وكلا المعاملتين في الشهر الثاني. أظهر المستحلب النانوي تأثير مضاد للأكسدة في العينات المضاف إليها وعلى نقيض ذلك أظهرت العينات المضاف إليها الزيت الخام قيما مرتفعة من المواد المتفاعلة مع حمض الثيوباربتيوريك. أيضا أدى إضافة المستحلب النانوي إلى إنخفاض ملحوظ في المركبات النيتروجينية الطيارة وبالأخص في أخر شهرين من التخزين. كذلك إتضح عن طريق الفرق المعنوي بين العينة الضابطة وعينات المستحلب النانوي، تأثير المستحلب النانوي في كبح الزيادة بالأس الهيدروجيني. كما أظهرت نتائج قدرة المنتج على الاحتفاظ بالماء تأثرا معنويا عند إضافة زيت عشبة الليمون وقد إنخفض هذا التأثير تدريجيا مع التخزين، وأظهرت نسب المقدار المتبقي بعد الطهي زيادة ملحوظة عند إضافة تركيز المستحلب النانوي لزيت عشبة الليمون (١,٥٪)، وأظهرت نسب التناقص في القطر تذبذبا كبيرا بين العينات مع عدم وجود فرق معنوي بين المعاملات. خلصت الدراسة إلى أن إضافة المستحلب النانوي لزيت عشبة الليمون تركيز (١,٥٪) كان له أفضل النتائج على الخواص الحسية كما أظهر تأثيرا ملحوظا على تحسين فترة صلاحية المنتج وخواص الطهي.