EFFECT OF ESSENTIAL OIL EXTRACT FROM LEMONGRASS (*Cymbopogon citratus*) LEAVES ON VAIABILITY OF SOME PATHOGENIC BACTERIA AND SENSORY PROPERTIES OF FISH BALLS

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ABSTRACT

Lemongrass (*Cymbopogon citratus*) plant belongs to the Gramineae family. Lemongrass leaves essential oils was extracted by Clevenger method, antibacterial, *MIC* and *MBC* were evaluated against some gram positive and gram negative bacteria. *Bacillus cereus*, *Staphylococcus aureus* and *micrococcus* spp., recorded high sensitively to essential oil with inhibition zone reached (40, 32 and 28) mm respectively. While *Pseudomonas spp*., *Salmonella typhimurium* and *Escherichia coli* recorded (20, 20 and 22) mm respectivly. MIC and MBC values reached (3, 6.5) % respectively for gram positive bacteria and (25,50) % respectively for gram negative bacteria. *C.citratus* leaves essential oil showed superior efficiency in reduction count of total microorganisms, coliform bacteria, psycrotrophic bacteria, *Staphylococcus aureus* and molds and yeasts, as well as elongated shelf life for 15 days of fish balls treated with (5,10) µl\gram of essential oil under refrigerated storage compared with control treatment (no oil added) which excluded for test after 6 days of refrigerated storage because microbial load and bad quality. Fish balls samples Lg_{10} (treated with 10µl\g of essential oil) gained best sensorial properties of color, texture, flavor, taste and overall acceptability were recorded 9/9 at the end of storage compared with treatment Lg_5 (5µl\g essential oil added) which gained acceptable sensorial score through refrigerated storage periods.

Key words: fish balls, *Cymbopogon citratus*, MIC, MBC, Shelf life.

تاثير الزيت الاساسي المستخلص من اوراق نبات حشيشة الليمون *Cymbopogon citratus* على فعاليه الممرضات البكتيريه والخصائص الحسيه لكرات السمك

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المستخلص

يعود نبات حشيشة الليمون (*Cymbopogon citratus*) الى العائلة النيجيلية. أُستخلص الزيت الأساسي لأوراق حشيشة الليمون بطريقة Clevenger , أُختبرت الفعالية المضادة للبكتريا ، والتركيز المثبط الأدنى والتركيز القاتل الأدنى للزيت الأساسي تجاه بعض البكتريا الموجبة والسالبة لصبغة كرام. أظهرت بكتريا *Bacillus cereus* ، *Staphylococcus aureus* و.spp *Micrococcus* حساسية قوية تجاه للزيت ، أذ بلغ قطرهاله التثبيط (40 ، 32 و 28) مللمتر على التوالي، في حين بلغ 20 ملم لكل من بكتريا .spp *Pseudomonas* و *Salmonella typhimurium* وسجلت بكتريا *Escherichia coli* 22 ملم. بلغت قيم MIC و MBC (3 ، 6.5)% على التوالي تجاه البكتريا الموجبة لصبغة كرام وبلغت القيم (25 ، 50)% على التوالي تجاه البكتريا السالبة لصبغة كرام. كما أظهر الزيت الأساسي لأوراق حشيشة الليمون *C. citratus* كفاءة عالية في إختزال العدد الكلي للأحياء المجهرية، مثل بكتريا القولون، البكتريا المقاومة للبرودة، العنقوديات الذهبية والخمائر والأعفان فضلاً عن إطالة العمر الخزني لمدة 15 يوم في كرات السمك المخزونة تحت التبريد والمعاملة بـ (5، 10) مايكروليتر/غرام من الزيت الأساسي مقارنة مع معاملة السيطرة (لم يضاف الزيت) والتي أُستبعدت من الفحص بعد 6 ايام من الخزن المبرد بسبب الحمل المايكروبي العالي والنوعية الرديئة. حقق نموذج كرات السمك Lg_{10} (المعامل مع 10 مايكروليتر/غم من الزيت الأساس) أفضل درجات التقييم الحسي من ناحية اللون، القوام، النكهة، الطعم والتقبل العام والتي سجلت 9/9 في نهاية مدة الخزن مقارنة مع المعاملة Lg_5 (المضاف لها 5مايكروليتر/غم من الزيت الأساس) والتي حققت نتائج مقبولة في الخصائص الحسية خلال فترات الخزن المبرد.

الكلمات المفتاحية: كرات السمك، زيت حشيشة الليمون، MIC ،MBC، العمر الخزني .

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INTRODUCTION

Essential oils (EOs) are aromatic and volatile liquids derived from plant materials and shown antimicrobial properties(1, 15, 24). Oil Lemongrass is one of the essential oils, as it contains between 70 - 80 percent citral compound, citral is the raw material for vitamin A synthesis, and has an antibacterial and antifungal characteristics , particularly for Gram - positive bacteria (17). Compared to other EOs, Lemongrass EO was most effective against 11 bacterial strains: *Pseudomonas aeruginosa*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Bacillus cereus*, *Klebseilla pneumonia*, *Staphylococcus aureus*, *Lactobacillus plantarum , Lactobacillus acidophilus and two strains of yeasts (Candida albicans*, *Saccharomyces cerevisiae*) (19). Lemongrass and thyme can indeed be recommended as important antioxidants and antimicrobial agents in frozen beef burger and its ingredients, but lemongrass is the strongest one. Lemongrass oil was an amber oil containing approximately 75-85% of aldehydes, mostly citral, neral and geranial (11). The incorporations of lemongrass oil to the film reduced the bacterial contents of meat and extend the lifetime of the food products compared with the samples plated free from lemongrass oil (22). The aim of this study was to assess the antibacterial activity of lemongrass essential oil against bacterial isolates as well as test the impact of two concentrations of this oil on the microbiological and sensory properties of refrigerated fish balls.

MATERIALS AND METHODS

Extraction of Lemongrass oil

Oil was extracted in accordance with (10) by using a Clevenger-type apparatus with small modifications, as follows :100gm fresh lemongrass plant leaves were put in a round bottom flask and 1000 ml distilled water was added 6 hours prior of hydro-distillation process. The oil was collected and refrigerated until use.

Bacterial preparation

(Staphylococcus aureus, Bacillus cereus and *Micrococcus spp.),* and (*Escherichia coli, Salmonella typhimurium* and *Pseudomonas spp.)* were obtained from the college of Agricultural Engineering \ University of Basra \ Iraq. Bacterial isolates have been activated at 37°C for 24h by nutrient broth, yielding log 7 colony - forming unit (cfu)/ml in comparison with 0.5 McFarland solution (4).

Antibacterial activity

agar well-diffusion method was used to detremin antibacterial activity. The surfaces of the plates containing muller-hinton agar was inoculated through the spreading of the microbial inoculum over the entire agar surfaces. Then, holes with a diameter of 6 to 8 mm were punctured aseptically with a sterile tips, and a volume (100 µl) of the extract solution at a concentrations was introduced into the wells. Then, the agar plates were incubated under suitable conditions depending on the test microorganism. The extract solution was diffused in the agar medium and inhibited the growth of the tested bacterial strains, the diameter of the clear zones of inhibition was measured via a ruler.

MIC and MBC

the dilution method for the determination of MIC and MBC was used. Briefl lemongrass oil extract was diluted into various concentrations (5, 10) µg/ml. A loopful (10 µl) of each bacterial stock culture was inoculated into a sterile nutrient broth in test tubes containing 1 ml from each concentrations of lemongrass oil. Similarly, The tubes were incubated at 37°C for 18 to 24 hrs and later observed for growth or turbidity. Subsequently, a loopful of broth from each test tube not showing growth, was inoculated into nutrient agar plate. Thereafter, equal volumes of sterile nutrient broth were added into the test tube cultures and incubated further for 24 h at 37°C. Then, the tubes and agar plates were examined for growth or turbidity using unaided eye. These experiments were repeated three times.

Fish balls preparation

Attended Mixture from the fresh minced meat obtained from common carp *(Cyprinus carpio)* fish , salt, pepper powder, coriander, cumin, garlic and breadcrumbs were (100, 1.5, 0.25, 0.25, 0.25, 0.4 and 10)% respectively. The oil of Lemongrass leaves was added as two concentration to the previously prepared mixture (5, 10) µl\g and mixing them by hand using medical gloves to ensure mixing and

homogenization of all ingredients and the mixture was used for the preparation of fish balls. The products was stored at 4℃ until were subjected for quality evaluation. Fish balls were evaluated for microbial characteristics on the day of preparation and during the subsequent period of storagefrom (1-15) dayes while the control from (1-6) days (16). Total plate count, coliform bacteria, Psychrotrophic bacteria, Molds and Yeasts were determined by APHA (7), *Staphylococcus aureus* was determined by (21). *Salmonella bacteria* was determined according to (3). Sensory properties of fish balls which treated with C. *citratus* leaves extracted oil were evaluated on 5 scale in color, flavor, taste, texture and overall acceptability after were deep fried in refined sunflower oil until they were cooked before being presented to the panelists (13).

RESULTS AND DISCUSSION

Figure(1) showed the lemongrass (*C.citratus*) leaves oil antibacterial activity against studied bacteria by method of agar well-diffusion. The highest zone of inhibition (40 mm) was recorded against *Bacillus cereus*, while the lowest (20 mm) was recorded against *Pseudomonas spp.* and *Salmonella typhimurium*. In comparison with gram-negative bacteria, *C. citratus* leaves oil has been found to be more effective against gram-positive. The structural and compositional differences in the cell walls between the two groups are probably attributable to sensitive differences between them. the same results were also showed by (14) and (19).

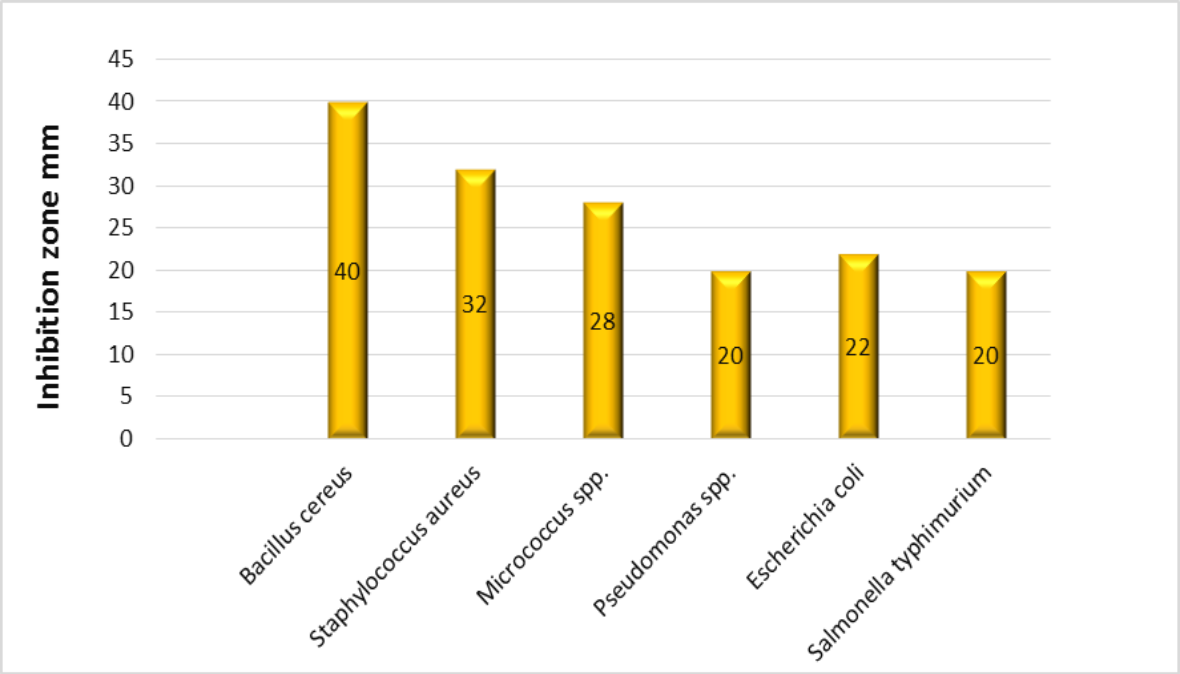

Figure 1. Antibacterial activity of *C. citratus* leaves essential oil against treated bacteria.

MIC, MBC and MFC of *C.citratus* Oil

MIC is defined as the minimum concentration of certain compound required for inhibiting the visible growth of microbes to at least 99%, while MBC or MFC is a minimum concentration antimicrobial concentration required to kill at least 99% of the growth (25). Table (1) showed the MIC, MBC value of *C. citratus* extracted oil against tested microorganisms. The MBC and MIC were determined by using sub-culturing dilution. The extracted oil of *C. citratus* showed the least MIC value (3%) and the highest MBC value (6.5%) against gram positive bacteria, while showed the highest MIC value (25%) and lowest MBC value against gram negative bacteria 50%. Same results had been recorded by (9), when they showed that lemongrass essential oil was detected as effective against six species of bacteria including *Pseudomonas aeruginosa*. The MIC of Lemongrass essential oil for all strains except *P. aeruginosa* was ranged from 0.016 to 0.5% (v/v). Naik and coworkers (14) referred to the MIC, MBC values of essential oil of lemongrass against *Staphylococcus aureus, Klebsiella pneumonia, Bacillus subtilis, Bacillus cereus and Escherichia coli* which ranging from 0.06 to 0.50% respectively.

Table 1. MIC, MBC values of *C. citratus* leaves essential oil against tested bacteria:

Microorganisms	MIC (%)	MBC (%)
Bacillus cereus	**3**	**6.5**
Staphylococcus aureus	**3**	**6.5**
Micrococcus spp.	**3**	**6.5**
Pseudomonas spp.	**25**	**50**
Escherichia coli	**25**	**50**
Salmonella typhimurium	**25**	**50**

Microbiological results of fish balls under refrigerated storage

Figure (2) showed the total plate count (TPC) of fish balls treated with (5, 10) µl\g of *C. citratus* essential oil and at different storage periods (1, 3, 6, 9 12 and 15) days. Results showed that the total number of microorganisms decrease gratually as storage days increase, while presence of total microorganisms for control sample (without adding oil) was reached 7.89 log cfu/g after 15 days of refrigerated storage, which exceeds the maximum limits of microbiological criteria for fish balls and full odor was observed. Amany et al, (6) reported that antibacterial effect of garlic, thyme and Lemongrass oils on refrigerated minced beef increase. Lemongrass contained active ingredients like citral which has antimicrobial activity and play a significant role as antibacterial and antioxidant agent in refrigerated chicken patties (12).

Figure 2. Total plate count (TPC) of fish balls treated with (5, 10) µl\g *C. citratus* leaves essential oil during refrigerated storage 4±2°C

Psychotrophic bacteria in fish balls samples which were treated with different concentrations of *C. citratus* leaves oil essential were presented in the figure (3). The sample on it showed decresed number of Psychotropic bacteria in last day as compared with first day, while the control receded 6.12 log cfu/g after 15 days of refrigerated storage which was exceeds the maximum limits of microbiological criteria from Psychrotrophic bacteria for fish balls.

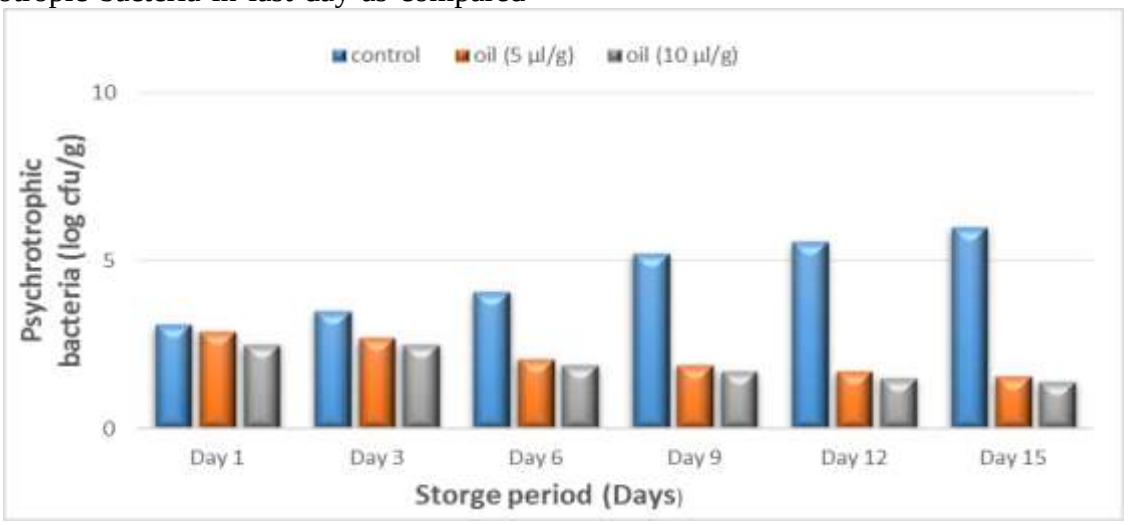

Figure 3. Psychrotrophic bacteria count of fish balls treated with (5, 10) µl\g *C.citratus* leaves essential oil during refrigerated storage 4±2°C

Figure (4) showed also reduced number of Coliform bacteria in fish balls samples that treated with two different concentrations of *C.citratus* leaves essential oil. While found a presence of these Coliform bacteria for control sample which reached 4.03 log cfu/g after 15 days of refrigerated storage, which exceeds the maximum limits of microbiological criteria (Coliform bacteria) for fish balls 2 log cfu/g. Antimicrobial properties of the *C.citratus* aqueous extract was also studied and showed a great inhibition of Coliform bacteria by another research at different concentrations (10).

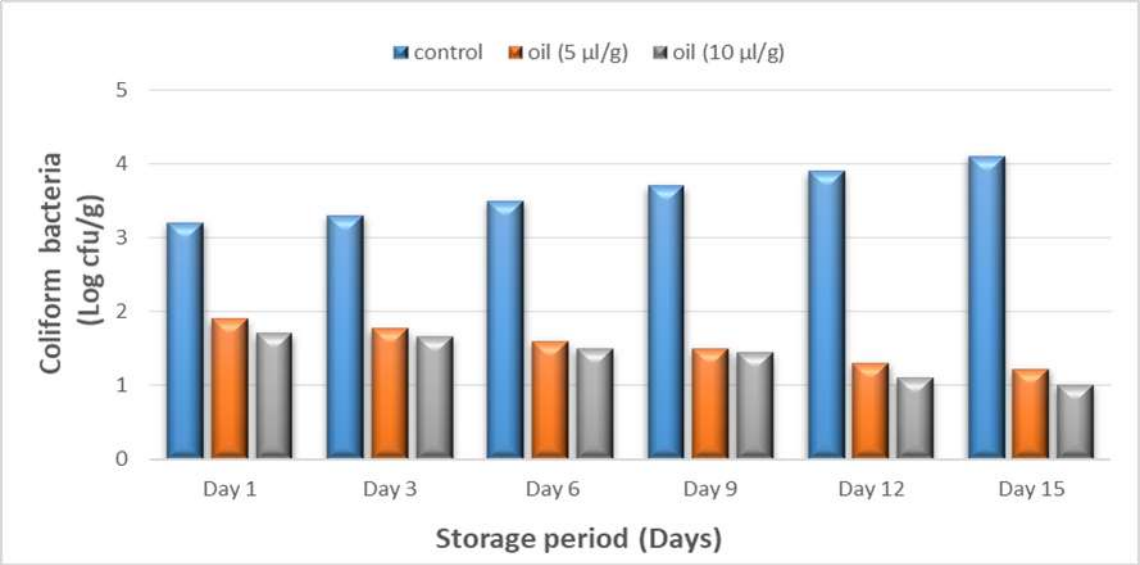

Figure 4. Coliform bacteria count of fish balls treated with (5, 10) µl\g *C.citratus* leaves essential oil during refrigerated storage 4±2 C°.

In a study conducted by (6), lemongrass, thyme and garlic oils at 1.5 percent have strong impacts on the growth of *Enterobacteriaceae* and Coliform, in cold storage minced beef. While (8) pointed that the effect of marjoram essential oil on raw chicken drumsticks during refrigerated storage for 12 days. *Staphylococcus aureus* count in fish balls treatments illustrated in (figure 5). Results showed decresed count of this bacteria as concentration increase, while presence in control sample (without adding oil) reached 4.5 log cfu/g after 15 days of refrigerated storage, exceeds the maximum limits of microbiological criteria (*Staphylococcus aureus*).

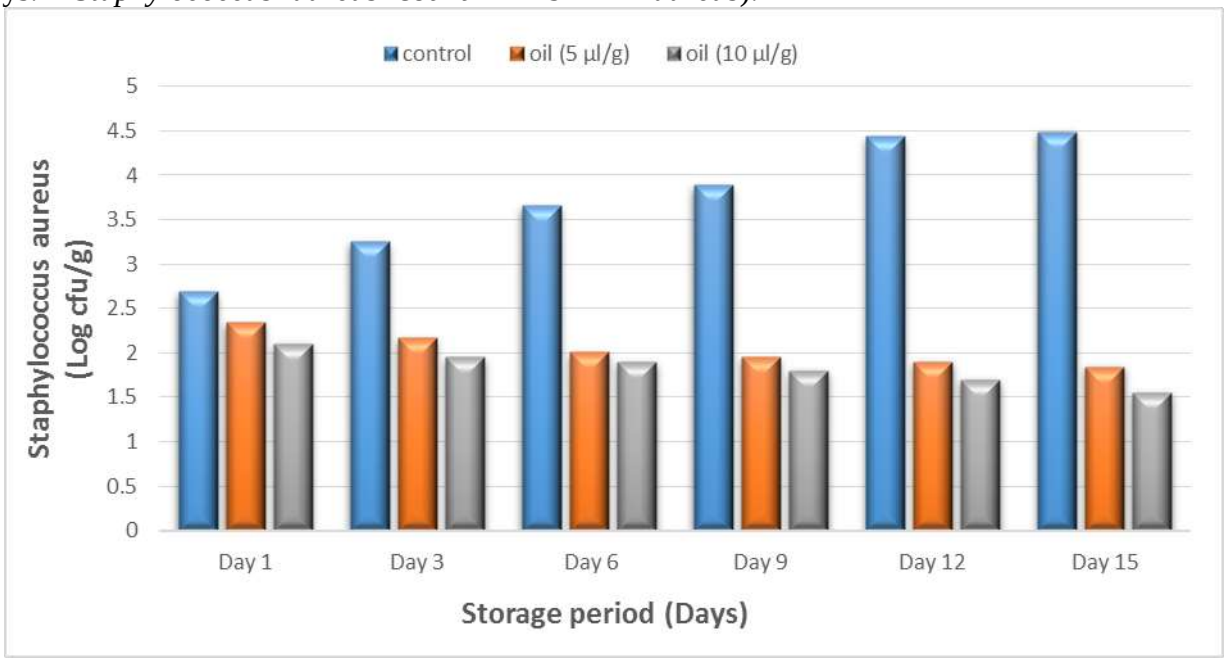

Figure 5. *Staphylococcus aureus* count of fish balls treated with (5, 10) µl\g *C.citratus* leaves essential oil during refrigerated storage 4±2°C.

In a research by (5) they noted the inhibitory effect of used essential oils (thyme, cumin and parsley) at three different concentrations (0.3, 0.6 and 1.2) % against *Staphylococcus aureus*, *Salmonella enterica*, artificially inoculated in beef burger stored at 4±1˚C for 4 days. However (20) referred that lemongrass essential oil had both antimicrobial activated against *Listeria monocytogenes*, *Staphylococcus aureus.* Figure (6) monitored molds and yeasts count in fish balls which reached 4.24 log cfu/g after 15 days of refrigerated storage in control treatment, while samples which treated with lemongrass essential oil showed decreased percentage of molds and yeasts. However (2) mentioned that increasing levels of lemongrass essential oil added to yoghurt either incubated at 5 or 28 ˚C for 28 days, an inhibitory effect was noted on the growth of all tested molds (*Aspergillus flavus* , *Aspergillus parasiticus* and *Aspergillus ochraceus*).

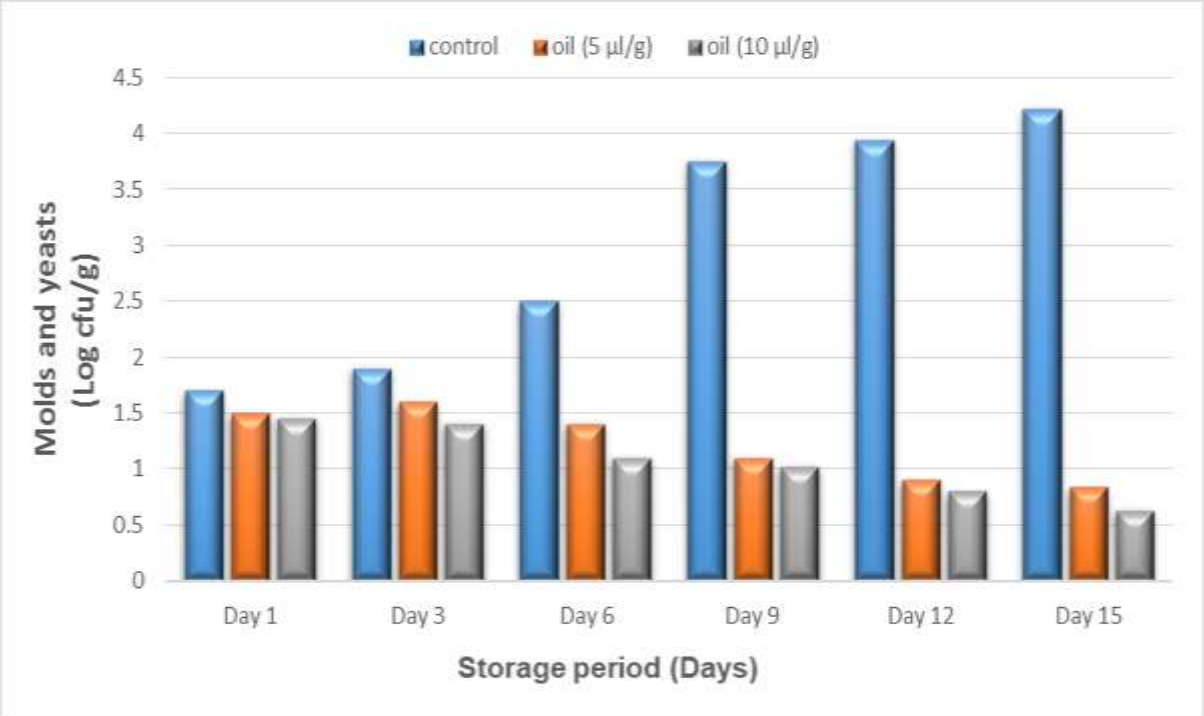

Figure 6. Molds and yeasts count of fish balls treated with (5, 10) µl\g *C. citratus* leaves essential oil during refrigerated storage 4±2˚C.

Organoleptic properties

Results in Table (2) observed increasing of sensorial values of samples were treated with *C.citratus* leaves essential oil during storage periods. Treatment Lg_{10} (treated with 10µl/g *C.citratus* oil) recorded highest score of color, texture, flavor, taste and overall acceptability which reached (9/9) compared with other treatments. Control treatment were out after 6 day of refrigerated storage, due to foreign color, watery texture and bad in flavor and taste. Taste, appearance and odor in a product could be the criteria for rejection of any kind in food if they differ significantly from what is expected by the consumers, among the positive effects of marjoram oil in retarding color loss, lipid oxidation, off-odor formation and microbial growth occurring during refrigerated storage (8). Treatment Lg_5 (treated with 5µl/g *C.citratus* oil) was record less than treatment Lg_{10} in all sensorial values which do not access (4/9) after 15 days of storage. Abd-El Fattah *et al*., (2) pointed that Lemongrass components may contained aldehydes, volatile compounds and high amounts of phenolic compounds which effected on colors stability and a sticky texture due to high level of extracts were possessed high amounts of phenolic compounds was refereed on texture.

Table 2. Sensory Evaluation scores(9=excellent , 1=bad) of fish balls treated with (5, 10) µl\g *C.citratus* leaves essential oil during refrigerated storage 4±2°C

Treatment	Storage period	Color	Texture	Flavor	Taste	Overall acceptability
Control	1	6	4	5	5	4
	3	4	3	5	5	5
	6	5	2	3	2	2
Lg_5 (5µl/g essential oil)	1	6	5	7	4	6
	3	6	5	6	4	6
	6	5	4	4	3	5
	9	3	4	5	4	4
	12	4	5	5	3	4
	15	4	4	4	4	4
Lg_{10} (10µl/g essential oil)	1	9	8	8	9	9
	3	8	7	8	8	9
	6	7	8	7	9	9
	9	8	9	9	9	9
	12	9	9	9	9	9
	15	9	9	9	9	9

Conclusion

Lemongrass (*Cymbopogon citratus*) leaves essential oil has a key role in extended the shelf life of fish balls during refrigerated storage throughout decreasing microbial load and improving of sensory attributes (Flavor, Taste, Color, Textures and Overall acceptability) during refrigerated storage. Treatment (treated with 10µl/g essential oil) showed the best properties in maintain of Microbiological and sensory properties during 15 days of refrigerated storage.

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