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The Effect of thyme, rosemary, and lemongrass oils on extension of the shelf life and qualitative characteristics of Iraqi soft cheese

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ABSTRACT

Background: In the case of Iraqi soft cheeses, researchers have noted that the moisture content of these cheeses decreases over time in storage and reaches its maximum depletion in the final storage period. This information was found to be true for all samples that were tested. As storage times increased, this decline was followed by a rise in the ratio of fat and protein, which reached the maximum at 21 days for all samples under investigation. Additionally, research was done on the impact of thyme, rosemary, and lemongrass oil additions on the microbiological and sensory properties of the soft cheese generated during storage.

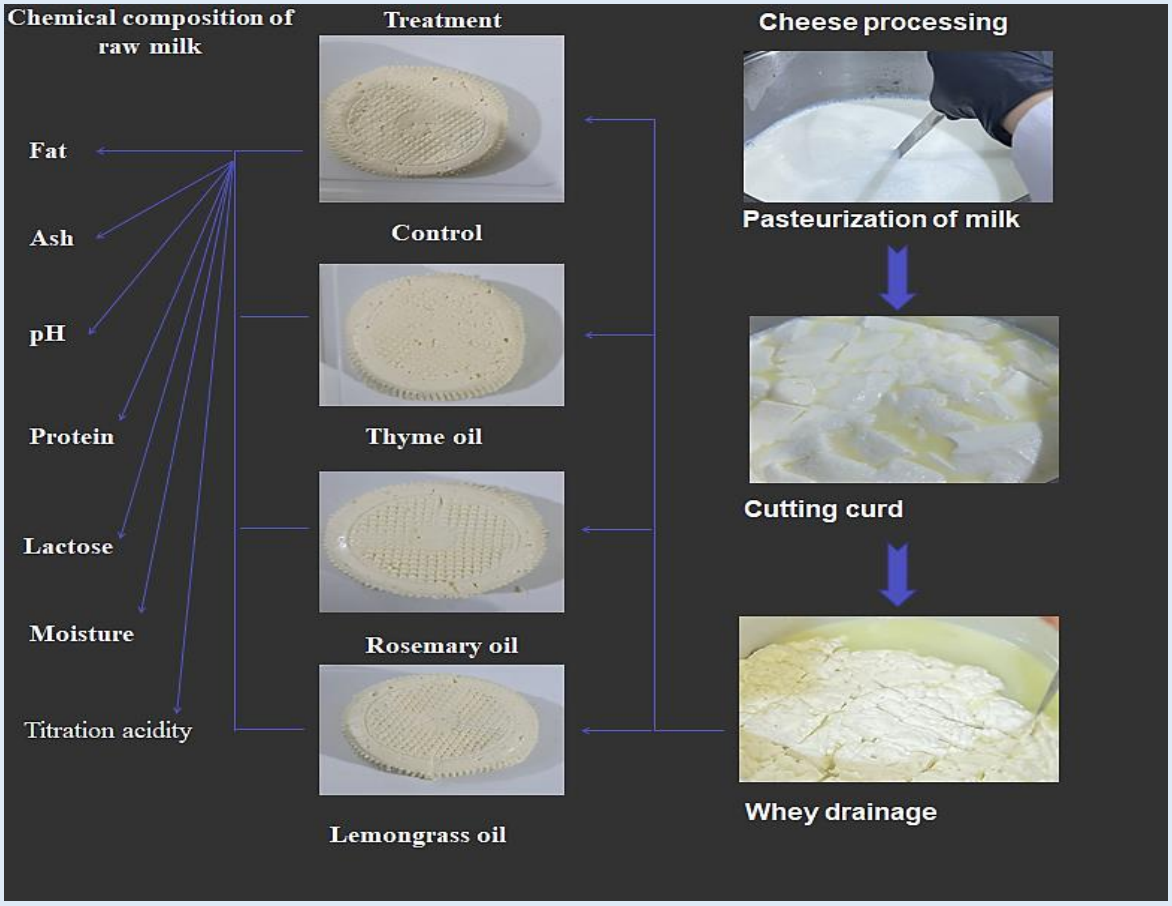
Objective: This study set out to ascertain the impact of adding oils such as *thyme*, *rosemary*, and *lemongrass* on the chemical, microbial, and sensory properties of Iraqi soft white cheese made from cow's milk and stored for 21 days at 1 ± 5°C.

Materials and Methods: The wild *thyme* and *rosemary* were obtained from local markets in Baghdad, while the *lemongrass* was obtained from one of the nurseries in Abu Ghraib / Baghdad in December 2022. The essential oils were extracted by water distillation according to the method described.

Results: As storage went on, the number of *Staphylococcus aureus* bacteria increased; on day 21, they reached 1.75, 3.54, 5.41, 6.75, 1.20, 2.14, 3.21, and 4.20 CFU / g of cheese, respectively. This was the largest rise in the count. By contrast, these values dropped when *thyme* oil was added, coming down to 1.54, 1.48, 2.10, and 2.88 CFU/g of cheese, respectively. In the sample to which *rosemary* oil was added, the quantity of *Staphylococcus aureus* bacteria fell as

well, reaching 1.54, 1.76, 2.88, and 3.31 CFU /g cheese, respectively, despite the fact that they were not present at all. Additionally, there was no *Staphylococcus aureus* germs present, in contrast to the cheese sample that had *lemongrass* oil added, which had both *Staphylococcus aureus* and *coliform* bacteria entirely missing. The findings also demonstrated that, when compared to the cheese samples treated with wild *thyme* oil, *rosemary* oil, and the control sample, the cheese sample treated with *lemongrass* oil exhibited superior sensory qualities, such as flavor, color, texture, and bitterness over various storage times.

Keywords: Soft Cheese, *Thyme* Oil, *Rosemary* Oil, *Lemongrass* Oil, Chemical composition, Storage period



INTRODUCTION

The salted white cheese industry is widespread in the Mediterranean and Balkan countries and is traditionally made because the high percentage of salt acts as a great preservative [1-2]. The process of making cheese transforms milk into a cohesive food substance with a low moisture content that can be preserved for an extended period [3]. Iraqi soft white cheese is one of the most essential local cheeses widely produced in Iraq due to its high nutritional value. It is produced traditionally

in the countryside and in dairy factories. Due to the lack of liquid milk production locally, the supplementation of imported powdered milk has been used to produce or fortify some dairy products. In previous studies, powdered milk has been used in the production of soft cheese, as reconstituted milk was used in manufacturing Egyptian Damietta cheese [4]. Concern has increased regarding the safety of chemical food additives and doubts surrounding the extent of their safe use as artificial antioxidants that inhibit microorganisms. Researchers have turned to preserving foods in ways that ensure their survival naturally, preserving their properties for the most extended possible period by finding natural extracts that can scavenge free radicals or their effect on microorganisms contaminating food [5]. Thymoquinone, considered an antioxidant compound [6], and its derivatives serve as antidotes to various microorganisms and fungi [7]. Lemongrass extract demonstrates efficacy against a range of bacteria, including Gram-negative and Gram-positive types, as well as fungi such as Staphylococcus aureus, Shigella, E. coli, Aspergillus flavus, and Aspergillus niger [8]. Studies have indicated that both alcoholic and aqueous extracts from the rosemary plant exhibit antibacterial activity against Gram-positive bacteria like Staphylococcus aureus and three Gram-negative species, namely E. coli, Proteus mirabilis, and Salmonella typhimurium [9].White cheese is defined as a fresh product obtained from coagulating fresh, whole or partially defatted milk, or from recovered or reconstituted milk with the addition of rennet or acidification [10]. Herbs that contain essential oils are usually used in foods and cheeses to add special flavors, prolong their shelf life, and improve human health [11]. Cheese is typically eaten in many meals due to its high nutritional value and its availability in all regions and in multiple forms. The white cheese industry is widespread in various parts of the world and is produced in some countries traditionally without health control resulting in contamination with pathogenic microorganisms that cause food poisoning in humans [12]. Therefore, the study aimed to prolong the shelf life of soft white cheese by using oils of *wild thyme*, *lemongrass*, and *rosemary*, and to demonstrate the effect of these oils on the chemical, microbial, and sensory characteristics of the cheese produced.

MATERIALS AND METHODS

The *wild thyme* and *rosemary* were obtained from local markets in Baghdad, while lemongrass was obtained from one of the nurseries in Abu Ghraib / Baghdad in December 2022. The essential oils were extracted by water distillation according to the method described by [13]. The milk was obtained from a milk processor in the city of Ramadi. In conjunction with the sensory tests, the production of soft white cheese took place. The manufacturing process occurred at the Dairy Processing Laboratory within the Department of Food Sciences, College of Agriculture, University of Anbar.

Production of soft white cheese: Soft white cheese was made using cow's milk, the content of which was calculated with the Danish Milkoscan equipment [14]. The milk underwent a 30-minute pasteurization process at 63°C. After cooling the milk to 35°C, the providing firm's instructions were followed in order to produce the microbial rennet, which was obtained from the Danish SACO business. After allowing the mixture to coagulate for 35 to 45 minutes, it was sliced both lengthwise and crosswise, left for 5 minutes, and then agitated until the coagulation was observed and the whey was extracted. It was then infused with 10 ml/1 kg of curd, thyme, rosemary, and lemongrass oils, respectively. Subsequently, the curd was compressed to

create cheese molds and refrigerated at 1±5°C. The cheese was tested at one, seven, fourteen, and twenty-one days.

Tests of the gross composition of cheese:

pH measuring: The pH of the cheese was estimated according to weighing 10 g of cheese and mixing it with 90 ml of distilled water in a ceramic mortar. The pH was then estimated using a pH meter [15].

Acidity measuring: The acidity percentage of cheese was estimated by weighing 3 grams and mixed well in a ceramic mortar with 10 ml of distilled water, then titrated with a 0.1 N sodium hydroxide solution, and the percentage of acidity was estimated according to the following equation [16].

$$Total\ acidity(TA) = \frac{(NaOH)volume \times 0.09 \times 0.1}{sampleweight} \times 100$$

Estimation of fat percentage: 3 grams of cheese were carefully weighed and combined with 10 milliliters of distilled water in a Gerber tube. Next, 10 milliliters of concentrated sulfuric acid and 1 milliliter of amyl alcohol were added. The tube was then well-shook and placed in the Gerber centrifuge for three to five minutes, at which point the percentage of fat in the cheese was measured from the fat column [17].

Estimation of the moisture percentage: Sterilized and clean drying racks were used to assess the cheese's moisture content. After drying and cooling, it was weighed empty. Then, 3 grams of cheese were added, and it was baked for 3 hours at 1 ± 105 °C. It was then weighed again after cooling to a constant weight, and the percentage of moisture was calculated using the following formula [18].

Estimation of total nitrogen percentage the percentage of total nitrogen was calculated by weighing 0.4 g of cheese. Next, 5 ml of concentrated sulfuric acid was added to the cheese. Finally, 1 g of digestion powder was added to finish the sample digestion process. This powder contained 25 g of potassium sulphate, 2 g of blue copper sulphate, and 1 g of selenium oxide. A 430 Buchi device was used to finish the digestive process, and a 320 Buchi device was used for the distillation [16]. The following equation was utilized to determine the proportion of nitrogen present overall:

$$TN\% = \frac{(V_1 - V_2) \times 0.014 \times 0.1}{sampleweight} \times 100$$

V_1= The volume of HCL consumed in titration with the sample

V_2=volume of HCL consumed in blank titration

Microbial examinations: Soft white cheese samples were prepared for Microbial examinations by weighing 11 grams of the samples to be examined and mixing them with 99 ml of Ringers solution prepared by the British company BDH using a sterile glass electric blender, then prepared the decimal dilutions, and calculated the coliform and *Staphylococcus aureus* bacteria using the pouring plate method according to the [20] using the culture media: Mannitol Salt Agar and MaCconkey Agar supplied by the Indian company Himedia [19-20].

Sensory evaluation: Sensory evaluation of white cheese was conducted by specialists in the Department of Food Sciences, College of Agriculture, University of Anbar, as outlined in the questionnaire prepared by Al-Warshan SH et al [21]. The assessment involved modifications, encompassing attributes such as flavor, texture, color, and taste (Table 1a). Each characteristic received a score ranging from 0 to 10 [21].

Table 1a. Sensory evaluation form

Treatments	Sensory characteristics	Storage period (day)			
	Flavor (0-10)				
	Texture (0-10)				
	Color (0-10)				
	Bitterness (0-10)				
	Total (40)				

RESULTS AND DISCUSSION

Chemical composition: Table 1 shows the chemical composition of the raw milk prepared used to produce soft white cheese, and if it was of good quality in terms of chemical composition, titration acidity, and pH [22].

Table 1. Chemical composition of raw milk used in the study.

Chemical composition of raw milk						
Moisture%	Protein%	Fat%	Ash%	Lactose%	pH	Titration acidity%
87.85	3.81	3.23	0.63	4.48	6.40	0.14

Chemical composition of processed cheese: The soft white cheese samples, which were stored at 1±5 °C for 21 days, were mixed with thyme, rosemary, and lemongrass. The control sample's chemical composition is displayed in Table 2.

Table 2. Chemical composition tests of soft white cheese treatments during storage

Treatment	Age of cheese (Day)	Moisture %	Fat %	Protein %	pH	Titration acidity%
Control	1	63.8	15.7	17.5	6.4	0.17
	7	63.4	15.8	17.9	6.1	0.18
	14	63	15.86	18.4	5.7	0.34

Treatment	Age of cheese (Day)	Moisture %	Fat %	Protein %	pH	Titration acidity%
	21	58.7	16.2	18.6	5.3	0.46
***Thyme oil* 10 ml/kg**	1	62.9	15.9	18.1	6.4	0.17
	7	62.4	16.2	18.5	5.95	0.23
	14	62.1	16.5	18.7	5.42	0.31
	21	59.7	16.6	18.95	5.32	0.40
***Rosemary* oil 10 ml/kg**	1	62.5	15.8	17.8	6.4	0.18
	7	61.5	15.96	18.3	6	0.22
	14	60.1	16.64	18.6	5.7	0.33
	21	59.8	16.67	18.89	5.4	0.41
***Lemongrass* oil**	1	63.1	15.7	18.1	6.35	0.17
	7	62.4	15.9	18.3	6.14	0.24
	14	61.9	16.3	18.7	5.6	0.35
	21	60.01	16.5	18.9	5.5	0.42

The white cheese and control samples had moisture contents of 63.8 and 63.4, 63 and 58.7%, after 1, 7, 14, and 21 days, respectively, according to the data. After adding wild thyme to white cheese samples, their moisture percentages were 62.9, 62.4, 62.1, and 59.7% during the aforementioned storage times, in that order. For the same prior storage periods, the white cheese samples that had rosemary added had moisture percentages of 62.5, 61.5, 60.1, and 59.8%. In contrast, the samples of white cheese to which lemongrass was added had moisture percentages of 63.1, 62.4, 61.9, and 60.01% [22-23]. The variations in the pressing operations across the different treatments might be the reason for the disparity in the moisture content in the initial stages of cheese production The ripening of the whey was thought to be the cause of the moisture content reduction during the storage periods. Alternatively, the pH may have dropped, tightening the curd and causing moisture to leak out of the cheese. These findings corroborated those of [24-26]. The Iraqi standard specification for soft cheese (1988) stipulated that the moisture level in all treatments must be at least 50%. The study's findings, displayed in Table 2, demonstrate the proportion of fat in soft cheese for each treatment. For the control treatment, the percentage of fat was, respectively, 15.7, 15.8, 15.86, and 16.2% after 1, 7, 14, and 21 days, which is the lowest amount. For the preceding storage periods, the highest percentages for cheese made with 10% rosemary addition were 15.8, 15.96, 16.64, and 16.67%,

respectively. Regarding the cheese treatment that included lemongrass and thyme, the results were 15.9, 16.2, 16.5, 16.6%, and 15.7, 15.9, 16.3, and 16.5% for the aforementioned storage times, respectively. It was found that several soft cheeses imported from Iraq have a fat content ranging from 15.87 to 16.35%. The findings of that the fat content of Iraqi soft cheese made from cow's milk varied between 15.87-16.43% and 16.67%, respectively, were also supported by the results. The findings corroborated those of [27-28], who showed that the fat content of Iraqi soft cheese rose from 16.9% on the first day of storage to 20.13% on the fourteenth. The study's findings, however, differed with those of [29], who claimed that the fat content of Iraqi soft cheese in the Basra Governorate ranged from 10.54 to 15.91%. It is important to remember that the [10] specification for soft cheese stated that the product's fat content should be 1±16%. The quality of the milk used in manufacturing and the proportions of its constituents may be to blame for the variations in fat percentages. Furthermore, it is evident that the production process has an impact on the fat percentage since some fat may be lost along with the whey during drainage. Additionally, the other ingredients in the cheese, particularly the moisture percentage, have an impact on the fat percentage. The percentage of titration acidity in soft white cheese samples, the control sample, and the samples that were treated with *thyme*, *rosemary*, and *lemongrass* oils and kept at 1±5°C for 1, 7, 14, and 21 days are all displayed in Table 2. As storage extended, the titration acidity results increased for every sample examined; for the aforementioned samples, respectively, and for the aforementioned storage periods, they reached 0.17, 0.18, 0.34, 0.46, 0.17, 0.23, 0.31, 0.40, 0.18, 0.22, 0.33, 0.41, 0.17, 0.24, 0.35, and 0.42%. The existence of non-pathogenic microorganisms such as lactic acid bacteria, which were not eliminated during pasteurization procedures, may be the cause of the increase in titration acidity after storage. For every sample examined, the highest rise occurred during the final storage phase, and the control sample performed better. The increase in titration acidity was ascribed to the growth and reproduction of lactic acid bacteria, which increases their metabolic efficiency and raises the phosphate ion concentration; alternatively, the accumulation of lactate or other organic acids could have caused [30]. Table 2 makes it evident that the pH values attained during the current investigation were within the range of 5.32 to 6.4 on the first day of storage and for all treatments. As the storage period extended, these numbers dropped until they reached the twenty-first day (5.5 5.3). The pH of soft cheese from Iraq is 6.1. Given that the values varied from 5.3-6, the findings were similar with [31], who discovered that Sudanese white cheese produced with 0.5% gum Arabic had a pH of 5.1. The findings are consistent with those published by [29], which stated that Mexican soft cheese made from pasteurized milk had an average pH of 6.04. The present investigation yielded lower findings than [6], which examined the chemical composition of soft cheese produced by adding inulin and discovered that the pH values varied between 6.76 and 6.71. It is important to note that the for soft cheese required the pH of the cheese to be no more than 0.2±6.4. The rise in the number of bacteria during storage may be the cause of a drop in pH values. This rise in organic acid production results in high acidity and low pH [32-35]. The proportion of protein in the soft cheese produced for each treatment is displayed in Table 2. On the first day of storage, the protein percentage values for the different treatments varied from 17.5 to 18.1%, and on the twenty-first day of storage, they increased from 18.6 to 18.95.19%.

These findings corroborated the findings of those who reported that the protein content of soft cheese rose throughout the course of the 21-day storage period, from 22.65-24.18% on the first day to 17.93-18.63% on the last day. Furthermore, the observed outcomes closely align with previous findings that investigated the protein content in soft cheese made from cow's milk. In that study, it was revealed that the protein content increased from 18.11% on the first day of storage to 21.71% on the fourteenth day." The findings also matched the findings of [36,37], which stated that the protein content of soft cheese ranged from 18.34 to 19.35%. However, the results of this investigation were not as high as the 26.27% protein.

Microbial tests: Table 3 shows the number of *coliform* and *Staphylococcus aureus* bacteria in the control sample and the samples to which thyme, rosemary, and lemongrass oils were added (stored at five °C for storage periods of 1, 7, 14, and 21 days). The logarithmic numbers of coliform bacteria of the control sample at the above periods amounted to 1.75 and 3.54, 5.41, and 6.75 CFU /g of cheese, respectively, increasing with the progression of storage periods and for all treatments, and their numbers exceeded the rest of the samples. While the samples of cheeses to which *thyme* and *rosemary* oil were added in the storage periods of 0, 7, 14, and 21 days reached (1.4, 1.48, 2.10, 2.88) and (1.54, 1.76, 2.88, 3.31) CFU /g of cheese, respectively, at the same time, the cheese *treatment* with *lemongrass* oil did not show any growth of *coliform* bacteria throughout the period.

Table 3. Number of *coliform* and *Staphylococcus aureus* bacteria in different cheese treatments

Treatments	Sensory characteristics	Storage period (day)			
		1	7	14	21
Control	*Coliform bacteria*	1.75	3.54	5.41	6.75
	Staphylococcus aureus	1.20	2.14	3.21	4.20
Thyme oil	*Coliform bacteria*	1.41	1.48	2.10	2.88
	Staphylococcus aureus	-	-	-	-
Rosemary oil	*Coliform bacteria*	1.54	1.76	2.88	3.31
	Staphylococcus aureus	-	-	-	-
Lemongrass oil	*Coliform bacteria*	-	-	-	-
	Staphylococcus aureus	-	-	-	-

The logarithm of the *Staphylococcus aureus* bacteria numbers appeared to be affected by the oil of *thyme*, *rosemary*, and *lemongrass* added to soft white cheese. This was evident because the control sample had higher numbers of *Staphylococcus aureus* bacteria, reaching 1.20, 2.14, 3.21, and 4.20 CFU / g cheese for storage periods of 1, 7, 14, and 21 days, respectively. However, in cheese samples that had oils of *thyme*, *rosemary*, and *lemongrass* applied, respectively, no *Staphylococcus aureus* germs appeared throughout the storage times indicated above [38-39] found that in comparison to the control sample, *black seed, ginger, cinnamon, thyme, mint*, and *cloves* have an impact on extending the shelf life of soft white cheese. The presence of hydrophobic or lipophilic molecules contributes to the inhibitory effect that essential oils have in lowering or suppressing the activity of microbes through a variety of methods. It has been demonstrated that lemongrass essential oil efficiently inhibits the development of several bacteria, including *Staphylococcus aureus*. Additionally, fish balls' shelf life while refrigeration is significantly increased with *lemongrass oil* [40-43].

Sensory tests: The sensory assessment findings for the soft white cheese treatments (the control sample and the cheese made with the addition of *thyme, rosemary*, and *lemongrass* oils) are displayed in Table 4. The results indicate that the soft white cheese treated with lemongrass oil is superior.

Table 4. The effect of adding aromatic herbs on the sensory characteristics of soft white cheese during the storage period

Treatments	**Sensory characteristics**	**Storage period (day)**			
		1	7	14	21
Control	Flavor	8	7	-	-
	Texture	8	7	-	-
	Color	9	8	-	-
	Bitterness	10	8	-	-
	Total	35	30	-	-
Thyme oil	Flavor	9	9	8	7
	Texture	9	8	8	8
	Color	9	9	8	8
	Bitterness	10	10	8	8
	Total	37	36	32	31
Rosemary oil	Flavor	8	8	7	6

Treatments	Sensory	Storage period (day)			
	Texture	9	7	6	5
	Color	9	8	7	7
	Bitterness	10	8	5	3
	Total	36	31	25	21
Lemongrass oil	Flavor	9	9	9	8
	Texture	10	10	9	9
	Color	10	10	9	8
	Bitterness	10	10	9	9
	Total	39	39	36	34

Characteristic received eight marks. Due to the growth of acidity and the bitter taste in comparison to cheese made with *thyme, rosemary*, and *lemongrass oils* added, its flavor grew intolerable as the storage age increased, ranging from 1 to 14 days. The enzymes of psychrophilic bacteria that were not destroyed by pasteurization, which are often proteolytic and convert proteins into short-chain peptides, may be responsible for the change in flavor and taste. The unwanted flavor is also caused by lipolytic enzymes, which degrade volatile short-chain fatty acids. Regarding the lactic acid bacteria, these are responsible for of the acidity that develops and the pH shifts while storage goes on.

Furthermore, the flavor and bitterness of the cheese samples made with *lemongrass oil* outperformed those made with thyme and *rosemary oil*. This is because the oils of these herbs have a preservative effect that inhibits the growth of microscopic organisms when compared to a control sample. The cheese samples that had *thyme*, *rosemary,* and *lemongrass* oils added to them had a better texture than the control sample. Regarding the color characteristic, it was observed that the cheese sample treated with *lemongrass* oil performed better than the samples treated with thyme, *rosemary*, and the control treatment. Hypothesized that the acrid flavor in cheese is attributed to minuscule enzymes catalyzing the hydrolysis of proteins into peptides. The impact of spice extracts on soft white cheese was investigated in a number of studies, one of which focused on the essential oil of *lemongrass*. This study found that the oil significantly increased the shelf life of fish balls during refrigerated storage, decreased the microbial load, and improved the fish balls' flavor, taste, color, texture, and acceptability over the course of 15 days [43-46].

CONCLUSION

The essential oils of thyme, rosemary, and lemongrass play a crucial role in influencing the chemical, microbial, and sensory properties of Iraqi soft white cheese. These oils contribute to extending the shelf life of the cheese during refrigerated storage by reducing microbial load and enhancing sensory attributes, particularly overall acceptability. Treatment (treated with 10 ml/kg oil)

showed the best properties in maintain of Microbiological and sensory properties during 21days at 1 ± 5°C of refrigerated storage.

Abbreviations: CFU: colony forming unit; °C: Degree Celsius**.**

Authors Contribution: Firas Najm Ismael: Formal analysis; Methodology; Project administration; Funding acquisition; Validation; Writing-original draft. Sara Thamer Hadi: Data curation; Formal analysis; Methodology; Project administration.

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