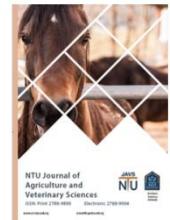




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## Studying the effect of lactoferrin on some sensory attributes and properties Cream stored for 21 days

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### ABSTRACT

The study included determining the effectiveness of lactoferrin at concentrations (0, 3, 6, 12) mg/ml over extending the storage period of cream (0, 7, 14, 21) days. The cream was manufactured, and was fortified with lactoferrin, after which (peroxide number, viscosity, pH, sensory evaluation) of the cream was estimated and in a storage period of (0, 7, 14, 21) days of storage at a temperature of 5 °C. The results show that the peroxide number increases with the increase in storage time, the results reached (6.32, 6.25, 6.23, 6.14) mEq O<sub>2</sub>/g fat at a concentration of (0, 3, 6, 12) mg/ml respectively on the first day. The results of the viscosity showed a slight increase in viscosity in the first period of storage and then usually decreased viscosity. As for the PH, where it was in the first storage PH of the comparison sample, 6.73. At the end of storage, it reached 5.92. The storage period led to a change in taste and flavor. On the first day of manufacturing, the product received taste evaluation scores of (9.0, 8.3, 9.3, 8.6) for concentrations of (0, 3, 6, 12) mg/ml, respectively, and at the end of the storage period the product obtained grades (7.6, 8.0, 8.6, 8.6) for concentrations (0, 3, 6, 12) mg/ml respectively for taste grades, and in terms of flavor, no noticeable changes occurred, while the effect of adding Lactoferrin changes the color of the product.



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## Introduction

Milk proteins are the richest source of nutritional and functional components in the diet. Nutritionally, it is considered a building material and a good source of essential amino acids for growth and develop the body. The functional properties of proteins affect some non-nutritional qualities produced, including binding to water, crystallization properties, emulsification or foaming, and physical, chemical, and sensory properties[1]. Milk proteins also have important biological functions, one of the bioactive milk proteins is lactoferrin (LF), which is one of the whey proteins, and its percentage is higher in the milk of the first birth (colostrum), and its level is not constant and decreases with the passage of the lactation period, reaching below in mature milk [2]. is a polyglycoprotein Functions such as immunoregulation, anti-inflammatory, antibacterial, antifungal, and antioxidant These broad functions are largely due to its ability to hold iron and interfere with cellular receptors of pathogenic microorganisms [3].

Thus, lactoferrin fortification can have a positive effect on the qualities of the product as well as the health of the consumer, and is considered safe under appropriate conditions, standards, and levels according to the limits recommended by the European Union Commission Implementing Regulation [4]. The maximum addition in food for drinks and milk is 200 mg /100 g, fermented milk products can contain 50 mg /100 g, cheese 2000 mg/100 g, and ice cream 130 mg/100 g, and since then it has appeared in the markets especially Japanese fortified products, including milk and milk fortified with lactoferrin, so the study aimed to evaluate the ability of lactoferrin to maintain the properties of cream for the longest period of storage. Lactoferrin is a safe and powerful food and pharmaceutical protein that can be used as a potential therapeutic agent alternative to antibiotics for the treatment of intestinal disorders, and can be used as a nutritional and functional supplement [5],[6].

Lactoferrin works to reduce the formation of cytokines by reducing iron absorption, which in turn works to reduce oxidative stress, which reduces cytokines [7]. Lactoferrin peptides are antioxidants with high strength in the process of inhibiting lipid peroxides because they contain OH groups at the end of the molecular chain at each aromatic ring. This gives it advantages in its ability to form ester compounds and high reducing power in binding metal ions, and thus its effectiveness against antioxidants [8].

## Methods and Materials:

The study included determining the effectiveness of lactoferrin at concentrations (0, 3, 6, 12) mg/ml over extending the storage period of cream (0, 7, 14, 21) days. The cream was manufactured to know the effect of lactoferrin reinforcement on some of the characteristics and sensory properties of the cream, as the milk was filtered by gauze to get rid of impurities, then heated to a temperature of 40 ° C to perform the sorting process by the electric sorter, the percentage of fat in the cream was estimated by the Kerber method, then the percentage of fat in the cream was adjusted to 25% using the Pearson square, the cream pasteurization process was performed at a temperature of 80-85 ° C for 15-20 seconds and cooled to 20-25 ° C, after which the reinforcement was done Lactoferrin in ratios (0, 3, 6, 12) mg/ml [9].

The following tests were performed for fortified cream:

### 1- Estimation of the peroxide number:

The peroxide number was estimated according to the method[10] by dissolving 5 g of fat extracted from the solvent, then adding ice acetic acid and chloroform in a ratio of (3:2) with mixing and add 0.5 ml of saturated potassium iodide solution and leave in a dark place for 5 minutes, then adding 30 ml of distilled water and powder the mixture with sodium thiosulfate 0.1 carats with continuous shaking until the yellow color disappears, then add 0.5 ml of starch solution (0.5%) with Shaking and brushing with sodium thiosulfate solution until the disappearance of the blue color with the preparation of the Planck sample using distilled water instead of oil according to the equation:

$$\text{Peroxide number (ml Eq/g fat)} = \frac{(ml \text{ sodium thiosulfate for sample} - ml \text{ sodium thiosulfate for blank} \times \text{Normality} \times 1000)}{ml \text{ Eq / g fat}}$$

### 2- Determination of pH in lactoferrin-fortified cream:

The pH of the cream samples and storage times was estimated by the PH-meter device type HANNA

### 3- Estimation of viscosity in cream fortified with lactoferrin:

The viscosity of the cream samples were estimated at a temperature of 20 ° C during the storage period of 1-12 days of refrigerated storage at 7 C° and using the Brook field Engineering Lab.st ongton (Mass) device and according to the method mentioned by [11]. if the use of spindle size 12 and the number of cycles 20 rpm and the size of a sample 50 ml and let

the spindle rotate in the sample for 60 seconds and then took the reading unit centipoise.

#### 4- Sensory assessment :

The samples were evaluated to clarify the effect of lactoferrin reinforcement in the cream factory in terms of Taste values, color, and texture after the passage of (21) days of refrigerated storage and at a temperature of 5 °C The evaluation was according to what was stated in [12]. by a number of the Department of Food Science, College of Agriculture - University of Mosul, and the grades were given by (0-10) so that the degree is 10 for the best evaluation and the lowest evaluation gets a zero degree for the most adjective.

#### 5- Statistical analysis:

The results were analyzed using the SPSS Social program Statistic System according to the C.R.D analysis test and the arithmetic means were compared using the Duncan test to compare the means at the level (p< 0.05) [13].

### Results and discussion:

#### 1- The effect of strengthening cream with lactoferrin on the value of the peroxide number:

Table (1) shows the effect of cementing cream samples with lactoferrin in proportions of (0, 3, 6, 12) mg/ml in the peroxide value of cream after processing and for a storage period of (0, 7, 14, 21) days at a temperature of 5 C°. The results showed that there were no significant differences in the peroxide number values for all cream samples on the first day, as they amounted to (6.32, 6.25, 6.23, 6.14) mEq O<sub>2</sub>/g fat at a concentration of (0, 3, 6, 12) mg/ml, respectively. The increase in the storage period led to an increase in the value of the peroxide number. The lowest values were for a sample concentration of 12 mg/ml of lactoferrin and for a storage period of 21 days, as the value was 10.93 mEq O<sub>2</sub>/kg fat. Where it was found that there were significant differences in all samples on day 21 compared to the first day. It is clear from this that storage has a direct effect of increasing the peroxide number as a result of the decomposition of free fatty acids in the cream. The concentration of lactoferrin has an adverse effect on the peroxide number, where the peroxide number is lower with an increase in the concentration of lactoferrin in the cream, and the reason for this is that lactoferrin is an antioxidant that protects against fat oxidation through the presence of peptides that can inhibit lipid peroxides and these peptides contain some amino acids such as histidine, tyrosine and methionine, if the amino acid histidine contains the amidazole ring responsible for donating the hydrogen atom to peroxides and then

stopping its work[14]. As well as the ability of lactoferrin in inhibit many types of bacteria, including the production of lipolytic enzymes (lipase enzyme), and thus reduce the decomposition of fat in cream by these organisms and thus reduce the number of peroxides.

**Table 1.** Effect of Lactoferrin Cementation on Peroxide Number Value for 21 Days of Storage.

Concentrations mg/ml	Time (Day) 0	7	14	21	Effect of concentrations
0	6.32	15.02	15.50	15.82	13.1650
	h	b	ab	a	a
3	6.25	11.50	11.68	11.84	10.3150
	h	cde	cd	c	b
6	6.23	11.01	11.05	11.13	9.8558
	h	defg	defg	def	b
12	6.14	10.40	10.47	10.93	9.4833
	h	g	fg	efg	b
Time effect	6.2	12.06	12.1	12.4	
	b	a	a	a	

\*Averages marked with different letters horizontally and vertically indicate significant differences at a significance level of 0.05.

\*The numbers represent the average of three replicates

#### 2- The effect of lactoferrin reinforcement on the viscosity of cream:

Table 2 shows the effect of strengthening cream samples with lactoferrin in proportions of (0, 3, 6, 12) mg/ml in the viscosity of cream after manufacturing. For a storage period of (0, 7, 14, 21) days at a temperature of 5 °C and from the table there are significant differences below the level of 0.01 in the comparison sample and the addition sample 3 mg/ml during the storage period and the significant differences in the sample 6 in the storage days were 7 and 21 and sample 12 were the significant differences in the days of storage 7 and 14. The viscosity of the cream was shown to increase with an increase in the concentration of lactoferrin where it was (2300, 2533, 3200, 4267) centipoise at concentrations (0, 3, 6, 12) mg/ml respectively on the first day. After that, there was an increase in viscosity in the storage period of 7 days and reached (2733, 3000, 4133, 4733) centipoise in concentrations (0, 3, 6, 12) mg/ml respectively, and usually the viscosity decreased with the increase of the storage period in 14 days, where it reached (1867, 2467, 067, 3533) centipoise in concentrations (0, 3, 6, 12) mg/ml respectively. The decrease increased with The end of storage until it reached 1200 centipoise for the comparison sample on day 21, and the highest viscosity for the sample was 12 mg/ml of lactoferrin concentration, where it was 3133 centipoise for the same time of storage, as a slight increase in viscosity was observed in the first storage period after that decreased, the viscosity of the comparison sample without adding lactoferrin

was lower at the storage period of 21 days 1200 centipoise.

It is noted that the viscosity increases with the increase in the percentage of lactoferrin addition, and the viscosity decreases with the increase in the storage period as a result of high acidity and the result of lactic acid bacteria and with the increase in the storage period induces separation in the components of the cream and thus the viscosity decreases with the increase in the storage period. The presence of proteins in milk is very important because it improves viscosity, binds water, acts as stabilizers and limits the ionization of water during storage at high temperatures, proteins are added in dairy products in the form of powdered milk or whey proteins to modify the texture of the products, thus adding protein to dairy products is useful for technological purposes[15],[16] stated that increasing the protein content leads to an increase in the gel lattice and increased viscosity with a semi-solid consistency. With increased storage duration and increased acidity, serum separation occurs due to the breakdown of the gelatin reticulum and the rearrangement of protein molecules[17]. Also due to the decomposition of fatty acids, which causes high acidity[18],[15] mentioned that viscosity increases with increasing storage time as a result of increased acidity resulting from the development of lactic acid bacteria, as it was initially 850 centipoise and reached 1370 centipoise in lactoferrin-fortified milk after a storage period of 28 days, where lactic acid bacteria convert multiple sugars into monosaccharides so that they increase viscosity.

**Table 2.** Effect of Cementing Cream with Lactoferrin on the viscosity of cream for 21 days of storage.

Concentration mg/ml	Time (Day)				Effect of concentration s
	0	7	14	21	
0	2300	2733	1867	1200	3916.7
	I	fg	j	k	a
3	2533	3000	2467	1933	3300
	ghi	def	hi	j	b
6	3200	4133	3067	2800	2483.3
	D	b	de	efg	c
12	4267	4733	3533	3133	2025
	b	a	c	d	c
Time effect	3,07	3,65	2,73	2,26	
	5	0	3	7	
	ab	a	bc	c	

\*Averages marked with different letters horizontally and vertically indicate significant differences at a significance level of 0.05.

\*The numbers represent the average of three replicates

### 3-The effect of lactoferrin reinforcement on pH:

Table 3 shows the effect of strengthening lactoferrin cream samples in proportions of (0, 3, 6, 12) mg/ml in the pH of the cream after manufacturing and for a storage period of (0, 7, 14, 21) days at a temperature of 5 C° and from the table it was found that there are significant differences in the level of 0.05 as the pH decreased with the

increase in the storage period in the comparison sample. There were significant differences in all storage days in order to increase the acidity in the cream with Increased storage time, while no significant differences were found on days (0, 7, 14, 21) for all samples to which lactoferrin was added in all addition ratios. As for the storage period of 21 days, it was found that there were significant differences from the comparison sample on the first day, where it was (6.73, 6.40, 6.41, 6.46) at concentrations (0, 3, 6, 12) mg/ml, respectively, where a decrease in pH is observed with increasing storage time and the increase in the concentration of lactoferrin has an effect, as the pH decreases with an increase in the concentration of lactoferrin, and we note that from day 7 of storage this decrease (6.49, 6.37, 6.39, 6.40) at concentrations 0, 3, 6 and 12 mg/ml, respectively. The lowest pH value for the comparison sample was 5.92 at the end of storage. It was noted that the increase in the concentration of lactoferrin is inversely proportional to the pH, as the sample was 12 mg/ml of lactoferrin, the least low than the rest of the samples, as it reached at the end of the storage period to 6.37, the low pH is attributed to the effect of natural and advanced acidity from the process of fermentation of lactose sugar and its transformation into lactic acid and this occurs due to enzymes resulting from types of lactic acid bacteria, as mentioned[19]. that lactoferrin inhibits the activity of microorganisms and thus reduces the activity of lactic acid bacteria that increase the acidity of the cream, leading to a decrease in pH in the samples of lactoferrin added.

**Table 3** Effect of Lactoferrin Cementation on pH Value for 21 Days of Storage.

Concentrations mg/ml	Time (Day)				Effect of concentrations s
	0	7	14	21	
0	6.73	6.49	6.03	5.92	6.3145
	a	b	g	h	a
3	6.40	6.37	6.33	6.23	6.4083
	bcd	cd	de	f	a
6	6.41	6.39	6.36	6.28	6.3625
	bcd	cd	de	ef	a
12	6.46	6.40	6.39	6.37	6.3317
	bc	cd	cd	de	a
Time effect	6.5	6.4	6.3	6.2	
	a	a	b	b	

\*Averages marked with different letters horizontally and vertically indicate significant differences at a significance level of 0.05.

\*The numbers represent the average of three replicates

### 4-Sensory evaluation of lactoferrin-fortified cream:

Table 4 shows the results of sensory evaluation by people in the Faculty of Agriculture. According to the evaluation of the different opinions of the evaluators and their experience of the evaluation process, it is noted from the table that the evaluation scores were high on the first day due to

the absence of changes in the sensory qualities of the cream, after the addition of lactoferrin, where the taste obtained degrees (9.0, 8.3, 9.3, 8.6) for concentrations (0, 3, 6, 12) mg/ml, respectively. With the increase in storage period, the evaluation scores decreased for all samples, and this is the result of changes in The sensory qualities of the cream such as high acidity, changes in texture and separation of components and this is a result of the action of microorganisms and their development during the storage period, where the taste obtained the degrees of the end of storage were (7.6, 8.0, 8.6, 8.6), for concentrations (0, 3, 6, 12) mg/ml respectively. As for the flavor, the sensory evaluation scores were close on the first day, and when the storage period passed after 21 days, the sensory evaluation scores decreased, so the samples got the grades (8.0, 8.3, 8.3, 8.0). for concentrations (0, 3, 6, 12) mg/ml respectively, where changes in flavor resulting from advanced acidity occurred. As for the strength, the evaluation scores were close at the beginning of manufacturing and with the passage of the storage period the lack of degrees due to the separation of the components of the cream and the sample with a concentration of 12 mg/ml outperformed all samples, where it got a score of 8.3 in the strength because lactoferrin improved the strength of the cream and the effect was with increasing concentration, where the higher the concentration of lactoferrin, the greater the viscosity and thus obtain a better texture, and the effect of lactoferrin on the color of the cream was not a clear effect and the degrees of color were close. Where the evaluation of the color at the beginning of manufacturing obtained degrees (9.3, 9.0, 9.0, 8.6) for concentrations (0, 3, 6, 12) mg/ml, respectively, and the evaluation of the grades decreased for the color of the last day of storage and the highest for the comparison sample was (8.0, 8.0, 8.6, 7.3) for concentrations (0, 3, 6, 12) mg/ml respectively. Rheology has high health quality and more stability during the refrigerated storage period and gave lactoferrin a positive effect on the cohesion of the product. [20]. stated that fortification of yoghurt with lactoferrin does not affect the flavor.

**Table 4.** Sensory evaluation of lactoferrin-fortified cream samples for 21 days storage

Taste	Concentrations mg/ml	0	3	6	12
1day		9.00	8.33	9.33	8.66
21day		7.66	8.00	8.66	8.66
Flavor	Concentrations mg/ml	0	3	6	12
1day		9.00	9.00	9.33	8.33
21day		8.00	8.33	8.33	8.00
Textures	Concentrations mg/ml	0	3	6	12
1day		9.00	8.66	8.66	8.66
21day		7.33	8.00	8.33	8.33
Color	Concentrations mg/ml	0	3	6	12

1day	9.33	9.00	9.00	8.66
21day	8.00	8.00	8.66	7.33

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