

Adding concentrations of lactoferrin as a fortifier to yogurt and studying its chemical and microbial properties

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Abstract

Manufacture of yogurt fortified with lactoferrin using three concentrations (10, 20, and 30 mg/liter) and conducting a set of chemical tests represented by estimating the percentage of moisture, percentage of protein, percentage of fat, percentage of ash, percentage of carbohydrates, and physical tests represented in estimating the acidity and pH for a storage period that included 14 days. The results of the thiobarbituric acid values for yogurt fortified with lactoferrin and stored in refrigeration at a temperature of 5 °C for a storage period (1, 3, 7, 14) days and the results of the statistical analysis showed that there were significant differences between the treatments to which lactoferrin was added with the control treatment. Conducting microbial count tests for yogurt fortified with lactoferrin with three concentrations (10, 20, 30) mg / liter and a control treatment for a storage period that included 14 days.

Keywords: Lactoferrin, yogurt fortified, Thiobarbituric acid

INTRODUCTION

Attention has been focused recently on natural sources for their vital benefits, and among these materials is lactoferrin, which is considered one of the non-specialized immune proteins characterized by high effectiveness through its association with iron and also has an antibacterial effect against many types of gram-negative and gram-positive bacteria (Abbas, 2015). Lactoferrin is one of the proteins found in whey. It has many applications as natural food additives, antimicrobials, multiple antimicrobials, antioxidants, anti-infectives, and anti-cancer agents. Lactoferrin was used because it is used to treat anemia in pregnant women and newborns. On the other hand, there is an urgent need to develop appropriate and cost-effective industrial technology to isolate high-value protein from the milk of various local species and to use it to extend the storage life of milk, meat, and fruits. chopped fresh vegetables and their products to increase shelf life, control disease, and promote general health (Kevion et al., 2022).

Lactoferrin protein (Lf) is a multifunctional glycoprotein with many roles associated with iron. It belongs to the Transferrin family. Lactoferrin has many functions related to its iron-binding properties. LF because of its ability to bind Fe³⁺ ions. Research has shown that LF has a high affinity for ferric iron and plays a key role in regulating the level of free iron in body fluids (Zhang et al., 2021). It is found abundantly in mammalian milk. It was isolated from cow's milk in 1939 (Srensen and Groves, 1960) and breast milk in 1960 (Montreuil, 1960, and Johansson). It was also isolated from various external secretions in mammals, such as tears, nose, and saliva. Bronchial mucus, plasma, and digestive system (Marnila & Korhonen, 2009) Lactoferrin protein is found in lactating milk and is present in all exocrine secretions such as tears, saliva, vaginal secretions, seminal fluid, nasal secretions, bronchial secretions, and bile secretions (Baker et al., 2005; Legrand et al., 2008). Lf is similar to the serum protein transferrin and the egg white protein ovotransferrin; lactoferrin is found in the milk of most mammals, including many rodents (Masson, 1971).

Oxidation it is the process of transferring electrons from the donor part to the receiving part, which leads to exchanges in the properties of both the donor and the receiver parts, and we can describe the oxidation process as containing a

smaller number of electrons, through the conversion of chemicals into other materials (Costantini, 2019). The oxidation process due to the accumulation of free radicals, which cause many human diseases, such as Alzheimer's (Badhani, 2015). Fat oxidation is a common problem in foods, especially due to the formation of oxidation products from free radicals, as foodstuffs such as milk, meat, fish, and wheat are subjected to oxidative decomposition to varying degrees. With unacceptable odors, oxidative decomposition begins with exposure to several factors such as heat treatment, high energy, ultraviolet radiation photons, ozone and metal ions, in addition to the ability to oxidation affects different foodstuffs, depending on their fat content, especially unsaturated fatty acids, as the problem of spoilage. Food is the oxidation of fats and makes them unacceptable to the consumer. Therefore, the process of fat oxidation is of great importance at that present (Madi and Ismail, 2010; Duh and Yen, 1999). A free radical is a molecule or atom that contains a non-double electron in its outer orbit. These ions may be organic or inorganic. The term oxidizing agent is applied to free radicals. The electrons remain in normal conditions in double molecules. When the molecule loses one of them, it becomes unstable and harmful to the molecules. The other neighboring ones, as the presence of one electron in its outer orbit makes it in a continuous and active search for the missing electron. The electron is a pair which is, and this is what makes it take an electron from neighboring molecules, causing damage to normal cell molecules in the body (Phaniendra et al., 2015).

Yogurt is a food made by fermenting milk. Milk is generally fermented using bacterial cultures; *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp are commonly used to prepare yogurt. Bulgarian When these starter cultures are added to milk, the lactose is fermented to form lactic acid, which reacts with milk proteins to give the yogurt its tart texture and taste. The production of lactic acid lowers the pH of the milk, which leads to coagulation of the milk proteins, which gives a gel-like appearance to the yogurt (Ahmad, 2022; Al-Hamid, 2022). Yogurt is an important healthy food, as it is one of the most widespread and symbolic dairy products in various countries (Aryana and Olson, 2017). It is the second most popular snack for children all over the world (Han et al., 2016), which is widely consumed, and its popularity is mainly due to its multiple health benefits (Shah and Champagne, 2016). Yogurt is defined as the food product that is produced by the action of starter bacteria and includes one or more of the following lactic ingredients: cream or milk and skimmed milk, wholly or partially, using a bacterial culture containing lactic acid-producing organisms *Streptococcus thermophilus* and *Lactobacillus delbrueckii* Subsp. *bulgaricus*, as full-fat yogurt contains no less than 3.00% of milk fat and not less than 2.00% of non-fat milk solids, and it has a calibrated acidity of not less than 5.0% expressed in lactic acid. As for the fat-free yogurt, it contains less than 5.0% of milk fat and not less than 2% of non-fat milk solids, and it has a calibrated acidity of not less than 5.0%, expressed as lactic acid (FDA 2021). Yogurt is characterized by high nutritional value, contains important mineral salts and essential amino acids for human nutrition, and is known in most countries of the world. One of the factors affecting the yogurt industry is the incubation period, the storage period, the increase in the starter ratio, the addition of water to the milk, the decrease in total solids, and the bitter taste. And the watery or tartish texture, as the tart taste is produced as a result of the increase in acidity exceeding 0.9%. These effects can be controlled by following the correct manufacturing methods (Tamime, 2003). Many studies have shown that yogurt has an important role, starting with its nutritional value, such as improving lactose tolerance (Chandan, 2015; Madjd et al., 2016). The innovation of different products and the availability of different flavors and types of yogurt have significantly contributed to the increase in its consumption during the past few decades (Chandan et al., 2017). On the other hand, several standards for the properties of yogurt were set in the United States of America for the first time in 1981 AD (Chandan et al., 2017), as defined by the US Food and Drug Administration (FDA). Removing fat from yogurt leads to defects in the product such as reduced fat perception, grainy texture, increased whey run-off during storage, and product inhomogeneity (Krzeminski et al., 2013). One of the most important proposed solutions to increase the hardness and reduce whey translucency in fat-free yogurt is to add whey protein powders to reduce the proportion of casein: whey, whey protein isolate (WPI), or whey protein concentrate (WPC), which have different effects on the properties of the yogurt gel (Delikanli and Ozcan, 2014).

MATERIAL AND METHODS

Starters used in the manufacture of yogurt

The source of milk Buffalo milk was used from farms in Kufa district. Strains of bacteria from Subsp *Salivarius* *Streptococcus* *Lactobacillus delbrueckii* Subsp *bulgaricus* and *thermophilus* produced by the French company Danisco were used, in addition to the direct addition to the yogurt mixtures, where the starter was activated by taking 20 g of screening milk per 200 ml of distilled water. It was sterilized using an autoclave at 121 °C for 5 minutes. Then the milk is cooled to 42 °C, a quantity of lyophilized starter is placed and incubated at 42 °C until coagulation is complete and kept in the refrigerator until the second day, then the activation process is completed again with the same steps, with the addition of the previously prepared coagulated coffee at a rate of 5% per 200 ml, then placed in the incubator at a temperature of 5%. The temperature is 42 C until it coagulates, and it is placed in the refrigerator and used in the manufacture of milk for special transactions.

Manufacture of functional yogurt

Yogurt was made according to the method used in Robinson and Tamime (1999). Buffalo milk was received from the local markets of the city of Najaf, and it was used in the manufacture. The milk was brought to a temperature of 90 °C for 10 minutes, then cooled to a temperature of 42 °C, then divided into two halves, leaving the first half without treatment and was used in the manufacture of yogurt with the To control treatment. As for the second half, it was divided into three sections to which lactoferrin was added at the rate of (10, 20, 30) micrograms / kg, represented by treatments T1, T2 and T3, and inoculated with the initiator after activation of *Lactobacillus* and *Streptococcus Salivarius* Subsp *thermophilus bulgaricus* Subsp *delbrueckii* and the quantity indicated by the producing company at a rate of 3%. It was packaged in plastic containers with a capacity of 150 milliliters and incubated at (42 ± 2) degrees C until coagulation was complete within 3-5 hours until the pH decreased to 6.4, then it was removed from the incubator and transferred to the refrigerator for cooling and preservation. At a temperature of (5±1) °C until the necessary tests are carried out after 1, 3, 7, 14 days of manufacture.

Estimate the percentage of moisture

Moisture was estimated according to the method of Ling (2008) with a weight of 2 g of yogurt, which was dried in an electric oven at a temperature of 105 °C until the weight stabilized, according to the following equation.

Humidity % = $\frac{\text{Weight of the sample before drying} - \text{Weight of the sample after drying}}{\text{Sample weight}} \times 100\%$.

Determination of Ash %

The ashes were estimated by the direct burning method described in A.O.A.C (2005) by taking 2 g of the sample and placing it in a dry ceramic flask with a known weight. It was then inserted into the incineration oven for 6 hours at a temperature ranging between 500-550 °C or until white ash was obtained. In order to facilitate the process of evaporating water from the sample, a few drops of concentrated acetic acid are added, which causes precipitation of proteins, thus preventing the formation of a layer on the surface that impedes the rapid evaporation process. Then the sample is transferred to a desiccant. The percentage of ash is calculated from the following equation:

$\frac{\text{Weight of the lid with ash} - \text{the weight of the lid empty}}{\text{sample weight}} \times 100\%$.

Determination of Fat %

The percentage of fat in yogurt was estimated using the Babcock method, according to Ling (2008).

Estimation of carbohydrates in milk

Its percentage was estimated mathematically according to what Naoddy (1985) & Ihekoronye (1985) reported using the difference method % of carbohydrates = 100% (ash + protein + fat + moisture) 3-12-5: Determination of pH Determine the pH using a pH meter Crison with an accuracy of one unit directly into the yogurt after mixing it well.

Determination of the acidity percentage

The acidity was estimated according to the method mentioned in A.O.A.C (1980) by withdrawing 9 milliliters of milk and placing it in a Mayron flask and adding a few drops of phenolphthaleine reagent to it and then flushing with 0.1 titer of NaOH solution. The ratio was calculated Percentage on the basis of lactic acid according to the following equation:

Acidity % = $\frac{\text{Volume of base consumed (ml) Standard base Gram equivalent weight of lactic acid (90)}}{\text{Sample weight}} \times 100\%$.

Total nitrogen estimate

The total nitrogen was estimated according to what was stated in (AOAC, 2005) by weighing 2 g of the yogurt sample and transferring it to the digestion vial of the microcalcification device, to which 25 ml of concentrated sulfuric acid H₂SO₄ was added with the addition of the digestion powder consisting of aqueous copper sulfate CuSO₄ 5H₂O with a substance that raises the temperature Boiling heat, potassium sulfate K₂SO₄ and hydrogen peroxide (H₂O₂ 30%) are often used. During the digestion process, all proteins and organic materials are oxidized and ammonia is liberated, which in turn combines with concentrated sulfuric acid. Then the ammonia is liberated from the formed ammonium sulfate by making the reaction medium alkaline and receiving the liberated ammonia in a container. On boric acid, the solution is then eluted with 0.1 M hydrochloric acid and in the presence of Tashiro's reagent consisting of 0.2 g of methylene red and 0.1 g of methylene blue dissolved together in 100 cm³ of ethyl alcohol with a concentration of 96%. The digestion and distillation process was completed using the Behr analysis system, Model S2. German origin and according to the amount of nitrogen according to the following equation. % protein = % N 6.

Thiobarbituric acid value (TBA)

The value of TBA is calculated according to Malonaldehyde, which is the main carbonyl that decomposes and is affected by the oxidation process. The compound unsaturated fats are affected by the oxidation process more than the saturated

fats (Estevez et al., 2009,2022., Ajami) The thiobarbituric acid TBA was estimated according to the method that Mentioned by A.O.C.S(1990)) by soaking 10 g of the yogurt sample in 47.5 ml of distilled water in a volumetric flask with the addition of 2.5-3 ml of a 4 N solution of hydrochloric acid (HCl), then complete the volume to 100 ml with distilled water, then heat it on an electric heater for 5 minutes Then the sample was filtered using Whatman No. filter paper until 5 ml of filtered liquid was collected and mixed with 5 ml of the prepared TBA reagent (0.2883 g/100 ml of 90% TCA) in a test tube. The comparison sample Blank was prepared by placing 5 ml of distilled water. With 5 ml of the reagent, then the tubes were closed well and placed in a boiling water bath for 35 minutes, after which the tubes were cooled for 10 minutes at room temperature 25 °C, then the absorbance was measured by a spectrophotometer at a wavelength of 538 nm, and the TBA number was calculated as follows, according to the following equation: TBA mg malondehyde/kg sample = absorbance x 7.8 3-13: Microbiological analysis Microbiological tests were conducted for the yogurt samples after (1,3,7,14) days of refrigerated storage at a temperature of 5 °C, which included the total number of bacteria, coliform bacteria, cold-loving bacteria, yeasts, and molds, by taking 10 grams of yogurt and adding it to 90 ml of peptone water. The sample was mixed well, then 1 ml was taken from it and transferred to the first test tube containing 9 ml of peptone water, thus obtaining the first dilution 1:10, then 1 ml of the first dilution was transferred to a test tube containing 9 ml and mixed well to obtain the dilution the second 1:100 and so on until reaching the sixth dilution (APHA, 1984).

Microbiological analysis

Microbiological tests were conducted for the yogurt samples after (1,3,7,14) days of refrigerated storage at a temperature of 5 °C, which included the total number of bacteria, coliform bacteria, cold-loving bacteria, yeasts, and molds, by taking 10 grams of yogurt and adding it to 90 ml of peptone water. The sample was mixed well, then 1 ml was taken from it and transferred to the first test tube containing 9 ml of peptone water, thus obtaining the first dilution 1:10, then 1 ml of the first dilution was transferred to a test tube containing 9 ml and mixed well to obtain the dilution the second 1:100 and so on until reaching the sixth dilution (APHA, 1984).

Total Bacterial Count

The total number of bacteria was estimated according to the pouring method, as 1 ml was taken from the pre-prepared sample, and decimal dilutions were made from it. 0.1 ml of each dilution was transferred and planted on a Nutrient agar medium. The dishes were incubated at a temperature of 37 °C for a period of (24-48) hours. Count the colonies according to the method mentioned by Moghimi and Mogadam., 2017).

The total number of cold-loving bacteria

Psychrophiles Bacteria The total Number of cold-loving bacteria was estimated according to the pouring method, as 1 ml was taken from the previously prepared sample, and decimal dilutions were made from it. 0.1 ml of each dilution was transferred and planted on Nutrient agar medium, and the dishes were incubated at a temperature of 7 °C for a period of 10 days according to the method mentioned. Moghimi & Mogadam, 2017).

The total number of coliform bacteria

The total number of coliform bacteria was estimated according to the pouring method, as 1 ml was taken from the previously prepared sample, and decimal dilutions were made from it, and 0.1 ml of each dilution was transferred and planted on MacConkey Agar medium, and the dishes were incubated at a temperature of 37 °C for a period of (24-48) hours (Moghimi and Mogadam ., 2017).

Mold and Yeast Count

The total number of yeasts and molds was estimated according to the pouring method, as 1 ml was taken from the pre-prepared sample, and decimal dilutions were made of it. 0.1 ml of each dilution was transferred and planted on Potato Dextrose Agar medium, and the dishes were incubated at a temperature of 22 °C for a period of 5 days (Saleh et al., 2011). 3-14: Sensory evaluation of yogurt Sensory tests were conducted for yogurt treated with lactoferrin at concentrations (10, 20, 30, mg/L) in addition to a control sample by a group of specialists from (1-5) according to the sensory evaluation form mentioned by (Ramdhani & Setiadi, 2019) that included the appearance Appearance, Taste, and Consistency, which are replaced by Acidity. Chemical composition and physical properties of yogurt fortified with lactoferrin protein The tables below show the chemical composition of the manufactured yogurt with lactoferrin added at a ratio of (10, 20 and 30 mg/L) represented by coefficients To, T1, T2 and T3, respectively

RESULTS AND DISCUSSION

Humidity estimate

The results shown in Table 1 indicate the percentage of moisture for each of the control treatment To and the yogurt treatments with lactoferrin added (T1, T2, T3) when kept at a temperature of 5 ° C, as the percentage of moisture for the

control treatment To reached the direct manufacturing process, which was (87.01%) As for the yogurt treatments with lactoferrin added, they amounted to (86.94, 86.85, 86.75), respectively, and from observing the results of the statistical analysis, it is clear that there are no significant differences between the different treatments immediately after manufacturing, but in days (7.3) and the humidity was (68.68). (86.87,86.95)% and (86.63,86.85,86.92)%, respectively, compared to the control treatment, which was (86.68,87.00)%.

Table 1: The percentage of moisture overlap between the control treatment and the yogurt to which lactoferrin was added at different concentrations and between the days of storage.

		transactions		
30mg/L T3	20mg/L T2	10mg/L T1	Control To	Storage days
86.75 abcde	86.85 abcde	86.94 cde	87.01 e	1
86.68 abc	86.87 abcde	86.95 cde	86.68 abcd	3
86.63 a	86.85 abcde	86.92 bcde	87.00 e	7
86.64 ab	86.80 abcde	86.91 abcde	86.97 ce	14

* The different letters indicate that there are significant differences between the treatments

*Each number in the table represents an average of three replicates

As for the percentage of humidity for the fourteenth day, the control treatment was (86.97)% for the control yogurt, while the percentage of the treatments T1, T2, and T3 recorded (86.80, 86.91, 86.64)%, respectively. Storage at a temperature of (5) °C, and the third concentration excelled by giving the lowest percentage of moisture compared to the rest of the treatments. The decrease in percentage is due to the rate of evaporation of moisture content during storage (Al-Sheikh, 2018).

Estimation of protein content:

The percentage of protein was estimated according to the results shown in Table 2, the percentage of protein for each of the control treatment and the yogurt treatments to which lactoferrin (T1, T2, T3) was added when kept at a temperature of 5 °C, as the percentage of protein for the control treatment reached To after the manufacturing process directly, which was (4.31)%. As for the yogurt treatments with lactoferrin added, they amounted to (4.33, 4.36, 4.41) respectively, and from observing the results of the statistical analysis, it is clear that there are significant differences between the different treatments immediately after manufacturing, as the protein percentage was (4.30, 4.40, 4.33) % (4.44,4.41,4.37)%, respectively, for 3.7 days compared to the control treatment (4.33,4.32)%, respectively On the other hand, the results showed that the percentage of protein after 14 days was (4.35)% for the control treatment, where there is a significant difference between the first and day 14 of storage at a temperature of (5) °C, while the percentage of the treatments T1, T2, and T3 was recorded as (4.45). (4.42, 4.39)% for the 14th day in a row. It is noted from the results that the T3 treatment was superior to the rest of the treatments by giving the highest percentage of protein, and it was found that there was a significant difference between T2 and T3.

Table 2: The percentage of protein overlap between the control treatment and the yogurt to which lactoferrin was added at different concentrations and between the days of storage

		transactions		
30mg/L T3	20mg/L T2	10mg/L T1	Control To	Storage days
4.41 fg	4.36 d	4.33 b	4.31 a	1
4.43 hi	4.400 ef	4.33 b	4.32 b	3
4.44 ij	4.41 fg	4.37 d	4.33 b	7
4.450 j	4.420 gh	4.390 e	4.35 c	14

* The different letters indicate that there are significant differences between the treatments

*Each number in the table represents an average of three replicates

The reason may be due to the variation in the percentage of protein in the T1, T2 and T3 treatments compared to the To treatment, because lactoferrin is of a protein nature and will contribute to the increase in the percentage of protein in the manufactured yogurt, even if it is small. From the results of the statistical analysis, it is clear that there are significant differences between the different yogurt treatments immediately after manufacturing and when storing

Estimating the percentage of fat:

Table 3 shows the percentage of fat for each of the control treatment and yogurt treatments with lactoferrin added (T1, T2, T3) when preserved at a temperature of 5 ° C, as the percentage of fat for the control treatment To after the manufacturing process directly reached (3.52)% in When the yogurt added to lactoferrin was (3.58, 3.64, 3.57)%, respectively, and it is clear from the results of the statistical analysis that there are significant differences in the percentage of fat immediately after manufacturing, as well as during storage at a temperature of (5) °C for a period of 14 days, and it was in agreement with what Sengupta et al., (2014) found that the percentage of fat in yogurt made from whole milk was 3.67%.

Table 3 Percentage of fat overlap between the control treatment and yogurt to which lactoferrin was added at different concentrations and between days of storage.

		transactions		
30mg/L T3	20mg/L T2	10mg/L T1	Control To	Storage days
3.57 bcd	3.64 e	3.58 cd	3.52 a	1
3.75 f	3.62 de	3.58 cd	3.53 ab	3
3.80 g	3.64 e	3.57 bc	3.51 a	7
3.81 g	3.66 e	3.58 cd	3.50 a	14

* The different letters indicate that there are significant differences between the treatments

*Each number in the table represents

An average of three replicates On the other hand, the percentage of fat per day was 3,7,14 for the treatments T1, T2, T3 (3.75,3.62,3.58)% for the third day, (3.80,3.64,3.57)% for the seventh day, and (3.81,3.66,3.58)% for the next day. Fourteenth in a row and (3.53, 3.51, 3.50)% and for the control treatment, respectively. An increase in the percentage of fat is observed during storage days, and T3 is superior to the rest of the treatments by giving the highest percentage of fat. The reason may be due to the evaporation of the water content and this leads to the concentration of total solids, including fat (Al-Zarfi, 2022) From the results of the statistical analysis, there are significant differences.

Calculation of carbohydrates

The results showed in (Table 4) the percentage of carbohydrates for each of the control treatment and the yogurt treatments with lactoferrin added (T1, T2, T3) when preserved at a temperature of 5 ° C, as the percentage of carbohydrates for the control treatment reached To immediately after the manufacturing process, which was (4.437)% As for the yogurt treatments with lactoferrin added, they amounted to (4.413, 4.383, 4.467) respectively, and the results showed that there were no significant differences for the first day, and storage days decreased by 3.7 days, as they were (3.97, 4.31, 4.38)% and (4.29, 4.30). (4.37)% compared with the control treatment (4.74,4.42%). It is clear from the results of the statistical analysis that there are no significant differences between the treatments. Sengupta et al., (2014) explained in his study that the percentage of carbohydrates in yogurt made from full-fat cow's milk is 4.47%.

Table 4: Percentage of carbohydrates overlap between the control treatment and yogurt with lactoferrin added at different concentrations and between days of storage.

		transactions		
30mg/L T3	20mg/L T2	10mg/L T1	Control To	Storage days
4.46 bc	4.38 bc	4.41 bc	4.43 bc	1
3.97 a	4.31 ab	4.38 bc	4.74 c	3
4.29 ab	4.30 ab	4.37 bc	4.42 bc	7
4.30 ab	4.30 ab	4.35 b	4.44 bc	14

* The different letters indicate that there are significant differences between the treatments

*Each number in the table represents an average of three replicates

The control treatment To, after 14 days of refrigerated storage at a temperature of 5°C, reached (4.44)%, while for the yogurt treatments to which concentrations of lactoferrin were added, it amounted to (4.35, 4.30, 4.30)%, respectively. It is clear from the results of the statistical analysis that there are no significant differences between T3 and T2 A decrease in the percentage of carbohydrates is observed due to the activity of starter bacteria that convert lactose into lactic acid (Yalmaz-Ersan, 2014).

Ash percentage estimation

Table 5 shows the percentage of ash for each of the control treatment and the yogurt treatments with lactoferrin added (T₁, T₂, T₃) when kept at a temperature of 5 °C, as the percentage of ash for the control treatment T₀, after the manufacturing process directly, was (0.71)% As for the yogurt treatments with lactoferrin added, they amounted to (0.72, 0.75, 0.79)%, respectively. From observing the results of the statistical analysis, it was found that there were significant differences between all the treatments with lactoferrin added to it and the control treatment for the first day. As for the days (3,7), the percentage was Ash (0.82, 0.78, 0.74)% (0.83, 0.79, 0.75)%, respectively, compared with the control treatment, which was (0.72, 0.71)%, respectively.

Table 5 Examination of the percentage of ash for the interaction between the control treatment and the yogurt supplemented with lactoferrin

		transactions		
30mg/L T ₃	20mg/L T ₂	10mg/L T ₁	Control T ₀	Storage days
0.79 gh	0.75 e	0.72 c	0.71 a	1
0.82 i	0.78 f	0.74 d	0.71ab	3
0.83 i	0.79 fg	0.75 de	0.72 bc	7
0.84 j	0.80 h	0.75 e	0.73 c	14

* The different letters indicate that there are significant differences between the treatments

*Each number in the table represents an average of three replicates

The results of the statistical analysis indicate that there are significant differences between the treatment and the control treatment T₀ to which lactoferrin was added, a significant increase was also observed in the average percentage of ash with increasing days of storage and for all treatments, as it reached 14 days of refrigerated storage at a temperature of 5 °C for treatments (T₃, T₂, T₁), 0.75 (0.80, 0.84)%, respectively, compared to the treatment of Control (0.73)%. This is due to a decrease in the humidity and an increase in the total solids. The process of adding lactoferrin and the growth of microorganisms were factors that led to an increase in the ash percentage, or the reason may be due to the composition of the milk used in the manufacture of yoghurt. When the fat is removed, the percentages of both moisture and protein rise, which leads to an increase The amount of dissolved mineral salts leads to an increase in the percentage of ash (2000, Madadlou et al.)

TBA thiobarbutyric acid test

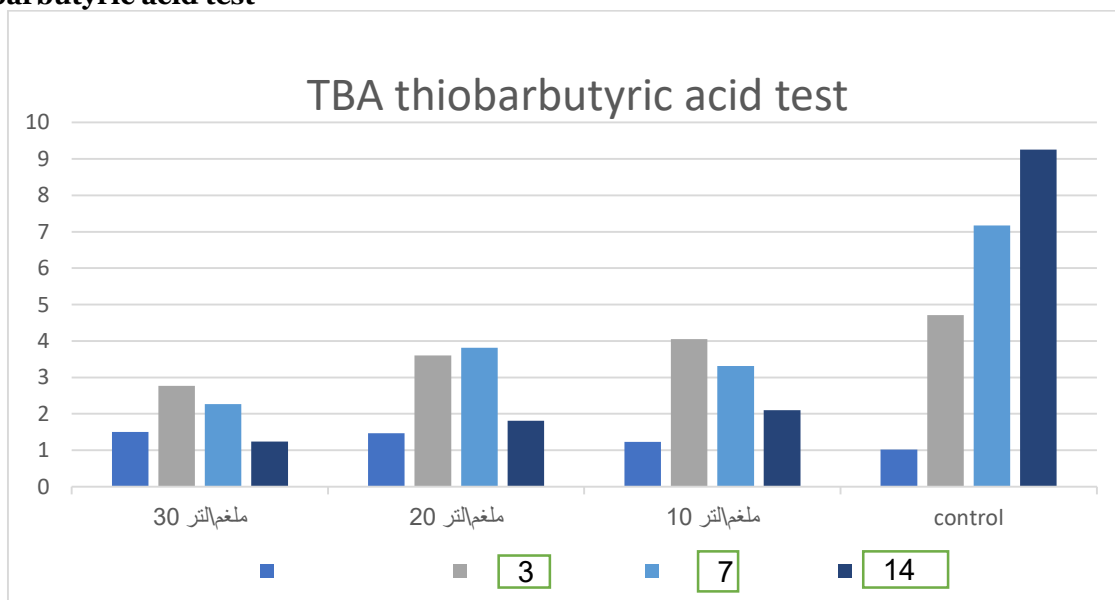


Figure 1: The effect of adding different percentages of lactoferrin on the TBA acid values of yogurt stored at 5°C for 14 days

The results showed that there were significant differences after manufacturing between the control treatment and the rest of the treatments to which different concentrations of lactoferrin were added. The control amounted to (1.02) mg of malondehyde / kg, respectively On the third day of refrigerated storage, significant differences were observed between the preserved samples to which different concentrations of lactoferrin were added, compared to the control sample. 2.77 mg malondehyde / kg, while the rest of the treatments recorded (3.60, 4.05) mg malondehyde / kg, respectively, for

concentrations (10,20) compared to the control treatment, which reached (4.71) mg malondehyde / kg, respectively. On the seventh and fourteenth day, a decrease was observed in the acid value of the treatments to which lactoferrin was added, as it reached (1.24, 1.81, 2.10) and (3.31, 3.81, 3.81) mg of malondehyde / kg, respectively, compared with the control treatment, as it reached (9 17, 7, 26 (mg malondehyde / kg for days 7, 14, respectively) This is due to the fact that lactoferrin is an efficient antioxidant that has the ability to hinder the growth of free radicals that are formed by the action of peroxides. Lactoferrin also has a high reducing power and is thus able to convert iron from Ferric ions $Fe + 3$ to the form Ferro ions $F + 2$, which is one of the encouraging factors. To oxidation, it works similarly to ferricyanide in converting $[Fe(CN)_6]^{-3}$ to $[Fe(CN)_6]^{-4}$ (Perenlei et al., 2011). The well-known thiobarbituric acid assay was adopted by many researchers to estimate fat oxidation in milk and it can be performed to monitor the level of oxidative deterioration in milk and dairy products, as the results of the TBA test showed that milk samples were not higher than 0.023. There was no perceptible off-taste, although the TBA values were increasing during the storage period, but still the highest value did not exceed (0.025) (Abdulghani and Ali 2017). Among the results of the statistical analysis, it was found that there were significant differences between the treatments to which lactoferrin was added with the control treatment. It was also noted that the T3 treatment, to which lactoferrin was added at a concentration of 30 mg / L, was superior to all treatments in maintaining the value of TBA within the limits of acceptance Physical properties of yogurt fortified with lactoferrin protein.

pH

Figure (2) indicates the pH values of the different yogurt treatments, as these values were (4.56), immediately after the manufacturing process, for the control treatment T0, while for the treatments with lactoferrin (T1, T2, T3) added (4.52, 4.46, 4.32), respectively For the first day, while the pH values were ((4.45,4.48,4.52) and (4.50,4.50,4.55) for days 3,7 respectively and the control treatment (4.57,4.56), but on the 14th day the pH of the control treatment T0 was 4.57 () and the rest of the treatments (T1, T2, T3 (are) 4.56, 4.53, 4.51) respectively, as it was observed that the pH values increased for the treatments to which the lactoferrin protein was added, and the reason for this is due to the ability of the lactoferrin protein to partially delay the growth of *Streptococcus thermophilus* and push it to the production of lactic acid in a smaller amount, as these bacteria provide the appropriate conditions for the growth of *Lactobacillus bulgaricus* (Franco et al., 2010)) and the results of the statistical analysis indicate that there are significant differences between all the treatments to which lactoferrin is added compared to the control treatment T0 and superiority T2 by giving the highest pH values.

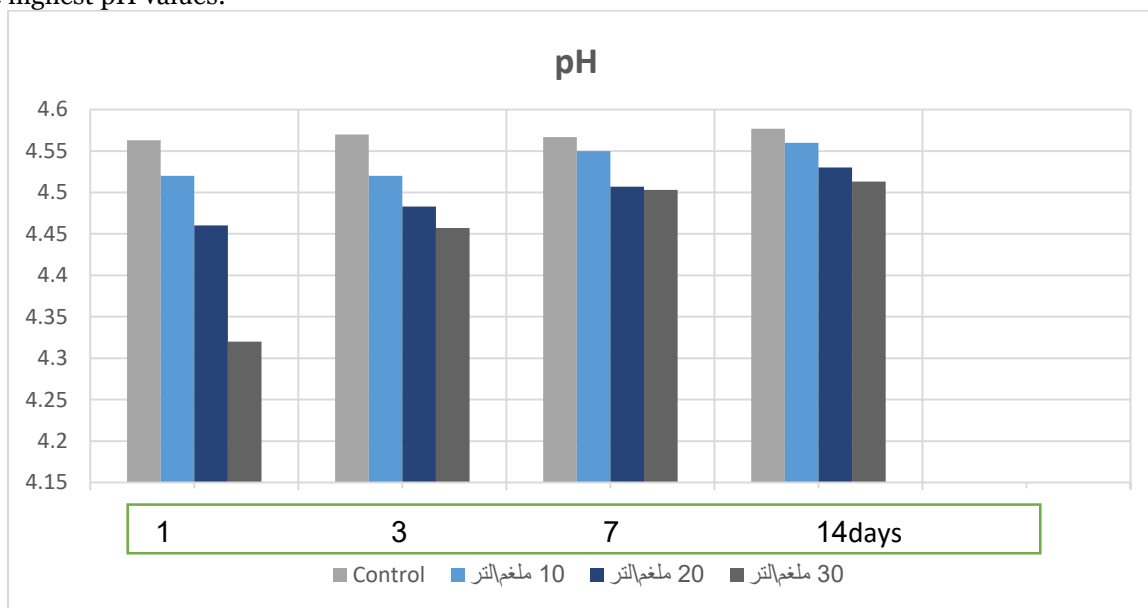


Figure 2: The effect of adding different percentages of lactoferrin on the pH values of yogurt stored at 5°C for 14 days

Total Titratable Acidity (TA)

The results in Figure (3) indicate the pH values calculated on the basis of lactic acid for the different treatments, immediately after manufacturing and when stored at a temperature of (5) °C. Lactoferrin added to it (T1, T2, T3) reached (0.94, 0.96, 1.01)%, while the percentage of acidity was (0.98, 0.95, 0.92) and (0.95, 0.92, 0.89), respectively, for days 3,7 and reached (0.91). 0.89) in the control treatment.

Figure (3) The acidity of the overlap between the control treatment and the yogurt to which lactoferrin was added at different concentrations and between storage days

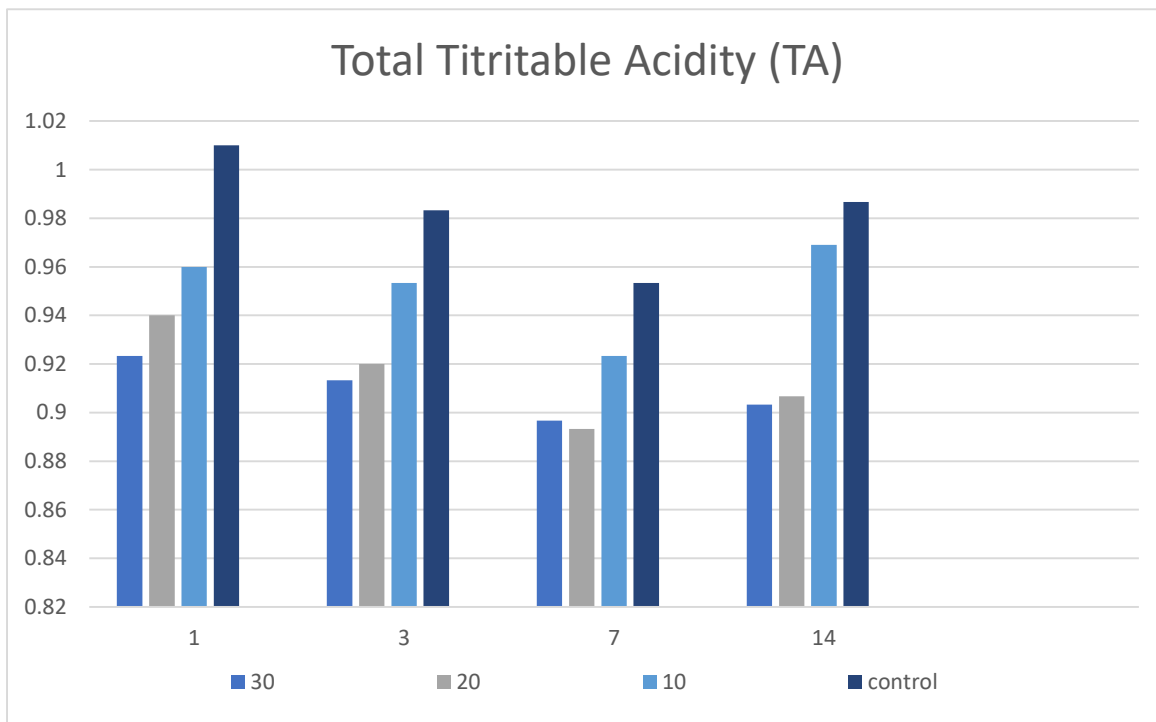


Figure 3: The acidity of the overlap between the control treatment and the yogurt to which lactoferrin was added at different concentrations and between storage days

From the results of the statistical analysis, it is clear that there are significant differences between the treatments immediately after manufacturing. As the results show in the same table, there is a rise in the pH values during storage, as after 14 days for the control treatment T_0 it was (0.90) %, while it was in the treatments (T_1 , T_2 , T_3) are (0.96, 0.90, 0.98)%, respectively. It is noted from the results the effect of lactoferrin protein in preserving the yogurt from the high acidity, as the acidity being higher than the normal limit is one of the defects in making the yogurt. The results of the statistical analysis also indicate that there are significant differences. Among the different treatments, T_3 excelled by giving the lowest percentage of.

Microbiological tests for yogurt.

Table (6) shows the results of estimating the number of microorganisms, which included each of the total number of starter bacteria, the number of coliform bacteria, the number of cold-loving bacteria, the number of yeasts and molds, for the control treatment and the treatments with added lactoferrin protein, immediately after manufacturing and when stored at a temperature of (5) C It is clear from the results in Table (4-12) that the total number of starter bacteria after manufacturing directly for the control treatment was $10^6 \times 161$ u.m.t / g.

Table 6: Microbiological analysis results of the control yogurt treatment and the yogurt treatments supplemented with lactoferrin protein at different concentrations.

Number of yeasts and molds wtm/gm	Number of thermophilic bacteria wtm/g	Number of coliform bacteria wtm/g	Number of starter bacteria wtm/g	Yogurt age/day	transactions
0	0	0	$10^6 \times 161$	1	Control T_0
0	0	0	$10^6 \times 168$	3	
0	$10^6 \times 1$	0	$10^5 \times 150$	7	
0	$10^6 \times 2$	0	$10^5 \times 170$	14	
0	0	0	$10^6 \times 145$	1	10 mg/L T_1

0	0	0	$10^5 \times 150$	3	20 mg/L T2
0	0	0	$10^5 \times 145$	7	
0	0	0	$10^5 \times 130$	14	
0	0	0	$10^6 \times 143$	1	
0	0	0	$10^6 \times 142$	3	
0	0	0	$10^6 \times 139$	7	
0	0	0	$10^4 \times 1$	14	
0	0	0	$10^6 \times 131$	1	30 mg/L T3
0	0	0	$10^5 \times 130$	3	
0	0	0	$10^6 \times 132$	7	
0	0	0	$10^5 \times 90$	14	
--	-----	---		0.5453 0.5453 1.0906	L.S.D for the days L.S.D for transactions L.SD to overlap

It was within the limits set by the international standards (FAO / WHO, 1994; FIL / IDF, 1997), which stipulates that the number of live cells of starter bacteria should not be less than 10^7 uT / g. As for the numbers of starter bacteria for the treatments to which the lactoferrin protein was added, they were $10^6 \times 161$, $10^6 \times 145$, $10^6 \times 143$, and $10^6 \times 131$ w.m / g, respectively. From the results of the statistical analysis, it is clear that there are no significant differences in the number of starter bacteria immediately after manufacturing. between all transactions During storage, the number of starter bacteria increased until it reached $10^6 \times 168$ upm/g after three days for the control treatment, and for the treatments to which lactoferrin protein was added, it reached $10^5 \times 15$, $10^6 \times 142$ and $10^5 \times 13$ upm/g. respectively This indicates that the lactoferrin protein does not affect the growth of starter bacteria, and this result is consistent with what was found by Kim et al., ., (2004) who showed that the addition of lactoferrin to milk does not affect the starter bacteria *Lactobacillus acidophilus* and *Bifidobacterium* spp., as lactoferrin provides support for the growth of bacteria, due to the amino acids lactoferrin contains that increase the activity of starter bacteria And that the increase in bacterial growth increases the availability of non-protein nitrogen and some peptides, which are important growth factors for starter bacteria, and it is also noted that a gradual decrease in the number of starter bacteria, as it was after 14 days for the previous treatments $10^5 \times 17$, $10^5 \times 13$, $10^4 \times 1$ and $10^5 \times 9$ atm/g, respectively This is consistent with what was found by Dave, (1998) who indicated that the number of bacteria in yogurt starter culture decreased from 99.1×10^7 Utm/g to 65.3×10^7 Utm/g on day 15 in storage at refrigerator temperature. The reason for this is due to the increase in the acidity of the yogurt, as the high percentage of lactic acid leads to a decrease in the number of the starter revival. This is consistent with what Michael et al., ., (2010) indicated that the growth of *Streptococcus Salivarius* Subsp thermophiles in the yogurt declines from 9 cycles. Logarithmic to 8 log cycles over the 50 day storage period And from the results of the statistical analysis, it is clear that there are significant differences between the treatments to which lactoferrin protein is added, with the control treatment at a significant level ($P < 0.05$). As for the numbers of coli bacteria *E. coli*, it did not show any growths throughout the storage period in all treatments. As for the numbers of cold-loving bacteria, they were for the control treatment C immediately after manufacturing, and for the treatments to which lactoferrin was added were zero, zero, zero, zero, and T.M / g, respectively. The results indicate that these numbers increased after 14 days to reach $10^6 \times 2$ U.M.T / gm for the control treatment, while no growths appeared in the treatments to which lactoferrin protein was added, which indicates the ability of lactoferrin to inhibit the growth of cold-loving bacteria, and from the results of the statistical analysis It is clear that there are significant differences between the different treatments and treatment C at the level ($P < 0.05$). As for the preparation of yeasts and molds for the different treatments of yogurt, no growths appeared during the storage stages and for all treatments. The reason is that lactoferrin is anti-microbial, germs and micro-organisms, including yeasts and molds, and thus deprives it of the elements necessary for growth such as *L. monocytogenes*, *Salmonella* spp., *Escherichia coli*, *Bacillus stearothermophilus*, *Shigella dysenteriae*, and *Bacillus subtilis*. (Kevion et al., 2022).

CONCLUSION

The results of the chemical tests of yogurt added to concentrations of lactoferrin (10, 20, 30) mg/liter showed that the storage period was prolonged and the yogurt spoiled for a period of 14 days. Adding lactoferrin to yogurt to prolong storage life at three concentrations (10, 20, and 30) mg/L was able to inhibit the growth of coliform bacteria, cold-loving bacteria, molds, and yeasts.

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