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Evaluate the effect of using a whey protein membrane loaded with zinc nanoparticles, lactoferrin and neptomycin on microbial growth in laboratory-made soft white cheese

Muhammad Jamel Muhammad[1*], Somaya Khalaf Badawi[2], Muhammad Yaqoub Atallah[2]

[1]*Department of Food Sciences, College of Agriculture, Tikrit University, Tikrit, Iraq.*

[2]*Department of Food Sciences, College of Agriculture and Forestry, University of Mosul, Mosul, Iraq.*

[*]Corresponding Author
Muhammad Jamel M, Department of Food Sciences, College of Agriculture, Tikrit University, Tikrit, Iraq.

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Abstract

The study was conducted in the laboratories of the Department of Food Sciences-College of Agriculture and in the animal house of the College of Veterinary Medicine at Tikrit University for the period from 1/2/2021 to 15/3/2022. The aim of this study was to manufacture edible whey protein membranes with concentrations of 10%, as well as fortify these membranes with zinc nanoparticles, natamycin, and lactoferrin with different concentrations and interactions. The treatments were as follows: T1:control, T2:membrane, T3:0.25 mg nanoparticle zinc and membrane, T4:60 mg lactoferrin and membrane, T5:50 mg bacteriocin and membrane, T6:0.25 mg nanoparticle zinc, 60 mg lactoferrin and T7:0.25 mg zinc. Nano and 50 mg bacteriocin and T8:60 mg lactoferrin and 50 mg bacteriocin and T9:0.25 mg nano zinc and 60 mg lactoferrin and 50 mg bacteriocin. Soft cheese was made from cow's milk and coated with whey proteins, and the cheese was stored for 21 days at 5±2 °C. The microbial evaluation of cheese coated with whey protein films and fortified showed that the number of bacteria on the first day of storage ranged between 4.13-5.65 Log/g compared with the control samples 1T and 2T at 7.59 and 6.63 Log/g, respectively. At the end of the storage period, treatment 9T was the lowest in terms of growth, reaching 4.26 Log/g, as well as for lipolytic bacteria, as treatment 9T was the least in terms of growth, reaching 1.12 Log/g. This applies to protein-analyzing bacteria, as the results showed that the transaction 9T was the least in terms of growth, reaching 1.64 Log/g.

Keywords: Dairy Industry, Ultraviolet Radiation, Microbiological Contamination, Natamycin, Lactoferrin

Introduction

Whey it is a by-product of the dairy industry that is yellowish-green in color, soluble in water, and is derived after extraction of casein from cheese [1]. Whey proteins are an excellent source for the production of edible biopolymers in the packaging industry, as the main organic part of whey protein is carbohydrates (lactose), followed by protein, minerals, lactic acid and fats [2]. Packaging is one of the ancient industries with a long history, as it has been known since the beginning of mankind and has expanded over the years and generations. The primary purpose of food packaging is to protect food from oxygen, water vapor, ultraviolet radiation, chemical and microbiological contamination [3], that packaging materials must possess reliable features such as microbial stability and non-toxic as well as mechanical properties, reservation, appropriate sensory qualities and inexpensive production and product compatibility [4], there is a great interest in the development of biodegradable polymer-based packaging materials, whose functionality is improved by incorporating active compounds such as plasticizers, antimicrobials, antioxidants, and sensors P [5].

Bacteriocins are several antimicrobial peptides with low molecular weight and high activity against bacteria [6]. Natamycin, also known commercially as bimarcin, belongs to polyene antibiotics, as it is synthesized by fermentation. The submerged antenna of Streptomyces natalensis, and other related species, is used as an antimicrobial on the surface of cheese due to its activity against yeasts and molds. Although natamycin has been shown not to have toxic effects even at high levels of ingestion, its use as a food additive is still restricted by law in all countries. In addition, bacteriocins are attractive because they are natural preservatives that do not require the addition of synthetic materials to the food [7].

As for lactoferrin, it belongs to the family of multifunctional transferrin proteins, and it participates in regulating the level of free iron, which is considered one of the proteins that inhibit bacteria and is beneficial to health, (Marchewka et al., 2012). Lactoferrin has gained much attention for its bioactive properties that boost the immune system [8]. Lactoferrin is an inherently multifunctional molecule due to its high affinity for iron, which deprives microbes of free iron necessary for their growth, and its tendency to interact

with host cell surfaces and microbes [9].

The current study aimed to know the effectiveness of edible coatings of whey protein fortified with zinc nanoparticles (ZnNPs), natamycin and lactoferrin on the microbial growth of microorganisms in soft white cheese.

Materials and Methods

Materials Used

Lactoferrin: Obtained from the American Jarrow Company, Entamycin: Obtained from the Belgian Handray Company, Nano Zinc:Obtained from the American Research Nanomaterials Company, Whey Protein: Obtained from the American Isolabs Company.

Preparation of whey protein membranes Whey protein membrane solutions were prepared according to the method of Carvalho and Grosso [10], with a weight of 10 g of whey protein powder, dissolved in 80 ml of distilled water, and mixed all components using a hot plate-Magnetic Stirrer at a temperature of 60°C for a period of time 15 minutes, then glycerol was added at a rate of 3% of the dry weight of the whey, and the volume was added to 100 ml of distilled water and the pH was adjusted to 7.

Prepare Cheese Samples

The method of making soft cheese: The cheese was made according to the method of Fox et al. [11] which was modified as follows:

The cow's milk obtained from one of the milk processors in Salah Al-Din Governorate was pasteurized at a temperature of 7°C for 30 minutes, and after cooling to a temperature of 35-40°C, rennet (1 g/25 liters of milk) prepared from Meito Sangyo CO. was added to it. LTD of Japan and left for 45 minutes until it reached the stage of curd, after which the curd was cut to get rid of the whey, and the curd was placed in a wet cloth to get rid of the largest amount of whey. The curd was packed in special molds for each sample, then the samples were marked and kept in the refrigerator for microbial and chemical tests which were confirmed in the study directly at the age of 1, 7, 14 and 21 days.

Packaging of cheese samples: Cheese samples were cut in a rectangular shape with a weight of 50 g per sample to ensure that the envelopes completely contained the samples and were covered with whey membranes loaded with zinc nanoparticles ZnNPs, lactoferrin, natamycin and their interactions, according to the following additions.

✓ T1 cheese without casing (comparison sample 1).
✓ T2 cheese coated with whey protein film without any addition (comparative sample 2).
✓ T3 cheese coated with whey protein film, with zinc nanoparticles (ZnNPs) added at a concentration of 0.25 mg/100 ml.
✓ T4 cheese coated with whey protein film, with lactoferrin added at a concentration of 60 mg/100 ml.
✓ T5 cheese coated with whey protein film, with natamycin added at a concentration of 50 mg/100 ml.
✓ T6 cheese coated with whey proteins, with ZnNPs and lactoferrin added at a concentration of 0.25 and 60 mg/100 ml.
✓ T7 cheese coated with whey protein membrane, with ZnNPs and natamycin added at a concentration of 0.25 and 50 mg/100 ml.
✓ T8 cheese coated with whey proteins, with lactoferrin and natamycin added at a concentration of 60 and 50 mg/100 ml.
✓ T9, whey protein membrane-coated cheese with ZnNPs and lactoferrin natamycin added at 0.25, 50 and 60 mg/100 ml.
✓ Then it was stored in refrigeration until examinations were conducted on it, according to the suggested time periods.

Microbiological Examinations

Estimation of total bacterial numbers contaminated with cheese: Take 10g of each cheese sample using a clean, sterilized spoon. The weighed quantity was mixed with 90 ml of distilled water in a volumetric flask. The mixture was shaken well to obtain a homogeneous solution and the first dilution was considered 1:10, then the appropriate dilution was carried out to obtain the ideal numbers in the plates. Agar, then the plates were incubated at 37°C for 24 hours. Then the numbers of growing bacterial colonies were calculated [12].

Estimation of the number of proteolytic bacteria: Estimation was made using the method mentioned in Harrigan and McCence [13], using Milk Agar Skimmed nutrient medium consisting of (100 ml of Nutrient agar +10% skimmed milk). The plates were inoculated with a volume of 0.1 ml of the fourth dilution of cheese samples, then the plates were incubated at a temperature of 21±2ºC for 24-48 hours, then 1% hydrochloric acid was added to it and left for one minute, then the colonies surrounded by clear zones were counted.

Estimation of the number of lipolytic bacteria: The method mentioned in Harrigan and McCence, [13] was used using a Bacterial Lipolytic Media consisting of (100 ml Nutrient agar + 1 ml of sunflower oil + emulsifier (glycerol). The dishes were inoculated with a volume of 0.1 ml of the fourth dilution of the cheese samples and then incubated at At a temperature of 21±2ºC for a period of 24-48 hours, after the incubation period, a quantity of 20% copper sulfate solution was added to cover the surface of the nutrient medium for 5 minutes, then the copper sulfate was removed by distilled water and the colonies stained in a bluish-green color were counted using a colony Counter.

Estimation of the total number of yeasts and molds in cheese samples: The total number of yeasts and molds was estimated after spreading 0.1 ml of the sixth dilution on the molded plates using PDA medium, and after incubating the plates at 25ºC for 5-7 days, the total numbers of colonies formed on the surface of the culture medium were calculated [12].

Statistical Analysis

The experiment was implemented under a complete randomized design (CRD) and analysis of variance was carried out using the general linear model within the ready-made statistical program (SAS, 2001). In the case of significant differences, Duncan's test [13] was used to determine the significance of the differences between the different means at a probability level of 0.05.

Results and Discussion

Microbial Evaluation of Cheese

The total number of bacteria: Table 1, shows the total number of bacteria in soft cheese samples coated with whey films loaded with ZnPNs, natamycin and lactoferrin, which were coated only with the film and the control sample, and stored for (21) days at a temperature of (5±2)°C. The results showed that the number of bacteria The total on the first day at the beginning of storage for the transactions T1, T2, T3, T4, T5, T6, T7, T8, T9 were 7.59, 6.63, 5.14, 5.62, 5.65, 4.56, 4.84, 4.84, 4.13 Log/g respectively and with continuation In the storage process, there was a significant increase in the total bacterial numbers of all treatments, as it was highest on the fourteenth day for the treatments T3, T4, T5, T6, T7, T8, and T9, reaching 5.60, 5.95, 6.14, 4.53, 5.21, 5.13, 4.38 log/g. Respectively, compared with the control sample T1 and T2, which were at 8.57 and 7.33 Log/g, respectively, and when following up the increase in total bacterial numbers until the end of the storage period, it was found that the treatment T12 was the lowest at 4.26 Log/g compared with the two control samples T1 and T2. Results with Kavas et al. [14] reported that the number of total bacteria in cheese samples that were coated with protein films was 4.3-12 Log/g.

The results showed that the total number of bacteria is within the permissible range, and the reason for this is due to the pasteurization process of milk before the soft cheese making process, which eliminated large numbers of microorganisms at the beginning of storage in cheese Bachmann et al. [15]. It also agrees with what Bellamy et al. [16] found, where they found a decrease in the numbers of microorganisms caused by lactoferrin containing Lactoserricin peptide, as it shows strong antimicrobial activity, which leads to a reduction in its numbers. It also agrees with what was mentioned by Al-Badrani et al. [17] that the total number of bacteria in coated Monterey cheese treated with neptomycin and lysozyme decreased growth by 2-3 log stages CFU/g compared to uncoated Monterey cheese coated with wax only. Also with Saral et al. [18] the addition of zinc nanoparticles to a polyurethane/poly urthane membrane containing Mahua oil reduced bacterial growth by about 0.6 to 0.3 (cells/ml) in packed carrot slices compared to with a control sample not packed in membranes.

The reason may be attributed to the correct methods used in the manufacturing process, the cleanliness of the utensils, the sterilization of the utensils and the equipment used, and the joint effect of each of the whey protein membrane coating process on the one hand, and the effect of anti-microbial agents on the other hand, whether by the effect of nanoparticles or lactoferrin or neptomycin alone or with each other, as it reflects The role of these antibiotics in limiting the microbial growth of these organisms in cheese during its ripening and storage period.

Transactions	Day 1	Day 7	Day 14	Day 21
T1	7.59 ± 0.052^{a}	7.94 ± 0.043^{a}	8.57 ± 0.075^{a}	8.23 ± 0.046^{a}
T2	6.63 ± 0.046^{b}	6.97 ± 0.046^{b}	7.33 ± 0.046^{b}	7.13 ± 0.055^{b}
T3	5.14 ± 0.043^{f}	5.41 ± 0.043^{f}	5.60 ± 0.055^{f}	5.44 ± 0.052^{g}
T4	5.62 ± 0.058^{e}	5.81 ± 0.043^{de}	5.95 ± 0.043^{e}	5.85 ± 0.046^{e}
T5	5.65 ± 0.046^{e}	5.94 ± 0.041^{d}	6.14 ± 0.038^{d}	6.03 ± 0.043^{d}
T6	4.56 ± 0.040^{h}	4.48 ± 0.046^{h}	4.53 ± 0.043^{h}	4.42 ± 0.052^{i}
T7	4.84 ± 0.041^{g}	5.07 ± 0.046^{g}	5.21 ± 0.046^{g}	5.00 ± 0.046^{h}
T8	4.84 ± 0.041^{g}	5.05 ± 0.055^{g}	5.13 ± 0.038^{g}	5.04 ± 0.041^{h}
T9	4.13 ± 0.049^{i}	4.45 ± 0.043^{i}	4.38 ± 0.049^{i}	4.26 ± 0.038^{j}

*The numbers in the table are the average of three replicates and represent the mean values ± the standard deviation.

*Different lowercase letters within one column indicate significant differences ($p \leq 0.05$) between treatments.

T1: Control; T2: Whey Protein Membrane; T3: Membrane Reinforced with 0.25 mg Zinc Particles: T4 :Membrane Fortified with Lactoferrin At A Concentration of 60 mg; T5: Membrane Fortified with 50 mg of Antamycin; T6: 0.25 Membrane Fortified with Zinc Nanoparticles at a Concentration of 0.25 mg + 50 mg of natamycin; T7: Membrane Fortified with Zinc Nanoparticles at a Cconcentration of 0.25 mg + 60 mg Lactoferrin; T8: Membrane Fortified with Lactoferrin at a Concentration of 60 mg + 50 mg of Neptomycin; T9: Membrane Fortified with Zinc Nanoparticles at a Concentration of 0.25 mg + 60 mg Lactoferrin + 50 mg Natamycin.

Table 1: Effect of wrapping with whey protein films and treatment with different treatments on Log/g the total number of bacteria in soft cheese stored for (21) days at a temperature of (5±2)°C.

4-3-2 Lipolysis Bacteria

Table 2, shows that lipolytic bacteria in soft cheese samples did not grow in all treatments at the beginning of the storage time one day, and when storage continued and when examined on the seventh day, growth was observed in treatments T3, T4, T5, T6, and T7. T8, T9, with numbers of 1.83, 1.84, 3.29, 1.36, 2.06, 1.20, 1.09 Log/g, respectively, compared with the control samples T1 and T2, which were at 3.46 and 3.26% Log/g, respectively. From observing the results, it was found that treatment T9 is The lowest in its content of the number of lipolytic bacteria (1.09Log/g) compared to the control sample T1, where the highest number was 3.46Log/g, and when the storage process continued, and on day 14, the number of lipolytic bacteria increased, as the T5 treatment was the highest at 3.29 Log/g While treatment T9 recorded the lowest number of 1.21 Log/g compared to the control samples T1 and T2, as the number of lipolytic bacteria reached 4.26 and 4.10 Log/g, respectively. At the end of the storage period, the results showed that the total number of lipolytic bacteria in treatments T3, T4, T5, T6, T7, T8, and T9 on day 21 was 2.08, 2.01, 3.35, 1.35, 2.04, 1.51, 1.12 log/g. Respectively, compared with the control treatments T1 and T2, which were at 4.07 and 3.93 Log/g, respectively, the results showed that the best treatment was T9, as it was the least significant compared to other treatments, reaching 1.12 Log/g at the end of storage.

Transactions	Day 1	Day 7	Day 14	Day 21
T1	0.00 ± 0.000^{f}	3.46 ± 0.046^{a}	4.16 ± 0.052^{a}	4.07 ± 0.046^{a}
T2	0.00 ± 0.000^{f}	3.26 ± 0.046^{c}	4.10 ± 0.052^{ab}	3.93 ± 0.064^{ab}
T3	0.00 ± 0.000^{f}	1.83 ± 0.046^{g}	2.13 ± 0.052^{e}	2.08 ± 0.049^{e}
T4	0.00 ± 0.000^{f}	1.84 ± 0.050^{g}	2.07 ± 0.049^{e}	2.01 ± 0.043^{e}
T5	0.00 ± 0.000^{f}	3.29 ± 0.062^{bc}	3.73 ± 0.046^{c}	3.35 ± 0.055^{c}
T6	0.00 ± 0.000^{f}	1.36 ± 0.040^{h}	1.58 ± 0.046^{g}	1.35 ± 0.046^{g}
T7	0.00 ± 0.000^{f}	2.06 ± 0.052^{ef}	2.18 ± 0.049^{e}	2.04 ± 0.052^{e}
T8	0.00 ± 0.000^{f}	1.20 ± 0.052^{e}	1.86 ± 0.046^{f}	1.51 ± 0.055^{f}
T9	0.00 ± 0.000^{f}	1.09 ± 0.055^{i}	1.21 ± 0.052^{h}	1.12 ± 0.049^{h}

* The numbers in the table are the average of three replicates and represent the mean values ± the standard deviation.

*Different lowercase letters within one column indicate significant differences ($p \leq 0.05$) between treatments.

T1: Control; T2: Whey Protein Membrane; T3: Membrane Reinforced with 0.25 mg Zinc Particles; T4: Membrane Fortified with Lactoferrin at a Concentration of 60 mg; T5: Membrane Fortified with 50 mg of Antamycin; T6: 0.25 Membrane Fortified with Zinc Nanoparticles at a Concentration of 0.25 mg + 50 mg of Natamycin: T7: Membrane Fortified with Zinc Nanoparticles at a Concentration of 0.25 mg + 60 mg Lactoferrin; T8: Membrane Fortified with Lactoferrin at a Concentration of 60 mg + 50 mg of Neptomycin; T9: Membrane Fortified with Zinc Nanoparticles at a Concentration of 0.25 mg + 60 mg Lactoferrin + 50 mg Natamycin.

Table 2: Effect of wrapping with whey protein films and treatment with different treatments on the number of lipolytic bacteria log/g in coated soft cheese stored for (21) days at a temperature of (5±2)°C.

The reason for the low rate of lipolytic bacteria in coated cheeses is due to the fact that the coating process contributed to preventing the proliferation of microorganisms [18], between Wahab et al. [19] that zinc nanoparticles (ZnONPs) are effective in killing Gram-positive and Gram-negative organisms, and the reason for the presence of Lipolytic bacteria indicate an increase in the percentage of fat in cheese resulting from a decrease in moisture content. Ollé Resa et al. [20] also indicated that when using natamycin and nisin with edible films and cheese packaging, there was stability in microbial growth, as well as changes in cheese samples, as well as with Duran and Khave [21]. The use of lactoferrin in the manufacture of meat, wine and dairy as a natural preservative that works to inhibit bacteria that cause spoilage, as well as to the new environmental conditions that were formed by the packaging itself, through its retention of gases, especially oxygen, which has a significant effect on the respiration process.The reason for the presence of lipolytic bacteria is due This leads to a high percentage of fat in the cheese and the storage period, in addition to a decrease in the percentage of moisture in the cheese samples.

4-3-3 Proteolytic Bacteria

Table 3, shows the total number of proteolytic bacteria in soft cheese. The results show that no growth occurred on the first day in all treatments, and with the continuation of the storage period, the growth process took place. On the seventh day, significant growth occurred for treatments T3, T4, T5, and T6. T7, T8, and T9 had growth rates of 1.59, 1.96, 3.46, 1.81, 2.27, 1.11, and 1.45 Log/g, respectively, compared with treatments T1 and T2, where they were 3.76 and 3.61 Log/g, respectively, and the results agreed with what Bonilla found and Sobra [22], indicating that the proteolytic bacteria did not show any growth during the first days of storage, that the low growth rate of the proteolytic bacteria

until the end of the 21-day storage period could be due to the whey membranes and their ability to trap gases, as well as the role of the nanocomposite as well. The most important reasons that led to the growth of proteolytic bacteria are due to the high percentage of protein in soft cheese with low moisture content, as well as the changes that occurred during the storage period that provide the appropriate conditions for growth. The end of the storage period is at 21 days and the temperature is (5 ± 2)°C. It was observed that the numbers of proteolytic bacteria increased significantly, reaching the highest rate at the end of the storage period for the treatments T3, T4, T5, T6, T7, T8, T9 were 1.97, 2.65 , 3.62 , 2.16 , 2.51 , 2.26 , 1.64 Log/g, respectively, these results converged with what was found by El-Sisi et al. [23] that the numbers of proteolytic bacteria were at 1.5-6.4 Log/g during the specified storage period, and it can be attributed to The reason for the low increase rates of proteolytic bacteria was due to the presence of AgNPs, ZnNPs and natamycin added to gelatin membranes, which proved their role in inhibiting the growth of microorganisms.

Transactions	Day 1	Day 7	Day 14	Day 21
T1	0.00 ± 0.000^{g}	3.76 ± 0.052^{a}	4.58 ± 0.043^{a}	4.66 ± 0.049^{a}
T2	0.00 ± 0.000^{g}	3.61 ± 0.058^{b}	4.16 ± 0.043^{b}	4.26 ± 0.038^{b}
T3	0.00 ± 0.000^{g}	1.59 ± 0.052^{h}	1.67 ± 0.043^{h}	1.97 ± 0.046^{j}
T4	0.00 ± 0.000^{g}	1.96 ± 0.046^{e}	2.34 ± 0.046^{f}	2.65 ± 0.043^{f}
T5	0.00 ± 0.000^{g}	3.46 ± 0.049^{c}	3.52 ± 0.046^{d}	3.62 ± 0.043^{d}
T6	0.00 ± 0.000^{g}	1.81 ± 0.043^{g}	2.08 ± 0.052^{g}	2.16 ± 0.043^{i}
T7	0.00 ± 0.000^{g}	2.27 ± 0.046^{f}	2.33 ± 0.052^{f}	2.51 ± 0.043^{g}
T8	0.00 ± 0.000^{g}	1.11 ± 0.043^{f}	2.15 ± 0.049^{g}	2.26 ± 0.038^{hi}
T9	0.00 ± 0.000^{g}	1.45 ± 0.049^{i}	1.55 ± 0.046^{h}	1.64 ± 0.046^{k}

* The numbers in the table are the average of three replicates and represent the mean values ± the standard deviation.

*Different lowercase letters within one column indicate significant differences ($p \leq 0.05$) between treatments.

T1: Control; T2: Whey Protein Membrane; T3: Membrane Reinforced with 0.25 mg Zinc Particles; T4: Membrane Fortified with Lactoferrin at a Concentration of 60 mg; T5: Membrane Fortified with 50 mg of Antamycin; T6: 0.25 Membrane Fortified with Zinc Nanoparticles at a Concentration of 0.25 mg + 50 mg of Natamycin; T7: Membrane Fortified with Zinc Nanoparticles at a Concentration of 0.25 mg + 60 mg Lactoferrin; T8: Membrane Fortified with Lactoferrin at a Concentration of 60 mg + 50 mg of Neptomycin; T9: Membrane Fortified with Zinc Nanoparticles at a Concentration of 0.25 mg + 60 mg Lactoferrin + 50 mg Natamycin.

Table 3: Effect of wrapping with whey protein films and treatment with different treatments Log/g number of proteolytic bacteria in coated soft cheese stored for (21) days at a temperature of (5±2)°C

In addition to the role of lactoferrin in inhibiting bacterial growth by seizing or restricting the iron element from it, or depriving it of the most important nutrients because of its iron-holding property, as well as the lactoferrin molecule is a glycoprotein carbohydrate characterized by an abundance of basic amino acids that possess amino groups -NH2, which are ionized and The interactions interfere or attract and form bonds with the anionic negative charges present or possessed by one of the components of the outer membrane of the bacteria, and this interference in turn leads to a change in the ability of the membrane to exchange and the natural permeability of the membrane permeability of the bacteria, meaning that the bacterial membrane is damaged and leads to its decomposition and as a result the exit and liberation of this component Natural in the membrane, which is a lipopolysaccharide, which, as a result, leads to the death of bacteria [24]. It also agreed with Kallinteri et al. [25], when using the antimicrobials Natamycin and Nisin together gave good results in extending the shelf life of Galotyri cheese. On the other hand, natural antimicrobial agents [18], and simple membranes treated with antimicrobial materials had an effect on the aerobic microorganisms present on the surface of the cheese, meaning that the activity of the proteolytic bacteria continued because the antimicrobial agents were not Able to migrate inside the cheese mold and remain confined to the organisms present on the surface of the cheese and prevent the development of these organisms, and thus the activity of the internal microorganisms that depend on the internal conditions of water activity and oxygen remains, and this is why the growth of proteolytic bacteria [26].

4-3-4 Yeasts and Molds

Table 4, shows the total number of yeasts and molds in soft cheese. We note from the results that no growth occurred on the first day as well as on the seventh day in all studied treatments of the sample of soft cheese coated with whey membranes loaded with ZnPNs, lactoferrin, and entomycin, which were coated without loading, and the uncoated control sample, and when continuing the storage process until the end of storage on day 21, yeast and mold growth was observed. In the treatments T3, T4, T9, they were 0.62, 1.19, 1.79, and 0.58 Log/g, respectively, which gave significant

differences when compared with the control treatments T1 and T2, as they were 2.41 and 1.14 Log/g, respectively, and when these treatments were compared with each other Where the best treatment was T3 in terms of significant decrease in the content of yeasts and molds from growth, as it reached 0.62 Log/g. between 4.70-2.20 Log/g, and the results also agreed with Henriques et al. [27] as they found a decrease in the number of yeasts and molds in cheese coated with whey proteins membrane with natamycin for 45 days at 11°C and 85% humidity compared to uncoated cheese, and also agreed with Matan [28] who confirmed the decrease in the number of molds of Aspergillus niger and Penicillium spp. of dried fish coated with whey proteins with natural oils and an increase in the storage life of the fish. It agreed with Isinmahan and Bostan [29] who found an increase in molds and yeasts from 1.66 to 3.28 UTC/g during storage of sauce coated with chitosan with acetic acid compared with uncoated samples in which the numbers increased from 2.33 to 6.72 UTC/g./gloom. It also agrees with Hao et al. [30] where they indicated that lactoferrin has many health benefits as well as antifungal and other antibacterial properties.

Transactions	Day 1	Day 7	Day 14	Day 21
T1	0.00 ± 0.000^{a}	0.00 ± 0.000^{a}	2.15 ± 0.052^{a}	2.41 ± 0.052^{a}
2T	0.00 ± 0.000^{a}	0.00 ± 0.000^{a}	1.03 ± 0.043^{b}	1.14 ± 0.046^{c}
T3	0.00 ± 0.000^{a}	0.00 ± 0.000^{a}	0.00 ± 0.000^{e}	0.62 ± 0.023^{e}
T4	0.00 ± 0.000^{a}	0.00 ± 0.000^{a}	0.00 ± 0.000^{e}	1.19 ± 0.038^{c}
T5	0.00 ± 0.000^{a}	0.00 ± 0.000^{a}	0.00 ± 0.000^{e}	0.00 ± 0.000^{f}
T6	0.00 ± 0.000^{a}	0.00 ± 0.000^{a}	0.00 ± 0.000^{e}	0.58 ± 0.040^{e}
T7	0.00 ± 0.000^{a}	0.00 ± 0.000^{a}	0.00 ± 0.000^{e}	0.00 ± 0.000^{f}
T8	0.00 ± 0.000^{a}	0.00 ± 0.000^{a}	0.00 ± 0.000^{e}	0.00 ± 0.000^{f}
T9	0.00 ± 0.000^{a}	0.00 ± 0.000^{e}	0.00 ± 0.000^{e}	0.00 ± 0.000^{f}

*The numbers in the table are the average of three replicates and represent the mean values ± the standard deviation.

*Different lowercase letters within one column indicate significant differences ($p \leq 0.05$) between treatments.

T1: Control; T2: Whey Protein Membrane; T3: Membrane Reinforced with 0.25 mg Zinc Particles; T4: Membrane Fortified with Lactoferrin at a Concentration of 60 mg; T5: Membrane Fortified with 50 mg of Antamycin; T6: 0.25 Membrane Fortified with Zinc Nanoparticles at a Concentration of 0.25 mg + 50 mg of Natamycin; T7: Membrane Fortified with Zinc Nanoparticles at a Concentration of 0.25 mg + 60 mg Lactoferrin; T8: Membrane Fortified with Lactoferrin at a Concentration of 60 mg + 50 mg of Neptomycin; T9: Membrane Fortified with Zinc Nanoparticles at a Concentration of 0.25 mg + 60 mg Lactoferrin + 50 mg Natamycin.

Table 4: Effect of packaging with whey protein films and treatment with different treatments Log/g on yeast and mold count in soft cheese wrapped and stored for (21) days at a temperature of (5±2)°C.

The reason for these significant differences between comparison samples and other samples is due to the use of anti-microbial agents loaded with whey membranes, which contributed to limiting the growth of yeasts and molds compared to treatments that did not contain them, as the nanocomposite and active compounds proved to destroy all hyphae and plaques of molds D Amato and Sinigagia [31].

The reason for the decrease in the number of the studied bacteria, yeasts, and molds is due to the correct conditions used in the cheese manufacturing process, following the sanitary conditions and the pasteurization process for milk, as well as the sterile laboratory conditions. It may also be attributed to the new environmental conditions that were formed by the packaging process itself, because of its importance in seizing Gases, especially oxygen, which have a significant impact on the respiration process on the one hand, and on the other hand, in the aquatic activity suitable for these organisms on the other hand, and this leads to a prolongation of the lag phase [32], as well as the role of nanoparticles that reduce or inhibit These organisms through their effect on the cell wall and this causes an increase in permeability and inhibition of respiratory enzymes and stopping the replication of the genetic material. As for the role of neptomycin in inhibiting these organisms, it is due to its direct association with ergosterol with its functional group (Mycosamine), which may lead to its immobilization and prevent functions normal cell and then leads to cell death [33-35].

It was observed through the study that the treatments that were coated with films treated with nanoparticles, lactoferrin, and entomycin with added concentrations resulted in high effectiveness in eliminating and reducing the growth of the studied microorganisms, as well as the effectiveness of the whey protein membrane in preserving samples from external conditions, which led to a prolongation of storage life compared to other treatments. Others are uncoated or coated without any additives, and this indicates that whey protein membranes are highly effective and advantageous in this field.

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