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# Preparation of a nanolaminate of alginate and nisin in LBL method and its use in prolonging shelf life of Cheddar cheese

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## ABSTRACT

This study was carried out to evaluate the preparation of five-layer nanolaminates that were introduced by Layer By Layer (LBL) technique by the use of two solvents: the sodium alginate and the other antimicrobial agent, Nisin. The halo diameter was estimated on a petri dish containing *Staphylococcus aureus* and *Bacillus subtilis* or negative gram bacteria *E. coli* and *Pseudomonas aeuroginosa, Enterococcus ssp. Aspergillus niger* and *Fusarium* were used to estimate the effectiveness of antimicrobial extracts. The results showed that 0.2% concentration of nisin solution showed inhibitory activity against these microorganisms. The scanning electron microscope was used to detect the thickness of prepared nanolaminates, The total thickness of the alginate and nisin was 119.55 nm , The Zeta Potential of the alginate solution was -28.49 mV at pH 7 and the nisin solution 42.00 mV, The WVP water permeability values for the nanolayered PET film without any addition to the charged PET (treatment 1) were 29.091 g.m²/24h) and for the nanolayered PET-charged for sodium alginate and nisin solution 58.182 g.m²/ 24h, OTR was obtained for the nanolayered with no addition of the charged PET, 14.78 ml / m².day), and for the PET-charged, covered with sodium alginate and nisin solution 29.61ml / m².day. Three treatments were made of cheddar cheese, the first treatment was covered with the paraffin wax as control T1, the second was covered with gelatin (T2) and the third was coated with a nanolayered film consisting of the sodium alginate and the nisin solution (T3). The results showed a significant decrease in the moisture content and the correct acidity of the treatment T3 and evolution in the values of ADV by the duration of maturation and the body of the nisin solution in the nanolayered was determined from the microbiological growth of the treatment (T3), making it superior in the sensory characteristics of the comparison treatments.

**Keywords:** Nisin, Nanolaminate, Cheddar cheese.

## 1. INTRODUCTION

Nanotechnology is derived from the Greek word "nanos", meaning dwarf. Nanotechnology has been defined as a technique for existing materials, systems and processes that operate on a scale of 100 nanometers (nanometers) or less, or nanotechnology [1], Nanotechnology is one of the most promising ways to develop technology in materials and industry for the 21st century. Nanoscience is the study of the basic principles of molecules and compounds that do not exceed 100 nanometers. The diameter of the head hair is approximately 75,000 nm, and the red blood cell is up to 2000 nm. It is the boundary between the world of atoms and molecules and the world of water. Crow, or the particles and particle sizes within the nano (1-100) nm [2, 3], Nanoscience involves controlling substances and creating structures and systems on the scale of atoms and molecules. Nanotechnology is also a way of controlling matter at near atomic standards to produce unique or improved materials, products, and devices [4].

The progress of nanotechnology has opened hopes for food packaging by increasing conservation time, as packaging has shown more safety than normal

packaging and food has become healthier [5], The need to respond specifically to the need to reduce Packaging and transformation instead of producing alternative materials / technologies for packaging of food products that have a relative effect on environmental pollution [6].

Nisin is one of the most important bactericides produced from lactic acid bacteria. It consists of 34 amino acids with a molecular weight of 3354 dalton and has inhibitory effect against a group of microorganisms and is widely used in food preservation, especially cheese [7, 8]. and it is effective against most of the Gram-positive bacteria, including *Lactococci, bacilli, micrococci, Staphylococcus aureus, Listeria, Clostridium botulinum* and does not appear to be effective or low-effective against Gram-negative bacteria[9]. Nisin was discovered in 1928 and the Food Standards Committee of WHO / FAO confirmed that Nisin was a safe substance in 1969 and was permitted to be used. In 1988, Nisin was safe from the FDA and It has been used in more than 50 countries, including the United States of America, which is one of the most stringent countries in the legislation of food additives and not exceeding 10000IU /g in the conservation of a number of food products [7], The Layer By Layer (LBL), Which consists of electrostatic self-assembled layers on the surface of the material, is the technology used in the distribution of the nanolaminate, applied in various fields such as biomedicine and food processing, the Layer By Layer (LBL) technology has been proposed as a suitable method for obtaining a nanolaminate It is made up of natural polymers as the material is used to consume the nano-sensitive casing food packaging, which must be electrostatic charged with important functional properties including antibiotics, antioxidants and functional gas tolerances [10], The use of nanolaminate for food packaging has been tested on fruits [11] and has never been used on processed cheese, which is a complex food product consisting mainly of water, casein and fat as well as a large consumption product. (LBL), made up of sodium alginate with nicotine for the purpose of cheese packaging, [12] pointed out that the nanolaminate consists of two or more layers of materials with nanoparticles that are associated with some Chemical or physical bonds that improve the properties of water retention starch, which enters into the covers edible food to protect against Microorganisms taking into account the affected properties cover the functional water content industry.

A multilayered nanolaminate casing was produced by LBL by coupling Chitosan with cashew gum through electrostatic reactions and by the success of the cover through the results of the Zeta and FTIR effort. These nanoparticles can be assembled and stabilized from covalent bonds in an alternative to conventional electrostatics and expand their potential in biomedical, Food industries, or environmental applications [13].

## 2. MATERIALS AND METHODS

### 2.1 Nanolaminate Preparation

Nanolaminate was prepared for the purpose of labeling it in two phases. The first phase included polymer polyethylene packaging (PET), which was obtained from Sigma Aldrich/USA as a supporting membrane for coating solutions [14]. The Agar well diffusion method was used by [15] using the Muller Hinton agar medium to measure the effectiveness of antimicrobial extracts. The characterization process was performed on the UV spectrum as described by [16] (FIR), Zeta Potential [17], WVP measurement according to [18], (OTR) according to [19], the cheese was made according to the prescribed method of [20] and kept in the refrigerator at 10°C ± 2°C for 6 months. The above steps were followed to coat the cheese samples using the Dipping method to prepare the T3 treatment. T1, T2, T1 and T1 were used in paraffin wax at 118°C for several seconds and then remove to dry, T2 was coated with gelatin. After drying the cheese pieces, the gelatin solution was submerged after the packaging process was completed. The models were placed in sterile sealed plastic containers and stored in the refrigerator at 10 °C ± 2 for 6 months for chemical, microbial and sensory tests. After drying, the previously weighed models were weighed to estimate the ratio of the coating material to the weight of the coated cheese. In order to follow changes in loss of moisture in storage and under storage conditions, the refrigerator temperature is ± 10°C and the relative humidity is (54 ± 1 % and 75 ± 1%) By using a humidified liquid with saturated sodium bromide solution to obtain the relative humidity of 54 ± 1% or saturated sodium chloride to obtain moisture of 75 ± 1% according to the method mentioned from [21] Monthly and for 6 months Storage For cheese, the cheese samples were taken in periods 0, 1, 2, 3, 4, 5, 6 months and 75 g for each model. The samples of some chemical tests were placed in polyethylene bags and sealed and kept at a temperature (- 20°C) until the required analysis.

The percentage of moisture for cheese was estimated before the plating process and during the maturation stage of the cheese according to the method [22] and modified by. [23] and the ash in the Muffle furnace, the fat content of the cheese was estimated according to [24] and the pH of the cheese according to the method mentioned in [25]. The acidity according to the method described in AOAC [26] was estimated as total nitrogen nitrogen [22]. The protein ratio was calculated by multiplying the total nitrogen ratio by 6.38, The soluble nitrogen (SN) was calculated according to the method described in [27] and completed digestion Distillation according to the method mentioned above [22], estimated non-protein nitrogen according to the method mentioned in [27] and modified from [28] and completed the process of digestion and distillation according to the method mentioned by [22], the acid density value (ADV) method was determined by Bureau of Dairy Industry (BDI) of [29], the method of dishwashing in [30] using the nutrient agar medium in estimating the total number of animals and use the PDA medium to estimate the number of yeasts and yeasts.

The center of salt agar Mannitol was used to estimate the number of *Staphylococcus aureus* [31] and used the Blatimore Biological Laboratory (BLL) Milk agar according to the method described in [32] to estimate the number of protolytic bacteria using food center (100-cm $^3$ Nutrient agar +1 g of sun flower oil + emulsion) and use food center Nutrient agar to estimate lipolytic bacteria in the cold following the method mentioned in the [30], These tests were performed after manufacturing and monthly through storage. The statistical evaluation of the cheese samples was conducted by experienced evaluators and the proposed cochlear evaluation form was used by [33]. The Statistical Analysis System (SAS) [34] was used in the analysis of the data to study the effect of the different factors in the studied traits (CRD) and the probability level of 0.05.

## 3. RESULTS AND DISCUSSION

The effect of the addition of nicotine at 0.2% (weight/volume) after addition to the used alginate in the preparation of the multilayered nanolaminate was studied in inhibiting the growth of a group of bacteria and the fungus and its use in the cheese packaging for the purpose of studying the effect of this effect in reducing the growth of Gram positive. The results in Table (1) showed that the nisin solution at a concentration of 0.2% showed a negative effect in the Gram negative bacteria, as the diameter of the inhibition of the *E. coli* and the *Enterococcus ssp* bacteria was 15 and 22 mm respectively. The inhibitor diameter of the growth of Gram positive bacteria increased by 25 mm for *Staphylococcus aureus* and Bacillus bacteria, The effect of nisin in inhibiting the growth of Gram-positive bacteria is due to its effectiveness in fracturing the type-1-4-ß-erythrocyte matrix that binds the N-acetyl muramic acids and acetyl glucose amine N-constituents of the peptidoclacane layer, breaking the cell wall and destroying it. Inhibiting the growth or discontinuation of these bacteria [35]. These results are consistent with [36] that nisin inhibits a large group of Gram positive bacteria causing food damage, including the constituent bacteria of spores and some negative bacteria, In yeast and mold, results also showed to check that the solution nisin concentration of 0.2 mg /l also had an effect on the effectiveness of molds *Aspergillus niger* and *Fusarium* diameter, the rate has reached a halo of inhibition of two 25 mm, and these results are consistent with [37], which showed that the addition of lysozyme to the casein or gelatinous causing inhibited the growth of Gram positive bacteria except for *Staphylococcus aureus* when lysozyme was used at 1 mg /L concentration in both the casein and gelatin casings. The diameter of the halo inhibition of bacterial growth Positive for the chromium dye in general when increasing the concentration of lysozyme to 2 mg /l added.

**Table 1:** Effect of nisin in the effectiveness of microbiology.

| Species | Name | The rate of the inhibition diameter (mm) of the nisin solution |
|---|---|---|
| **Gram negative bacteria** | ***E.coli*** | **15** |
| | ***Enterococcus ssp*** | **22** |
| | ***Pseudomonas aeuroginosa*** | **–** |
| **Gram postive bacteria** | ***Staphylococcus aureus*** | **25** |
| | ***Bacillus subtillis*** | **25** |
| **Molds** | ***Aspergillus niger*** | **25** |
| | ***Fusarium*** | **25** |
| *** 4.82 LSD** | | |

* The numbers in the table represent a rate for repeaters.

Using the FTIR infrared measurement to confirm the presence of active groups on the surface of PET as in Fig. 1, the zeta potential of alginate and nisin solutions was calculated based on its comparison with the supporting PET surface, reaching the solution of alginate – 28.49 mV at pH = 7 and nisin solution 42.00 m The values of the alkyla groups These results are consistent with what found [10], where the values of the Zeta potential -62.13 ± 4.10 mV for the sodium alginate solution were based on pH = 7 and -58.28 ± 4.18 mV for Chitosan solution at pH = 3.8 which showed that it could interfere with the pH Electrostatic and lysozyme was 29.27 ± 3.18 mV at pH = 3.8 the presence of the Secretary totals as well as with [38], where the values of the Zeta voltage for alginates were 62.13 mV at pH =7 and for lysozyme at 2.27 ±25.67 mV at pH = 3.8. Figure 2 shows an increase in absorbance at a wavelength of 260 nm by depositing the five layers of alginate and nisin on the surface of the charged PET. As the absorption value increases by depositing layer after layer, this confirms the successful deposition of multiple layers, More for these materials [14] These results are consistent with [10] who also found a significant increase in absorption values by depositing five layers of alginate and chitosan on the charged PET surface. SEM images confirmed that the nanolaminate was constructed on the charged PET surface; Also mentioned [39] showed that the increase in the number of nanolaminate layers caused a significant increase in optical absorption values. The results of Fig. 3 (WVP)

indicate that the water vapor permeability of the film without any addition to the charged PET only (transaction 1) is (29.091 g.m²/ 24h) and for PET charged and covered with alginate and nisin solution (treatment 2) (58.182 g.m²/24h),The OTR value of the nanolaminate was obtained as in Fig. 4 the covered without any addition to the charged PET (transaction 1) was (14.78 ml / m².day) and to the PET loaded and encapsulated with the gene and the nisin solution (Treatment 2) (29.61ml/m².day).

The values of both WVTR and OTR for the nanolaminates were within the mean of the recorded values. The affinity of treatment 2 (nisin and alginate) is observed from the comparison treatment. The multi-electrolytic interaction between the antimicrobial strata and the compatible alginate and the amino acids The malignant pathway can be formed between the layers of alginate, which form the nanolaminate, which reduces or permits the molecules of water and oxygen. This is in line with what was stated [10; 39; 40]. Figure (6) shows the pictures of the microscopic electronic survey (SEM) for different spectra of the multilayered nanolaminates deposited on the layer by layer (LBL) PET. The alginate layer, which represents the upper surface layer, is soft and crystalline and the appearance of multiple layers and different sizes for these covers in the first cover, Figure (a) showed that the thickness of the three alginate layers was 7.8, 8.8 and 10.10 nm, The thickness of the two nisin layers was 50.7 and 90.32 nm while the total thickness was 167.72 nm. The results agree with [10] that the thickness of the five-tiered nucleus of the alginate and chitosan, and physical physics 121.28 nm. [40] reported that the thickness of the total nanolaminates obtained from the integration of 3 layers of alginate including two layers of lysozyme was 198.2 nm and The results agree with [41] that the size of nanoparticles should not exceed 500 nm.

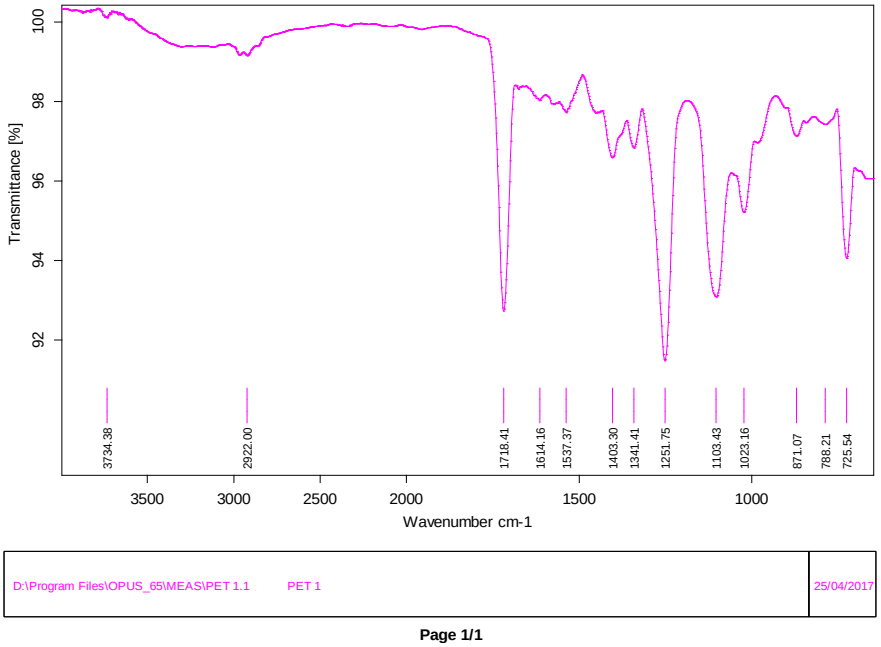


**Figure 1:** Analysis of the FTIR spectrum of the PET loaded and coated with alginate and nisin.

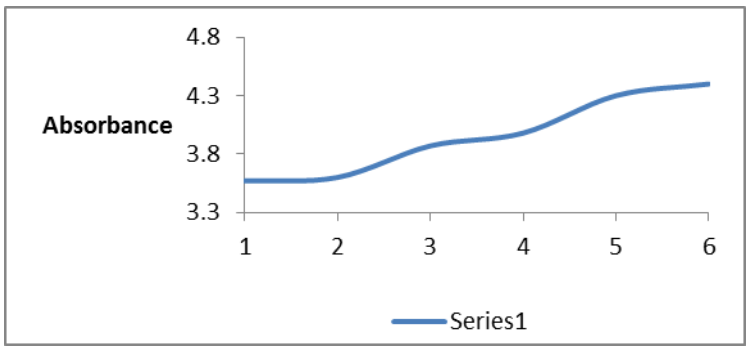


**Figure 2:** Series 1= Absorbance for nisin solution

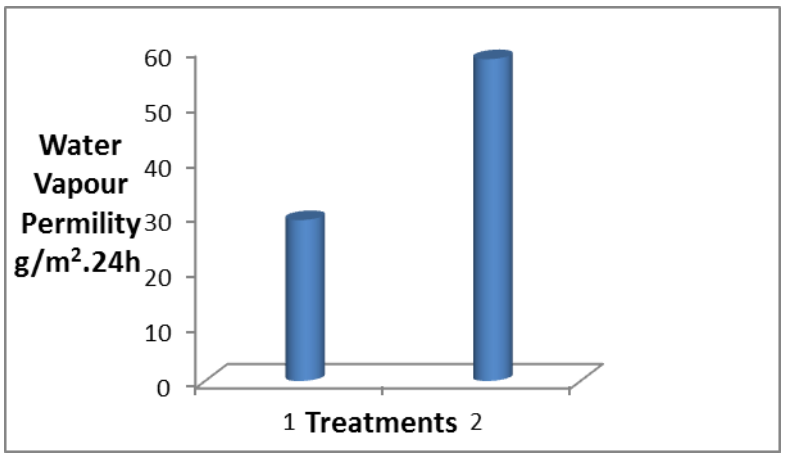


**Figure 3:** Water Vapor Permility for the prepared nanofilm (g / m².24h)

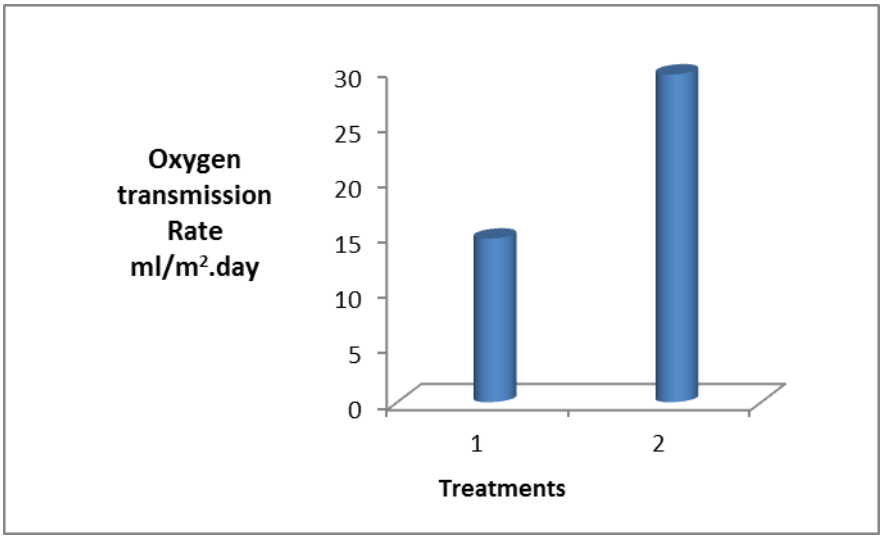


**Figure 4:** Oxygen Transmission Rate for the prepared nanofilm (g / m².24h)

Three treatment coefficients of cheese samples were performed, including treatment T3, in which the multicellular nanolaminates were synthesized with a 0.2% concentration of nano-protein nanolaminates, as well as the first control of the cheese in the paraffin wax. It promised to treat T1 negative control and the other wrapped cheese with a gelatin cover containing sorbitol as a plasticizer with a concentration of 30% of the dry weight of the gelatin and a positive T2 control treatment, all of these treatments were subjected to chemical, microbial and sensory tests and as follows:

The results shown in Figure 6 show a decrease in weight loss at P <0.05 level from zero to 6 months. The results shown in Fig.6 show the lipid-coated lipoprotein (T1), gelatin film gelatin Treatment (T2), the nanoparticle cover made of alginate and nisin (treatment T3) is lower than that of waxed cheese coated with gelatin cover, This is in combination with multiple sugars and / or proteins that reduce the loss of mass of cheese. This corresponds to [40], which showed a significant reduction in mass loss in 'Coalho' Brazilian cheese of non-coated cheese (comparison treatment) from day to day until 20 days of packaged cheese at the end of the storage period.

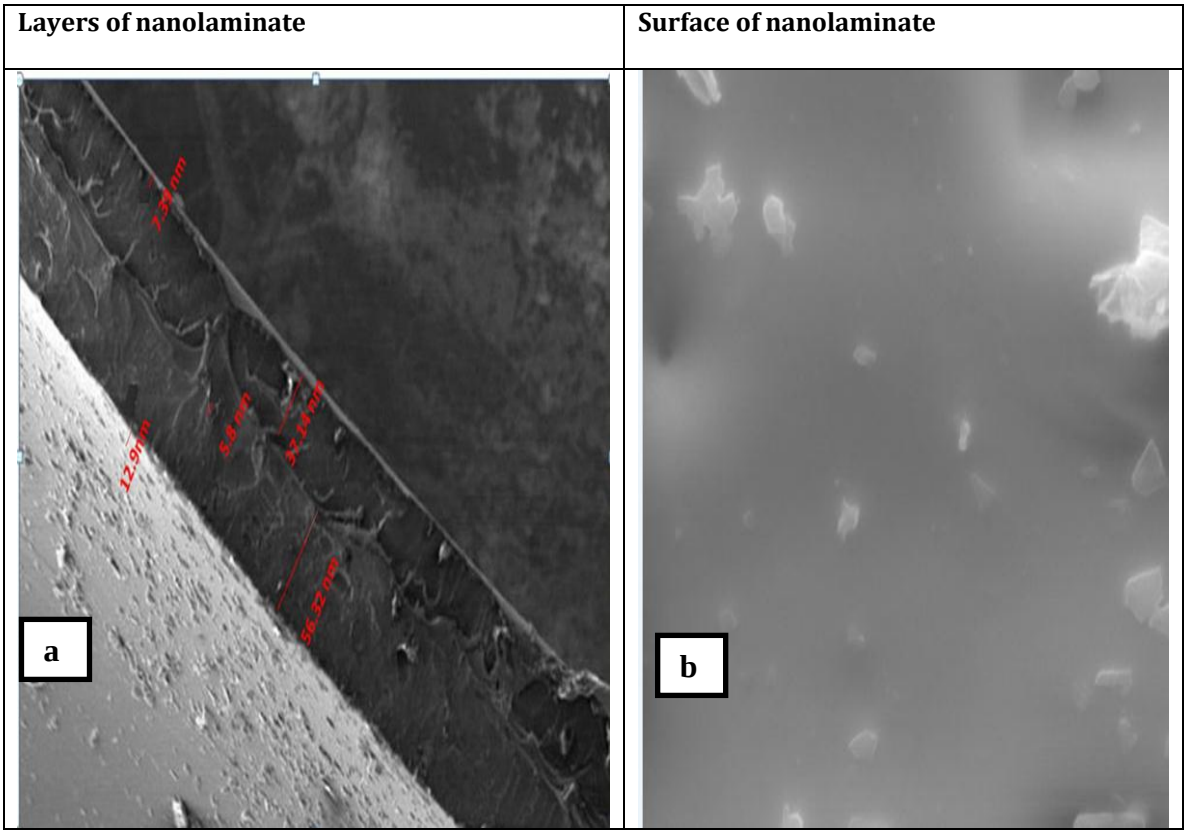


**Figure 5:** (a) A picture of the nanolayers of the alginate and nisin cover and (b) of the surface using the Scanning Electron Microscope SEM

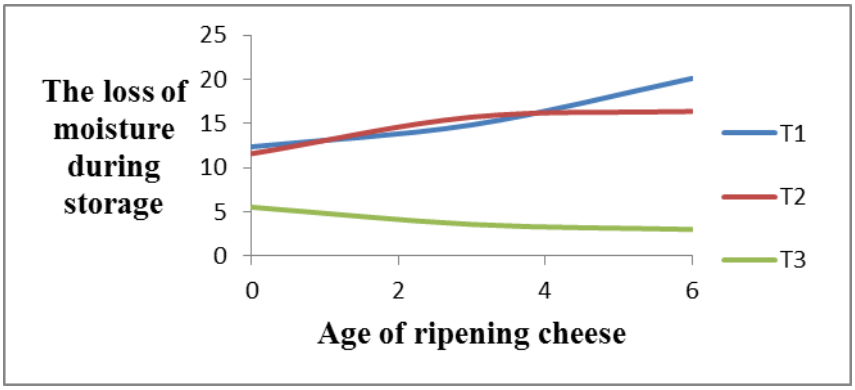


**Figure 6:** Moisture loss during storage in the nanolayers coated of nisin during the 6-month maturation period and at 10 ± 2 ° C

Table (1) shows that moisture content values for all models decreased with age of evaporation due to evaporation. Humidity decreased to 31.00%, 29.00% and 31.00% in T1, T2 and T3 respectively. The differences were minor among the packaging models, and these percentages are within the limits of the moisture content set by the Iraqi standard [42] for dry whey, which is 30-40%, This is due to the moisture content of the moisture content during the storage period and this is supported by [43; 44], and increase in the ratio Percentage of fat in the treatment with the progress of maturation as the readings show that the proportion of fat in all coefficients of cheese was close, ranging between 28.00% - 33.50%, and may be due to this slight difference in fat ratio to the difference in the proportion of moisture content between these factors and Comparing these results with what other researchers have found, they are consistent He was found by [45], [46] and [47].The results showed that the percentage of protein at the time of zero was 27.60%, 27.00% and 27.49% for T1, T2 and T3 respectively, while the percentage of protein in the sixth month of maturation was 30.86%, 30.09% and 30.00% [44] and [48] showed a difference in the

percentage of protein between the different treatments due to differences in moisture content values and the chemical composition of the microbial agents added to the casing and the existence of the protein analysis bacteria. 2.617, 2.629 and 2.643% for T1, T2 and T3 respectively, and the percentage of ash increased The ratio of T1 to 3.5673 and T2 to 4.039 and T3 to 3.845 was the same as [50] of the high ash ratio with age, The results were identical to those reported by [51] from the increase in ash percentage (T3) from 0.70 to 0.90%. In the cheese coated with red paraffin (T1), it increased from 0.77 to 0.88% and in gelatin coated cheese (T2) from 0.75 to 0.85%. These results are consistent with the findings of [50], which indicated an increase in acidity in the maturation of cheese ripening due to lactic fermentation, as well as the results of [53] and [54] during the storage period of 6 months.

The results of the statistical analysis showed a significant difference (P <0.05) in acidity values at age 1 and 6 months between gelatin coated with gelatin and coated with gelatin cover, pH reduction in T1 from 5.45 to 5.15 and T2 from 55.5 to 5.20 Treatment T3 from 5.45 to 5.10. The results showed that the pH level during the maturation period of the alginate-nisin-coated alginate cheese was between 0.64-3.00 indicating that there were no significant differences in values between all the treatments at the beginning of the ripening period. As the maturation process progressed, The ADV was the most significant change in the value of the ADV in T1 and T2, at 4.80 and 5.00 Ml equivalent/100 g fat While the evolution was lower in nano-encapsulated cheese processing, it indicates the role of the casing in moisture retention within the cheese block, providing a more favorable environment for initiatory bacteria activity, particularly for aquatic activity (aw) to produce enzymes, especially lipid-converting enzymes. As found in a number of studies conducted by [53], [49] and [47].

**Table 2:** Total Composition of nanolayers Coated of Nisin during the 6 Month Maturation Period and at 10 ± 2 °C

| Treatments | | Moisture | Fat | Protein | Ash | Acidity | pH | ADV |
|---|---|---|---|---|---|---|---|---|
| **T1** | **0** | 38.66 | 28.00 | 27.60 | 2.617 | 0.77 | 5.45 | 0.67 |
| | **3** | 36.94 | 29.00 | 25.56 | 2.845 | 0.83 | 5.22 | 1.32 |
| | **6** | 31.00 | 30.00 | 30.86 | 3.567 | 0.88 | 5.15 | 4.80 |
| **T2** | **0** | 38.70 | 29.00 | 27.00 | 2.629 | 0.75 | 5.55 | 0.65 |
| | **3** | 34.12 | 30.50 | 30.00 | 3.064 | 0.80 | 5.35 | 1.35 |
| | **6** | 29.00 | 33.50 | 30.09 | 4.039 | 0.85 | 5.20 | 5.00 |
| **T3** | **0** | 38.70 | 28.00 | 27.49 | 2.643 | 0.70 | 5.45 | 0.64 |
| | **3** | 36.70 | 29.00 | 29.45 | 2.215 | 0.84 | 5.32 | 1.93 |
| | **6** | 31.00 | 33.00 | 30.00 | 3.845 | 0.90 | 5.10 | 3.00 |
| LSD | | *4.77 | *4.18 | *2.33 | *0.971 | 0.753 NS | 0.281 NS | 0.341 NS |

*P<0.05

Table (3) shows the percentages of soluble nitrogen and non-protein nitrogen for nanoparticles covered with alginate and nisin, as well as the paraffin coated cover (T1) and gelatin (T2). The SN ratios each for T1, T2 and T3 were at the beginning The process of maturation was 0.26%, while at the end of the maturation of 1.10, 0.981, 0.926% for the above treatments respectively, as well as with the non-protein nitrogen NPN, which at the end of the process of maturation of all transactions ranging between 0.98 to 0.920%,The reason for the evolution in the percentages of SN and NPN is based on the added promoter proteases as well as the rennet proteins used in the cheese industry. These results were consistent with the finding of the high percentage of soluble nitrogen and non-protein nitrogen the duration of ripening due to the role of enzymes for the analysis of protein produced from bacteria and other initiates in the analysis of cheese proteins.

**Table 3:** Percentage of soluble nitrogen and dissolved nitrogen of total nitrogen and non-protein nitrogen N-protein of total nitrogen in nanolayers for nisin during the 6-month maturity period and 10°c ± 2.

| Treatments | | SN | SN/TN | NPN | NPN/TN |
|---|---|---|---|---|---|
| **T1** | **0** | 0.26 | 6.01 | 0.19 | 4.935 |
| | **3** | 0.85 | 16.84 | 0.78 | 18.35 |
| | **6** | 1.10 | 22.77 | 0.98 | 20.28 |
| **T2** | **0** | 0.26 | 6.14 | 0.183 | 4.320 |
| | **3** | 0.87 | 18.51 | 0.802 | 17.06 |
| | **6** | 0.981 | 20.28 | 0.912 | 19.40 |
| **T3** | **0** | 0.26 | 6.23 | 0.185 | 4.30 |
| | **3** | 0.844 | 18.30 | 0.832 | 18.04 |
| | **6** | 0.926 | 19.70 | 0.920 | 19.57 |
| LSD | | 0.558* | 5.416* | 0.492* | 4.198* |

*P<0.05

Table 4 shows a decrease in the total number of microorganisms in niacin-coated cheese after the 6-month storage period in the treatment of the cheese coated with Nisin T3 decreased by approximately two logarithmic cycles and 1 µg / g compared with the two comparison treatments for the waxed cheese The total number of bacteria was 3.2 x $10^7$ and 5.41 × $10^6$ cfu / g for T1 and T2. The reason for the decrease in the total number of bacteria is due to the combined effect of both the effect of microbial agents and the packaging process On the other hand, whether as an anti-abrasive agent J towards microscopic bacteria positive for the dye cram or the combined effect against negative bacteria

The high level of contamination in treatment (T1) may be due to cracks in the wax cover, as well as the lack of antimicrobial agents, as the process of packaging alone contributes to the prevention of reproduction of airway by preventing oxygen entry, Which reduced their numbers or lengthened the phase of their growth, thus reducing the growth rates of bacteria. Oxygen also plays an important role in controlling aerobic growth through the large role it plays in the water activity (aw) necessary for the activity of these microorganisms. [54] Suggests that some organisms such as *Staphylococcus aureus* have their minimum requirements of water activity (aw) dependent on the concentration of oxygen

In anaerobic conditions, the minimum water activity (0.91) is minimal while in aerobic conditions (0.86).The effect of antimicrobial agents is through the effectiveness of these factors in inhibiting the growth or prevention of these organisms. For example, lysozyme is a strong antagonist for the growth of Gram positive bacteria, but it has no direct degradation effect of the Gram negative bacteria. This supports [55].

The lipid and protein-containing bacteria, including lead bacteria, were not affected by inhibitory activity. At the end of the ripening period, the number of lipid-tolerant bacteria was 9.1 x $10^2$ cfu / g in T3, 2.0 × $10^2$ and 1.4 × $10^3$ for T1 and T2. [56], noting that some strains of lactate-lactic acid, proteins and acid-producing proteins, such as *Lactobacillus delbrueciii ssp*, were not affected. *bulgaricus, Lactobacillus lactis*, and *Lactococcus lactis*, as well as It is also due to the role of packaging in restricting antimicrobial agents, including nisin [57]. The bacteria and the kidneys did not notice any growth during the maturation stages in the treatment of alfalfa cheese, which was coated with a membrane containing antimicrobial agents (T3) (Table 4), indicating that the use of nisin with these casings contributed to reducing the growth of the molds compared to the treatments with which these factors were not used (Table 4), which played an important role in preventing the growth of bacteria and therefore is widely used in food preservation, and Milena), Table (4) showed the microorganisms growing in the cold after storage time (P<0.05) in the cheese coated with the wax and gelatin cover of the cheese coated with nisin, in the cheese coated with wax and cheese coated with gelatin cover from 6.8 × $10^2$ to 1.3 × $10^2$ cfu /g and from 6.6 × $10^2$ cfu /g to 1.3 × $10^2$ cfu/ g respectively, while the cheese coated with alginate and nisin was 8.0 to 4.8 × $10^2$ for the coefficients T1, T2 and T3, respectively, the lower total numbers of microorganisms growing in the cold in cheeses coated with anti-microbial growth factors have already been associated with bacterial resistance to nicotine Which allows the degradation of peptidoglycan, causing cell wall dissolution and the $O_2$ carrying properties of coagulated cheese, which reduced the rate of $O_2$ transmission and its manufacture with the lowest growth of fungi [58].These results were identical to those of [59] to reduce the microbial contamination of the Italian Stracciatella cheese and are consistent with [38] and [60]. The results (Table 4) indicate that the differences in the number of migratory organisms between the nano-coated cover (T3) and nisin-tolerant cheese samples were somewhat minor and within the permissible limits of this type of cheese indicating that cheese industry is acceptable in terms of nutrition and health.

**Table 4:** Results of microbiological tests cfu/gm in the treatment of cheddar cheese for nisin during the 6-month maturation period and at 10°C ± 2 at the beginning and end of ripening.

| Treatments | | Total Count | *E. coli* | Molds | Lipolytic | Protolytic | Psychrophilic |
|---|---|---|---|---|---|---|---|
| T1 | **0** | **$10^4$ ×6.3** | Nill | Nill | **$10^2$ ×0.4** | **$10^2$ ×1.2** | **$10^1$ ×6.8** |
| | **3** | **$10^5$ ×4.8** | **$10^1$ ×5.70** | Nill | **$10^2$ ×1.0** | **$10^2$ ×3.0** | **$10^1$×7.7** |
| | **6** | **$10^7$ ×3.2** | **$10^2$ ×1.21** | **$10^2$ ×3.0** | **$10^2$ ×2.0** | **$10^3$ ×1.2** | **$10^2$ ×1.3** |
| T2 | **0** | **$10^3$ ×4.1** | Nill | Nill | **$10^2$ ×4.6** | **$10^2$ ×5.0** | **$10^1$ ×6.6** |
| | **3** | **$10^5$ ×5.12** | **$10^1$ ×4.4** | Nill | **$10^2$ ×7.5** | **$10^2$ ×8.1** | **$10^1$ ×7.5** |
| | **6** | **$10^6$ ×5.41** | **$10^1$ ×9.92** | **$10^2$ ×2.0** | **$10^3$ ×1.4** | **$10^3$ ×1.5** | **$10^2$ ×1.3** |
| T3 | **0** | **$10^5$ ×2.91** | Nill | Nill | **$10^2$ ×9.1** | **$10^2$ ×9.6** | **$10^1$ ×8.0** |
| | **3** | **$10^3$ ×2.20** | Nill | Nill | **$10^2$ ×7.3** | **$10^2$ ×7.7** | **$10^1$ ×7.30** |
| | **6** | **$10^2$ ×1.4** | Nill | Nill | **$10^2$ ×4.5** | **$10^2$ ×4.8** | **$10^1$ ×4.8** |
| LSD | | ***87.31** | ***22.64** | ***36.91** | ***3.77** | ***19.61** | ***12.63** |
| | | | | *P<0.05 | | | |

* The numbers in the table represent a rate for repeaters.

Figure (6) shows the images of gelatinous gelatin (T2) and Nisin-containing capsule (T3) taken at the beginning and end of the ripening period. Table (5) shows the results of the sensory evaluation of the cheese-processing treatments, which included the cheese wrapped in the wax cover (T1) (T2), T2-encapsulated gelatin cover containing Nisin T3 over the course of 6 months The high grades given to flavor and

taste of the nano-coated cheese model and the maturation process in these models Free of odors and exotic odors, This is due to the role played by the antimicrobial agents added to the nanolaminate despite their small quantities in preventing pollution and thus preventing the production of undesirable taste such as calories, rotten and acidic taste, and other factors leading to the highest grades of these models, while noting the low degrees The evaluation of the two comparative models for taste and flavor was shown by the scores obtained by this score, which reached 6.0 and 6.0 at the age of 5 and 6 months This may be due to the action of proteolytic enzymes, which are the result of the high microbial load in cheese for these treatments because they do not contain antimicrobial agents, as well as some short-chain peptides produced by renal proteolysis and proteolytic enzymes, which are derived from the starting bacteria [59].

In terms of strength, appearance, cohesion and adhesion, the similarity of the cheese samples in the coefficients was observed to some extent in the first months of maturation. However, with the progress of ripening, especially in the last month, the grades for these traits were relatively low in the treated treatment T1 and T2, The grades obtained from these qualities were relatively high in the nanocoated cheese (T3) as the ripening stage progressed. This may be due to the role played by the casings in reducing moisture loss and thus providing an aquatic environment suitable for the work of the initiator bacteria In turn increases the level of protein and fat degradation which is reflected in improving the strength and consistency of the appearance of cheese during the ripening period, and this is consistent with what [60].

As for the separation of fat, there was no separation of fat in treatment T3, while T2 treatment was observed in fat separation in the last month of maturation as well as for treatment T1. This may be due to the role of substances and components of multiple packaging in the oxygen storage, and the consistency of hydrogen bonding between polymer chains, which encourages the use of these packaging in food preservation, particularly those susceptible to oxidative damage [31].

The results of the sensory evaluation of Table (5) indicate that there was slight growth of the leaves on the cheese surface of T1 and T2 in the last month of maturation. The coagulant cover model containing the antimicrobial agents (T3) showed no growth of the eggs during the maturation period, and these results are consistent with [38] when using the lysozyme nanoparticle in Brazil's Coelho cheese packaging. Fungi contamination was observed in the free-cover comparison cheese at the age of 20 days, while no growth was observed in the coated cheese Nanoparticles

The results are consistent with [61] that the addition of lysozyme has improved the antibacterial effect of the casing and with what [62] found that the covers containing chitosan and lysozyme used in mozzarella cheese packaging had been reduced by microorganisms, especially *E. coli*, *P. pylori, P. fluorescence* and *L. monocytogenes* bacteria. These findings indicate the possibility of using an anti-microbial protective cover in the cheese packaging as well as the anaerobic conditions provided by the packaging which also contribute to preventing the growth of the mold on the cheese surface as an aerobic microorganism.

**Table 5:** Results of Sensory Evaluation in nanolayers coated of cheddar cheese during the Maturation Period of 6 Months and at 10°C ± 2 at the Beginning and End of Maturation.

| Treatments | | Appearance | Cohesion and adhesion | Textures | Taste and flavor | Separation of fat | Growth of the molds |
|---|---|---|---|---|---|---|---|
| T1 | **0** | 9 | 9 | 10 | 9 | 10 | 10 |
| | **1** | 10 | 9 | 10 | 9 | 10 | 10 |
| | **2** | 9 | 10 | 10 | 10 | 10 | 10 |
| | **3** | 9 | 9 | 9 | 8 | 10 | 10 |
| | **4** | 8 | 6 | **8** | 6 | 10 | 10 |
| | **5** | 8 | 7 | 7 | 6 | 10 | 10 |
| | **6** | 6 | 5 | **7** | 6 | 10 | 8 |
| T2 | **0** | 9 | 9 | 10 | 9 | 10 | 10 |
| | **1** | 10 | 9 | 9 | 8 | 10 | 10 |
| | **2** | 9 | 10 | 10 | 9 | 10 | 10 |
| | **3** | 8 | 9 | 9 | 8 | 10 | 10 |
| | **4** | 9 | 9 | 9 | 8 | 7 | 10 |
| | **5** | 8 | 6 | 8 | 7 | 7 | 10 |
| | **6** | 7 | 6 | 7 | 6 | 6 | 10 |
| T3 | **0** | 10 | 9 | 10 | 10 | 10 | 10 |
| | **1** | 9 | 9 | 9 | 9 | 10 | 10 |
| | **2** | 8 | 8 | 8 | 9 | 10 | 10 |
| | **3** | 9 | 8 | 8 | 8 | 10 | 10 |
| | **4** | 9 | 8 | 9 | 9 | 10 | 10 |
| | **5** | 10 | 8 | 10 | 9 | 10 | 10 |
| | **6** | 10 | 8 | 10 | 8 | 10 | 10 |

*P<0.05

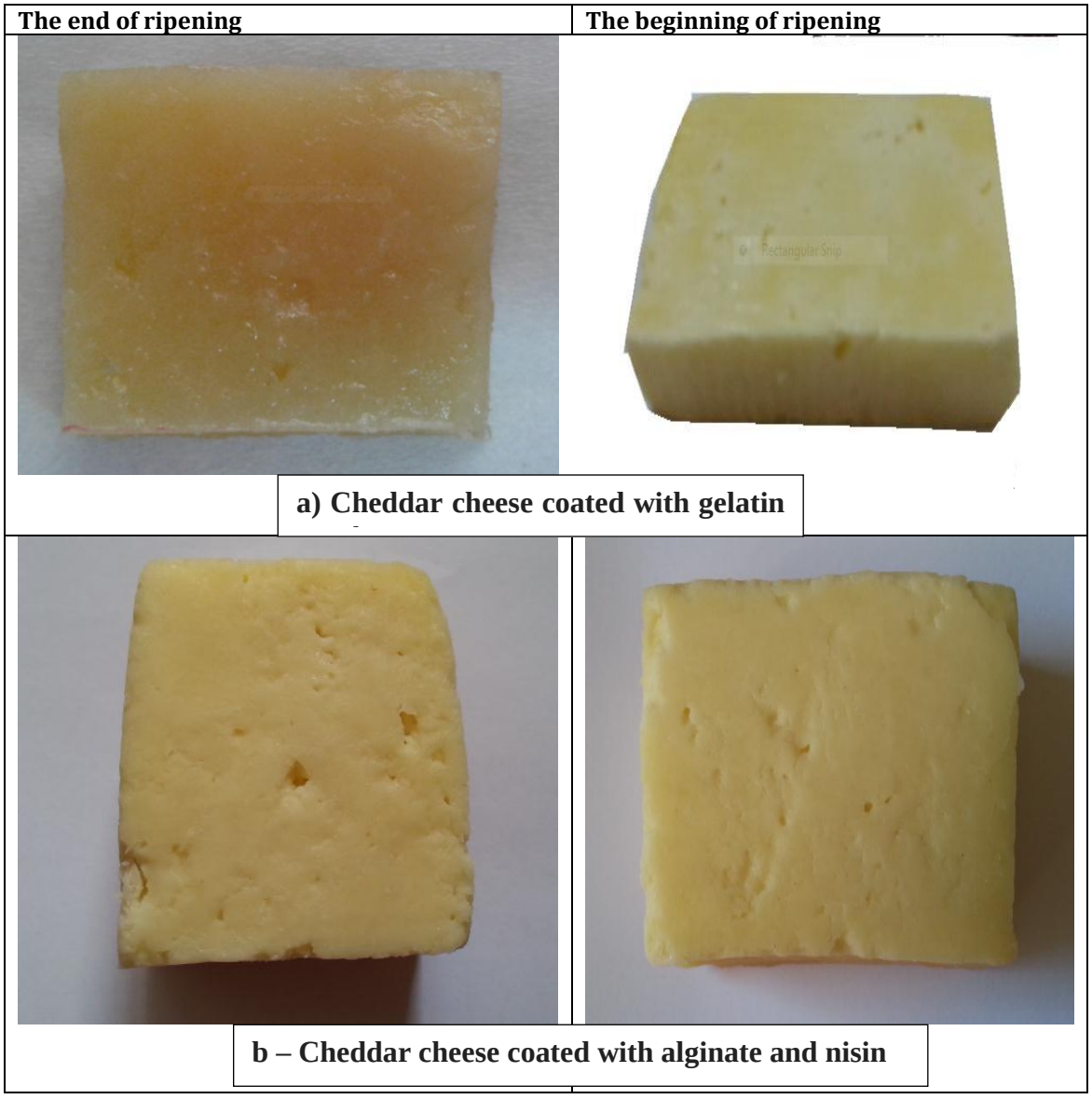


**Figure 6:** The nanolayers containing alginate and nisin at the beginning and end of the maturity period of 6 months.

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