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Study of the Physicochemical and Microbiological Properties of Mozzarella Cheese Coated with Edible Casings Subsidized with Green Tea

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Abstract

In this study, mozzarella cheese covered with edible and biodegradable films was manufactured from whey proteins reinforced with green alcoholic extract to preserve the environment from degradable plastics surrounding it. Two types of mozzarella cheese were manufactured. The first made unwrapped mozzarella cheese (control sample), and the second treatment was mozzarella cheese coated with whey proteins fortified with green tea at a concentration of (3%) (envelope sample), and its effect on prolonging the storage life of the processed cheese was evaluated by chemical and microbiological tests, as the moisture content on the first day was 53.26 and 53.30% for each of the control sample and envelope sample, respectively. Moreover, this percentage was decreased with significant differences on the last day (P≤0.05) between the two samples to 47 and 51.06%, respectively. As for the pH decreased during the storage period, and it was found that there was a development in acidity during storage and for the two samples with insignificant differences(P < 0.05), The Acid value (AV) for both control and envelope sample was 0.13 (mEq / 100 gm fat). And the value of AV increased with significant differences in storage samples, control sample, and envelope sample until it reached 2.0 and 1.1 (mEq / 100 gm fat). As for the microbiological tests, the total number of bacteria on the day of storage was 3.5 x 102 and 2.7 x 102. control and envelope samples, respectively, decreased at of the storage phase 3-6 logarithmic cycles in the envelope sample compared to the control sample. The numbers of Gram-negative E. coli bacteria were lower by two logarithmic cycles in the envelope sample compared to the control sample. The envelope sample was free of lipolytic bacteria, proteins, Salmonella sp, yeasts, and samples, for the length of the storage period.

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1. Introduction

Packaging is an essential part of food manufacturing operations to contain and facilitate the transportation, distribution, and sale of food comfortably and to maintain its natural specifications throughout the period between manufacturing, packaging, and consumption locally or after export without the food being exposed to chemical [1], physical and biological damage. Plastic wrapping represents 70% of the total packaging materials used in packaging food products [2], and most plastic materials are not biodegradable and are derived from nonrenewable materials. Their durability makes them useful, but their presence in the environment constantly and the difficulty of disposing of their waste, which is raised annually, constituted a major obstacle to its use and the need came to find an alternative, the food packaging industry in the last two decades has received great attention from those interested in food safety and environmental preservation, as attention has been directed to how to create edible and biodegradable packaging materials [3]. In general, proteins are considered suitable polymers for forming edible and biodegradable films and covers because they have excellent mechanical properties as well as their ability to retain moisture, gas and odour and act as a barrier to deter the invasion of microorganisms in the food product and their ability to carry food additives [4]. In addition, it is edible and is produced and formed under cold conditions. The edible packaging, or the so-called biofilm, includes cellulose derivatives, carrageenan, alginates, caseins, and whey proteins, incorporating antimicrobial factors into them [5]. Among the antimicrobial factors that can also be used in this type of packaging is an extract. Green tea acts as an antioxidant. The consumption of green tea has shown various health benefits. Scientific research has shown that the compounds in tea have anti-cancer, anti-microbial, and anti-viral properties. In addition, these compounds protect against heart disease and have anti-diabetic properties [4]. The secret of the many benefits of green tea is due to the multiple phenols contained in its leaves, the most important of which are flavonoids, specifically the catechins group, which constitutes from 90-80% of the flavonoids and about 40% of the water-soluble solids, which makes it a valuable substance in Oral Health Benefits [6]. Mozzarella is one of the unripe soft cheeses belonging to the Pasta-Filata family. Its original homeland is Battipaglia in Italy. This type of cheese has been used for over 50 years in the pizza industry [7]. A soft white cheese characterizes mozzarella cheese with a shiny and attractive surface, slight saltiness, and a high stretchability in hot water. - 5.4 Most of it turns into Monocalcium paracasienate, responsible for the processed cheese's shiny appearance and high extensibility. Among the main characteristics of mozzarella cheese is the bright white colour; buffalo milk is preferred over cow's milk in its manufacture [7]. This study aimed to develop the functional character and increase the ability to preserve the biodegradable wrapping mozzarella cheese produced from whey proteins and its inclusion in green tea extract, which is essential in limiting microbial growth.

2. Materials and Experimental Work

2.1. Green Tea Extracts Preparation

Dried green tea leaves (Mahmoud) were purchased from the local markets in Baghdad. The dried leaves were ground using an electric grinder, and then the ground leaves were sifted by using a sieve (70/100 mesh) to get green tea powder. Finally, extraction was carried out according to the method mentioned [8].

2.2. Preparation of Casings isolated Whey Proteins and Green Tea Extract

The casing solution was prepared according to the method described by [9] with some modifications as follows: Dissolve 10 g of whey protein isolate in 100 ml of non-ionic distilled water while stirring the solution until dissolution using a magnetic stirrer with a hot plate for 30 minutes, then heat the mixture at a temperature of 90 C° with continuous stirring, then leave the solution to cool at room temperature and then Filter the solution using a piece of gauze to avoid the presence of any lumps or insoluble materials in the solution, then adjust the pH to 7 using one standard sodium hydroxide solution, then add 5% glycerol to the previous solution and mix it for 5 minutes, then add the alcoholic extract of green tea at a concentration of 3% to The prepared solution and to get rid of the air bubbles inside the casing solution, use a vacuum pump for 10 minutes, then keep the solution in the refrigerator under dark conditions to prevent oxidation processes.

2.3. Mozzarella Cheese Production

Making mozzarella cheese according to the method described by [10] which includes the following with some modifications: Receiving 5 kg of the bulk milk from the diary mill- food science division- agriculture college-Baghdad university of 63° C / 30 minutes and cool the milk to 4° C and add citric acid until the pH is reached 5.3

The temperature was gradually raised, and microbial rennet (chymosin enzyme) prepared by Danish company CHR-HANSIN was added after dissolving it with distilled water and according to the instructions of the producing company and leaving it for half an hour until coagulation occurred, The clot was cut longitudinally and transversely, and then left for 5 minutes without moving, then the whey was drained. The curd was collected and then served (pulled and stretched) inside the hot sauce at 90 °C until it became a ball with a diameter of 5-10 cm and the balls were placed in water at a temperature of 9-11 °C, then placed in a saline solution with a concentration of 15% for 10 hours. The cheese section was divided into two parts, the first was left unwrapped by the control treatment (control sample), and the second section was covered with casings reinforced with green tea extract at a concentration of 3% by immersion method (envelope sample) so that the weight of the sample was approximately 100 g. The samples were left at a refrigerator temperature of 4 °C \pm 1 and humidity a proportion of 40-40% until the film hardened on the surface of the cheese after stirring from time to time. The cheese was subjected to the necessary tests (chemical and microbiological) after 1, 30, 60 and 90 days of storage at a temperature of (5 \pm 1) C.

2.4. Chemical Tests for Mozzarella Cheese

2.4.1 Moisture Analysis

The percentage of moisture of cheese was estimated after packaging during the preservation period during days 1, 30, 06, 09, according to the method modified by [11] 3 g of cheese was taken and dried in the oven at a temperature of 105 ° C after reaching the dryness stage and until stability was measured, the weight was measured the dry matter and the initial weight were subtracted to extract the percentage of moisture according to the following equation:

% humidity = Weigh the sample before drying - Weigh the sample after drying / Weigh the sample x 100%

2.4.2. Estimation of the Percentage of Fat

The percentage of fat in cheese was estimated by Babcock method by taking 4.5 g of cheese and using Babcock bottles graded from zero to 40 [12].

2.4.3. pH Determination

Weigh 3 g of cheese and mix well with 10 ml of distilled water, then the pH value was estimated after the mixing process using a pH meter according to the method mentioned [12].

2.4.4. Titratable Acidity

The acidity represents the number of milliliters consumed from the base for an equation of 100 gm of cheese in the presence of a drop of phenolphthalein alcohol solution (2%), the acidity was estimated according to the standard method mentioned in [13] by weighing 3 g of cheese sample prepared for the examination and mixing it well with 10 ml of distilled water. After that, the model was smeared with (0.1) molar NaOH, and the percentage of acidity was calculated based on lactic acid according to the following equation:

Note that the gram equivalent weight of the lactic = 90

Acidity percentage %= Equivalent gram weight of lactic acid* base caliber* base volume consumable/ Sample Weight (gm)

2.5. Microbiological Analysis of Mozzarella Cheese

The total number of bacteria, molds and yeasts was estimated according to APHA [14] using Nutrient agar, *E.coli* bacteria using MacConkey agar, *Salmonella* bacteria using SS agar medium, lipolytic bacteria and protein using Milk agar component. (100 cm 3 of 10% + Nutrient agar screened milk) as stated in [15].

2.6. Statistical Analysis

All data were analyzed using the SAS statistical analysis system using the least significant difference (LSD) test.

3. Results and Discussion

3.1. Chemical Assays of Mozzarella Cheese Unwrapped and Coated with Whey Proteins fortified with Green Tea Extract

3.1.1. Checking Moisture Content

The results of Table (1) for the moisture content of unwrapped mozzarella cheese samples, treated as control sample and cheese samples coated with whey proteins casings fortified with green tea extract at (3%) envelope sample indicated a decrease with significantly different (P< 0.05). in the moisture percentage values for all samples with the progression of the storage period of 90 days as a result of the loss of water vapor during storage, as the moisture percentage on the first day was 53.26 and 53.30% for each of the control sample and envelope sample, respectively, and this percentage is higher than what was found by [15] for mozzarella cheese, where the moisture percentage was 47.79%, as well as higher with What he found [16] for mozzarella cheese, which amounted to 47.74%, and close to what was found by [17] in Sudanese soft cheese, where the moisture content was 55.69%, These differences depend on the manufacturing method, the temperature and acidity used, and the moisture content of the resulting cheese. This result is considered in conformity with the Iraqi standard specification for soft cheese [18], which indicates that the moisture content of soft cheese is not less than 50%. As for storage, a gradual decrease in the percentage of moisture is observed after the passage of 30, 60 and 90 days Figure 1, where the percentages of moisture reached 52.12, 51.04 and 47.00% for control sample respectively, while for envelope sample they were 52.78, 52.00 and 51.06%, respectively, and it is noted that there are A difference between the percentages of moisture loss in cheese samples and that the reason for these large differences between the two treatments is due to the composition of the whey proteins casings Fortified with green tea extract, which plays a role in retaining moisture and reduces the amount of evaporated water lost from it during storage to an extent in which it is efficient in the moisture-reserving properties and this supports what was mentioned by [19] Al-Mudhafra cheese, where the moisture content decreased from 56.5 to 52.5% and also agrees with [20] The moisture content decreases during the storage period.

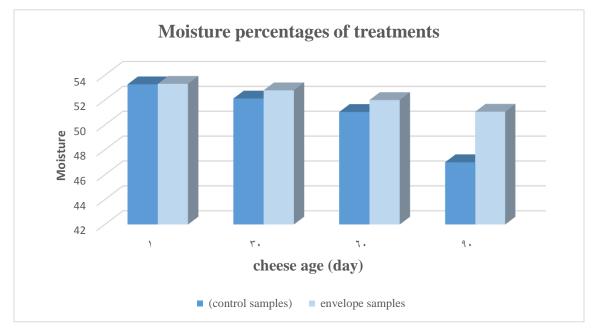


Figure 1: Moisture percentages of treatments for unwrapped mozzarella cheese (control sample) and cheese samples coated (enveloped sample) during storage period.

3.1.2. Percentage of Protein in Different Cheese Treatments

The protein substance is a source of many changes in the sensory characteristics related to the flavor and texture of cheese during the storage period because of this relationship to proteolysis [21]. The proportion of protein during storage is affected by the proportions of other components, especially the moisture content [22]. The results of Table 1 show the percentage of protein in content control sample and enveloped sample during the storage period (90) days, as the treatment control sample after a day of manufacture was 25.30%. This result is identical to what found [15], which indicated that the percentage of protein in mozzarella cheese made from

cow's milk was 25.30% and higher than what was found [23] in mozzarella cheese made from whole cow's milk, amounting to 18%, and close to what was found [24] in Sudanese al-Mudhafra cheese, which amounted to 22.30%. As for the envelope sample immediately after manufacturing, it was 25.22%, and these percentages are less than the result mentioned by [17], which amounts to 30.22%. During storage, an increase in the percentage of protein is observed with insignificant differences. (p<0.05), and this is due to the loss of moisture during storage, which leads to an increase in the percentage of total solids present in cheese, including protein, but the losses The moisture in the packaged cheese treatment was less than what it is in the unwrapped cheese, where the protein percentage at the end of the storage period for both treatments control sample and envelope sample was 26.85 and 26.23%, respectively Figure 2, These results agree with his findings in [25]which confirmed that the high The percentage of protein during the storage period is due to the decrease in the moisture content of the cheese, and the increase in the decrease in the moisture content in the cheese during the storage period affects the state of balance of the proportions of other components, including protein, which leads to an increase in the proportions of these components and is consistent with what I found [15] and also with what was found by [26], who noted that the rate of increase in protein ratios during maturation.

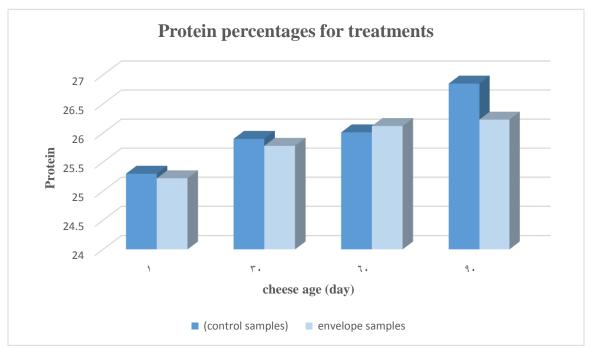


Figure 2: Protein percentages for treatments of unwrapped mozzarella cheese (control sample) and cheese samples coated (enveloped sample) during storage period.

3.1.3. Percentage of Fat

Table 1 shows the percentage of fat in the cheese of the different control sample and treated enveloped sample mentioned previously, as the percentage of fat one day after manufacturing for the control sample cheese was 22.35%, and this result was closed to what [18] found for mozzarella cheese made from cow's milk, which amounted to 20.7%. He found it and found it [16] and found it and found it in the treatment of 23.21%. As for the percentage of fat in the enveloped sample treated, it was 22.35%. The statistical analysis results indicate that there are no significant differences (0.05 (P<0.05) between the control and envelope samples. 25.12% Figure 3, and the result was found, close to what it found [27] for mozzarella cheese made from cow's milk 24.50%. The percentage of the total chemicals that were viewed during the storage period and the results are also consistent with his finding [28] who noted the high storage period, and this is due to the composition of the casing s of whey proteins supported on green tea, which plays a role in sequestering and storing the amount of evaporated water for moisture, which leads to increase the proportion of fat.

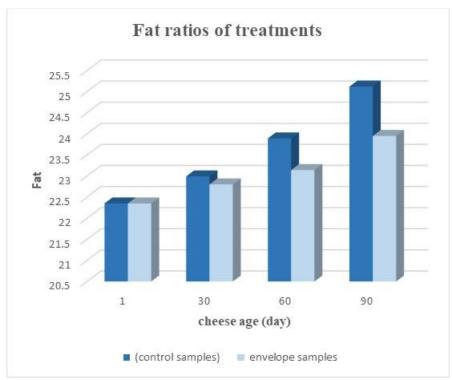


Figure 3: Fat ratios of treatments for unwrapped mozzarella cheese (control sample) and cheese samples coated (enveloped sample) during storage period.

3.1.4. Total of Titratable Acidity in Chees

The results shown in Table 1 show the values of acidity (calculated based on lactic acid) and express the natural acidity and the developed acidity of the cheese of the different treatments. The results showed an increase in the acidity values, as these values were a day after manufacturing for the control sample. 0.27% and then increased until it reached 0.87% within 90 days of storage. As for the envelope sample treated, the acidity percentage was 0.35% initially, and it reached 0.63% at the end of storage.

It is also noticed that there are significant differences (0.05 (P<), and these results agree with what It was reached by [19], [15] and [29] that the percentage of acidity of the covered and unwrapped Triumphal cheese samples rises during the treasury period, and [30] and [19] indicated that the acidity of the unwrapped Triumphant cheese increased with the increase in the storage period, as It was higher than the acidity of the packaged cheese, and this reflects the superiority of the packaged treatment over that of unwrapped cheese.

Table 1: Chemical analysis of mozzarella cheese packaged with green tea extract casings during storage period.

		Moisture	protein	Fat	total acidity
Control sample	1	53.26 ±3.14 a	25.30 ±2.05 a	22.35 ±2.47 a	0.27 ±0.08 d
	30	52.12 ±3.06 a	25.90 ±1.39 a	22.99 ±1.94 a	0.58 ±0.bc
	60	51.04 ±2.17 b	26.01 ±2.42 b	23.89 ±2.62 ab	0.66 ±0.25 b
	90	47.00 ±2.02 c	26.85 ±1.27 b	25.12 ±2.97 c	0.87 ±0.33 a
	1	53.30 ±3.48 a	25.22 ±1.94 a	22.35 ±2.08 a	0.35 ±0.09 cd
Envelope sample	30	52.78 ±3.82 a	25.78 ±1.66 a	22.80 ±2.56 a	0.43 ±0.12 bc
	60	52.00 ±3.68 a	26.12 ±2.07 b	23.14 ±2.82 b	0.58 ±0.18 bc
	90	51.06 ±3.08 b	26.23 ±1.38 ab	23.95 ±2.04 b	0.63 ±0.27 b

Means in the same column not bearing similar superscript letters are significantly difference (P>0.05).

3.1.5. pH Values in Different Cheese Samples

The pH represents the natural acidity and the developed acidity resulting from the fermentation of lactose sugar and its transformation into lactic acid by the action of enzymes from the initiator bacteria. The acidity shares a balance with other compounds to give the cheese taste and flavour and thus affect the degree of consumer acceptance of cheese. The results are shown in Figure 4 show the pH values of the coefficients For control sample and envelope sample cheese, the pH values a day after processing for T1 were 5.75, and this result was higher than what was found by [16] for full-fat mozzarella cheese, which was 5.32 and very close to what was reached by [24] for Sudanese Muzaffar cheese. As for the envelope sample treated, it was also 5.75. It is close to what was found by [31], which estimated the pH of Al-Mazfour cheese was 5.45, and close to what was found by [32], which estimated the pH of Al-Mazfour cheese, which ranged between 5.50 -5.45. During storage, a decrease in pH values was observed for all treatments. After 90 days, for cheese, control and envelope samples were 5.09 and 5.12, respectively. The reason was due to the decrease in the pH of lactose consumption by microorganisms producing lactic acid. It is also noted that there were significant differences (P<0.05) within one treatment between the beginning and end of the storage period.

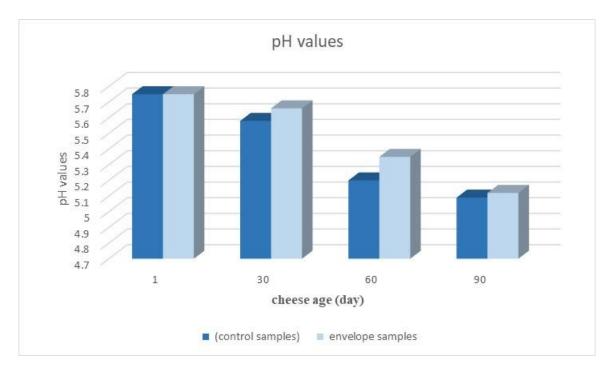


Figure 4: pH values of treatments for unwrapped mozzarella cheese (control sample) and cheese samples coated (enveloped sample) during storage period.

3.2. Results of Microbial Tests for Mozzarella Cheese 3.2.1. Total Count Bacteria

The results shown in Table 2 indicate that the number of treated bacteria (control sample) for unwrapped mozzarella cheese (control treatment) was higher than the number of bacteria of treatment (envelope sample) for cheese coated with whey protein casing s reinforced with green tea extract and these differences persisted between control sample and envelope sample During the storage period until its end, which led to the appearance of signs of spoilage in the control sample model faster than the envelope sample wrapped cheese model. It was noted that the bacterial numbers decreased by approximately 3-1 logarithmic cycle in the envelope sample treated compared to the unwrapped cheese, where the total number of bacteria for the treated cheese was control sample on the first day was 10^2 x 3,5 CFU/gm and reached 10^6 x 5.2 CFU/gm at the end of the storage period. The envelope sample on the first day of manufacture was 10^2 x 2.7 CFU/gm and reached 10^3 x 8. 9 CFU/gm, which is still within the standard specification for soft cheese, which states that cheese is sensually acceptable and edible when the total bacterial count in it does not exceed 10⁵ CFU/gm. The higher microbial numbers in control sample than in envelope sample may be due to non-encapsulation, cheese and expose it directly to the atmosphere, which is more susceptible to contamination [33], as well as to casing s of whey protein isolates enriched with green tea extract As it leads to the inhibition of aerobic bacteria by reducing the entry of oxygen, which is essential for the growth of microorganisms, and on the other hand, the anti-microbial activity of the green tea extract, which supported the coating against bacterial species (positive and gram-negative) because it contains the phenolic compounds it contains. Green tea extract, especially catechins and ECGC determination, [34,19] reported that the total number of bacteria was decreased in whey protein-coated strands cheese compared to non-coated cheeses.

Table 2: microbial tests of mozzarella cheese packaged with green tea extract casings during the storage period

Samples	age in days	Total count /CFU gm	E-coli CFU CFU / gm		ipolytic eria CFU / gm	Proteolytic bacteria CFU / gm	Salmonella.sp CFU / gm	Molds & Yeasts CFU / gm
control sample	1	102 x 3,5 d	10 x 3 b		0 c	0 c	0 b	0 b
	30	103 x 2,5 c	102 x 3.6 a	0 с		0 c	0 b	0 b
	60	104 x 3,3 b	102 x 4.2 a		10 x 2.4 b	10 x 3.1 b	0 b	102 x 3.4
	90	106 x 5,2 a	102 x 6,3 a		102 x 3.7 a	102 x 4.5 a	10 x 3.1 a	102 x 8.1 a
	1	102 x 2,7 d	0 с	0 c		0 c	0 b	0 b
envelope sample	30	102 x 3,1	10 x 2,9 b	0 с		0 c	0 b	0 b
	60	103 x 2,9 c	10 x 3,6 b		0 c	10 x 1 b	0 b	0 b
	90	103 x 8,9 c	10 x 5,0 b		0 c	10 x 1.4 b	0 b	0 b

Mean \pm SD. Having different superscript letters on columns are significantly different (P \leq 0.05)

3-2-2 Preparation of *E-coli* Bacteria

The results are shown in Table 2 showed that the number of *E-coli* bacteria in the control sample treatment was 10 x 3 CFU/gm after one day of manufacture, while the envelope sample treated representing the packaged cheese was devoid of growth, and it was observed that the number of bacteria increased during the progression of the storage period. For cheese workers, where for control sample and envelope sample at the end of the storage period CFU/gm were 10² x 6.3 and 10 x 5.0, respectively, it was noted that there were significant differences (p<0.05) between the control sample and envelope sample, as it was found in two logarithmic cycles and the reason for this is due to the effectiveness of The casing s in the inhibition of *E-coli* bacteria, especially the anti-bacterial action of green tea extract against microorganisms, and these results came close to what was mentioned by [35] that it was shown that the non-packed cheeses contained more *E-coli* bacteria compared to their numbers in packaged cheeses. The reason for this rise is due to the lack of cheese packaging in this treatment and its exposure to the direct atmosphere, which may be a direct cause of its contamination.

3.2.3. Number of Lipolytic Bacteria

The results of Table 2 indicate that no countable lipolytic bacteria growths (i.e. less than 30 colony-forming units/gm) appeared in the control sample and envelope sample at the beginning of the storage period, as the numbers appeared in the last period of storage, as these growths appeared bacteria when detected in control sample only, where it reached 10^2 x 3.7 CFU/gm. The reason for the absence of growth in cheese for the envelope sample factor is attributed to the activity of the alcoholic extract of green tea against lipolytic bacteria;

that is, the reason is due to the encapsulation process that contributed to preventing the proliferation of microorganisms [30].

3.2.4. Number of Proteolytic bacteria

The results are shown in Table 2 indicate that no numbers of *proteolytic* bacteria appeared in each of the control sample and envelope sample, at the beginning of the storage period, but it appeared in the last period of storage in the control sample only, it reached 10^2 x 4.5 CFU/gm, and for the envelope sample treated it is 10 x 1.4, that the absence of growths in the envelope sample treated is due to the new environmental conditions that were formed by the packaging process and the effect of the natural antimicrobial agents present in the green tea extract and added on the other hand [35].

3.2.5. Salmonella Bacteria Number

The results shown in Table 2 indicate that the envelope sample treated was free of *Salmonella.sp* bacteria, in which whey protein wrappers were used, reinforced with green tea extract throughout the storage period, in contrast to the control sample, which showed a clear growth in the last period of storage after it was free in the first period, where it was in The end of the storage period CFU/gm 10 x 3.1. These results agree with what was found by [26], as it was shown that the casein-coated mouterine cheese was free of *Salmonella.sp* bacteria throughout the cheese storage period. These results indicate the effectiveness of the casing s of whey protein isolates enriched with green tea extract in preventing the growth and proliferation of microorganisms due to its anti-microbiological activity because it contains active phenolic compounds such as catechins and determination of the (ECGC) compound present in the green tea extract that supported the casing against bacterial species (gram-positive and gram-negative) [34].

3.2.6. Preparation of Yeasts and Molds

It is possible that yeasts and molds are present in dairy products through contamination, especially after the pasteurization process[36], because pasteurization in itself is a determining factor for the presence of this type of microorganisms[37], and this group of organisms that can lead to protein and fat decomposition, which is usually accompanied by the production of substances that affect the taste The flavor[38], of cheese is evident from Table 2 that no growth of molds and yeasts appeared on the first day in the control sample and envelope sample, but with the progression of the storage period for cheese, growth began to appear in the unwrapped control sample until the end of the storage period, as it was $10^2 \times 8.1$ CFU/gm on the Unlike the envelope sample treated of coated cheese, growth did not appear in it throughout the storage period, and these results are in agreement with [19] where it was found that the number of molds in triumphant cheese coated with whey protein casing s is less than in unwrapped cheese Molds and yeasts prevent the entry of oxygen and the effectiveness of the natural antifungal agents found in whey proteins and anti-microbial additives. It also agrees with [39] that a decrease in the number of molds and yeasts was found during storage of cheese with whey proteins with natamycin for 45 days at 11 °C and 85% moisture compared to unwrapped cheese.

4. Conclusions

Whey protein casings enriched with alcoholic extract of green tea have proven their ability to inhibit a number of microorganisms and yeasts and molds, making them efficient for food preservation purposes.and that the use of isolate casings of whey proteins and their support with the alcoholic extract of green tea had the effect of reducing the microbial content of the coated samples of cheese compared with the unwrapped samples, as well as in slowing the chemical and physical changes of the cheese during storage, which in turn was reflected in the sensory evaluation. and the ease of conducting the packaging process with isolated proteins and whey-bearing anti-microbial growth factors compared to wax encapsulation.

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Conflict of Interest

Authors declare that their present work has no conflict of interest with other published works.

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