



Application of Active Biodegradable Packaging Technologies using Curcumin-enriched Chitosan Films to Prolong the Shelf Life of Soft Cheese

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ABSTRACT

Background: Soft cheese is a perishable dairy item that may experience chemical alterations and an escalation in microbial content during storage. Research is being conducted on edible coatings derived from natural substances like chitosan and curcumin to enhance quality and extend shelf life. These materials possess antimicrobial and antioxidant properties, potentially mitigating spoilage during refrigeration.

Methods: Composite membranes composed of chitosan and curcumin were fabricated and utilized in locally produced soft cheese. Coated samples and an uncoated control were preserved at 4°C for 28 days. The moisture content and pH of the cheese were assessed during storage. A microbial assessment was performed by observing the emergence of bacterial and fungal colonies at various storage intervals.

Result: The coated cheese samples exhibited a marginal increase in moisture content relative to the control group. A minor increase in pH was also noted in the coated samples. Bacterial colonies emerged after 21 days in all samples; however, the coated cheese exhibited generally reduced microbial growth compared to the control. The composite membranes demonstrated efficacy against fungal proliferation, as no fungal colonies were observed after 28 days on the cheese coated with chitosan containing 0.2 g of curcumin.

Key words: Chitosan, Curcumin, Films, Microorganism, Packaging technologies.

INTRODUCTION

The demand for creative food packaging solutions has significantly increased in recent years due to the need to increase food safety and prolong shelf life. Multilayer biopolymer films are a promising alternative among these innovations due to their environmental friendliness. Because they are composed of environmentally safe biopolymers, these films are entirely biodegradable. Their ability to incorporate intelligent or effective ingredients, which makes it easier to create packaging materials that extend product shelf life, is what sets them apart (Mayuri *et al.*, 2023; Tkaczewska *et al.*, 2024).

Because sugars have hydroxyl and polar aggregates that promote hydrogen bonding, which is essential for membrane formation and gives the membrane its unique final shape, polysaccharides are useful substances in the creation of edible membranes (Roman and Gomez, 2018).

Chitosan, alginates, carrageenan, cellulose and starch are some of the most frequently utilized sugars in membrane composition (Nesic *et al.*, 2019; Alkhashab *et al.*, 2024). According to Giaconia *et al.* (2020), chitosan has numerous applications in food industry magazines, food preservation, food packaging, biodegradability and as a health enhancer for prebiotic products. Various studies have confirmed that chitosan has efficacy against bacteria and fungi, since antimicrobial properties are closely related to its structure, physical and chemical

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properties and environmental conditions (Rivera *et al.*, 2020; Anantharaman, 2025).

Curcumin is a carotenoid pigment and a phenolic compound that is mostly present in the turmeric plant. Curcumin serves as a food preservative due to its anti-inflammatory, anti-cancer, cardiovascular stimulant, antibacterial, antifungal and antioxidant properties. Oral administration of up to 12 g per day is advised by the World Food and Drug Administration (FDA) (Gereltuya *et al.*, 2015; Kharat and McClements 2019; Benzineb *et al.*, 2025).

Cheese is classified as a dairy product with high nutritional value and its production is primarily based on

the coagulation of milk with the enzyme rennet. In response to the increasing demand for functional foods, research has focused on enhancing cheese with natural antioxidants, such as turmeric (*Curcuma longa* L.), due to its richness in phenolic compounds and flavonoids. Studies have demonstrated the effectiveness of these compounds as antimicrobial and antioxidant agents, making them an ideal choice for developing healthy and sustainable food products (Arkan *et al.*, 2024).

By examining how curcumin-enriched chitosan coating inhibits microbial growth and ensures an extended shelf life under various storage conditions, this study seeks to assess the efficacy of this active packaging technique for soft cheese preservation.

MATERIALS AND METHODS

This study was conducted in the labs of the Environmental Research Center at the University of Mosul in Iraq in 2025. We bought chitosan powder from Biorigin in the US and the UK and curcumin from Central Drug House (CDH) in India.

Preparation of chitosan coating: prepared membrane solutions weighing 20 g of dry chitosan and dissolved it in one liter of distilled water according to the method used before (Vásquez *et al.*, 2009) and used a hot plate with a magnetic stirrer to combine all the ingredients at 55°C for 15 minutes. Next, add 17 ml of glacial acetic acid and 20 ml of glycerol and thoroughly mix the mixture. One liter of distilled water was added to the volume, the PH was set to 6.5 and it was refrigerated until needed.

Preparation of solutions

- Chitosan solution alone by 2%.
- 2% chitosan solution with 0.1 g of curcumin with blending for two hours using (Hot plate -Magnetic Stirrer).
- 2% chitosan solution with 0.2 g of curcumin with blending for two hours using (Hot plate-Magnetic Stirrer).

Preparation of soft cheese and the process of dipping in the previous solutions

Soft cheese was made from cow's milk using the procedure outlined by Fox (2017). The cheese was then cut into 2×2 cm cubes, immersed in each of the three solutions for 30 minutes, dried in an air oven at 30 M for two hours (Maghsoudlou *et al.*, 2012) and refrigerated at 4°C for 28 days, with tests on the coefficients conducted every seven days.

Moisture percentage determination

Moisture content was estimated according to the AOAC (2005) method by drying 3 grams of the sample in a vacuum oven at 100°C until the weight was constant and then the mass loss was calculated as a percentage to represent the moisture content.

The pH determination

The pH value of the cheese samples was estimated using a digital pH meter by mixing 10 g of the sample with 90 ml

of distilled water and homogenizing them well, then measuring the extract at a temperature of 25°C, as stated by Larionov *et al.* (2020).

Microorganism analysis

Microbiological examinations of cheese samples were performed using the pour-in-plate method. The total aerobic bacterial count (TCB) and total coliform count were estimated using nutrient agar and MacConkey agar, respectively, according to the methodology described by Frank and Yousef (2004). Psychrotrophic bacteria were also estimated using nutrient agar with incubation at 4-7°C for 48 hours (Apha, 2004). To detect lipolytic bacteria, nutrient agar supplemented with sunflower oil and glycerol was used. The plates were inoculated with 0.1 ml of the prepared dilutions and incubated at 21±1°C for 5-7 days. After incubation, the plates were immersed in a 20% copper sulfate solution for 5 minutes and then washed with distilled water to count the blue-green colonies (Al-Khashab *et al.*, 2024). For proteolytic bacteria, skim milk agar (10% skimmed milk) was used and the plates were incubated at 21±1°C for 2-3 days. Hydrochloric acid (1%) was then added to identify and count the colonies surrounded by transparent halos (Al-khashab *et al.*, 2024). Finally, the total number of molds and yeasts was estimated using potato dextrose agar (PDA) as described by Uaboi-Egbenni *et al.* (2010). All microbial results were expressed in colony-forming units per gram of sample (CFU/g).

Statistical analysis

A completely randomized design (CRD) was used to statistically analyze the data and Duncan's Multiple Range Test at a probability level of 0.05 was used to assess the significance of mean differences. The Statistical Package for the Social Sciences (SAS 2012) was used for all analyses.

RESULTS AND DISCUSION

Moisture

The moisture content for the treatments S, S1, S2 and S3 during the storage periods is displayed in Fig (1). Moisture content values remained stable at 55.50% for all treatments during the first seven days of refrigerated storage (4°C). By day 14, a significant decrease in moisture content was observed in the control sample (S) compared to treatments (S1, S2, S3), which maintained their stability. This stability is attributed to the protective role of the chitosan film in limiting moisture loss (Al-Khashab *et al.*, 2024). By day 28, treatments (S2) and (S3) showed statistical superiority, maintaining a moisture content of 54%. This can be explained by the synergistic effect of curcumin, a hydrophobic compound, associated with chitosan, which enhances the film's efficiency in preventing moisture loss from the cheese samples (Roy and Rhim, 2020). Fig 1 illustrates the detailed changes in moisture content of the packaged and unpackaged cheese samples at the end of the storage period.

The pH values

Fig 2 shows the pH values of the treatments S, S1, S2 and S3. The pre-preservation result was 6.65, similar to what was previously reported (Jaafar, 2023). No statistically significant differences were observed after 7 and 14 days of preservation between treatments S1, S2 and S3, while statistically significant differences were found between the coated (S1, S2 and S3) and uncoated (S) treatments. Furthermore, the pH values of treatments S and S1 were significantly higher than those of treatments S2 and S3 after

21 and 28 days of preservation, respectively, due to the higher microbial content in treatments S and S1 compared to treatments S2 and S3. Higher microbial content leads to protein degradation and increased nitrogenous compounds cause higher pH values (Kim *et al.*, 2019).

Total count bacteria

According to the results shown in Table (1), the absence of any bacterial growth was observed after the manufacturing process for all transactions, the absence of growth of bacterial colonies of transactions (S1, S2 and S3) was

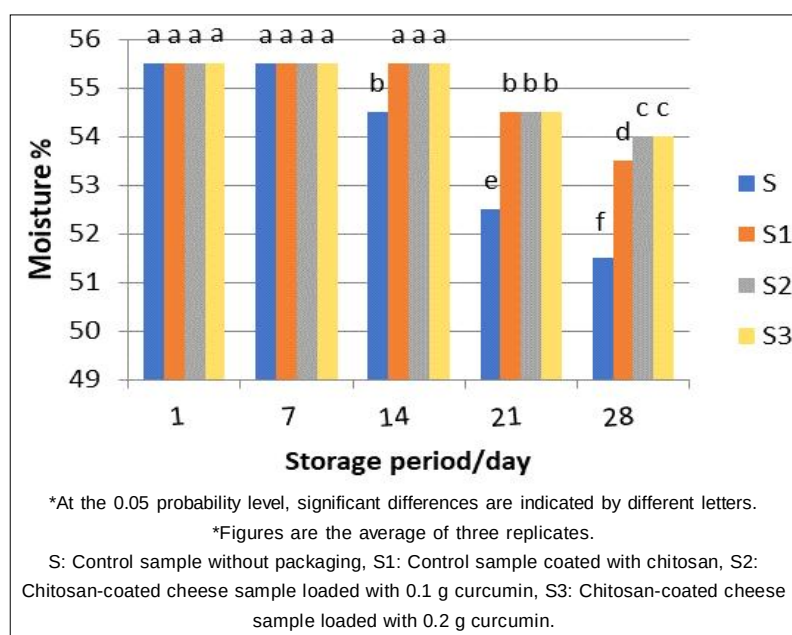


Fig 1: Curcumin-chitosan coated soft cheese's moisture content after 28 days in cold storage.

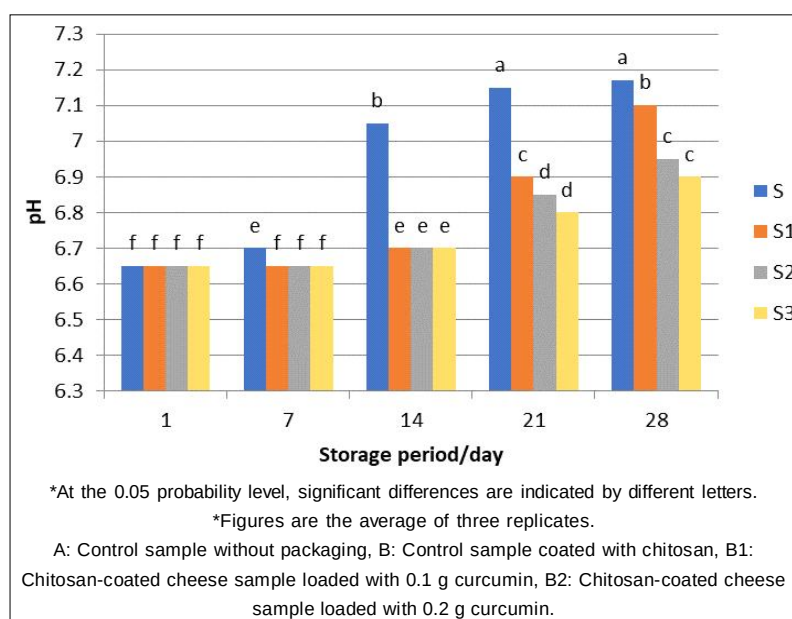


Fig 2: Curcumin-chitosan coated soft cheese's pH value after 28 days in cold storage.

also observed after 14 days of preservation compared to the transaction (S), the reason is the lack of growth of bacterial colonies to the anti-membrane effectiveness of chitosan (Kravanja *et al.*, 2019), while the growth of bacterial colonies was observed after 21 days of transactions (S1 and S2) and a significant with treatment (S3), no growth of bacterial colonies was observed, due to the combined effectiveness of chitosan with curcumin (Salazar-sesatty *et al.*, 2024).

Coliform bacteria

Table (2) shows that there was no growth of colon bacteria after 14 days of preservation for transactions (S1, S2 and S3) compared to transaction (S), coliform bacteria colonies were observed There, estimated at 3.60 (log₁₀ cfu/g), while colon bacteria colonies were observed after 21 and

28 days of preservation for all transactions, while transaction (S3) recorded the lowest number of coliform bacteria growth after 28 days of preservation, estimated at 3.60 (log₁₀ cfu/G). This is attributed to the high concentration of curcumin in the chitosan coating, which plays a key role in inhibiting the growth of these bacteria, as previously reported (Yun and Le, 2016).

Psychrotrophic bacteria

Table (3) shows that no bacterial colonies grew in treatments (S1 and S2) after 14 days of storage, while no colonies grew in treatment (S3) after 21 days. In contrast, bacterial colonies grew in treatment (S) after 14 days of storage, thanks to the anti-chitosan and anti-curcumin effect on cold-resistant bacteria, most notably *Pseudomonas* sp., which infects food and cheese. Even when bacteria

Table 1: Total bacterial count of coated soft cheese in cold storage for 28 days.

Treatment	Total counts (log CFU) Storage period/day				
	1	7	14	21	28
S	0.00 i	3.48 f	4.62 c	4.87 b	5.10 a
S1	0.00 i	0.00 i	0.00 i	3.60 e	4.17 d
S2	0.00 i	0.00 i	0.00 i	3.01 h	3.36 g
S3	0.00 i	0.00 i	0.00 i	0.00 i	3.48 f

*At the 0.05 probability level, significant differences are indicated by different letters.

*Figures are the average of three replicates.

A: Control sample without packaging, B: Control sample coated with chitosan, B1: Chitosan-coated cheese sample loaded with 0.1 g curcumin, B2: Chitosan-coated cheese sample loaded with 0.2 g curcumin.

Table 2: Total coliform bacterial count of coated soft cheese in cold storage for 28 days.

Treatment	Total counts (log CFU) storage period/day				
	1	7	14	21	28
S	0.00 g	0.00 g	3.60 c	4.00 a	3.25 e
S1	0.00 g	0.00 g	0.00 g	3.47 d	4.00 a
S2	0.00 g	0.00 g	0.00 g	3.00 f	3.90 b
S3	0.00 g	0.00 g	0.00 g	3.00 f	3.60 c

*At the 0.05 probability level, significant differences are indicated by different letters.

*Figures are the average of three replicates.

A: Control sample without packaging, B: Control sample coated with chitosan, B1: Chitosan-coated cheese sample loaded with 0.1 g curcumin, B2: Chitosan-coated cheese sample loaded with 0.2 g curcumin.

Table 3: Total psychrotrophic bacterial count of coated soft cheese in cold storage for 28 days.

Treatment	Total counts (log CFU) storage period/day				
	1	7	14	21	28
S	0.00 h	0.00 h	3.47 e	4.25 b	4.47 a
S1	0.00 h	0.00 h	0.00 h	3.30 f	4.04 c
S2	0.00 h	0.00 h	0.00 h	3.00 g	3.84 d
S3	0.00 h	0.00 h	0.00 h	0.00 h	3.30 f

*At the 0.05 probability level, significant differences are indicated by different letters.

*Figures are the average of three replicates.

A: Control sample without packaging, B: Control sample coated with chitosan, B1: Chitosan-coated cheese sample loaded with 0.1 g curcumin, B2: Chitosan-coated cheese sample loaded with 0.2 g curcumin.

appeared on day 28, (S3) recorded the lowest bacterial count (3.30 log₁₀ cfu/g) compared to the control sample (4.47 log₁₀ cfu/g), indicating a very significant reduction in microbial load. These results are consistent with the study by Khairi and Al-Mousawi (2018), which demonstrated the effectiveness of curcumin when added to cheese packaging.

Protolysis bacteria

It is noted from Table (4) that there was no growth of colonies in transactions (S1, S2 and S3) from 1 to 14 days, while the growth of colonies was observed in transaction (S) after 14 days of preservation, due to the strength of the Shell, which acts as a barrier preventing oxygen, which has an important role in controlling the growth of aerobic biota through the large role it plays in the aquatic activity necessary for the activity of these microorganisms (Jalilzadeh *et al.*, 2015), Treatment (S3) recorded the lowest bacterial colony growth rate at 3.60 log₁₀ CFU/g by the end of the storage period (28 days), while the control sample (S) recorded the highest growth rate at 4.41 log₁₀ CFU/g. This difference is attributed to the high inhibitory activity of the chitosan membrane combined with curcumin and its superior ability to suppress the activity of proteolytic bacteria compared to the untreated sample.

Lipolysis bacteria

Table (5) shows the total number of proteolytic bacteria, as no colony growth was observed after 21 days of

preservation for the treatment (S2, S3) compared to the treatment (S and S1), which reached the number of colonies 3.47 and 3 (log₁₀ cfu/g), respectively, due to the effectiveness of the antibacterial membrane of chitosan (Duran and Kahve, 2020) and the effectiveness of curcumin also as an antibacterial (Buniowska-Olejnik *et al.*, 2023). While the growth of bacterial colonies was observed after 28 days for all transactions, reaching the lowest value of 3 (log₁₀ cfu/g) for transaction (S3) and the highest value of 4.25 (log₁₀ cfu/g) for transaction (S).

Total number of fungi

Table (6) presents the results showing changes in the total number of fungi during the preservation period. Treatment (S1) demonstrated significant efficacy in inhibiting fungal growth up to day 21, compared to the control sample (S), which exhibited growth of 3 log₁₀ CFU/g. This inhibition is attributed to the functional properties of the chitosan membrane and its ability to penetrate fungal cells and inhibit protein and nucleic acid synthesis, as well as disrupt mitochondrial activity (Shih *et al.*, 2019; Ke *et al.*, 2021). On the other hand, treatments (S2) and (S3) showed a complete absence of fungal colonies throughout the preservation period, thus outperforming treatments (S) and (S1). This maximum efficacy is attributed to the synergistic effect between chitosan and curcumin, which enhanced resistance against fungal growth and provided extended protection for the product (Jassim, 2021).

Table 4: Total protolysis bacterial count of coated soft cheese in cold storage for 28 days.

Treatment	Total counts (log CFU) storage period/day				
	1	7	14	21	28
S	0.00 i	0.00 i	3.30 g	3.84 c	4.41 a
S1	0.00 i	0.00 i	0.00 i	3.47 f	3.95 b
S2	0.00 i	0.00 i	0.00 i	3.00 h	3.77 d
S3	0.00 i	0.00 i	0.00 i	3.00 h	3.60 e

*At the 0.05 probability level, significant differences are indicated by different letters.

*Figures are the average of three replicates.

A: Control sample without packaging, B: Control sample coated with chitosan, B1: Chitosan-coated cheese sample loaded with 0.1 g curcumin, B2: Chitosan-coated cheese sample loaded with 0.2 g curcumin.

Table 5: Total lipolysis bacterial count of coated soft cheese in cold storage for 28 days.

Treatment	Total counts (log CFU) Storage period/day				
	1	7	14	21	28
S	0.00 f	0.00 f	0.00 f	3.47 c	4.25 a
S1	0.00 f	0.00 f	0.00 f	3.00 e	3.77 b
S2	0.00 f	0.00 f	0.00 f	0.00 f	3.30 d
S3	0.00 f	0.00 f	0.00 f	0.00 f	3.00 e

*At the 0.05 probability level, significant differences are indicated by different letters.

*Figures are the average of three replicates.

A: Control sample without packaging, B: Control sample coated with chitosan, B1: Chitosan-coated cheese sample loaded with 0.1 g curcumin, B2: Chitosan-coated cheese sample loaded with 0.2 g curcumin.

Table 6: Total fungi count of coated soft cheese in cold storage for 28 days.

Treatment	Total counts (log CFU) storage period/day				
	1	7	14	21	28
S	0.00 d	0.00 d	0.00 d	3.00 c	3.90 a
S1	0.00 d	0.00 d	0.00 d	0.00 d	3.47 b
S2	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d
S3	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d

*At the 0.05 probability level, significant differences are indicated by different letters.

*Figures are the average of three replicates.

A: Control sample without packaging, B: Control sample coated with chitosan, B1: Chitosan-coated cheese sample loaded with 0.1 g curcumin, B2: Chitosan-coated cheese sample loaded with 0.2 g curcumin.

CONCLUSION

The results indicated that the curcumin-fortified chitosan coating preserved the soft cheese for 28 days under a temperature of 4°C by acting as an antidote to the various microorganisms studied in the experiment, thereby prolonging the preservation period. The development of edible wrappers should be carried out through the use of natural materials supporting these wrappers for the purpose of preserving various foods and reducing the risks of polyethylene wrappers.

Conflict of interest

All authors declare that they have no conflict of interest.

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