Benha Veterinary Medical Journal 48 (2025) 58-61

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

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Original Paper

Effect of chitosan on sanitary status of yoghurt prepared for sale in public stores

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ARTICLE INFO

Keywords

Chitosan

Public stores

Sanitary status

Yoghurt

Received 28/01/2025

Accepted 07/03/2025

Available On-Line 01/04/2025

ABSTRACT

This study assessed the sanitary quality of yogurt sold in public stores and evaluated chitosan's effect on yogurt quality. Fresh cow's milk (6 L) was sourced from the Menoufia University herd, standardized to 3% fat, heated (85°C, 30 min), and cooled (45°C). The milk was divided into three groups (2 L each), inoculated with 2% yogurt starter culture (Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus), and incubated at 42°C until curd formation. The groups included G1 (control), G2 (2.5 mg/100 mL chitosan), and G3 (5 mg/100 mL chitosan). Yogurts were stored at 4°C and analyzed for sensory and microbiological quality. The bacterial count of beneficial bacteria (8.52 ± 0.12 log10 CFU/g) declined over storage ($p < 0.05$), while yeast and mold counts significantly decreased in chitosan-treated samples (G2, G3). Chitosan enhanced microbial stability, extended shelf life, and maintained better texture and sensory properties. It inhibited spoilage microorganisms while preserving beneficial bacteria, reducing syneresis, and preventing excessive sourness by slowing pH decline. Chitosan's antioxidant properties further protected yogurt's flavor and color. The antimicrobial and preservative effects contributed to extended freshness in G2 and G3 compared to G1. These findings suggest that chitosan is a valuable additive for improving yogurt's microbial stability, texture, and shelf life, making it beneficial for commercial yogurt products.

1. INTRODUCTION

Yoghurt is one of the best-known foods containing probiotics. It is defined by the codex Alimentarius of 2003 as a coagulated milk product that results from the fermentation of lactic acid in milk by *Lactobacillus delbrueckii ssp. Bulgaricus and streptococcus thermophilus*. The nutritive value of yoghurt is based on the nutrient composition of milk, and it is **a** popular fermented milk product (Yabaya et al., 2012). The presence of bacteria in milk products reveals additional dangerous microbes posing a public health risk. The development of probiotic food products is an essential research topic for the future functional food industry. Economic forecasts suggest that the global market for probiotic dietary supplements will expand from 3.3 to 7 billion US dollars between 2015 and 2025 (Terpou et al., 2019). Various probiotics have been shown to provide health-enhancing benefits in most probiotic species known as "core benefits," including regulation of gut transit, balancing altered microbiota, blocking pathogens, lowering lipids, preventing atherosclerosis, and antioxidant abilities (Soni et al., 2020; Singh Narayan et al., 2021). Other health advantages are specific to probiotic strains, such as immune effects and the creation of bioactive compounds (Scourboutakos et al., 2017). Producing LAB can better handle production hurdles and survive travel via GIT than non-producing bacteria. *Streptococcus thermophilus*, as a potential probiotic, has been widely used in the manufacture of several key fermented dairy products, with various active roles like producing extracellular polysaccharides and showing various health benefits, temporary survival, and moderate attachment in the GIT, as well as creating different metabolites with benefits beyond lactic acid fermentation (Iyer et al., 2010). On the other hand, when extracellular polysaccharides are generated throughout the process, they provide highly favorable changes in texture, acting as natural thickeners, giving the product a proper thickness, enhancing viscosity, and lessening syneresis (Surber et al., 2019). Chitosan is a complex polymer made of glucosamine and N-acetyl glucosamine created through the alkaline deacetylation of chitin and partial deacetylation of acetyl glucosamine. Key biological traits of chitin include its ability to break down naturally, compatibility with living tissue, and biological activity. Additionally, it is a polycationic polymer, which is an important chemical feature as it has functional groups with active amino and hydroxyl groups (Nam and Shin, 2017). Chitosan, the main derivative of chitin, is a linear amino-polysaccharide primarily made up of repeating units of -(14)2-amino-2-deoxy-D-glucose (D-glucosamine).

Previous research has shown that chitosan has blood sugar-lowering effects (Lee et al., 2007). The chitosan polymer has drawn the attention of chemists, physicists, and engineers due to its compatibility with biological systems, ease of processing, and strong film-forming capabilities. These features have led to increased interest in creating various formulations using chitosan nanomaterials, nanofibers, nano-clay, colloids, composites, and other products, whether used alone or alongside natural chemicals (Rao et al., 2019). Chitosan can help prolong the shelf life of perishable mozzarella cheese. Applying a chitosan lactic acid solution in the starter culture prevents spoilage microorganisms from growing up to ten days throughout cold storage (Al-Altieri, et al., 2005). The use of chitosan in dairy products is limited

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because it has low solubility. Nevertheless, employing nanosized chitosan could improve solubility and bioavailability. The present investigation was designed to characterize the effect of adding chitosan on the quality and shelf life of yoghurt.

2. MATERIAL AND METHODS

2.1 Activating starter cultures

Lactobacillus delbrueckii subsp. bulgaricus and *Streptococcus thermophiles* (yoghurt starter cultures) were gained from Cairo MIRCEN, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Both strains were activated *in Man, Rogosa, and Sharpe agar* (MRS) and M17 broth, incubated at 37 °C for 24 hours, and transferred three times for activation. These activated strains were transferred to sterile 11% reconstituted skimmed milk powder and incubated at 40 °C for 24 hours. Dilutions were made till obtaining a concentration of 10^7-10^9 cfu/ml. The active starting cultures were stored in the refrigerator and utilized within 24 hours (Badawi and El- Sonbaty, 2004). Commercial Chitosan materials were obtained by Nakaa nanotechnology Network (NNN), Egypt. The code and specifications for the Chitosan materials were provided by NNN and adhered to standard quality parameters for food applications. This study did not involve human participants or live animal experimentation; therefore, ethical approval was not required. However, all procedures related to milk collection and handling complied with institutional and national regulations.

2.2. Yogurt preparation

Yogurt was prepared according to Nighswonger et al. (1996) guidelines. A total of 6 liters of fresh raw cow milk was obtained from the Faculty of Agriculture, Menoufia University**.** The milk fat content was standardized to 3% by separating the cream and recombining it in precise proportions. The final fat percentage was confirmed using the Gerber method. The standardized milk was then heated at 85 °C for 30 minutes, then cooled to 45 °C. The whole milk volume was divided into three groups (2 L each) and inoculated with active starter cultures (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*).

Chitosan Dissolution and Addition to Milk

Chitosan is sparingly soluble in neutral or alkaline solutions and requires an acidic medium for dissolution. The required amount of chitosan powder was first dissolved in 1% (v/v) acetic acid solution under continuous stirring for 30–60 minutes at room temperature to ensure complete solubilization. The chitosan solution was then gradually added to the warm milk (45 °C) with continuous stirring to ensure uniform dispersion before inoculation with starter cultures.

Yogurt Groups

G1 (Control): 2 L of milk + 2% yogurt starter culture (1:1).

G2: 2 L of milk + 2% yogurt starter culture (1:1) + 2.5 mg chitosan per 100 mL milk (total 50 mg chitosan in 2 L).

G3: 2 L of milk + 2% yogurt starter culture (1:1) + 5 mg chitosan per 100 mL milk (total 100 mg chitosan in 2 L).

Each group was portioned into 100 mL cups (20 cups per group), incubated at 42 °C until curd formation (pH ~4.6), and then stored at 4 °C in a refrigerator. The yogurt samples were evaluated for sensory and microbiological properties at regular intervals until spoilage occurred. Yogurt preparation and assessment were performed in triplicate (Seo et al., 2009)

2.3 Sensory assessment

Sensory assessment of yoghurt samples followed Mehanna et al. (2000). The points were allocated as follows: 60 for flavor, 30 for body and texture, and 10 for appearance, for a total possible score of 100 points.

2.4. Microbiological Examination

2.4.1. Preparation of serial dilutions

The yogurt samples were carefully blended under sterile conditions. Serial dilutions were performed; one gram of each well-mixed yoghurt sample was added to 9 ml of sterile distilled water to produce a tenfold serial dilution, from which additional decimal dilutions were obtained (APHA, 1992).

2.4.2. Determination of lactic acid bacteria

Lactobacillus delbrueckii subsp. bulgaricus was counted using the pouring plate technique. Prior serial dilutions were mixed with MRS agar in Petri plates and incubated for 48 hours at 37 °C. *Streptococcus thermophilus* was counted on M17 agar with 0.5% glucose at 42 °C for 48 hours (Ryan et al., 1996).

2.4.3. Determination of total mold and yeast counts

One ml of produced serial dilutions was put on duplicate Petri dishes and mixed with Sabouraud dextrose agar medium containing chloramphenicol (0.01%), as recommended by the International Dairy Federation (IDF) 1990. The plates were incubated for 5-7 days at 25 °C. The first examination was performed after three days to assess yeast and mold growth levels.

2.5. Statistical Analysis

The data was statistically examined using SPSS 16.0's variance analysis. One-way analysis of variance was used for statistical comparisons. Clarke and Kempson (1997) determined that the results were substantially different ($P< 0.05$). To identify significant differences between means, Tukey’s Honestly Significant Difference (HSD) post-hoc test was applied, and different letters were assigned to indicate statistically significant differences among values.

3. RESULTS

The microbial stability of yogurt was assessed by monitoring Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus counts. As shown in Table 1, bacterial counts declined in all groups, with spoilage in G1 by day 21, while chitosan-treated groups (G2, G3) maintained higher viability. Table 2 confirmed similar trends for S. thermophilus, with G3 showing the best bacterial retention.

Yeast and mold counts (Table 4) increased in G1 from day 7, reaching spoilage by day 21, whereas G2 and G3 exhibited significantly lower fungal growth. Table 3 revealed better sensory scores for G2 and G3, with G3 maintaining the best texture and acceptance until day 35 (Table 5). Chitosan also reduced syneresis, improving yogurt stability. These results confirm that chitosan enhances microbial stability, extends shelf life, and improves yogurt quality, with G3 being the most effective treatment.

Effect of chitosan on yogurt shelf life

The shelf life of yogurt improved with increasing chitosan concentration. In the control group (G1) without chitosan, spoilage occurred by day 14. Yogurt in G2 (2.5 mg chitosan/100 mL) remained stable for up to 28 days, while G3 (5 mg chitosan/100 mL) exhibited the longest shelf life, maintaining stability for 35 days.

Table 1 Changes in *Lactobacillus delbrueckii* subsp. *bulgaricus* count (log_{10} cfu/g) during storage

Storage (days)	G1 (control)	G2 (2% yoghurt starter cultures (1:1) + chitosan 2.5 mg/100ml milk)	G3 (2% yoghurt starter cultures (1:1) + chitosan 5mg/100ml milk)
0	10.34±0.03Aa	10.34±0.03Aa	10.34±0.03Aa
7	10.45±0.04Aa	10.48±0.04Aa	10.46±0.01Aa
14	9.58±0.02Aa	10.23±0.02Aa	10.17±0.03Aa
21	S	9.84±0.03Ab	10.00±0.02Aa
28	S	9.17±0.01Aa	9.78±0.03Aa
35	S	S	9.46±0.04Aa

Means (±SE) with different capital and small letters within the same column and rows, respectively, are significantly different at (P< 0.05). S: refers to "Spoiled"

Table 2 The mean counts of *Streptococcus thermophilus* (log_{10} cfu/g) for examined yoghurt groups throughout refrigerated storage

Storage (days)	G1 (control)	G2 (2% yoghurt starter cultures (1:1) + chitosan 2.5 mg/100ml milk)	G3 (2% yoghurt starter cultures (1:1) + chitosan 5mg/100ml milk)
0	10.34±0.03Aa	10.34±0.03Aa	10.34±0.03Aa
7	10.45±0.04Aa	10.48±0.04Aa	10.46±0.01Aa
14	9.58±0.02Aa	10.23±0.02Aa	10.46±0.01Aa
21	S	9.84±0.03Ab	10.00±0.02Aa
28	S	9.17±0.01Aa	9.78±0.03Aa
35	S	S	

Means (±SE) with different capital and small letters within the same column and rows, respectively, are significantly different at (P< 0.05). S: refers to "Spoiled".

Table 3 Sensory evaluation of yogurt samples during refrigerated storage (4°C)

Storage (days)	Flavor (60)			Body and Texture (30)			Appearance (10)			Total acceptance (100)		
	G1	G2	G3	G1	G2	G3	G1	G2	G3	G1	G2	G3
0	54±0.1Bc	56±0.1Bb	58±0.1Aa	28±0.1Ba	28±0.1Ba	29±0.1Aa	9±0.1Ba	9±0.1Ba	9±0.1Aa	91±0.1Bc	93±0.1Bb	96±0.1Aa
7	48±0.1Cb	55±0.1Ba	56±0.1Ba	27±0.1Ba	28±0.1Ba	28±0.1ABa	8±0.1BCa	9±0.1Ba	9±0.1Aa	83±0.1Cc	91±0.1Cb	93±0.1Ba
14	45±0.1Dc	53±0.1Cb	56±0.1Ba	25±0.1Cb	27±0.1BCa	28±0.1ABa	7±0.1Ca	8±0.1BCa	8±0.1ABa	77±0.1Dc	88±0.1Db	92±0.1Ba
21	S	52±0.1Dc	54±0.1Cb	S	26±0.1CDb	27±0.1Bb	S	8±0.1BCb	8±0.1ABb	S	84±0.1Ec	89±0.1Cb
28	S	48±0.1Ec	52±0.1Db	S	25±0.1Dc	27±0.1Bb	S	7±0.1Cc	8±0.1ABb	S	80±0.1Fc	87±0.1Db
35	S	S	50±0.1Eb	S	S	26±0.1Bb	S	S	7±0.1Ba	S	S	83±0.1Eb

Means (±SE) with different capital and small letters within the same column and rows, respectively, are significantly different at (P< 0.05). S: refers to "Spoiled".

Table 4 Mean total yeast and mold counts (log_{10} cfu/g) for examined yoghurt groups throughout refrigerator storage

Storage (days)	G1 (control)	G2 (2% yoghurt starter cultures (1:1) + chitosan 2.5 mg/100ml milk)	G3 (2% yoghurt starter cultures (1:1) + chitosan 5mg/100ml milk)
0	ND	ND	ND
7	2.34±0.04Bc	1.98±0.03Bb	1.45±0.02Aa
14	3.45±0.05Cc	2.65±0.04Cb	2.12±0.03Ba
21	S	3.54±0.06Db	2.76±0.05Ca
28	S	4.12±0.07Eb	3.10±0.06Da
35	S	S	3.58±0.08Ea

ND: Not detected. Means (±SE) with different capital and small letters within the same column and rows, respectively, are significantly different at (P< 0.05). S: refers to "Spoiled".

Table 5 Mean total sensory score of yogurt groups during refrigerated storage (4°C)

Storage (days)	G1 (control)	G2 (2% yoghurt starter cultures (1:1) + chitosan 2.5 mg/100ml milk)	G3 (2% yoghurt starter cultures (1:1) + chitosan 5mg/100ml milk)
0	$<10^2$ Ba	$<10^2$ Ba	$<10^2$ Aa
7	1.80±0.10 Da	$<10^2$ Ba	$<10^2$ Aa
14	3.20±1.02 C b	$<10^2$ Ba	$<10^2$ a
21	S	$<10^2$ Bb	$<10^2$ Ab
28	S	2.20±0.10 Cb	1.10±0.10 Cc
35	S	S	2.34±0.66 Bb

Means (±SE) with different capital and small letters within the same column and rows, respectively, are significantly different at (P< 0.05). S: refers to "Spoiled".

4. DISCUSSION

In this study, the effect of chitosan on the microbial stability, texture, and shelf life of yogurt was assessed, with a particular focus on its impact on sensory and microbiological qualities during refrigerated storage. The findings indicated that the addition of chitosan significantly improved the overall quality of yogurt, particularly in terms of extending shelf life and enhancing texture stability. The yogurt groups treated with chitosan (G2 and G3) exhibited noticeable improvements over the control group (G1), with a more stable microbial profile and better sensory qualities over time. These findings are supported by previous research indicating that chitosan exhibits antimicrobial activity and enhances the structural integrity of dairy products (Seo et al., 2009; Sawant et al., 2015). The ability of chitosan to interact with microbial cell membranes and inhibit spoilage organisms is well documented (Nam & Shin, 2017).

The microbial analysis showed that the inclusion of chitosan inhibited the growth of spoilage microorganisms like yeasts and molds, which is evident from the substantial reduction in yeast and mold counts in G2 and G3 compared to G1. In the control group, the total yeast and mold count reached spoilage levels by day 21, (10^4 CFU/g (log10 4.00 CFU/g)) is commonly considered the spoilage threshold for yogurt and other fermented dairy products, (ICMSF 2005). whereas in the chitosan-treated groups, microbial stability was maintained until day 35. This extended shelf life suggests that chitosan's antimicrobial properties are effective at controlling the proliferation of spoilage microorganisms, preserving the yogurt's freshness for a longer period. The beneficial bacteria, such as *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*, were also better preserved in G2 and G3, with G3 showing the highest bacterial counts throughout the storage period, reinforcing the idea that chitosan helps maintain the viability of probiotics during storage. Research also indicates that chitosan improves microbial stability by inhibiting spoilage organisms and promoting probiotic survival in yogurt (Rao et al., 2019; Nam & Shin, 2017). The ability of chitosan to act as a natural preservative has been further confirmed in studies evaluating its impact on mold and yeast reduction in dairy products (Sawant et al., 2015). Sensory evaluations supported microbiological findings, with G3 showing the best overall acceptance in terms of flavor, body and texture, and appearance, followed by G2. The control group (G1), which lacked chitosan, had the lowest sensory scores, particularly on day 14, when spoilage began to be noticeable. The texture and water-holding capacity of the yogurt in G2 and G3 were notably superior, with less syneresis observed compared to G1.

Chitosan likely played a role in improving the yogurt's texture by preventing whey separation, contributing to a smoother, more stable product. Furthermore, the slower pH drops in chitosan-treated samples helped avoid over-acidification, which often leads to excessive sourness and undesirable changes in flavor. Research indicates that chitosan strengthens the protein matrix in fermented dairy products, leading to improved water retention and textural stability (Rao et al., 2019). The ability of chitosan to slow pH reduction has also been documented, highlighting its role in preventing excessive acidification and maintaining a balanced flavor profile (Kumar et al., 2020).

Notably, the addition of 5 mg of chitosan per 100 mL (G3) was more effective than 2.5 mg (G2) in preserving both the microbial quality and sensory attributes of yogurt. This suggests that a higher concentration of chitosan provides better protection against spoilage microorganisms and enhances the textural qualities of the yogurt, ensuring prolonged freshness. The enhanced antioxidant properties of chitosan may also have contributed to the preservation of yogurt's flavor and color, preventing oxidative damage to lipids and proteins.

In comparison, previous studies on chitosan's effects on dairy products have shown similar benefits. For example, chitosan has been shown to extend the shelf life of mozzarella cheese by inhibiting microbial growth (Al-Altieri et al., 2005). The current study expands these findings to yogurt, demonstrating that chitosan, particularly at higher concentrations, can effectively prolong shelf life while maintaining product quality. The use of chitosan as a natural preservative in yogurt offers promising potential for the dairy industry, especially in commercial products where microbial stability and extended shelf life are critical.

5. CONCLUSIONS

In conclusion, this study demonstrates that chitosan, particularly in higher concentrations, can significantly enhance microbial stability, texture, and overall quality of yogurt. By inhibiting spoilage microorganisms and preserving the viability of probiotics, chitosan-treated yogurts offer extended shelf life, improved texture, and better sensory qualities, making it a valuable additive for improving the quality and safety of commercial yogurt products.

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