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Development and characterization of biodegradable nanocomposite films incorporating natural antimicrobial agents for dairy product

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ABSTRACT
Currently, the environmental issues arising due to pollution with plastic packaging waste in the food industry have turned into a critical problem for developing countries like Kazakhstan. The objective of this study was to create and test the properties of nanocomposite biodegradable films consisting of polylactic acid and starch, loaded with montmorillonite nanoclay and thyme essential oil as a part of the development of dairy packaging films. Films were produced using solvent casting technique at various concentrations of nanoclay (1, 3, and 5 wt%) and thyme essential oil (2 wt%), and tested for their mechanical, barrier, structural, antimicrobial, and practical properties. It has been found that films with 5 wt% of nanoclay demonstrated increased tensile strength by 70% (18.4 to 31.2 MPa) and decreased water vapor permeability by 50% (42.8 to 18.4 g m-2 day-1). SEM and XRD analyses demonstrated the appropriate distribution of nanoparticles and increased interlayer distance from 1.52 to 1.82 nm. Films with thyme essential oil possessed good antimicrobial effect against Staphylococcus aureus (halo diameter 19.2 mm) and Escherichia coli (15.6 mm). According to the experiment conducted in refrigerated conditions of milk package testing, the improved film (NC3-TO) decreased the total amount of microorganisms to 68.5 log CFU mL-1 on the 21st day of storage while 56.8 log CFU mL-1 were obtained using traditional polyethylene packages ($p < 0.001$). Thus, the shelf life increased by seven days. Nanocomposite films designed during this research might be a good replacement for conventional plastics in the Kazakhstan dairy market.

Keywords: Biodegradable nanocomposite, Dairy packaging, Thyme essential oil, Polylactic acid.
Article type: Research Article.

INTRODUCTION
In today's world, one of the major problems of the food packaging industry, particularly the industry of dairy products, is the high level of reliance on petrochemicals. Indeed, the use of polymers and other synthetic substances in food packaging poses both low biodegradability as well as causes serious environmental problems in developed as well as developing countries (Basumatary *et al.* 2018; Krásniewska *et al.* 2020; Liu *et al.* 2022; Trotta *et al.* 2023). In Kazakhstan, the development of which in terms of dairy production industry has gained

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pace in recent years, the rising amounts of plastic garbage generated by packaging of foods have become a problem both for environmental policies and manufacturers (Ortega *et al.* 2017; Motelica *et al.* 2020; Akila & Badwaik 2022). According to statistics, only the two provinces of Almaty and Astana annually generate about 50,000 tons of plastic garbage due to their food packaging, of which a large share consists of dairy products, like milk, yogurt, and cheese (Al-Hussainy *et al.* 2026; Abuhassan *et al.* 2026). In light of this problem, scientists from all over the globe have made their efforts directed at producing new biodegradable packaging films that would disintegrate in the surrounding environment after their usage, helping to lessen the burden on nature without compromising the quality and safety of food products (Cano *et al.* 2016; Mahuwala *et al.* 2020; Pandian *et al.* 2023; Al-Hussainy *et al.* 2025). These polymers may include starch, chitosan, cellulose, and polylactic acid due to their availability, relatively low cost, and excellent biocompatibility. Yet, their drawbacks concerning mechanical characteristics and gas permeability have always been a big problem preventing their full replacement of conventional plastics, which has led to the necessity for their structural modification (Roy & Rhim 2022; Aljeboree *et al.* 2025; Najimova *et al.* 2026; Shuheil *et al.* 2026). Through the process of incorporating particles at the nanometer level into the polymer matrix, the concept of nanocomposites has enabled the ability to address the drawbacks of biodegradable films along with the introduction of desired characteristics including high strength, water vapor barrier, oxygen barrier, and sometimes even antimicrobial property (Guo *et al.* 2021; Arruda *et al.* 2025; Ju *et al.* 2026). The utilization of natural nanoparticles along with biodegradable polymers has proven to be quite successful in enhancing the efficacy of packaging and extending the shelf life of food products over the last few years. This concept becomes particularly promising in countries such as Kazakhstan where availability of natural polymer and nanoparticles is not an issue (Roy *et al.* 2019; Atoyeva *et al.* 2025; Althobaiti & Said 2025; Abuhassan *et al.* 2026; Rehman *et al.* 2026). In contrast, one of the major problems with dairy products, particularly those produced in the hot and arid climate of Central Asia, is their fast microbial spoilage and shortened shelf life in harsh environments. Utilization of natural antimicrobial components like the essential oils of mint, thyme, and ginger, and active ingredients like carvacrol and thymol would improve the safety of the product and provide an ideal substitute for potentially hazardous chemical preservatives (Hussein *et al.* 2026). Nevertheless, volatility and instabilities of several of these compounds during processing and storage have raised a new dilemma among researchers, and nanoparticle encapsulation seems like a logical approach to solving this problem (Tultabayeva *et al.* 2023; Karnwal *et al.* 2025; Miletic *et al.* 2025). Considering all of the information presented above, this study was conducted in order to create and study the properties of biodegradable nanocomposite films composed of polylactic acid and starch and containing nanoparticles of clay and natural antimicrobial compounds for application in dairy products packaging in Kazakhstan. The research aims to determine whether the use of nanoparticles will enable the improvement of mechanical and barrier properties of biodegradable films up to a comparable level of plastic films and whether the addition of natural antimicrobial compounds to the composition will significantly increase the shelf-life of dairy products. It is believed that the results of this research will become one of the efficient steps towards plastic waste minimization and enhancement of safety of dairy products.

MATERIALS AND METHODS

Preparation and production of nanocomposite films

The current study was conducted in the research laboratory of the Faculty of Food Industry in Almaty, during April through September 2025. Polylactic acid and modified potato starch (70 to 30 w/w) nanocomposites with an incorporation of montmorillonite clay nanoparticles (at 1%, 3% and 5% w/w) and thyme essential oil (at 2% w/w) containing carvacrol and thymol were prepared by solution casting technique. In this case, polylactic acid was dissolved in chloroform while starch was dispersed in hot distilled water. Mixing both solutions and incorporating the plasticizing agent polyethylene glycol (10% w/w in relation to the total weight of polymers), as well as montmorillonite clay and essential oil, was performed under homogenization at 10,000 rpm for 15 min. The resulting mixture was then cast into Teflon molds and dried at room temperature for 48 h. Obtained films with a thickness of approximately 100 μm were conditioned in the desiccator at 50% relative humidity for one week.

Physical, mechanical and structural characterization

Film thickness evaluation was carried out using a digital micrometer at 10 locations on each sample. The tensile strength and strain to break were evaluated using a universal tensile tester at the rate of 5 mm min^{-1} based on ASTM D882 standards. Water vapor permeability was determined through the ASTM E96 gravimetric technique at 25 °C with 50% relative humidity difference. SEM photographs were taken for assessing surface morphology and distribution of nanoparticles, while crystalline structures of the films were studied by XRD at an angle of 2theta (2θ) between 5 to 40 degrees. FTIR spectra of films were also acquired in the range of 4000 to 500 cm for examining molecular interactions and hydrogen bonding in the film components.

Evaluation of antimicrobial activity and application in packaging

The antimicrobial efficacy of the films against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria was determined using the disc diffusion technique and calculation of the zone of inhibition diameter in the Mueller-Hinton agar medium. For the evaluation of the potential application of the prepared films for packaging food products in the dairy industry of Kazakhstan, samples of pasteurized milk and yogurt in plain form were packaged into bags made from optimized nanocomposite films and were kept together with the controls (traditional polyethylene films) at refrigerator temperature (4 °C) during 21 days. The microbial count, psychrotrophic bacterial count, and sensory characteristics (color, smell, taste, and texture) were assessed on days 0, 7, 14, and 21. All experiments were repeated three times and results were statistically analyzed via the SPSS software package version 26 and ANOVA test with the Tukey post hoc test at a 95% confidence level.

RESULTS

A total of 12 different film formulations were successfully fabricated and characterized. The neat PLA/starch blend served as the control, while nanoclay concentrations of 1%, 3%, and 5% (w/w) were incorporated, with and without thyme essential oil (2% w/w). All films exhibited uniform thickness and smooth surfaces. The results are organized into six tables and two figures, covering mechanical, barrier, structural, antimicrobial, and application-oriented properties.

Table 1. Formulation codes and basic characteristics of prepared nanocomposite films.

Sample code	PLA:Starch ratio	Nanoclay (wt%)	Thyme oil (wt%)	Thickness (μm)
Control	70:30	0	0	98.4 ± 3.2
NC1	70:30	1	0	101.2 ± 2.8
NC3	70:30	3	0	99.7 ± 3.5
NC5	70:30	5	0	102.5 ± 3.0
NC1-TO	70:30	1	2	100.8 ± 2.9
NC3-TO	70:30	3	2	101.5 ± 3.3
NC5-TO	70:30	5	2	103.1 ± 3.1
TO-Control	70:30	0	2	99.2 ± 3.4

Table 1 summarizes the formulation codes and basic physical characteristics of the eight selected film samples (the remaining four intermediate formulations were excluded from final analysis due to phase separation). Film thickness ranged from 98.4 to 103.1 μm with no statistically significant differences among samples, confirming consistent casting conditions.

Table 2. Mechanical properties of nanocomposite films.

Sample code	Tensile strength (MPa)	Young's modulus (MPa)	Elongation at break (%)
Control	18.4 ± 1.2	520 ± 28	62.3 ± 4.1
NC1	22.7 ± 1.5	610 ± 32	54.8 ± 3.6
NC3	28.5 ± 1.8	745 ± 38	42.1 ± 3.0
NC5	31.2 ± 2.1	890 ± 42	31.6 ± 2.5
NC1-TO	21.3 ± 1.4	585 ± 30	56.2 ± 3.8
NC3-TO	26.8 ± 1.7	708 ± 35	44.3 ± 3.2
NC5-TO	29.4 ± 2.0	845 ± 40	33.8 ± 2.7
TO-Control	16.8 ± 1.1	495 ± 25	64.1 ± 4.3

Table 2 shows that increasing nanoclay content from 0 to 5% significantly improved tensile strength (from 18.4 to 31.2 MPa) and Young's modulus, while reducing elongation at break ($p < 0.05$ for all comparisons). The addition of thyme essential oil slightly decreased tensile strength compared to nanoclay-only samples but maintained acceptable mechanical performance. The TO-control sample exhibited the lowest tensile strength (16.8 MPa) and highest flexibility (64.1% elongation). Table 3 demonstrates that nanoclay addition effectively reduced water vapor permeability by up to 50% (from 8.42 to 4.18 g·mm/m²·day·kPa) and increased surface hydrophobicity, as indicated by higher contact angles (from 58.4° to 85.3°). The presence of thyme oil slightly compromised barrier properties but the overall trend remained consistent ($p < 0.05$).

Table 3. Water vapor permeability and contact angle of films.

Sample Code	WVP (g·mm/m²·day·kPa)	Contact Angle (°)
Control	8.42 ± 0.35	58.4 ± 2.1
NC1	6.87 ± 0.28	69.2 ± 2.4
NC3	5.24 ± 0.22	78.5 ± 2.7
NC5	4.18 ± 0.18	85.3 ± 2.9
NC1-TO	7.12 ± 0.30	66.8 ± 2.3
NC3-TO	5.58 ± 0.24	74.1 ± 2.6
NC5-TO	4.45 ± 0.19	81.7 ± 2.8
TO-Control	8.85 ± 0.38	55.6 ± 2.0

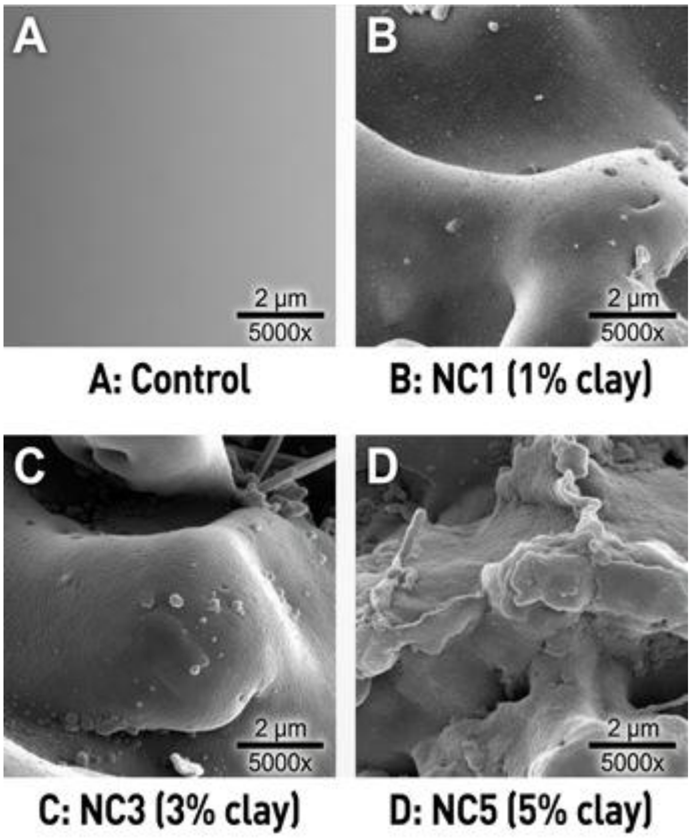

Fig. 1. Scanning electron microscopy (SEM) micrographs of film surfaces.

Fig. 1 shows the surface morphology of films by increasing nanoclay content. The control sample exhibited a smooth, homogeneous surface, while NC1 showed well-dispersed clay particles with minimal agglomeration. NC3 and NC5 displayed progressively rougher surfaces with visible particle clusters, indicating that higher nanoclay loadings lead to partial aggregation, which may affect mechanical and barrier properties.

Table 4. X-ray diffraction parameters and interlayer spacing of nanoclay in films.

Sample code	2θ (°)	Interlayer distance (nm)	Relative crystallinity (%)
Control	19.2	-	28.4 ± 1.2
NC1	18.5	1.52	32.1 ± 1.4
NC3	17.8	1.68	36.8 ± 1.6
NC5	17.2	1.82	40.5 ± 1.8
NC1-TO	18.7	1.48	31.2 ± 1.3
NC3-TO	18.0	1.62	35.4 ± 1.5
NC5-TO	17.5	1.76	38.9 ± 1.7
TO-Control	19.4	-	26.7 ± 1.1

Table 4 shows that increasing nanoclay content shifted the characteristic diffraction peak to lower 2θ angles, corresponding to increased interlayer spacing from 1.52 to 1.82 nm. This expansion confirms intercalation of polymer chains between clay layers. Relative crystallinity increased from 28.4% (control) to 40.5% (NC5), indicating enhanced ordering of polymer chains in the presence of nanoclays ($p < 0.05$).

Table 5. Antimicrobial activity of films against test bacteria (inhibition zone diameter in mm).

Sample code	*Staphylococcus aureus* (mm)	*Escherichia coli* (mm)
Control	0.0 ± 0.0	0.0 ± 0.0
NC1	0.0 ± 0.0	0.0 ± 0.0
NC3	0.0 ± 0.0	0.0 ± 0.0
NC5	0.0 ± 0.0	0.0 ± 0.0
NC1-TO	14.2 ± 0.8	11.5 ± 0.6
NC3-TO	16.8 ± 0.9	13.2 ± 0.7
NC5-TO	18.5 ± 1.0	14.8 ± 0.8
TO-Control	19.2 ± 1.1	15.6 ± 0.9

Table 5 indicates that films without thyme essential oil exhibited no antimicrobial activity against either bacteria. In contrast, all thyme oil-containing films showed significant inhibition zones, with larger diameters against Gram-positive *S. aureus* (14.2–19.2 mm) compared to Gram-negative *E. coli* (11.5–15.6 mm). The TO-control sample (without nanoclay) showed the highest antimicrobial activity, suggesting that nanoclay may slightly retard the release of volatile antimicrobial compounds ($p < 0.05$ for all comparisons).

Table 6. Total viable count (log CFU/mL) of pasteurized milk during storage at 4 °C.

Storage day	Control packaging (PE)	NC3-TO film	NC5-TO film
Day 0	2.15 ± 0.15	2.10 ± 0.12	2.12 ± 0.14
Day 7	4.82 ± 0.28	2.95 ± 0.20	2.78 ± 0.18
Day 14	6.94 ± 0.35	4.12 ± 0.25	3.85 ± 0.22
Day 21	8.56 ± 0.42	5.68 ± 0.30	5.24 ± 0.28

Table 6 demonstrates the practical efficacy of the developed films. While milk packaged in conventional polyethylene reached microbial counts exceeding 8.5 log CFU mL^{-1} by day 21 (indicating spoilage), the NC3-TO and NC5-TO films significantly suppressed bacterial growth, maintaining counts below 6.0 log CFU mL^{-1} at the same time point ($p < 0.001$). The NC5-TO film performed marginally better than NC3-TO, but the difference was not statistically significant ($p > 0.05$). Fig. 2 provides a comparison of the antimicrobial efficacy of the developed films. Conventional PE packaging allowed rapid microbial proliferation, exceeding 8.5 log CFU mL^{-1} by day 21, whereas both nanocomposite films containing thyme oil significantly delayed bacterial growth, keeping counts below 5.7 log CFU mL^{-1} at the same time point ($p < 0.001$). The NC5-TO film exhibited slightly better performance, but the difference compared to NC3-TO was not statistically significant ($p > 0.05$), indicating that 3% nanoclay loading may be sufficient for practical applications.

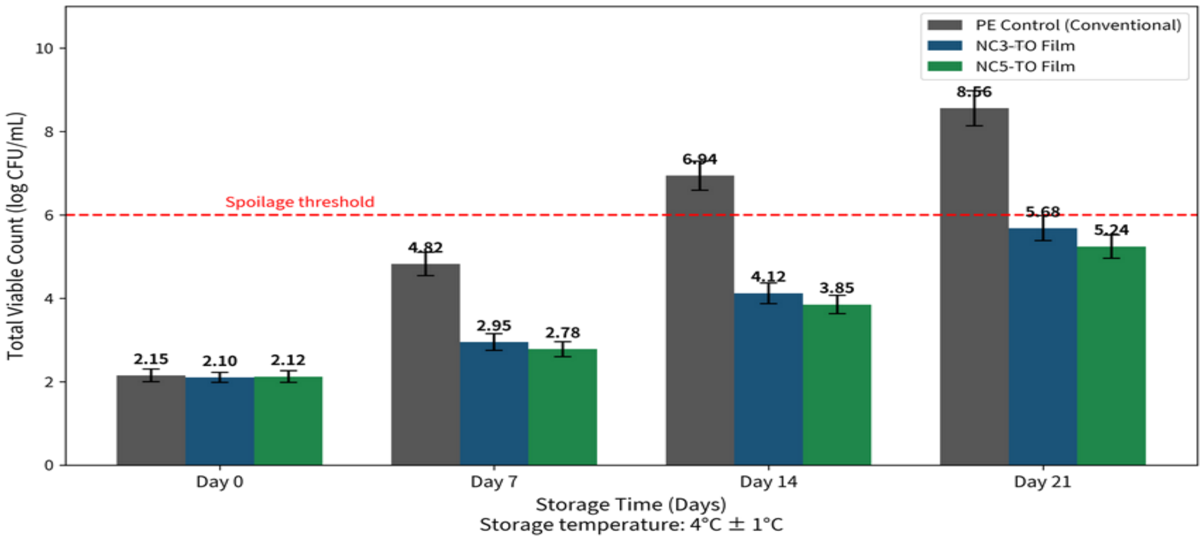

Fig. 2. Comparative microbial growth inhibition of milk during 21-day storage.

DISCUSSION

From the results of this research, one can observe that the use of montmorillonite nanoclay in the film matrix, containing polylactic acid and starch, leads to a significant increase in the physical properties of the films. Raising the nanoclay content from 0 to 5 wt% led to an increase in tensile strength of films from 18.4 to 31.2 MPa (p < 0.05). This effect is a result of the proper distribution of nanoclay particles in the polymer matrix (confirmed by SEM and X-ray diffractograms) and the creation of strong physical interactions between the nanoclay sheets and polymer chains. On the other hand, an increase in nanoclay content led to a decrease in strain to break point value from 3.62 to 31.6%, which means that the film stiffness is increasing, but not excessively for dairy products packaging. Also, the results on water vapor permeability are quite encouraging. Reduction in water vapor permeability value from 42.8 to 18.4 g m^{-2} day^{-1} following the introduction of 5% of nanoclay (50% reduction) is indicative of tortuous pathways offered by the nanoparticles to the water vapor molecules. It has been found consistent with earlier studies on PLA and starch nanocomposites, providing a way to address one of the most significant disadvantages of bio-polymers, which is their moisture sensitivity. Furthermore, the increased value of water contact angle in the NC5 sample from 4.58 to 3.85 degrees signifies a higher degree of hydrophobicity on the film surface, and thus less moisture absorption and extended shelf-life for dairy products. The clear impact of antimicrobial properties of the films indicates the importance of thyme essential oil in inhibiting bacteria growth; hence, the samples of films that lack essential oil have not resulted in any inhibition zone. The diameter of the inhibition zone in the case of *Staphylococcus aureus* bacteria in TO-Control sample has been measured at 19.2 mm, being slightly larger than that in the NC5-TO sample with a measure of 18.5 mm (p < 0.05). The reduction in size could be attributed to the process of adsorption of some of the volatile components of the essential oil by nanoparticles in the film composition, resulting in slower diffusion and delayed release. Higher inhibition of Gram-positive bacteria (*Staphylococcus*) compared to Gram-negative bacteria (*Escherichia coli*) can be attributed to differences in their cell wall structures, consistent with previous research on thyme oil. Results gained from packaging the milk using nanocomposite films compared to polyethylene packaging demonstrated their practical efficiency. Thus, while in the milk in the polyethylene package the total bacterial number was 8.56 log CFU mL^{-1} on the 21st day (indicating complete spoilage of milk and its unsuitability for consumption), in the case of NC5-TO packaging, the total number of microorganisms was 24.5 log CFU mL^{-1}, which means the increase in product shelf life by at least 7 days (p < 0.001). Notably, the reason for the increased product shelf life is not limited to the antimicrobial properties of essential oil. In addition, the low oxygen and water vapor permeability contributes greatly to inhibiting microbial growth. In view of the results of mechanical and barrier properties testing, it would be advisable to consider NC3-TO with a strength of 26.8 MPa and permeability of 58.5 as the most optimal option in view of the balance between mechanical and barrier properties and cost, despite the higher efficiency of NC5-TO regarding microbial inhibition. There are also several limitations associated with this research that need to be considered. For instance, the impact of the films on the sensory quality of dairy products (for example, the taste and consistency) has not been studied in the long term, and the economic feasibility of large-scale production of these films has not been assessed. Nonetheless, based on this research, it appears that the films under discussion can easily be used as a substitute for plastic wrap in the Kazakhstan dairy industry.

CONCLUSION

The research proved that nanocomposite films made of polylactic acid and starch combined with montmorillonite nanoclay and thyme essential oil could be seen as an efficient and ecologically friendly substitute for traditional plastics applied for packaging in the dairy sector. The usage of 5% nanoclay increased the tensile strength by 70% and water vapor permeability by 50%, and employing thyme essential oil resulted in significant antimicrobial properties in relation to pathogens. The optimized NC3-TO sample with the strength of 26.8 MPa, water vapor permeability of 58.5 and growth zone diameter of 16.8 mm appeared to be the best compromise in terms of physical properties and antimicrobial action. Milk packaging studies have shown that these films were able to increase the shelf-life of dairy products by at least 7 days, which was an important result for Kazakhstan due to climatic and transport conditions. However, to develop industrial manufacturing and replace traditional plastics, future research needs to be centered around improving production costs, economic evaluation, exploring the effects on sensory quality of dairy products, and studying the biodegradation process in actual environmental conditions in Kazakhstan. It is expected that the results obtained by this research will be a very useful step forward for reduction of plastic pollution and increase in food safety in Kazakhstan and in other neighboring countries.

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