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Anbar Journal of Agricultural Sciences
(University of Anbar – College of Agriculture)

Effect Of Alginate Film Containing Plant Extract with Nisin on The Properties and Extended Shelf Life of Minced Meat

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Article info

Received: 2025-01-22
Accepted: 2025-05-31
Published: 2025-12-31

DOI-Crossref:
10.32649/ajas.2025.189373

Cite as:
Hamarashid, Sh. H., Jafar, N. B., and Mirzan, N. A. (2025). Effect Of Alginate Film Containing Plant Extract with Nisin on The Properties and Extended Shelf Life of Minced Meat. Anbar Journal of Agricultural Sciences, 23(2): 1343-1362.

Abstract

This study investigated the development and application of sodium alginate film combined with nisin and pomegranate peel extract (PoPE) to improve the preservation of minced meat. Five different edible film levels were evaluated, namely control (T1), sodium alginate with 2.5% PoPE and nisin (T2), sodium alginate with 5% PoPE and nisin (T3), sodium alginate with 7.5% PoPE and nisin (T4), and sodium alginate with 10% PoPE and nisin (T5). It was then applied to minced beef and stored for examination at 0, 7, and 14 days at 4 °C. All coated meat except for the control were found to be significantly acceptable. The physical, antioxidant, and mechanical properties of the alginate films with varying concentrations of PoPE and nisin were characterized. They exhibited significant increases in antioxidant activity, reduced water solubility, and improved tensile strength in line with the amount of PoPE content. Over the 14 day period, these active films, particularly those with higher PoPE and nisin, significantly inhibited microbial growth including aerobic, *sychrotrophic*, and *Enterobacteriaceae* counts, and slowed lipid oxidation on the minced meat. This decrease TBARS and peroxide values. Active films substantially improved the color, aroma, appearance, and overall acceptability of the meat in

comparison to the control. The results suggest that alginate films containing PoPE and nisin provide a promising natural and biodegradable solution for extending the shelf life, maintaining quality, and enhancing the safety of minced meat. This solution has potential for enhancing food preservation in the meat industry.

Keywords: Pomegranate peel powder, Antimicrobial properties, Shelf life, Edible films, Minced meat.

تأثير غشاء الألجينات المحتوي على مستخلص نباتي مع النيسين على خصائص الغشاء وإطالة مدة صلاحية اللحم المفروم

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الخلاصة

بحثت هذه الدراسة تطوير استخدام أغشية الألجينات مع النيسين ومستخلصات قشور الرمان (PoPE) لتحسين حفظ اللحم المفروم الذي تم تخزينه في درجة حرارة 4 درجات مئوية لمدة 14 يوم. تضمنت الدراسة تحضير خمسة أغشية مختلفة صالحة للأكل (T1: فيلم تحكم، T2: صوديوم ألجينات مع 2.5% PoPE والنيسين، T3: صوديوم ألجينات مع 5% PoPE والنيسين، T4: صوديوم ألجينات مع 7.5% PoPE والنيسين، و T5: صوديوم ألجينات مع 10% PoPE والنيسين) ثم تم تطبيقها على اللحم المفروم وتخزينها في اليوم (0، 7، و14) عند درجة حرارة 4 درجات مئوية. كانت جميع اللحوم المغلفة مقبولة بشكل ملحوظ. باستثناء مجموعة التحكم. تم تحديد الخصائص الفيزيائية، والأنشطة المضادة للأكسدة، والخصائص الميكانيكية لأفلام ألجينات مع تركيزات مختلفة من PoPE والنيسين. أظهرت الأفلام زيادة ملحوظة في النشاط المضاد للأكسدة، وتقليلاً في القابلية للذوبان في الماء، وتحسينًا في قوة الشد مع زيادة محتوى PoPE على مدى فترة الـ 14 يومًا، أدت هذه الأغشية النشطة، خاصة تلك التي تحتوي على نسبة أعلى من مستخلص قشر الرمان والنيسين، إلى تثبيط نمو الميكروبات بشكل ملحوظ، بما في ذلك تعداد البكتيريا الهوائية واللبكتريا المحبة للبرودة والمعوية وتباطئ أكسدة الدهون عند تغليف اللحم المفروم بها. وقد ظهر ذلك من خلال انخفاض قيم TBARS والبيروكسيد. حسّنت الأغشية النشطة بشكل كبير كل من الخواص الحسية كاللون والرائحة والمظهر والقبولية العامة للحوم مقارنةً بعينات السيطرة. اشارت النتائج إلى أن أغشية

الجينات الصوديوم المدعمة بمستخلص قشور الرمان والنيسين ستكون حلول واعدًة طبيعية وقابلًا للتحلل الحيوي لإطالة العمر الافتراضي والحفاظ على الجودة وتعزيز سلامة اللحوم المفرومة. هذه الحلول تدعم اتجاه حفظ الأغذية في صناعة اللحوم.

كلمات مفتاحية: مسحوق قشر الرمان، خصائص مضادة للميكروبات، مدة الصلاحية، افلام صالحة للأكل، لحم مثروم.

Introduction

Minced meat is a highly perishable food product that provides an ideal environment for microbial growth, making it particularly vulnerable to spoilage. This deterioration leads to financial losses for producers and poses health risks to consumers owing to the potential presence of harmful microorganisms. Consumer concerns over the side effects of chemical preservatives and their strong preference for natural alternatives has made the use of natural preservatives in food products increasingly popular (3 and 14). The increased demand for sustainable preservation techniques has driven research into creative ways to minimize spoiling, prolong shelf life, and decrease dependence on synthetic chemicals while maintaining product quality.

Biopolymer-based edible films are one of the new solutions as they not only act as physical barriers but can also be functionalized with antimicrobial agents to actively combat microbial growth (6). For food packaging, most research has focused on replacing petrochemical-based polymers with biodegradable materials (25). Therefore, using biodegradable materials helps prolong the shelf life of foods (31). The use of edible films and coatings can potentially increase the shelf life of food products, as well as are biodegradable, biocompatible, recyclable, and derived from renewable sources to address environmental awareness (34). They have been shown to act as natural barriers to prevent the loss of lipids, flavoring chemicals, moisture, and gas exchange (oxygen, carbon dioxide).

As natural biopolymers are environmentally benign, biodegradable, and renewable, their use in the production of edible coatings has several advantages (28). Brown seaweed is a primary source of alginate, a naturally occurring polymer. It has special colloidal qualities as it can crosslink with calcium ions to form strong gels and insoluble polymers when exposed to calcium chloride. Various studies have shown alginate to be a good film-forming agent for food packaging because of its biodegradability and biocompatibility. Applying alginate as a coating to foods can help prolong shelf life, maintain functional qualities, and minimize negative changes such as weight loss during storage. Additionally, alginate films can serve as carriers for bioactive ingredients, such as antioxidants and antimicrobial agents (11 and 17).

Pomegranate peel extract is a sustainable and valuable ingredient for the development of health-promoting products and the reduction of agro-industrial refuse due to these bioactivities (17). Moreover, nisin, a bacteriocin produced by *Lactococcus lactis*, demonstrates potent antimicrobial activity against gram-positive bacteria, which include various spoilage and pathogenic organisms that are prevalent in meat products. The integration of nisin into alginate films provides dual benefits of extending the

microbial safety and storage life of minced meat while contributing to eco-friendly packaging solutions (35). The integration of nisin into alginate films influences the properties of multiple films. Adding nisin to alginate films does not significantly weaken their structure but might slightly lower their tensile strength because it disrupts the polymer matrix. Nisin in the film can improve the barrier properties of the film by lowering the water vapor permeability, which slows down the dehydration of food products (36).

Also, the film's ability to fight microorganism is improved because nisin stops the growth of gram-positive bacteria, which are the main contributors to spoilage and poisoning in meat products (31). It enhances the barrier characteristics of the film, thereby reducing its permeability to gases and moisture, which are critical factors in the spoilage of minced meat. Moreover, the mechanical properties of the film, such as tensile strength and elongation at break, play a crucial role in ensuring durability and ease of application, making it a viable option for industrial-scale food preservation (15).

The aim of this study was to prepare a sodium alginate edible film composite with nisin and evaluate the effects of nisin on the physicochemical and antimicrobial properties of the films. The principal objectives were to enhance the film formulation, describe its properties, and evaluate its capacity to prolong the shelf life of minced meat.

Materials and Methods

Sodium alginate (SA), calcium chloride/CaCl2, and glycerol as plasticizers were purchased from a local chemical market, gallic acid and Folin-Ciocalteu as reagents from Sigma-Aldrich, together with sodium carbonate, pomegranate peel, deionized water, distilled water and ethanol.

Extraction of pomegranate peel powder: To prepare the alcohol extract, 10 g of powdered pomegranate peel was dried and mixed with 250 mL of 80 % ethanol (v/v). The mixture was then stirred overnight at room temperature (39). Whatman No. 1 filter paper was used to filter the solution. The extract was poured into sterilized glass Petri dishes and incubated at 50 °C for 48 h. After drying, the extracts were stored in sealed bottles at 4 °C for use as natural antioxidants (13, 30 and 31).

Preparing the nisin solution: The nisin solution was prepared by dissolving 1000 IU in 50ml/ml sterilized water (37) and nisin was added to the film solution.

Preparation of film formation: 1 % (w/v) was added to 100 mL of deionized water and stirred for 30 min at 70 °C. After cooling to 55 °C, glycerol 0.75% (v/v) was added, followed by stirring for another 15 minutes (4, 12, 20 and 23). Pomegranate peel extract at concentrations of (2.5, 5, 7.5, and 10) % (w/v) was added to the film solution (39) and mixed thoroughly. Then, nisin was added. After that 50 ml of the solutions were poured into a 10 cm × 10 cm Petri dish. The film was re-dried for 1 minute after being immersed into the calcium chloride solution (45 mL) (23). Forceps were used to peel the films that had been formed and kept in desiccators before further testing (22). For each film, more than three replicates were considered. The different types of edible films are shown in Table 1.

Table 1: Different types of alginate films.

Edible film	Components
T 1 *	NaA
T 2	NaA+ 2.5%, PoPE+ Nisin
T3	NaA+ 5%, PoPE+ Nisin
T4	NaA+ 7.5%, PoPE+ Nisin
T5	NaA+ 10%, PoPE+ Nisin

*Control alginate film.

Characterization of the films:

Physical Properties:

Moisture content: Based on AOAC (5), the moisture content of the films was determined by dehydrating samples of approximately 0.5 g at 105 °C for 24 hours in triplicate. The moisture is expressed as a percentage of the total weight (23).

$$Moisture\% = \frac{W1 - W2}{W1 - W0} \text{x}100$$

Solubility: The water solubility of the edible films was measured using the method described by (11). Films were sliced into 2 cm × 2 cm squares and dried at 60 °C for 24 h, after which their initial dry weight was recorded. Each piece was then immersed in 25 mL of deionized water for 2 hours at room temperature (25±1 °C) (11). The films were then dried again in an oven at 60 °C for another 24 h. The final weight of the dried films was measured after cooling to room temperature (11).

$$Solubility\% = \frac{I.Weight - F.Weight}{I.Weight} \text{x}100$$

Thickness: Film thickness was determined using a hand-held digital micrometer (2 and 25). Measurements were taken at ten randomly selected spots on each film, and the average of these readings was used to calculate the final thickness value (22).

Antioxidant properties:

Free radical scavenging activity by DPPH radical scavenging assay: The antioxidant activity of the film extracts was assessed following the method described by (33), where Acontrol and Asample represent the absorbance readings for the control and sample, respectively.

$$DPPH\% = \frac{AControl - ASample}{Acontrol} \text{x}100$$

Radical Scavenging Activity (ABTS): A 100 µL solution from the film sample was taken and mixed with 0.9 mL ABTS solution and kept in the dark for 15 minutes. The ability of the film extract to scavenge ABTS radicals was then assessed (22).

$$ABTS\% = \frac{AControl - ASample}{AControl} \text{x}100$$

Mechanical Properties: An electronic universal tensile testing machine was employed following the ASTM D882-12 method, as outlined by (16).

Tensile strength: The film was sliced into rectangles measuring 2 cm × 7 cm, with a clamp distance of 40 mm and a tensile testing speed of 10 mm/min. (16).

$$TS(MPA) = \frac{MaximunTensileForceWhenTheFilmBroke(N)}{CrossSectinalArea(mm2)}$$

EAB elongation: Films were sliced into strips measuring 50 mm in length and 10 mm in width, ensuring they were free of air bubbles or defects. The grip separation was set to 30 mm, and the cross-head speed was adjusted to 25 mm/min. Each film strip was pulled in one direction until it broke, at which point the breaking force and elongation were recorded.

$$EAB\% = \frac{MaximumLenght\,\mathrm{Re}achedWhenTheFilmBroke(mm)}{InitialLengthOfFilm(mm)} X100$$

Water vapor permeability: Using a small glass cup for measurement, WVP was set to a uniform shape and diameter of 4, 4.1, 4.5 cm for internal depth, external depth, and length, respectively. The films were sealed at cup mouths and fixed completely by O-rings elastic placed around them. Then, 10 ml of distilled water was poured into each cup and weighed, and placed in a desiccator with RH of 55%±1% using silica dioxide. Eight measurements were made over 24 hours. The water vapor transmission rate was calculated by dividing the slope of the weight gain versus time graph by the exposed film area. Water vapor permeability (WVP) of the films was measured gravimetrically, in triplicate, following a modified version of the ASTM E96-01 method as described by (7). The films were sealed onto glass cups (4 cm outer diameter, 3.8 cm inner diameter, 4 cm depth) filled with silica gel using an O-ring elastic. The cups were then placed in desiccators containing saturated magnesium nitrate Mg (NO3)26HNO3 solution (50% relative humidity) and maintained at 25 °C in electronically controlled incubators. The relative humidity gradient across the film was 50:0 (outside: inside the cups). The cups were weighed to a precision of 0.0001 g at regular intervals once the rate of weight change stabilized (8).

$$WVP = \frac{WVTR.x}{P0.(RH2 - RH1)}$$

Application of film on minced meat: The minced meat was prepared and separated into portions of 5 gm each, and then packaged immediately according to edible film type (12). The packages were placed on a plate and refrigerated at 4 ºC for two weeks. Weekly tests were conducted on both the control and packed meat samples.

Physicochemical Analyses:

pH measurement: Ten grams of each raw minced beef sample were combined with 100 mL of water. The mixtures were homogenized, filtered, and the pH of the resulting filtrate was measured using a pH meter. The pH meter had been calibrated at values of 7.0 and 4.0 using standard buffer solutions kept at room temperature (14).

Thiobarbituric acid reactive substances (TBARS): A mixture containing 2 g of minced meat, 100 µL of butylated hydroxytoluene (1 g/L), and 16 mL of trichloroacetic acid (50 g/L) was filtered. Next, 2 mL of the filtrate were combined with 2 mL of thiobarbituric acid solution (20 mmol/L). The mixture was then heated to 100 °C and subsequently cooled. The TBARS values were reported as milligrams of malonaldehyde (MDA) per kilogram of sample (7).

Peroxide value (PV): Five grams of the sample was stirred into 30 mL of a solvent mixture of acetic acid and chloroform (3:2 v/v). Subsequently, 0.5 mL of a saturated solution of potassium iodide (KI) was added, and the mixture allowed to stand in the dark for 1 minute to free the iodine. After addition of 30 mL distilled water, the liberated iodine was titrated against 0.01 N sodium thiosulfate (Na2S2O3) solution. Thereafter, it used starch as an indicator for the blue color disappearance. The PV analysis was carried out and expressed as meq of peroxide/kg in the test sample (10).

Microbiological Analysis: Ten grams of meat from each sample were combined with 90 mL of sterilized peptone water and homogenized in a stomacher for this analysis. The samples were subsequently decimally diluted and plated onto solid culture media for inoculation. For APC it was incubated at 30 °C for 48 h in plate count agar (32), for PTC at 7 °C for 10 days in PCA (ISO 17410, 2001) (40), and for EB counts at 37 °C for 24 h in violet red bile glucose agar (34).

Sensory attributes: Eight non-trained participants from the university aged 23 to 53 and non-smokers were used to evaluate the sensory quality of the raw minced beef (fresh) packed with NaA films. The panelists evaluated each sample at various sampling durations of 0, 7, and 14 days. They scored color, appearance, odor, and overall acceptability on a 9-point hedonic scale (15).

Statistical Analysis: Analysis of variance (ANOVA) was used to assess statistical differences among group means, and the least significant difference (LSD) post hoc test was employed to identify significant differences between treatments.

Results and Discussion

Physical properties of the film: The integration of PoPE and nisin into alginate films had a substantial impact on their physical characteristics, such as thickness, water solubility, and moisture content (Table 2). A decrease in MC was observed as the concentrations of PoPE and nisin increased. For example, the hydrophobic nature of PoPE and potential interactions between it and nisin with the alginate matrix may have been responsible for the substantially lower MC values of the T3 to T5 films, which could have restricted moisture retention (20). In comparison to the control, the films formulated with NaA without PoPE and nisin exhibited higher moisture content at 36.53%. The MC of the alginate films decreased as the extract concentration and nisin increased. In a comparable study, (15) observed that the MC values of the control films were higher than those formulated with banana peel extract. Consequently, MC values may be diminished by the incorporation of plant extracts into packaging films.

The water solubility of the films decreased with the addition of POPE and nisin in T3, T4, and T5. T3 had the lowest WS (34.53%), indicating that the water barrier properties of the film improved. This improvement is due to the phenolic compounds in PoPE, which lower the solubility of the biopolymer films by enhancing their interaction. The incorporation of PoPE and nisin resulted in a decrease in WS from 55.84% to 43.2%. The WS controls the resistance and integrity of the film in aqueous media. A significant decrease in WS was observed with increasing incorporation of nisin and PoPE. This could be attributed to the increased hydrophobicity and fortification of the polymer network in the prepared films. Due to an increase in the phenolic content, WS in the films is lowered, which may enhance hydrophobicity. As

a result, resistant films were produced. In some possible applications, the WS of films plays an important role as it affects the integrity, moisture barrier properties, and shelf life of the food product (15 and 39).

The thickness of the films varied slightly, with T5 having the highest value (0.063 mm). The potential interactions within the polymer network and the inclusion of solid PoPE may be the reasons for the increase in thickness. The incorporation of plant extracts frequently produce an increase in the thickness of consumable films, due likely to their ability to modify the film forming behavior and contribute to the overall solid content (11). The results indicate that adding PoPE and nisin to alginate films can enhance film solubility by decreasing it because that result is useful for meat packaging, resists moisture content, and significantly increases their thickness. These improvements enhance food packaging applications by providing greater mechanical strength and lower water permeability.

The addition of pomegranate peel extracts at (2.5, 5, 7.5, and 10) % and nisin significantly modified the water vapor permeability (WVP) of sodium alginate films. Film T3 demonstrated the lowest permeability at 0.005 (g•mm/m²•h•kPa) while T1, T4, and T5 had somewhat elevated yet comparable values of 0.007, whereas T2 showed a considerable decrease (0.006). The WVP values varied from 0.005 to 0.007 (g•mm/m²•h•kPa). The decrease in WVP, especially in films with moderate PoPE, can be ascribed to the polyphenolic constituents of the pomegranate peel. These components are recognized for enhancing intermolecular interactions, leading to the creation of a denser polymer matrix, and thus, a more efficient barrier against moisture migration (18). Nisin may marginally modify the hydrophilic-hydrophobic balance, but the interaction of PoPE with alginate remains the primary influence. Nonetheless, the WVP did not diminish linearly as the PoPE concentration escalated. Certain therapies with elevated PoPE demonstrated slight elevations in WVP that reverted to baseline levels. Higher extract loading may cause aggregation or phase separation, which might create microvoids or structural discontinuities in the film that allow water vapour to pass through. The mechanical strength of the sodium alginate films was altered by the addition of nisin and PoPE (Table 2). The tensile strength values varied from 44.6 MPa to 46.0 MPa. The film with the highest PoPE concentration, T5, was 46.003 MPa and demonstrated the highest TS value, marginally increasing as PoPE levels increased. This enhancement in TS is likely the result of the polyphenolic content of PoPE, which can function as a natural cross-linker, thereby enhancing the cohesion of the biopolymer structure and fostering stronger intermolecular interactions and network formation within the film matrix (1).

The elongation at break (EL), a measure of film flexibility, also increased from 2.89% to 4.39% as the PoPE and nisin content rose. This might be because PoPE and nisin affect some of the hydrogen bonding between alginate chains, thus affecting their mobility and flexibility and reducing strength slightly. The higher EL causes the films to become less brittle and more ductile, which is advantageous for real-world uses such food packaging (1). (27) reported comparable enhancements in mechanical performance when phenolic extracts were combined with alginate edible film. The addition of PoPE and nisin makes the film stronger and more flexible, as shown by the higher tensile strength and elongation, especially at moderate to high amounts of PoPE.

Table 2: Physical properties of alginate film containing PoPE with nisin.

Films	MC (%)	WS (%)	THK (mm)	WVP(g•mm/m²•h•kPa)	Mechanical Properties	
					TS (Mpa)	EL (%)
T1	36.53 a	65.38b	0.041cde	0.007ab	44.650	2.893i
T2	29.2 c	50.6 def	0.046bcd	0.006bcd	44.6gfgh	3.440 g
T3	26.4 def	34.53 ijk	0.053abc	0.005ef	45.193cde	4.053d
T4	26.87 de	36.29 hij	0.046bcd	0.007bc	45.56abc	4.280 c
T5	24.13 gh	43.9 efg	0.063a	0.007bc	46.003a	4.393 b
LSD value	2.07	4.14	0.01	0.001	0.7	0.1

*MC: moisture content, WS: water solubility, THK: thickness, WVP: water vapor permeability.

Antioxidant properties of the film: The antioxidant activity of alginate films was considerably improved by the incorporation of nisin and (PoPE), as assessed by the DPPH and ABTS radical scavenging assays (Table 3). The control film, T1, contained only sodium alginate and exhibited negligible antioxidant activity (19). Nevertheless, films with progressively higher concentrations of PoPE (T2–T5) demonstrated a statistically significant, dramatic increase in their capacity to scavenge free radicals. The DPPH scavenging activity of T2 – T5 increased from 61.07% to 92.46%, whereas the ABTS scavenging activity increased from 28.14% to 71.98%. The polyphenol compounds in pomegranate peel are directly responsible for progressive antioxidant enhancement. These compounds are known to donate hydrogen atoms or electrons to neutralize free radicals (9).

Table 3: Antioxidant properties of alginate films containing PoPE and nisin.

Films	DPPH %	ABTS %
T1	0	0
T2	61.07f	28.14f
T3	75.18	40.73e
T4	87.59b	52.22b
T5	92.46a	71.98a
LSD value	1.05	1.15

The strong positive correlation between the film's antioxidant capacity and PoPE concentration is consistent with previous reports on edible films enriched with plant polyphenols (9, 19 and 24). The potential for the development of highly active antioxidant packaging materials was underscored by the films' near-complete DPPH inhibition at the highest PoPE level (T5). These results confirm that sodium alginate films can be functionalized with pomegranate peel extract to generate active packaging that offers substantial antioxidant advantages. It helps improve the safety of perishable food products and extends their shelf life by preventing oxidative spoilage (21).

Influence of alginate film on minced meat:

pH values: The natural progression of protein and lipid degradation over time is consistent with the increased pH values of minced meat wrapped with diverse edible films during refigerated storage (15). Nevertheless, the pH values of the samples coated with alginate film containing PoPE and nisin (specifically T4 and T5) were substantially lower than those of the control (T1) after 7 and 14 d (Table 4). The pH of

T1 reached 8.12 on day 14, which is indicative of significant spoilage. In contrast, T5 had a much lower pH of 6.06, suggesting better preservation. Polyphenols in PoPE and nisin are antimicrobial and inhibit microbial growth and further proteolytic activity. Consequently, the lower pH remained intact. This effect delays the alkalization of meat decomposition (21 and 30).

Lipid oxidation: LO as measured by TBARS increased in all samples over storage (Table 4). The control film (T1) exhibited the greatest TBARS values at 3.00 mg MDA/kg by day 14, suggesting that it had undergone extensive oxidation. In contrast, T4 and T5, which had higher PoPE and nisin, exhibited significantly lower TBARS values of 0.77 and 0.70 mg MDA/kg, respectively. The strong antioxidative effect of PoPE was demonstrated by this significant inhibition, which is consistent with previous research identifying the high content of polyphenols and flavonoids in pomegranate peel as capable of scavenging free radicals and reducing oxidative damage (3).

The primary oxidation products (PV) exhibited comparable tendencies. T1 achieved 8.85 meq peroxide/kg after 14 days, whereas T5 maintained the lowest values of 4.10 meq peroxide/kg (Table 4). The antioxidant compounds in PoPE provide both initial and sustained protection against lipid peroxidation during storage. These data indicate that sodium alginate films with different concentrations of PoPE and nisin (particularly T4 and T5) are highly effective in maintaining the quality of minced meat during refrigeration. These films significantly reduce both secondary (TBARS) and primary (PV) lipid oxidation products, delay the rise in pH, and thereby maintain chemical stability and extend the shelf life of meat. These results are consistent with reports that edible biopolymer films enriched with plant polyphenols or antimicrobials can be employed as active packaging to enhance the quality and safety of food (2).

Table 4: Effect of sodium alginate edible film with PoPE and Nisin on lipid oxidation and the physicochemical properties of minced meat stored at 4 °C.

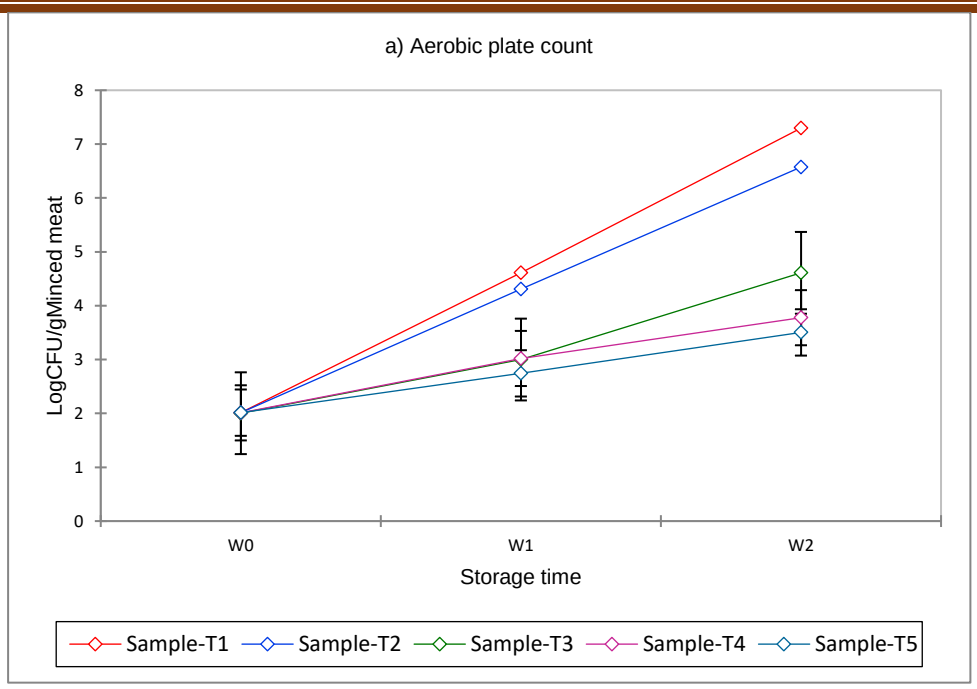
Parameters	Film	Storage (days)		
		0	7	14
pH	T1	5.663 ij	6.617 cde	8.123 a
	T2	5.520 j	6.530 def	7.463 b
	T3	5.947 ghi	6.773 cd	6.933 c
	T4	5.913 ghi	6.150 fgh	6.283 efg
	T5	5.797 hij	6.027 ghi	6.057 gh
	LSD value		0.4	
TBARS	T1	0.233 h	2.733 ab	3.000 a
	T2	0.267 h	2.100 c	2.667 b
	T3	0.333 gh	1.233 e	1.600 d
	T4	0.367 gh	0.700 f	0.767 f
	T5	0.400 gh	0.600 fg	0.700 f
	LSD value		0.3	
	T1	0.230 hi	6.217 c	8.850 a
	T2	0.400 h	5.297 d	7.110 b
PV	T3	0.343 hi	4.190 e	7.040 b
	T4	0.313 hi	3.777 f	5.237 d
	T5	0.340 hi	3.453 g	4.103 e
	LSD value		0.2*	

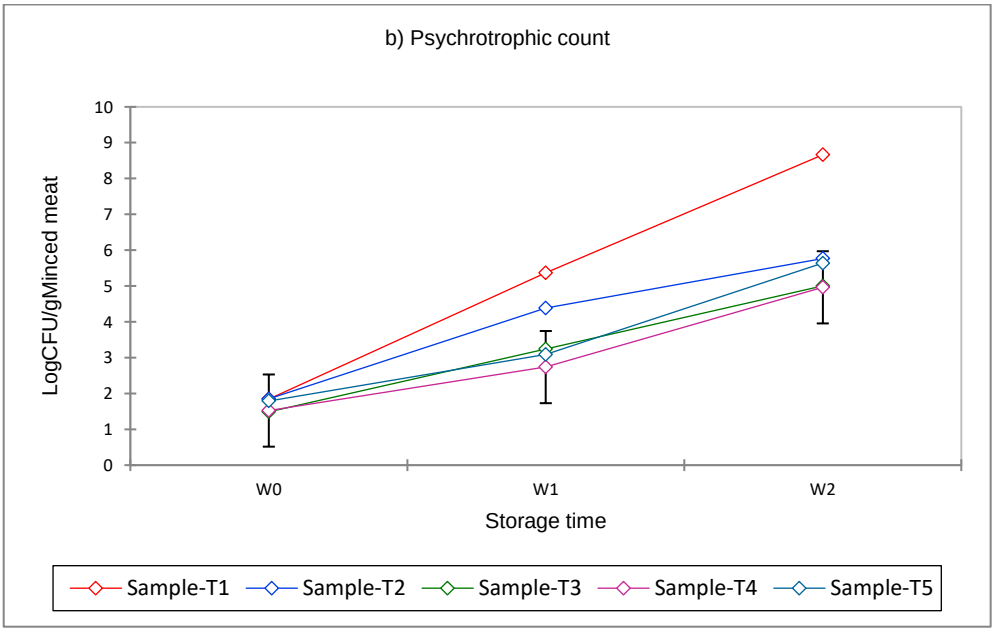
Least significant difference (LSD) value between treatments of 0.2, which indicates the difference between the means that can be considered statistically significant ($p < 0.05$). * Numbers in the table represent an average of triplicate.

Microbial analysis: The aerobic plate count (APC) is a key microbiological indicator of meat spoilage and microbial safety during storage. As shown in Figure 1, the APC (log_{10} CFU/g meat) increased in all the film-coated samples during refrigerated storage at 4 °C, consistent with typical microbial growth patterns in perishable meat products. Despite this, the control T1 film had the highest amount of APC because it was free from PoPE and nisin. This could be because nisin is known to be effective in inhibiting common pathogens, particularly on meat surfaces. Although nisin is an effective antimicrobial for food safety, its direct application becomes difficult owing to binding, degradation, and diffusion into food, resulting in reduced activity. Strong interactions between the polymers and between nisin and alginate can decrease the amount of free, available nisin in films (9 and 24).

On day 0 (W0), all samples showed similarly low APC values, reflecting effective initial microbial control, likely due to handling under hygienic conditions, with no significant differences among treatments. The microbial load of all treatments increased significantly as storage advanced from day 0 to day 7 (W1) and day 14 (W2). However, the respective rates of increase differed significantly. For T1 (control), all treatments recorded elevated APCs for both W1 and W2. The APC of T1 neared 7 log_{10} CFU/g by W2, suggesting fast spoilage and little antimicrobial effect from the control NaA film. T2 and T3 showed some antimicrobial activity owing to the presence of 2.5% and 5% PoPE and nisin, respectively. T4 and T5, which had higher concentrations of PoPE (7.5% and 10%) and nisin, exhibited the lowest APC throughout storage.

On day 14 (W2), these samples had about 4-5 times fewer bacteria per gram compared to the control (T1), showing that they were good at preventing bacterial growth. These results suggest that the increased levels of PoPE and nisin in NaA films significantly affect the inhibition of aerobic bacterial growth in minced meat during cold storage. The antimicrobial effect can be due to the action of pomegranate peel polyphenols that disrupt cellular integrity by interfering with cell wall synthesis. Maintaining APC values below the spoilage threshold (generally stated as 6–7 log_{10} CFU/g for meat) has a quality and safety rationale. The use of both POPE and nisin embedded in edible alginate films was found to efficiently prolong the microbial shelf life of minced meat and could be a good natural food preservative (29).


a) Aerobic plate count
LogCFU/gMinced meat
0
1
2
3
4
5
6
7
8
W0
W1
W2
Storage time
Sample-T1
Sample-T2
Sample-T3
Sample-T4
Sample-T5


b) Psychrotrophic count
LogCFU/gMinced meat
0
1
2
3
4
5
6
7
8
9
10
W0
W1
W2
Storage time
Sample-T1
Sample-T2
Sample-T3
Sample-T4
Sample-T5

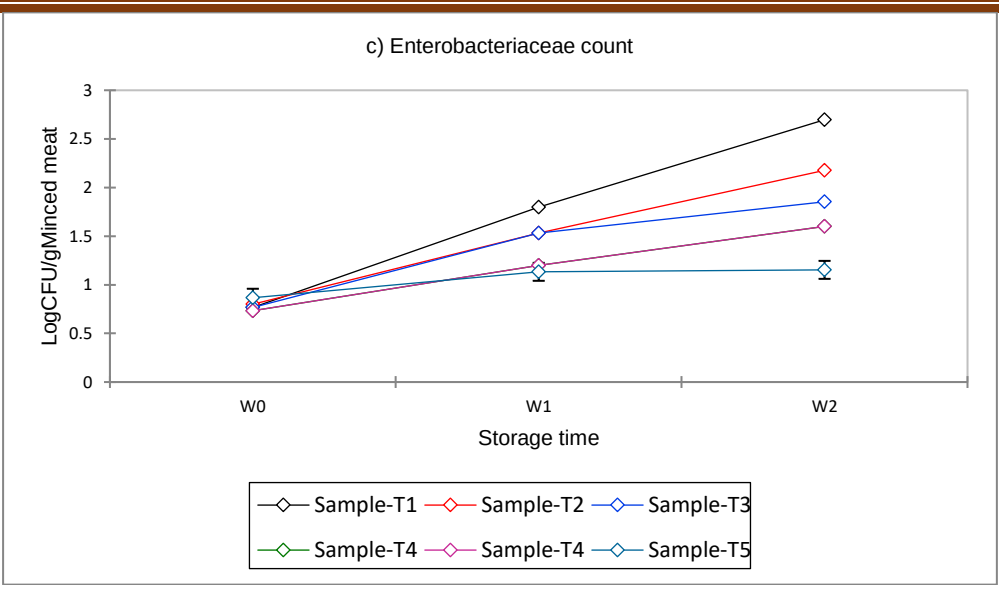


Figure 1: a) Aerobic plate count (log10 CFU) in minced meat with different types of NaA films tested at various storage times at 4 °C, b) *Psychrophilic* bacteria, and c) *Enterobacteriaceae* were evaluated. Each data point represents the mean value, with the error bars indicating the standard deviation.

Psychrotrophic bacteria are nonpathogenic and present as spoilage bacteria. Most are of the *Pseudomonas species*. While they spoil stored milk, they do not cause illness. On day 0, all samples, including those coated with NaA films with or without PoPE/nisin, showed low, similar *psychrotrophic* counts. Thus, they were of similar quality. Moreover, effective hygienic handling was performed prior to storage. During storage, the increase in *psychrotrophic* counts were measured, which varied significantly (Figure1). The control (T1) had the fastest and highest increase in *Psychrotrophic* (culturable) microbial count, reaching nearly 9 $\log_{10}$ CFU/g by week 2, indicating a rapid increase in spoilage microbes. This shows that the plain NaA film possesses minimal inhibitory effect against cold-tolerant spoilage microbes.

The expressions in T2 and T3 were moderately suppressed. During week 2, a steady growth pattern was observed in *psychrotrophic* counts, which were lower than that of the control. The *psychrotrophic* loads over the storage period were found to be significantly lower for T4 and T5 (films with higher concentrations of PoPE and nisin). In week 2, the sample count remained below 6 $\log_{10}$ CFU/g, reflecting slower growth. The reduced *psychrotrophic* growth in T4 and T5 was considerable, indicating the possibility of the combined antimicrobial action of PoPE and nisin within the NaA film.

Pomegranate peel extract is rich in polyphenols and flavonoids with known bacteriostatic and bactericidal actions, while nisin directly targets gram-positive psychrotrophs. This combination likely interferes with critical cellular functions of *psychrotrophic* spoilage organisms, thereby extending the microbial shelf life of the product. According to food safety guidelines and industry standards, a count above 6–7 $\log_{10}$ CFU/g is associated with unacceptable spoilage. The NaA films with higher PoPE and nisin content (T4 and T5) kept the *psychrotrophic* population well below these thresholds over two weeks, suggesting they can effectively extend the shelf life and maintain the microbiological quality of minced meat under refrigeration.

However, at week 0, the *Enterobacteriaceae* counts of all samples were similar (≈ 0.7–0.9 $\log_{10}$ CFU/g), indicating comparable initial microbial loads across treatments. The count in all samples experienced an increase during storage and was highly dependent on the type of film used. T1 experienced the largest increase at nearly 2.7 $\log_{10}$ CFU/g on the 2nd week. T2 and T3 also experienced an increase but were slightly lower than the control. T4 and T5 showed the lowest *Enterobacteriaceae* counts during storage time, with T5 having the lowest at about 1.2 $\log_{10}$ CFU/g. The slower increase in the bacteria count in T4 and T5 indicates more efficient antimicrobial action when more extract and nisin were added to the film. *Enterobacteriaceae* are spoilage and hygiene indicators, with their lower populations indicating the meat is more hygienic and safer during storage. PoPE and nisin could be used in alginate films to improve microbial control on food against gram-negative bacteria.

Sensory characteristics: Initially, all samples had high color scores of approximately 6- 8, with T1 and T2 exhibiting the highest values. On day 7, most treatments maintained good color, although slight declines were noted in T1, T3, and T4. However, on day 14 the control sample exhibited a dramatic loss of color quality at 3.63, likely due to oxidation and spoilage. T5, on the other hand, maintained a high color score of 7.5, indicating notable preservation in comparison to the other groups. Meat color was preserved during storage using NaA films with greater PoPE and nisin concentrations, especially T5. PoPE's antioxidant properties probably stopped discoloration and pigment oxidation. At day 0 all treatments had similar and favourable appearance scores. However, appearance maintained well in T2, while others showed slight declines on day 7. The control and T2 dropped below 6, while T3, T4, and T5 preserved higher appearance scores (~6- 6.75) on day 14. Films with PoPE and nisin better preserved the visual and surface quality of minced meat.

Table 5: Impact of NaA films with PoPE and Nisim on color, appearance, odor and the overall acceptability of coated minced meat stored at 4 °C.

Properties	Treatments	Storage (days)			LSD Value
		0	7	14	
Color	T1	8.125 ab	7.125 cde	3.625 j	0.4
	T2	8.000 ab	7.750 abc	5.250 i	
	T3	6.375 fg	7.000 def	5.625 hi	
	T4	6.375 fg	6.875 def	6.625 efg	
	T5	6.000 gh	7.250 cde	7.500 bcd	
LSD value					0.7
Appearance	T1	8.125 a	6.125 bc	5.250 d	0.4
	T2	8.125 a	7.875 a	5.625 cd	
	T3	6.375 b	6.750 b	6.125 bc	
	T4	6.500 b	6.500 b	6.125 bc	
	T5	6.375 b	6.750 b	6.750 b	
LSD value					0.7
Odor	T1	8.750 a	5.875 h	3.625 j	0.3
	T2	8.500 ab	7.250 de	5.125 i	
	T3	7.625 cd	6.500 fg	5.750 h	
	T4	8.000 bc	7.250 de	6.875 ef	
	T5	7.500 cd	7.375 de	7.250 de	
LSD value					0.6
Overall Acceptability	T1	9.000 a	6.125 d	3.500 f	0.3
	T2	8.500 a	7.625 b	5.375 e	
	T3	7.250 bc	7.000 c	6.125 d	
	T4	7.375 bc	7.125 bc	5.875 de	
	T5	7.125 bc	7.250 bc	7.375 bc	
LSD value					0.5

The active compounds minimize visible spoilage and dehydration. Accordingly, high odor acceptability was seen across all samples on day 0 (Table 5). Sharp decline occurred in odor acceptability for the T1 control at 3.625 by day 14, indicating spoilage. T5, and to a lesser extent T4, maintained much higher odor ratings (above 7 by day 14). Edible films with PoPE and nisin effectively restricted off-odors and spoilage aromas. This aligns with their antimicrobial effect, reducing the metabolic byproducts of spoilage bacteria. Eventually, on day 0 all coated minced meat had high acceptability. After one week at 4 °C, T1 dropped rapidly, but T2–T5 remained relatively high (over 7.0). On day 14, T1 was judged unacceptable (3.5) and rejected, while T5 was best retained (7.38). Other films with PoPE/nisin (T3, T4) showed moderate retention.

Incorporating higher levels of pomegranate peel extract and nisin into NaA edible films significantly improved the sensory quality and shelf life of minced meat at 4 °C. (26). These films outperformed the control in color stability, maintained more desirable appearance and odor, and achieved higher overall acceptability over two weeks. This supports prior findings that active edible films with natural extracts extend not only the safety but also the consumer acceptability of meat products. The antimicrobial agent nisin is incorporated to reduce bacterial spoilage on the food surface. This type of coating demonstrated improved preservation and shelf-life extension for mushrooms (24).

The combined treatment substantially inhibited microbial growth, reduced lipid oxidation, and maintained sensory qualities, thereby extending the shelf life. According to the investigations, these consumable coatings work well to maintain the quality of refrigerated beef, which is a potential natural preservation method. As a result, its shelf life is significantly longer than with conventional storage techniques. This method underscores the potential of natural preservatives to enhance the longevity and safety of fresh meat (38).

Conclusions

Alginate films with PoPE and nisin are biodegradable alternatives to plastic packaging that can protect the environment and food safety through antimicrobial behavior. Also, they can prolong the shelf life of food products, particularly perishable items such as meat. The results show that incorporating PoPE and nisin into sodium alginate edible films improves the preservation of minced meat in refrigerated storage. The addition of PoPE and nisin into alginate films also improved the physical properties of the films; that is, enhanced their tensile strength and flexibility, reduced water solubility and moisture content, and improved antioxidant capacity with increased concentrations of the agents.

When applied to minced meat, the films were highly effective at inhibiting lipid oxidation and microbial growth, especially at higher PoPE as natural compound and nisin levels (oregano). This effectiveness was evident from lower TBARS, peroxide values, and microbial counts of aerobic, psychrotrophic, and *Enterobacteriaceae* compared to the control. The improved chemical and microbiological properties resulted in more advantageous physicochemical qualities, such as lower pH and oxidation, and greater sensory scores of color, odor, appearance, and total acceptability throughout 14 days of chilled storage.

Overall, a natural biopreservation system based on sodium alginate films enriched with plant extracts and bacteriocin (nisin) could enable extending the shelf life and maintaining the quality of minced meat. The two-fold capacity for antioxidant and antimicrobial purposes creates enormous potential for active food packaging systems to satisfy consumer demand for clean labels and safer meat.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Shokhan Hidayat Hamarashid: conceptualization, investigation, methodology, project administration; Nehan Bahaaldden Jafar: writing – original draft, writing – review and editing; Naska Abdulqadir Mohammed Mirzan: writing – review and editing, conceptualization, and methodology.

Funding:

This research received no external funding.

Institutional Review Board Statement:

The study was conducted following the protocol authorized by the Head of the Ethics Committee, University of Sulaimani, Food Science and Quality Control Department.

Informed Consent Statement:

Not applicable.

Data Availability Statement:

Data available upon request.

Conflicts of Interest:

The authors declare no conflict of interest.

Acknowledgments:

The authors extend their sincere thanks to the College of Agricultural Engineering Science, University of Sulaimani, and College of Sciences, University of Kirkuk.

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