

APPLICATION OF CARRAGEENAN–PVA BIOPLASTIC REINFORCED WITH IONIC GELATION-DERIVED NANOCHITOSAN AS SUSTAINABLE PACKAGING FOR FISHBALLS

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Submitted: 20 March 2026/Accepted: 10 June 2026
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How to cite (APA Style 7th): Fadhallah, E. G., Febriati, N., & Rahmawati. (2026). Application of carrageenan–PVA bioplastic reinforced with ionic gelation-derived nanochitosan as sustainable packaging for fishballs. *Jurnal Pengolahan Hasil Perikanan Indonesia,* 29(6), 539-561. http://dx.doi.org/10.17844/c6jk3287

Abstract

Shrimp shell waste generated by seafood processing industries remains underutilized and may contribute to en-vironmental pollution. Valorization of this waste as a source of nanochitosan for carrageenan–polyvinyl alco-hol (PVA)-based bioplastic films represents a promising approach for sustainable food packaging development. This study aimed to synthesize and characterize carrageenan–PVA bioplastic films incorporated with na-nochitosan prepared via ionic gelation and evaluate their potential as packaging materials for fishballs during storage. The study employed a randomized block design with five nanochitosan concentrations (0, 0.5, 1.0, 1.5, and 2.0%) and three replicates. The evaluated parameters included mechanical properties, water vapor trans-mission rate (WVTR), swelling behavior, biodegradability, and application tests on fish balls. The synthesized nanochitosan exhibited an average particle size of 162.3±36.8 nm with a polydispersity index of 0.489. The incorporation of nanochitosan significantly increased the tensile strength and elongation while reducing the WVTR and swelling values. However, the film thickness and Young's modulus were not significantly affected. The bioplastic films exhibited tensile strengths of 20.40–24.94 MPa, elongations of 448.75–583.75%, and thicknesses ranging from 0.09 to 0.17 mm. The best performance was obtained at 2.0% nanochitosan incorpo-ration, resulting in the lowest WVTR value of 1.34 g/m²/day. Soil burial tests revealed that the bioplastic films completely degraded within 3–6 d. The application of the films as packaging showed potential to preserve the quality of fishballs by reducing weight loss and maintaining the moisture content, texture, and protein content during 14 days of storage at 4°C. These findings indicate that the developed bioplastic films have strong poten-tial as environmentally friendly packaging materials for fishery products.

Keywords: biodegradation, biopolymer, fish processing, shrimp waste, WVTR

http://dx.doi.org/10.17844/c6jk3287

Aplikasi Bioplastik Karagenan–PVA Berpenguat Nanokitosan Hasil Gelasi Ionik sebagai Kemasan Berkelanjutan untuk Produk Bakso

Abstrak

Limbah cangkang udang dari industri pengolahan hasil perikanan belum dimanfaatkan secara optimal dan berpotensi menimbulkan pencemaran lingkungan. Pemanfaatan limbah tersebut sebagai sumber nanokitosan untuk pembuatan bioplastik berbasis karagenan–polivinil alkohol (PVA) menjadi salah satu pendekatan dalam pengembangan kemasan pangan berkelanjutan. Penelitian ini bertujuan untuk menyintesis dan mengkarakterisasi film bioplastik karagenan–PVA terinkorporasi nanokitosan hasil gelasi ionik serta mengevaluasi potensinya sebagai kemasan untuk bakso ikan selama penyimpanan. Penelitian menggunakan rancangan acak kelompok dengan lima taraf perlakuan konsentrasi nanokitosan (0; 0,5; 1,0; 1,5; dan 2,0%) dan tiga ulangan. Pengujian meliputi sifat mekanis, laju transmisi uap air (WVTR), swelling, biodegradabilitas, dan aplikasi pada bakso ikan. Nanokitosan yang dihasilkan memiliki ukuran partikel rata-rata 162,3±36,8 nm dengan indeks polidispersitas 0,489. Penambahan nanokitosan meningkatkan kuat tarik dan elongasi serta menurunkan nilai WVTR dan swelling secara signifikan, sedangkan ketebalan film dan modulus Young tidak berbeda nyata. Film bioplastik memiliki kuat tarik 20,40–24,94 MPa, elongasi 448,75–583,75%, dan ketebalan 0,09–0,17 mm. Perlakuan terbaik diperoleh pada penambahan nanokitosan 2% dengan nilai WVTR terendah 1,34 g/m^2/hari. Film bioplastik terdegradasi dalam 3–6 hari melalui uji penguburan dalam tanah. Aplikasi film sebagai kemasan menunjukkan potensi dalam mempertahankan mutu bakso ikan melalui penekanan susut bobot serta mempertahankan kadar air dan tekstur, dan kadar protein selama 14 hari penyimpanan pada suhu 4oC. Hasil penelitian menunjukkan bahwa film bioplastik berpotensi sebagai kemasan ramah lingkungan untuk produk perikanan.

Kata kunci: biodegradasi, biopolimer, limbah udang, olahan ikan, permeabilitas kemasan

INTRODUCTION

Plastic waste from food packaging is a major global environmental concern. Synthetic plastics are widely used because they are lightweight, flexible, strong, and easily molded, making them suitable for applications ranging from food packaging to household products. However, conventional plastics are difficult to degrade and contribute to long-term environmental pollution. In Indonesia, the total amount of waste generated is estimated at 30.88 million tons per year, with approximately 17.5% being synthetic plastic waste (Zumira & Surtikanti, 2023). The degradation of plastics in the environment produces microplastics that contaminate soil and aquatic ecosystems and enter the food chain (Alberghini *et al*., 2022). Rahmawati *et al*. (2023) reported the presence of 108–199 microplastic particles per fish in several species caught in Indonesian waters, indicating the potential contamination of fishery products. In addition, hazardous compounds such as phthalates can migrate from packaging materials into food and act as endocrine disruptors that may cause reproductive disorders, anemia, and immune system abnormalities (Muncke *et al*., 2020; Chakori *et al*., 2021; Hlisníková *et al*., 2024). These concerns highlight the need to develop safer and more sustainable food-packaging materials (Najahi *et al*., 2025). Consequently, biodegradable packaging materials have been widely explored as environmentally friendly alternatives to conventional plastic.

Bioplastics are considered a promising solution because they are derived from renewable resources and can be degraded by microorganisms. Natural polymers, such as starch, cellulose, and chitosan, have been extensively investigated as biodegradable packaging materials (Hong *et al*., 2021). However, bioplastics based on single polymers often exhibit limited mechanical strength and water resistance. Therefore, polymer blending or reinforcement is commonly applied to improve the functional properties of these materials. Among various biopolymers, carrageenan has attracted considerable attention because of its film-forming ability and compatibility with food systems.

Carrageenan is a sulfated polysaccharide extracted from red seaweed (Rhodophyta) and is widely used as a thickener, stabilizer, and gelling agent in the food industry (Bhat *et al.*, 2020). The three main types of carrageenan are kappa, iota, and lambda. Iota carrageenan forms gels that are more elastic, flexible, and transparent than those formed by kappa carrageenan, making it more suitable for film packaging applications (Jabeen *et al.*, 2025). However, carrageenan films are highly hydrophilic and easily absorb moisture, which reduces their stability (Hidayati *et al.*, 2019). One strategy to improve carrageenan-based films is to blend them with polyvinyl alcohol (PVA). PVA is a synthetic polymer produced by the hydrolysis of polyvinyl acetate and is known for its excellent film-forming ability, biodegradability, water solubility, and mechanical strength (Kaynarca *et al.*, 2023). The combination of carrageenan and PVA has been reported to enhance the flexibility and mechanical properties of films (Liu *et al.*, 2022; Hanani *et al.*, 2023). Previous studies have also demonstrated that carrageenan–PVA-based bioplastics meet the international food packaging film standard Japanese Industrial Standard (JIS) Z 1707 (Fadhallah *et al.*, 2025). However, improving the barrier and functional properties of carrageenan–PVA films remains a challenge for food packaging applications.

The incorporation of reinforcing agents is a common strategy for enhancing the performance of bioplastic films. Chitosan, a natural biopolymer derived from crustacean shell waste such as shrimp shells, has significant potential as a sustainable packaging material. Chitosan is nontoxic, biodegradable, biocompatible, and exhibits antimicrobial and hydrophobic properties that can improve packaging performance (Koirala *et al.*, 2024). Its effectiveness can be further enhanced by reducing the particle size to the nanometer scale through the ionic gelation method, resulting in nano-chitosan. Nanochitosan can disperse more uniformly within polymer matrices and improve intermolecular interactions, while also exhibiting stronger antimicrobial activity than conventional chitosan (Safitri *et al.*, 2022). Moreover, nanochitosan derived from shrimp shell waste has been reported to improve the mechanical and functional properties of various biopolymer films (Koirala *et al.*, 2024), while simultaneously providing a strategy for valorizing seafood-processing by-products.

Despite these developments, carrageenan-based bioplastics have several limitations. Carrageenan films reinforced with nanocellulose have shown good mechanical properties but high water solubility (Nurhabibah & Kusumaningrum, 2021), while some formulations exhibit excessively rapid biodegradation, which may reduce practical stability during use (Timbuleng *et al.*, 2023). Other studies have developed chitosan-based bioplastics with antibacterial agents; however, their performance has not been evaluated in real food systems (Amanda *et al.*, 2020). Although nanochitosan has been widely investigated in biopolymer matrices such as cellulose, starch, and alginate (Ngo *et al.*, 2021; Deng *et al.*, 2022; Amaregouda & Kamanna, 2024; Guzmán-Pincheira *et al.*, 2025), its incorporation into carrageenan–PVA matrices remains limited, particularly in applications involving fishery products. Fishballs are highly perishable and sensitive to storage conditions, making them suitable models for evaluating the packaging performance (Tavares *et al.*, 2021). Therefore, this study aimed to synthesize and characterize carrageenan–PVA bioplastic films incorporated with nanochitosan prepared via ionic gelation and evaluate their potential as packaging materials for fishballs during storage. This approach also highlights the potential valorization of shrimp shell byproducts as sustainable resources for biodegradable food packaging.

MATERIALS AND METHOD

Preparation of Nanochitosan

The shrimp shell waste of *Litopenaeus vannamei* was obtained from a local shrimp processing industry in South Lampung, Lampung, Indonesia. The collected shells were washed under running water to remove adhering impurities and dried for 6 h prior

http://dx.doi.org/10.17844/c6jk3287

to chitosan extraction. Chitosan synthesis followed the method described by Barleany *et al*. (2023), which consists of deproteinization, demineralization, and deacetylation. During deproteinization, 50 g of shrimp shells were immersed in 1 M NaOH solution at a ratio of 1:10 (w/v) and heated at 80°C for 3 h with occasional stirring. The mixture was then filtered, washed with running water until a neutral pH was reached, and drained. Demineralization was performed by soaking the material in 2 M HCl solution (1:10 w/v) for 2 h. The sample was filtered again, washed with water until a neutral pH was achieved, and drained. At this stage, chitin was obtained as an intermediate product. Deacetylation was performed by treating chitin with 20% NaOH solution at 100°C for 60 min. The resulting crude chitosan powder was repeatedly washed with distilled water until a neutral pH was achieved, and then dried.

Nanochitosan was synthesized using the ionic gelation method following Bavel *et al*. (2023). Briefly, chitosan powder (4 g) was dissolved in 1% acetic acid solution (200 mL) and stirred until homogeneous. Subsequently, 20 mL of 0.1% sodium tripolyphosphate (STPP, Xingfa) solution was added dropwise to the chitosan solution while stirring at 50°C for 2 h using a hot magnetic stirrer. The resulting nanochitosan suspension was characterized for particle size and polydispersity index using a particle size analyzer (Beckman Coulter LS13-320 XR) based on the dynamic light scattering (DLS) method described by Oudih *et al*. (2023).

Bioplastic Film Formulation

Bioplastic films were prepared using a solution casting method modified from Fadhallah *et al*. (2025). A 10% (w/v) PVA solution was prepared by heating 100 mL of distilled water to 90°C, followed by the gradual addition of 10 g of PVA powder (88.7% hydrolyzed, Merck) while stirring until completely dissolved. After the solution cooled to room temperature, 2 g of carrageenan (Indo-Gum) and 1 mL of glycerol (OneMed) were added as plasticizers. The mixture was then stirred using a magnetic stirrer at 750 rpm for 30 min at 40°C until a homogeneous film-forming solution was obtained. Nanochitosan suspension was then added according to the formulation presented in Table 1, and the mixture was homogenized for 30 min. The resulting film-forming solution was poured evenly onto a glass plate measuring 20×20×3 cm and left at room temperature for 24 h to allow initial gel formation. The glass plate was then placed in a dehydrator and dried at 60°C for 3 h. After drying, the formed bioplastic film was carefully peeled from the glass plate and used for characterization and packaging applications.

Application of Bioplastic as Packaging

Commercial fishballs (Cedea, PT Citra Dimensi Arthali, Indonesia), composed of approximately 47% surimi and 30% tapioca flour, were used as the model food product to evaluate the application of the bioplastic film as a packaging for semi-moist foods. Packaging was carried out by folding the bioplastic film into two layers and sealing three sides (left, right, and bottom) using a heat sealer to form a pouch (Lai *et al.,* 2025). Approximately 60 g of fishballs (5-6 fishballs) were placed into the pouch, and the upper side was sealed using a heat sealer to ensure an airtight

Table 1 Formulation of bioplastic film

Code	Nanochitosan (mL)	PVA 10% (mL)	Gliserol (mL)	Carrageenan (g)
P0	0.0	100	1	2
P1	0.5	100	1	2
P2	1.0	100	1	2
P3	1.5	100	1	2
P4	2.0	100	1	2

package. The packaged samples were stored for 14 days under two storage conditions: room temperature (approximately 25°C) and refrigerated temperature (approximately 4°C). Unpackaged fishballs were used as the control. During storage, quality changes were evaluated by monitoring the weight loss, texture profile (hardness, springiness, and cohesiveness), moisture content, and protein content. Samples exhibiting severe spoilage on day 7 were excluded from further observation.

Mechanical Properties of Bioplastic Films

The mechanical properties of the bioplastic films were determined by measuring the thickness, tensile strength, elongation at break, and Young's modulus according to ISO 527-1 ([International Organization for Standardization] ISO, 2019). Tensile testing was performed using a universal testing machine (Instron 1026) equipped with a 25 kg load cell. The crosshead speed was set to 80 mm/min with a grip distance of 20 mm. The film samples were cut into strips measuring 5×50 mm and mounted vertically on the testing grips at 27°C and 70% relative humidity. During testing, the applied force (kgF) and elongation (mm) were measured. The thickness of the film (in mm) was measured using a thickness gauge (Mitutoyo 7301). The tensile strength (MPa) was calculated by dividing the applied force by the cross-sectional area of the sample. Elongation (%) was calculated as the ratio of the increase in length to the initial length. Young's modulus (MPa) was determined as the ratio of the tensile strength to the elongation. The bioplastic film formulation exhibiting the lowest WVTR value was selected as the optimal formulation and subsequently used for packaging applications and biodegradation tests.

Swelling and Water Vapor Transimission Rate

The film samples (2 × 2 cm) were weighed to obtain their initial weights (W_0). The swelling test was performed according to ASTM D570 ([American Society for Testing and Materials] ASTM, 2022) to determine the water absorption capacity of the films. The samples were immersed in 20 mL of distilled water at room temperature for 1 h. After immersion, the samples were drained for 10 min and gently wiped with tissue paper to remove any surface moisture. The samples were then reweighed to obtain their final weights (W_1). The water absorption (%) was calculated from the weight difference relative to the initial weight.

The water vapor transmission rate (WVTR) was determined using the gravimetric method according to ASTM E96 (ASTM, 2011). The film samples were cut into circular shapes corresponding to the diameter of the test cup (6 cm). Approximately 10 g of silica gel was placed in the cup, and the opening was sealed with a film sample. The specimens were then stored at room temperature for 24 h. The weight difference before and after storage was measured, and the WVTR was expressed in g/m^2/day.

Biodegradation Test

The biodegradability of the bioplastic films was evaluated using the soil burial test method (Rumi *et al.*, 2024). Film samples measuring 3×3 cm and 5×5 cm were buried in soil at a depth of approximately 5 cm in a pot. The soil was watered every two days to maintain the moisture. Observations were conducted every three days until the films showed a visible degradation.

Moisture Content

Moisture content was determined using the gravimetric method according to [Association of Official Analytical Chemists] AOAC) (2019). An empty weighing dish was weighed to obtain its initial weight. Approximately 2 g of fishball sample was placed in a dish and the initial sample weight was recorded. The samples were then dried in an oven at 105°C until a constant weight was reached. After drying, the sample was cooled in a desiccator and weighed again to obtain its final weight. The moisture content (%) was calculated based on the weight loss during drying relative to the initial sample weight.

http://dx.doi.org/10.17844/c6jk3287

Weight Loss

Weight loss was determined using a gravimetric method (Gautam *et al.*, 2025). The fishball samples were weighed using an analytical balance (Shimadzu AY220, accuracy 0.0001 g). The initial weight was recorded on day 0 before storage (W_0), and the weight at each observation time was recorded as W_1. Weight loss (%) was calculated by subtracting the final weight from the initial weight and dividing by the initial weight.

Texture Profile Analysis

The texture profiles of the samples, including hardness, springiness, and cohesiveness, were measured using a Brookfield CT-3 Texture Analyzer. Following Kunnath *et al.* (2022), the test condition was modified and set to a speed of 2.5 mm/s, deformation of 10.0 mm, and trigger force of 15.0 g. Each sample, prepared with dimensions of 20 mm in diameter and 20 mm in thickness, was placed under the probe for analysis. The results of each profile measurement were collected and recorded.

Protein Content

The total protein content of the fishball samples was determined using the Kjeldahl method (AOAC, 2019). Approximately 0.5–1 g of the sample was weighed and digested with concentrated H_2SO_4 in the presence of a selenium catalyst until the solution became clear. The nitrogen released during distillation was captured by an H_3BO_3 solution and subsequently titrated using a standardized 0.01 N HCl solution. The titration volumes of the sample (Va) and blank (Vb) were recorded. The protein content was calculated based on the nitrogen content obtained from titration, multiplied by the protein conversion factor (6.25) and the atomic mass of nitrogen (14.007).

Data Analysis

The mechanical properties, swelling, and WVTR data were analyzed using analysis of variance (ANOVA) to determine the effect of nanochitosan incorporation. Duncan's multiple range test was applied at a significance level of α=0.05. The results are expressed as mean ± standard deviation. Data from PSA characterization, biodegradation test, weight loss, texture profile, moisture content, and protein content analysis were evaluated descriptively. The processed data were presented as line graphs.

RESULTS AND DISCUSSION

Particle Size of Nanochitosan

Particle size analysis (PSA) was conducted to determine the particle size distribution of the synthesized nanochitosan. The PSA results are presented in Figure 1, which illustrates the distribution of the particle diameters in the nanochitosan suspension. In this study, the synthesized nanochitosan had an average particle size of 162.3±36.8 nm, with particle diameters ranging from 137.2 to 528.5 nm and a polydispersity index (PDI) of 0.489. The particle size distribution shown in Figure 1 indicates that most particles were

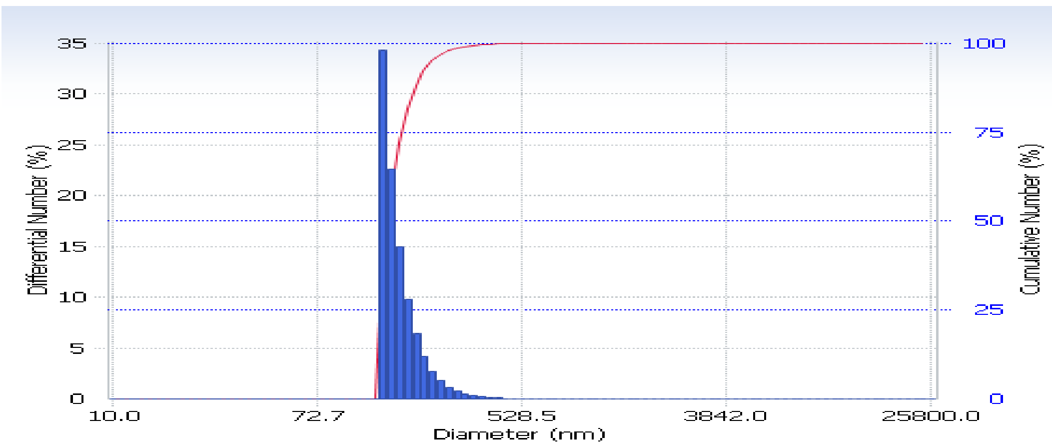

Figure 1 Particle size distribution of synthesized nanochitosan

concentrated within the nanometer range, confirming that the material produced using the ionic gelation method can be classified as nanochitosan. According to Daskar *et al.* (2022), nanoparticles are generally defined as particles with sizes ranging from 1 to 1000 nm, indicating that the nanochitosan produced in this study falls within this range. Previous studies have also reported nanochitosan particles with similar dimensions. Sam *et al.* (2022) reported particle sizes ranging from 54.44 to 230.1 nm when nanochitosan was synthesized using the sonochemical method. Similarly, ionic gelation has been reported to produce nanochitosan particles ranging from 39.65 to 105.8 nm in size.

The polydispersity index (PDI) obtained in this study was 0.489, indicating a moderately uniform particle size distribution. Warsito and Agustiani (2021) reported that PDI values closer to 0 indicate highly uniform particle dispersion, whereas values approaching 1 indicate broader particle size distributions. Therefore, the PDI value suggests that the synthesized nanochitosan exhibited relatively acceptable dispersion stability. The variation in nanoparticle size may be influenced by several synthesis parameters, including chitosan concentration, chitosan–TPP ratio, and stirring conditions during ionic gelation. Rajabimashhadi *et al.* (2025) reported that these factors significantly affect the formation and stability of chitosan nanoparticles. During ionic gelation, electrostatic interactions occur between the positively charged amino groups of chitosan and the negatively charged phosphate groups of TPP, leading to the formation of nanoscale particles. Overall, the results confirmed that the ionic gelation method successfully produced nanochitosan particles within the nanoscale range, which are suitable for incorporation into the carrageenan–PVA bioplastic matrix.

Mechanical Properties

Mechanical properties are critical for evaluating the suitability of bioplastic films for packaging applications. The mechanical properties evaluated in this study were tensile strength, elongation, Young's modulus, and thickness of the films. The results of these measurements are presented in Table 2.

Overall, the addition of nanochitosan improved the mechanical performance of the films, particularly the tensile strength and elongation at break. The tensile strength increased from 20.40 MPa in the control film (P0) to 24.94 MPa in the film with the highest nanochitosan concentration (P4). A similar trend was observed for the elongation at break, which ranged from 448.75% to 583.75%. In contrast, Young's modulus values varied only slightly within the range of 0.039–0.048 MPa, indicating no significant differences among the treatments. The film thickness ranged from 0.090 to 0.170 mm and showed no significant differences. These results indicate that the incorporation of nanochitosan mainly influenced the strength and flexibility of the bioplastic films, while having minimal impact on their stiffness and thickness.

The tensile strength results shown in Table 2 indicate a significant increase with increasing nanochitosan concentration ($p<0.05$). The incorporation of 0.5% did not substantially improve the tensile strength compared to the control film. However,

Table 2 Mechanical properties of bioplastic with variation of nanochitosan concentration

Nanochitosan concentration (v/v) (%)	Tensile strength (MPa)	Elongation (%)	Young's modulus (MPa)	Thickness (mm)
0	20.40±1.85[a]	448.75±28.5[a]	0.048±0.002[a]	0.090±0.025[a]
0.5	20.95±1.70[a]	527.50±31.2[bc]	0.039±0.007[a]	0.120±0.030[a]
1	22.65±1.40[b]	470.83±26.4[ab]	0.048±0.007[a]	0.150±0.028[a]
1.5	24.33±1.60[b]	583.75±22.8[d]	0.043±0.003[a]	0.140±0.027[a]
2	24.94±1.55[c]	561.67±24.1[cd]	0.044±0.005[a]	0.170±0.030[a]

http://dx.doi.org/10.17844/c6jk3287

increasing the concentration to 1% and 1.5% markedly enhanced the film strength, and the highest tensile strength was achieved at 2%. This trend suggests that a higher nanochitosan concentration promotes the formation of a stronger and more compact polymer network within the bioplastic matrix. The addition of nanochitosan improved the tensile strength of the films, with significant differences observed among the treatments. This improvement is likely related to the nanoscale particle size of nanochitosan, which enhances the formation of a compact polymer network within the bioplastic matrix. According to Suhartini *et al*. (2023), incorporating nanochitosan can improve the mechanical properties of packaging films owing to its reinforcing effect on the polymer matrix. Qadri *et al*. (2023) also reported that chitosan addition can increase tensile strength at concentrations below 6%, whereas higher concentrations may reduce tensile strength. The increase in chitosan concentration promoted stronger hydrogen bonding interactions within the polymer matrix, resulting in stronger chemical bonds and a denser film structure (Qadri *et al*., 2023). In the present study, the conversion of chitosan into nanochitosan likely improved its dispersion within the polymer matrix, thereby minimizing the brittleness of the resulting film. Furthermore, the tensile strength values obtained for all treatments met the minimum requirement for food packaging films, which is ≥ 0.39 MPa ([Japanese Standard Association] JSA, 2019).

The addition of nanochitosan also influenced the elongation of the bioplastic films ($p<0.05$). Increasing nanochitosan concentration generally increased the elongation values, although slight decreases occurred at certain concentrations, and the differences were not always significant. The elongation values increased after nanochitosan incorporation, particularly at concentrations above 0.5%. The nonlinear elongation trend may be attributed to variations in the nanochitosan dispersion and agglomeration within the polymer matrix, which can alternately enhance or restrict the polymer chain mobility. Films containing 1.5% and 2% nanochitosan exhibited similarly high elongation values compared to those with lower concentrations, indicating that nanochitosan incorporation at these concentrations maintained film flexibility while strengthening the polymer matrix. Higher nanochitosan concentrations may increase the mobility of the polymer chains within the matrix, thereby improving the film flexibility.

Ermawati and Adi (2023) reported that the highest elongation value was obtained in films containing 1 g of chitosan, while the lowest elongation was observed at higher chitosan concentrations. This is because increasing the chitosan concentration can strengthen the polymer structure, making the film more rigid and less elastic. However, in the present study, the use of nanochitosan rather than conventional chitosan allowed for better dispersion of the particles within the polymer matrix. Consequently, films containing higher nanochitosan concentrations exhibited relatively high elongation values compared to those containing lower nanochitosan concentrations. According to Qadri *et al*. (2023), excessive chitosan concentrations may reduce elongation due to stronger hydrogen bonding interactions that decrease the distance between polymer chains. Nevertheless, the elongation values obtained in this study still met the requirements specified by JIS Z 1707, which classifies biodegradable films with an elongation at break greater than 50% as being in the 'good' category (JSA, 2019).

Young's modulus reflects the stiffness of a bioplastic material, with lower values indicating greater elasticity and flexibility. As shown in Table 1, variations in the nanochitosan concentration did not significantly affect the Young's modulus values ($p>0.05$), indicating that nanochitosan incorporation did not affect the elasticity of the bioplastic films. The elasticity of the films was strongly influenced by glycerol, which acted as a plasticizer. Kholiq *and* Kusuma (2024) stated that glycerol increases polymer chain mobility and reduces intermolecular forces, thereby promoting greater flexibility in bioplastic materials. These findings are consistent with those reported by Brudzynska and Sionkowska (2025), who observed a significant reduction in the Young's modulus of chitosan-based bioplastics owing to variations in glycerol concentration. Furthermore, Kamaluddin *et al.* (2022) reported that increasing the plasticizer concentration can weaken intermolecular hydrogen bonding, thereby enhancing flexibility. Similarly, Putra *et al.* (2025) demonstrated that the addition of glycerol reduced both Young's modulus and tensile strength owing to the loosening of the polymer chain structure. According to JIS Z 1707, biodegradable plastic films are recommended to have a Young's modulus value of at least 0.35 MPa (JSA, 2019). The Young's modulus values obtained in this study ranged from 0.039 to 0.048 MPa, which were lower than the recommended value. This indicates that the films exhibited relatively low stiffness and high flexibility, which may be attributed to the plasticizing effect of glycerol in the bioplastic matrix.

The addition of nanochitosan did not significantly affect the thickness of the bioplastic films ($p>0.05$), with thickness values ranging from 0.090 to 0.170 mm (Table 2). This indicates that the nanochitosan concentrations used in this study were insufficient to cause substantial changes in film thickness. The observed thickness was likely influenced more by the processing conditions, such as the mold surface area and the total volume of the film-forming solution, which were maintained consistently across the treatments. Syamsyyah *et al.* (2023) reported that film thickness is primarily affected by the total solids content and the dimensions of the casting mold. Similarly, Susilowati *et al.* (2025) found that the volume of solution poured into the mold plays an important role in determining the thickness of the film. All films produced in this study met the thickness requirement specified by JIS Z 1707, which recommends a maximum thickness of 0.25 mm for food packaging films (JSA, 2019).

Swelling

The swelling behaviors of the bioplastic films are shown in Figure 2. The results showed that increasing the nanochitosan concentration significantly reduced the swelling values of the films. The control film (P0) exhibited the highest swelling value of 163.14%, followed by P1 (161.67 %). The films containing 0% and 0.5% nanochitosan exhibited similar swelling behavior, whereas higher nanochitosan concentrations resulted in markedly lower swelling values. The lowest swelling value was observed for the film containing 2% nanochitosan, indicating improved water resistance due to the formation of a denser polymer network. Although no specific swelling standard is provided in JIS Z 1707, Amran *et al.* (2025) suggested that water absorption values below 50% are desirable for bioplastics intended for packaging applications. In the present study, the swelling values ranged from 127.64% to 163.14%, indicating that although

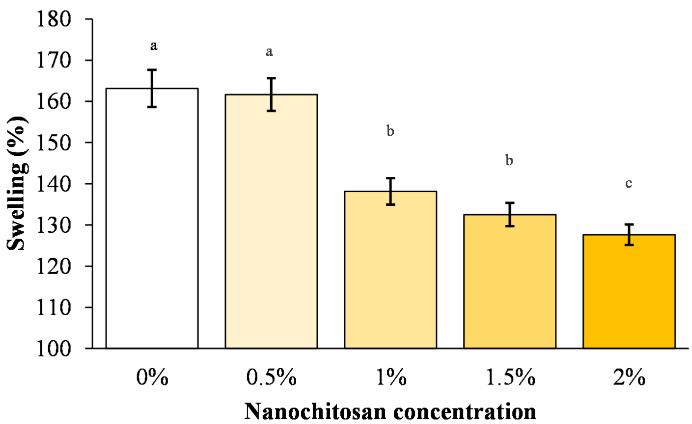

Figure 2 Swelling value of bioplastic films at different nanochitosan concentrations (□ =0%, □ =0.5%, □ =1%, □ =1.5%, □ = 2%). Different superscript letter showed a significant difference at $p<0.05$

http://dx.doi.org/10.17844/c6jk3287

nanochitosan significantly reduced water uptake, further optimization is still required to achieve the recommended level for packaging applications.

In this study, the addition of nanochitosan significantly improved the water resistance of the bioplastic films. Lower swelling values indicate a more compact polymer structure with stronger intermolecular interactions and a higher crosslinking density within the film matrix. Under these conditions, water molecules encounter greater resistance when they diffuse into the polymer network. The incorporation of nanochitosan may strengthen the hydrogen bonding and ionic interactions within the polymer matrix, resulting in a more stable and tightly packed structure. Furthermore, nanochitosan exhibits relatively more hydrophobic characteristics than several other biopolymers, such as starch, which tends to be highly hydrophilic. Therefore, the addition of nanochitosan effectively reduced the water absorption of the bioplastic films. According to Kholiq & Kusuma (2024), increasing the chitosan concentration can enhance the water resistance of bioplastic materials and improve their physical stability. Abdullah *et al.* (2023) stated that the incorporation of chitosan reduced swelling values due to the formation of a more compact polymer network.

Water Vapor Transmission Rate

The water vapor transmission rate (WVTR) of the bioplastic films incorporated with nanochitosan is presented in Figure 3. Overall, increasing the nanochitosan concentration significantly reduced the WVTR of the films ($p<0.05$). The control film (P0) exhibited the highest WVTR value of 4.02 g/m²/day, followed by P1 at 3.78 g/m²/day. A sharp decrease was observed at higher nanochitosan concentrations, where P2, P3, and P4 had WVTR values of 1.99, 1.74, and 1.34 g/m²/day, respectively. These results indicate that the addition of nanochitosan significantly improved the water vapor barrier properties of bioplastic films.

The decrease in WVTR suggests that the incorporation of nanochitosan produced a denser polymer matrix within the carrageenan–PVA bioplastic film. The interactions between the functional groups of nanochitosan, iota-carrageenan, and polyvinyl alcohol may enhance intermolecular bonding and reduce the free volume within the polymer structure. Consequently, the diffusion pathway for water vapor becomes more restricted, thereby lowering the permeability of the film. Similar results were reported by Widiastuti and Marlina (2022), who stated that the addition of chitosan-based nanofillers can improve the barrier properties of bioplastic films against water vapor. In addition, nanochitosan

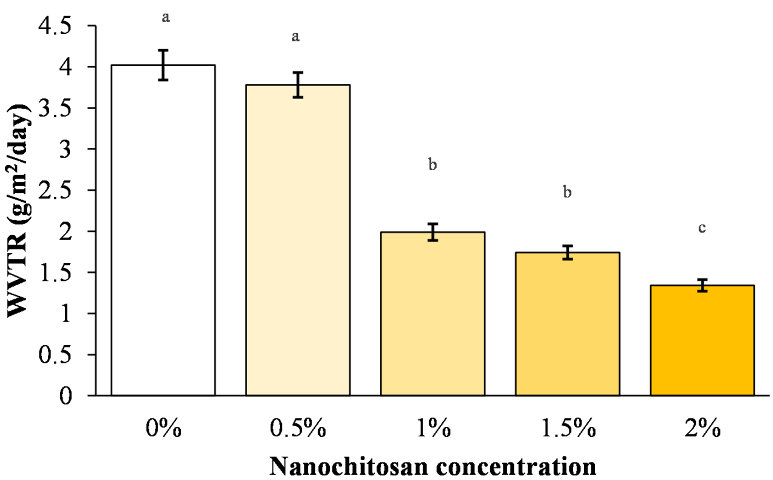

Figure 3 WVTR of bioplastic films at different nanochitosan concentrations (□ = 0%, □=0.5%, □=1%, □ =1.5%, □ = 2%). Different superscript letter showed a significant difference at $p<0.05$

particles may act as physical barriers within the polymer matrix, forcing water vapor molecules to travel through a more tortuous path, further reducing vapor transmission. Based on the classification according to JIS Z 1707, the WVTR values obtained in this study ranged from 1–5 g/m²/day, indicating that all treatments fall into grade 2 packaging material. This suggests that the developed bioplastic films possess adequate water vapor barrier properties for food packaging applications.

Application of Bioplastic as Food Packaging

The bioplastic film with treatment P4 (2% nanochitosan) was selected for food packaging application because it exhibited the lowest WVTR value of 1.34 g/m²/day, indicating superior water vapor barrier properties compared to the other treatments. Lower WVTR values indicate better resistance to moisture transfer, which is essential for maintaining food quality during storage. The resulting bioplastic film formed a thin, flexible, and transparent layer, indicating good film-forming ability and visual suitability for food packaging. In addition to its favorable appearance, the film containing 2% nanochitosan exhibited the best mechanical properties among all formulations, including a film thickness of 0.170 mm, tensile strength of 24.94 MPa, and elongation at break of 561.67%. These values meet the requirements specified by JIS Z 1707 for food packaging films, which recommends a maximum thickness of ≤0.25 mm, a minimum tensile strength of ≥0.39 MPa, and a minimum elongation of ≥50%. Therefore, the P4 bioplastic film can be considered suitable for application as a food packaging material. The combination of good mechanical strength, flexibility, and barrier properties allows the film to effectively protect food products from environmental influences during storage. The application of the bioplastic film as a packaging material for fishballs is illustrated in Figure 4.

Moisture Content

As shown in Figure 5, the initial moisture content of the fishballs was 56.87% for all treatments, and it decreased during storage. After 7 d, the moisture content of the unpackaged samples was 41.48% (25°C) and 39.49% (4°C), whereas the bioplastic-packaged samples retained 38.13% (25°C) and 37.12% (4°C). After 14 d, the moisture content further decreased to 31.80% in unpackaged samples and 28.98% in bioplastic-packaged samples stored at 4°C, indicating that bioplastic packaging slowed moisture loss during storage. The independent samples t-test showed no significant difference in moisture content between the packaged and unpackaged fishballs during storage ($p>0.05$), although the bioplastic-packaged samples tended to exhibit lower moisture loss.

According to SNI 7266:2017 for fishballs, the maximum permissible moisture content is 70% (Badan Standardisasi Nasional [BSN], 2017). The moisture content observed in the present study ranged from 28.98% to 56.87%, indicating that all treatments complied with the SNI requirement throughout the storage period. Although the moisture content

(A) (B)

Figure 4 Application of carrageenan–PVA bioplastic film incorporated with nanochitosan as packaging for fishballs; (A) formation of bioplastic pouch and (B) packaged fishballs.

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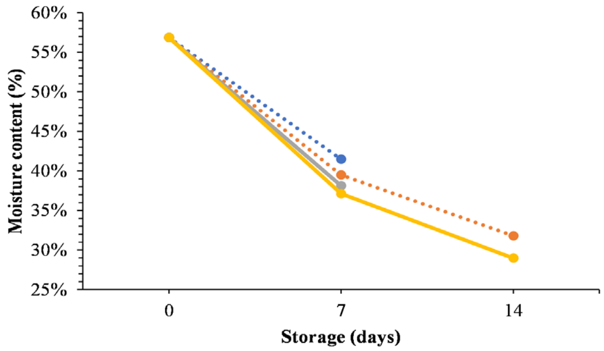

Figure 5 Changes in moisture content of fishballs during storage (=without packaging at 25°C; = without packaging at 4°C; = bioplastic film at 25°C; = bioplastic film at 4°C)

gradually decreased during storage, the values remained within acceptable limits, suggesting that the fishballs maintained their quality during storage.

The decrease in moisture content during storage is mainly associated with water evaporation and moisture migration from the product to its surrounding environment. Packaging materials act as physical barriers that reduce water vapor transfer and maintain product moisture levels. In the present study, the use of bioplastic packaging helped maintain moisture content in fishballs compared to unpackaged samples. This behavior is closely related to the barrier properties of the film, particularly its low WVTR, which limits water vapor diffusion from the food matrix to the environment. Chitosan-based films are widely reported to improve moisture barrier properties because the polymer network forms strong intermolecular interactions that reduce permeability and water diffusion through the film matrix (Cazón *et al.*, 2022).

The presence of chitosan or nanochitosan in packaging films has also been shown to enhance the preservation of fish and meat products by reducing moisture loss during their storage. For example, chitosan-based edible coatings applied to Atlantic bonito fillets have been reported to improve moisture retention and maintain overall quality during refrigerated storage (Ludtke *et al.*, 2025). Similarly, chitosan-based active films applied to chicken fillets significantly improved preservation performance by maintaining product quality and reducing deterioration during cold storage (Shiryanpour *et al.*, 2025). These findings support the results of the present study, indicating that bioplastic films containing nanochitosan can effectively reduce moisture migration and help maintain the quality of fish-based products.

Storage temperature also plays an important role in influencing moisture loss. Samples stored at 25 °C showed a greater decrease in moisture content than those stored under refrigerated conditions (4 °C). Higher temperatures increase molecular mobility and vapor pressure gradients between food products and the surrounding environment, thereby accelerating water evaporation and moisture migration. In contrast, refrigeration slows down diffusion processes and reduces the physicochemical deterioration of high-moisture foods, such as fish products. Similar observations have been reported in studies on bioplastic packaging for fresh fish and meat, where lower storage temperatures significantly reduced moisture loss and maintained product stability during storage (Uysal-Unalan *et al.*, 2024). Overall, the results demonstrate that the combination of nanochitosan-reinforced bioplastic packaging and refrigerated storage is effective for maintaining the moisture stability of fishballs during storage. The improved moisture retention observed in the packaged samples is consistent with the enhanced barrier properties of the chitosan-based bioplastic films, which limit water vapor diffusion and help preserve the quality of perishable fish products.

Weight Loss

Weight loss represents the reduction in product mass during storage caused by water evaporation and the loss of volatile components from the food matrix. The changes in the weight loss of fishballs during storage are presented in Figure 6. The independent samples t-test showed no significant difference in weight loss between packaged and unpackaged fishballs after 7 d of storage (p>0.05). However, after 14 days of storage, the packaged samples exhibited significantly lower weight loss than the unpackaged samples (p<0.05).

As shown in Figure 6, the highest weight loss was observed in fishballs stored without packaging at room temperature (50.37%), whereas the lowest weight loss occurred in fishballs packaged with bioplastic film and stored at 4°C (9.24%). The increase in weight loss is directly associated with the reduction in moisture content, as shown in Figure 5, indicating that water evaporation is the primary contributor to mass loss. The control treatment without packaging showed substantially higher weight loss due to the absence of a physical barrier, which allows water vapor evaporation and oxidative processes to occur more rapidly, particularly at the room temperature. The use of bioplastic packaging significantly reduced the weight loss during storage.

According to Umaraw *et al.* (2020), the application of film coatings can inhibit the transfer of water vapor from food products to the surrounding environment, thereby reducing weight loss in the food product. In this study, the combination of polyvinyl alcohol (PVA), iota-carrageenan, and nanochitosan played an important role in minimizing moisture loss. PVA acts as the primary film-forming matrix that limits water vapor diffusion, whereas iota-carrageenan contributes to the formation of a stable gel network. Nanochitosan generally improves barrier performance relative to bulk chitosan because nanoparticles disperse into the polymer matrix, fill micro-voids, and increase the tortuosity of vapor pathways; nanochitosan-containing films therefore show lower water vapor permeability and better moisture retention (Homayounpour *et al.*, 2020). These nano-effects are consistent with the superior performance of the PVA–carrageenan film reinforced with 2% nanochitosan in this study (lowest WVTR). Temperature amplifies moisture loss because higher temperatures increase vapor pressure and molecular mobility. Consequently, refrigerated storage (4°C) combined with barrier packaging produced the best outcome, in agreement with previous reports that chitosan-coated or chitosan-film-packaged fish/meat showed markedly lower weight loss under cold storage (Karsli *et al.*, 2021).

Texture Profiles

Food texture reflects the structural properties of food and its response to the applied force. In fishballs, texture is primarily influenced by myofibrillar protein interactions and processing conditions. Packaging can help maintain the texture of food by limiting microbial growth and oxidative changes (Meng *et al.*, 2022). In this study, the texture was evaluated using texture profile analysis (TPA), including hardness, springiness, and

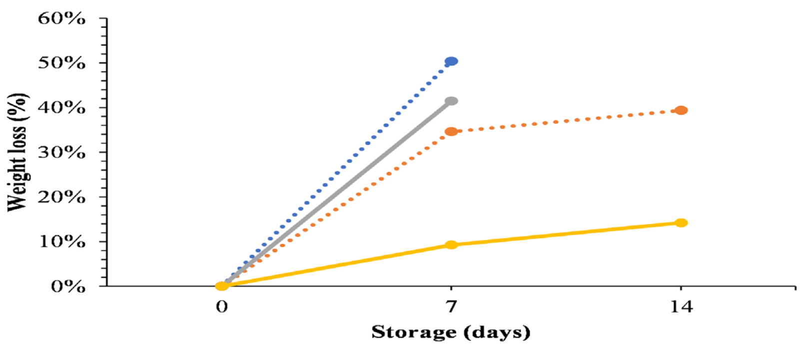

Figure 6 Weight loss of fishballs during storage (=without packaging at 25°C; =without packaging at 4°C; = bioplastic film at 25°C; = bioplastic film at 4°C)

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cohesiveness. The changes in the textural properties presented in Figure 7 are closely related to the reduction in moisture content (Figure 5) and the corresponding increase in weight loss (Figure 6), which affect the structural integrity of the fishball matrix.

Hardness represents the resistance of food to deformation and is related to the force required to compress or break its structure (Rosenthal, 2024). In this study, the hardness of fishballs increased during storage, ranging from 767.50–1,190.67 gF/cm^2 on day 7 to 2,177.67–2,285.33 gF/cm^2 on day 14 (Figure 7a). The independent samples t-test showed no significant difference in hardness between the packaged and unpackaged fishballs during storage (p>0.05). Nevertheless, the hardness values increased progressively during storage in all treatments. Fishballs packaged with bioplastic film also exhibited lower hardness than unpackaged samples. This increase occurred in both packaged and unpackaged samples and was associated with protein network strengthening through disulfide bond formation and non-covalent interactions, which reduced matrix flexibility (Indiarto *et al.*, 2020). Fishballs stored without packaging at 25°C exhibited higher hardness than those packaged with nanochitosan film and stored at 4°C, indicating that both packaging and storage temperature significantly influenced textural stability. Higher temperatures accelerate protein aggregation and moisture loss, resulting in a firmer structure. Similar trends were reported by Indiarto *et al.* (2020), who observed increased hardness in beef meatballs during storage, with strong correlation values (R^2=81.96–97.46%). In contrast, biopolymer-based packaging containing carrageenan and nanochitosan can help retain moisture and maintain structural integrity, thereby moderating the increase in hardness (Meng *et al.*, 2022). Overall, the results indicate that nanochitosan-based bioplastic packaging combined with low-temperature storage is more effective in maintaining the texture of fishballs by slowing structural changes during storage, which are

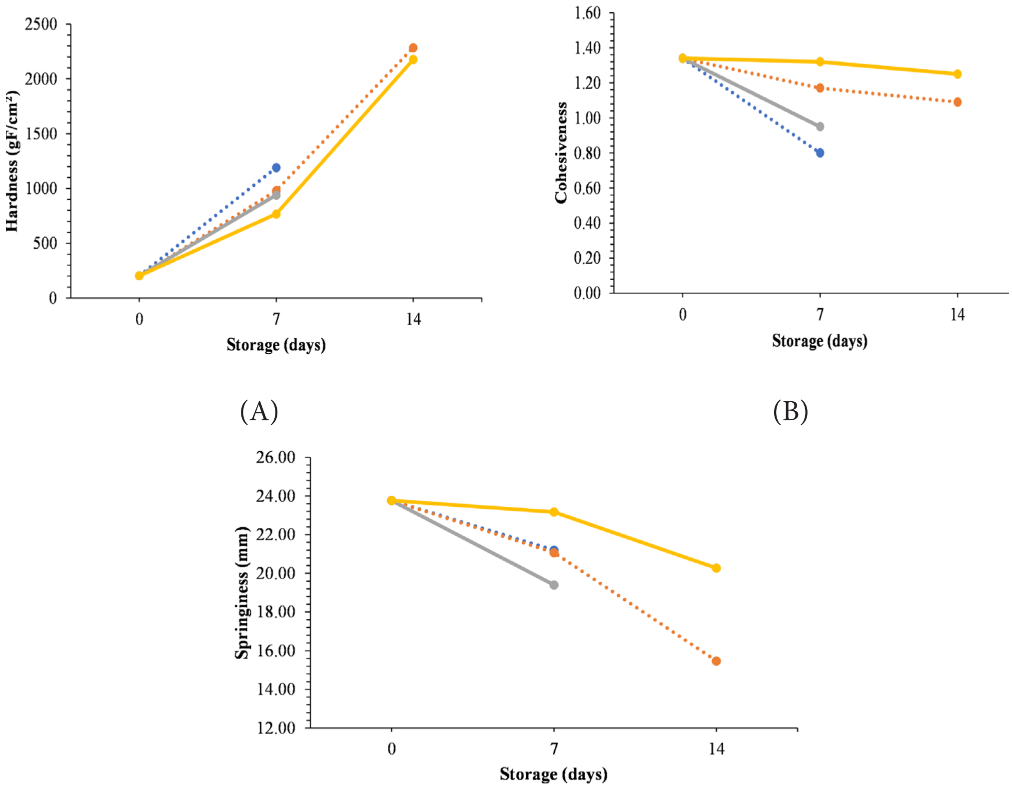

Figure 7 Changes in texture profile of fishballs during storage (=without packaging at 25°C; =without packaging at 4°C; = bioplastic film at 25°C; = bioplastic film at 4°C)

influenced by both packaging conditions and storage temperatures (Laksanawati *et al*., 2024).

Cohesiveness reflects the internal bonding strength of a food matrix and its ability to withstand deformation before its structural breakdown. In this study, the cohesiveness values ranged from 0.80 to 1.25 and generally decreased during storage (Figure 7b). The highest cohesiveness was observed in fishballs stored for 14 d at 4°C with nanochitosan bioplastic packaging (1.25), while the lowest value was found in unpackaged samples stored for 7 d (0.80). The independent samples t-test showed no significant difference in cohesiveness between packaged and unpackaged fishballs during storage ($p>0.05$). Nevertheless, cohesiveness values generally decreased during storage, with unpackaged samples showing a greater decline than bioplastic-packaged samples. This trend suggests that bioplastic packaging may help maintain fishball structural integrity during storage. The decrease in cohesiveness during storage is associated with the structural weakening of the protein matrix due to moisture loss and protein degradation. In contrast, samples packaged with nanochitosan bioplastic films exhibited higher cohesiveness, indicating better preservation of the internal structure. This effect is related to reduced water loss and stabilization of the protein network, which helps maintain the matrix integrity (Fadhallah *et al*., 2024). Similar findings were reported by Amalia *et al*. (2022), who demonstrated that variations in formulation and storage conditions significantly influenced the cohesiveness and other textural properties of meatball products.

Springiness describes the ability of a food product to recover its original shape after deformation. In this study, the springiness of the fishballs decreased during storage for both the packaged and unpackaged samples (Figure 7c). Fishballs stored at 4°C without packaging showed a lower springiness value (15.47 mm) than those packaged with nanochitosan bioplastic film at the same temperature (20.27 mm), indicating better structural resilience in the packaged samples. The independent samples t-test showed no significant difference in springiness between packaged and unpackaged fishballs after 7 d of storage ($p>0.05$). However, after 14 days of storage, the packaged samples exhibited significantly higher springiness values than the unpackaged samples ($p<0.05$), indicating that bioplastic packaging helped maintain the elastic properties of fishballs during storage. The decline in springiness during storage is associated with structural changes in the protein network, including moisture loss and protein denaturation, which reduce the elasticity. In contrast, the higher springiness observed in the packaged samples suggests that nanochitosan-based bioplastic films help maintain the integrity of the gel network by limiting water loss and structural damage. Chen and Rosenthal (2015) described that the fluctuations in the springiness of meat products during refrigerated storage are due to progressive structural rearrangement. In addition, the presence of hydrocolloid-based matrices and protein interactions contributes to the formation of a stable gel network that supports elasticity and water retention. The ability of such systems to bind water and fat plays a key role in maintaining their springiness during storage (Meng *et al*., 2022). Overall, nanochitosan-based bioplastic packaging combined with low-temperature storage was more effective in preserving the elastic properties of fishballs during storage.

Protein Content

Protein content analysis was conducted to evaluate the stability of fishball proteins during refrigerated storage (4°C). The changes in protein content are shown in Figure 8. The initial protein content was 6.65% for all the treatments. During storage, the protein levels gradually decreased. However, this decline was more pronounced in unpackaged samples. After 7 days, the protein content decreased to 5.78% in unpackaged fishballs and 6.13% in fishballs packaged with the bioplastic film. After 14 d, the values further decreased to 4.38% in unpackaged samples and 5.25% in bioplastic-packaged samples. These results indicate that bioplastic packaging effectively slowed the reduction in protein content during refrigerated storage. The independent

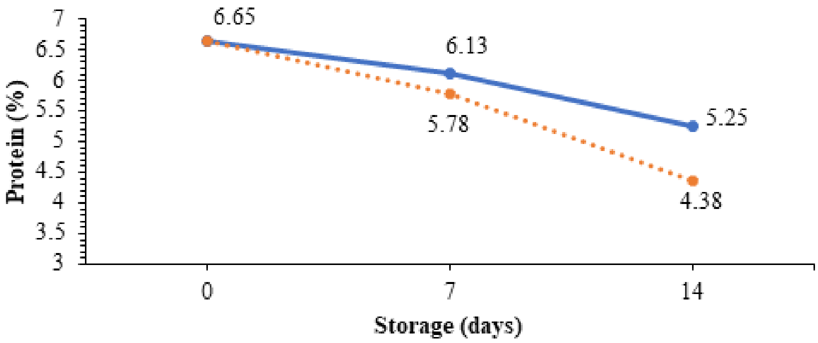

Figure 8 Changes in protein content of fishballs during storage at 4°C (= bioplastic film packaging; = without packaging))

samples t-test showed no significant difference in protein content between the packaged and unpackaged fishballs during storage (p>0.05). Nevertheless, the packaged samples tended to retain higher protein content than the unpackaged samples during storage.

According to SNI 7266:2017, fishballs should contain a minimum protein content of 7% (BSN, 2017). The protein content observed in this study ranged from 4.38% to 6.65%, which was below the minimum requirement specified by the standard. Although bioplastic packaging slowed the reduction in protein content during storage, the measured values remained lower than the recommended levels.

Although the moisture content also decreased during storage (Figure 5), the protein decline suggests that the change was not solely caused by water loss. This reduction may be attributed to protein denaturation, oxidative modification, and structural degradation of the fishball matrix during chilled storage. Chilled storage can promote protein oxidation and structural deterioration, whereas active preservatives help suppress these changes (Nguyen *et al.*, 2024).

The decrease in protein content shown in Figure 8 is closely linked to the changes in textural properties (Figure 7), as protein degradation and structural modifications of the gel matrix contribute to the decline in texture quality during the storage. The higher protein value in the bioplastic-packaged samples indicated a slower deterioration rate. Packaging materials can slow these processes by limiting oxygen transfer, reducing moisture migration, and inhibiting the growth of microorganisms. In the present study, the improved protein retention observed in fishballs packaged with bioplastic film is closely related to the barrier properties of the carrageenan–PVA matrix and the antimicrobial activity of nanochitosan, which suppress microbial activity and oxidative reactions that contribute to protein degradation. Recent studies have highlighted that chitosan-based packaging materials can improve the physicochemical stability and reduce the deterioration of seafood and meat products during storage (Chen *et al.*, 2026).

Similar findings have been reported in studies on seafood preservation using chitosan-based films and coatings. Rochima *et al.* (2025) found that nanochitosan-incorporated edible coatings maintained the quality of Pangasius fillets during storage by inhibiting microbial growth and reducing quality deterioration, thereby extending the storage life of the product. Teimourifard *et al.* (2024) reported that nanochitosan-based packaging films applied to rainbow trout fillets significantly delayed biochemical deterioration and improved quality stability during refrigerated storage. These findings support the results obtained in the present study, where the presence of nanochitosan in the film matrix likely inhibited microbial activity and slowed protein degradation in fishballs.

Furthermore, chitosan-based active packaging systems have been widely reported to enhance the preservation of aquatic products because of their antimicrobial, antioxidant, and barrier properties, which contribute to maintaining the nutritional and biochemical qualities of seafood during storage (Kurek *et al*., 2024; Liu *et al*., 2025). Lower storage temperatures also contribute to slowing protein degradation by reducing microbial metabolism and enzymatic activity in fish products. Overall, the results demonstrate that the combination of nanochitosan-reinforced bioplastic packaging and refrigerated storage effectively maintained the protein stability and nutritional quality of fishballs during storage.

Biodegradation Test

A biodegradation test was conducted for 6 days using the soil burial method to evaluate the degradation capability of the bioplastic films in soil. The sample used for this test was the best-performing bioplastic film (P4) containing 2% nanochitosan, which was previously selected based on its superior barrier and mechanical properties. The results of the biodegradation tests are presented in Table 3.

Based on the results presented in Table 3, the bioplastic samples underwent significant decomposition in the soil within 6 d. The degradation rate is influenced by the hydrophilic nature of the bioplastic components, which facilitates interactions between soil microorganisms, moisture, and the polymer matrix. These conditions enable microorganisms to break down polymer chains more rapidly. This finding is consistent with that of Rumi *et al.* (2024), who reported that biodegradable films derived from natural polymers are more easily degraded owing to the presence of hydroxyl (–OH) functional groups. These functional groups can absorb water from the soil, triggering hydrolysis reactions that accelerate the breakdown of polymer chains.

The biodegradation performance observed in this study also meets the criteria established by SNI 7188-7:2022 (BSN, 2022), which states that a plastic material can be categorized as biodegradable if it degrades by more than 60% within 7 d. The bioplastic films developed in this study demonstrated excellent degradation performance, as the samples were completely degraded (100% degradation) within six days. This degradation rate is faster than the general degradation range reported in the SNI standard, which typically indicates complete degradation within 20–60 d. Therefore, the bioplastic films produced in this study can be classified as environmentally friendly materials, as they exhibit rapid biodegradation under natural conditions while maintaining suitable mechanical and barrier properties for food packaging applications.

CONCLUSION

Carrageenan–PVA bioplastic films incorporated with nanochitosan were successfully synthesized using the ionic gelation method, and exhibited improved mechanical

Table 3 Results of biodegradation test of bioplastic films

Sample size	0 day	3 days	6 day
5×5 cm	 Sample intact	 Sample partially degraded	 Sample completely degraded
3×3 cm	 Sample intact	 Sample completely degraded	

and water vapor barrier performance. The application of the films for fishball packaging helped maintain product quality during storage. Therefore, nanochitosan-reinforced carrageenan–PVA bioplastic films show potential as biodegradable and sustainable packaging materials for fish-based food products.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Research and Community Service Institute of Universitas Lampung (LPPM Universitas Lampung) for funding this study through the 2025 Internal Research Grant under the Applied Research Scheme (No. 2103/UN26.21/PN/2025),awarded to Esa Ghanim Fadhallah, S.Pi., M.Si.

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