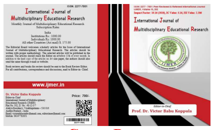
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EFFECT OF GLYCEROL CONCENTRATION ON THICKNESS, SOLUBILITY, AND MOISTURE CONTENT OF RABBIT SKIN GELATIN-BASED SMART EDIBLE FILM WITH PURPLE SWEET POTATO SOLUTION

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Abstract

Rabbit skin gelatin has the potential to form biodegradable edible films, while anthocyanins in purple sweet potato solution provide pH-responsive properties that support the development of smart food packaging. This study aimed to evaluate the effect of glycerol concentration on the thickness, solubility, and moisture content of rabbit skin gelatin-based smart edible films containing purple sweet potato solution. A completely randomized design was employed with five glycerol concentrations, namely 5%, 10%, 15%, 20%, and 25% (v/w, relative to gelatin weight), with four replications for each treatment. The films were prepared using 6.25% rabbit skin gelatin and 5% purple sweet potato solution. The data were analyzed using analysis of variance followed by Duncan's multiple range test. The results showed that glycerol concentration significantly affected all measured physical properties ($P < 0.01$). Increasing the glycerol concentration from 5% to 25% increased film thickness from 0.15 to 0.24 mm, solubility from 86.66% to 93.58%, and moisture content from 13.13% to 21.79%. The 5% glycerol treatment produced the lowest thickness, solubility, and moisture content, while all films had thickness values below 0.25 mm. Therefore, 5% glycerol provided the most favorable physical properties among the concentrations tested. This formulation has potential as a basis for environmentally friendly smart food packaging, although its pH-responsive performance and application to food products require further evaluation.

Keywords: Glycerol, Physical properties, Purple sweet potato, Rabbit skin gelatin, Smart edible film

INTRODUCTION

The development of sustainable food packaging is increasingly directed toward materials that are biodegradable, safe for consumption, and capable of providing information about food quality. Edible films made from natural polymers can act as thin protective layers that reduce the transfer of moisture, oxygen, lipids, and dissolved substances. Their function can be extended into smart edible films by incorporating pH-sensitive natural pigments that visually respond to quality changes in foods (Sani et al., 2021; Filho et al., 2021).

Rabbit processing generates skin as a by-product that has not been optimally utilized. Rabbit skin contains collagen that can be partially hydrolyzed into gelatin, a protein with favorable film-forming, transparency, adhesion, and gas-barrier properties. Gelatin obtained from rabbit skin therefore offers an opportunity to convert an animal by-product into a higher-value material for food packaging (Putro et al., 2019; Toniasso et al., 2022; Gumilar et al., 2024). However, gelatin-based films are naturally hydrophilic and may become rigid or brittle when formulated without an appropriate plasticizer (Gomez-Estaca et al., 2009; Gomez-Guillen et al., 2011).

Purple sweet potato is a potential source of anthocyanins for smart packaging. Anthocyanins are water-soluble pigments that display color changes as environmental pH shifts from acidic to neutral or alkaline conditions. When incorporated into a biopolymer film, these pigments can support visual monitoring of food freshness while also contributing antioxidant activity (Vo et al., 2019; Rahmadhia et al., 2023). The use of purple sweet potato solution in rabbit skin gelatin film therefore combines a biodegradable matrix with a natural pH-responsive component.

Glycerol is commonly used as a plasticizer because its small, hydrophilic molecules can enter the polymer network, weaken intermolecular forces, and increase chain mobility. Although this mechanism improves flexibility, it can also enlarge intermolecular spaces, increase total dissolved solids, attract water, and facilitate film dissolution. Consequently, glycerol concentration must be controlled because excessive addition may produce a film that is thicker, more soluble, and more moisture-sensitive (Sothornvit & Krochta, 2005; Aydin & Ilberg, 2016; Farahnaky et al., 2013).

Previous studies have examined glycerol in gelatin or starch-based edible films and anthocyanins in intelligent films, but the physical response of a rabbit skin gelatin-purple sweet potato smart edible film across a graded glycerol range still requires formulation-specific evaluation. This study aimed to determine the effect of 5%-25% glycerol on film thickness, water solubility, and moisture content and to identify the concentration that produced the most favorable physical properties among the treatments tested.

LITERATURE REVIEW

Rabbit skin gelatin as a film-forming matrix

Gelatin is produced through partial hydrolysis of collagen, which transforms the collagen triple helix into protein chains capable of forming a continuous network during drying. The resulting films are generally transparent and possess effective oxygen and carbon dioxide barrier properties. Rabbit skin is suitable for acid extraction because its collagen structure is relatively soft, and previous research has reported gelatin characteristics that meet food-related quality requirements (Gomez-Guillen et al., 2011; Putro et al., 2019; Gumilar et al., 2024). In a smart edible film, gelatin provides the structural matrix and polar functional groups that interact with glycerol and anthocyanin-containing purple sweet potato solution.

Purple sweet potato anthocyanins in smart edible films

Anthocyanins from purple sweet potato are sensitive to pH and can change from reddish-purple under acidic conditions toward blue-green under neutral to alkaline conditions. This behavior supports their use as natural indicators of biochemical changes associated with food deterioration (Vo et al., 2019; Rahmadhia et al., 2023). Incorporation of anthocyanins into gelatin-based matrices also introduces additional hydroxyl groups that can form hydrogen bonds with protein chains. These interactions may influence film compactness, water affinity, and indicator stability (Sitanggang et al., 2020; Etxabide et al., 2021).

Glycerol and the physical properties of edible films

Glycerol reduces the rigidity of biopolymer films by positioning itself between polymer chains and weakening protein-protein interactions. At the same time, its three hydroxyl groups make it strongly hydrophilic. Increasing glycerol may therefore increase film thickness through higher total solids and expansion of the polymer network, increase solubility by facilitating water penetration, and increase moisture content through hygroscopic water binding (Farahnaky et al., 2013; Aydin & Ilberg, 2016; Rusli et al., 2017). The optimum concentration depends on the intended use because a highly soluble film may be desirable as an edible coating, whereas a smart packaging film for moist foods requires adequate integrity and lower water sensitivity.

METHODOLOGY

Study design, time, and location

The experiment was conducted from 21 July 2025 to 8 January 2026 at the Animal Product Processing Laboratory and the Biotechnology Research and Testing Laboratory, Faculty of Animal Husbandry, Universitas Padjadjaran. A completely randomized design was applied with five glycerol treatments and four replications, resulting in 20 experimental units.

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Table 1. Experimental treatments

Treatment	Glycerol concentration (% v/w gelatin)
P1	5
P2	10
P3	15
P4	20
P5	25

Preparation of rabbit skin gelatin

Rabbit skins were cleaned and soaked in 7% lime solution for 48 h to facilitate hair removal. The skins were washed to pH 6-7, degreased in water at 90 degrees C for 15 min, and cut into approximately 3 x 3 cm pieces. Demineralization was performed using 1% HCl at a skin-to-solution ratio of 2:1 for 24 h, followed by washing to neutral pH. Gelatin was extracted in a water bath at 90 degrees C for 7 h, filtered, dried at 50 degrees C, and milled into powder using the modified procedure of Rahmi et al. (2022).

Preparation of purple sweet potato solution

Fresh purple sweet potatoes of the Biang variety were washed, peeled, cut into approximately 1 cm pieces, oven-dried at 65 degrees C for 24 h, milled, and sieved. A 5% w/v solution was prepared by dispersing 2.5 g of purple sweet potato flour in 50 mL distilled water. The mixture was heated at 65 degrees C for 30 min with periodic stirring and filtered to separate the liquid phase from residual starch, following a modification of Basuki et al. (2014).

Preparation of smart edible films

Gelatin powder (3.125 g; 6.25% w/v) was dispersed in 50 mL purple sweet potato solution. The mixture was heated at 55 degrees C for 30 min while stirring and then cooled to room temperature. Glycerol 85% was added according to the five treatment concentrations shown in Table 1. Each homogeneous film-forming solution was adjusted to 50 mL, cast into a 14 cm silicone mould, dried under lamp-assisted room conditions for approximately 48 h, and conditioned in a desiccator for 24 h. The dried films were carefully removed and stored in sealed plastic with silica gel.

Physical property measurements

Film thickness was measured at five different points using a micrometer with 0.01 mm precision, and the average was recorded (Ayranci & Tunc, 2003). For solubility, a 2 x 3 cm film specimen was weighed as W0. A filter paper was dried at 105 degrees C for 1 h and weighed as W1. The film was immersed for 24 h in 40 mL distilled water containing 0.02% sodium azide. After filtration, the filter paper and remaining insoluble film were dried at 105 degrees C for 24 h and weighed as W2 (Nairfana & Ramdhani, 2021).

$$Solubility\ (\%) = [W0 - (W2 - W1)] / W0 \times 100$$

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Moisture content was determined by the oven-drying method (AOAC, 2012). A 0.5-1.0 g film sample was weighed as the initial mass (A), dried at 105 degrees C for 3 h, cooled in a desiccator, and reweighed. Drying was repeated at 1 h intervals until a constant final mass (B) was obtained.

Moisture content (%) = (A - B) / A x 100

Statistical analysis

Data were analyzed using one-way analysis of variance in IBM SPSS software. When a treatment effect was detected, Duncan's multiple range test was applied at the 5% level to compare treatment means. Statistical significance in the analysis of variance was interpreted at $P < 0.05$, with the observed treatment effects for all three response variables reaching $P < 0.01$.

RESULTS AND DISCUSSION

Glycerol concentration had a highly significant effect on thickness, solubility, and moisture content ($P<0.01$). All three response variables increased as the glycerol level rose from 5% to 25%, indicating a consistent concentration-dependent change in the physical structure and water affinity of the film. The treatment means and Duncan groupings are presented in Table 2.

Table 2. Effect of glycerol concentration on the physical properties of smart edible film (n = 4)

Treatment	Thickness (mm)	Solubility (%)	Moisture content (%)
P1 (5%)	0.15^{a}	86.66^{a}	13.13^{a}
P2 (10%)	0.16^{a}	90.69^{b}	13.32^{a}
P3 (15%)	0.18^{b}	91.68^{bc}	15.03^{b}
P4 (20%)	0.20^{c}	92.66^{bc}	16.39^{c}
P5 (25%)	0.24^{d}	93.58^{c}	21.79^{d}

Note. Within each column, means with different superscript letters differ significantly at $P < 0.05$ according to Duncan's multiple range test. The analysis of variance was significant at $P < 0.01$ for all variables.

Thickness

Film thickness increased from 0.15 mm at 5% glycerol to 0.24 mm at 25% glycerol. P1 and P2 were not significantly different, whereas the 15%, 20%, and 25% treatments formed progressively higher and distinct Duncan groups (Table 2). This result indicates that the gelatin network initially accommodated a limited amount of glycerol without a measurable increase in thickness, but concentrations above 10% produced greater intermolecular spacing and expansion of the polymer matrix. All treatments remained below the 0.25 mm maximum thickness benchmark cited in JIS Z 1707:2019. Nevertheless, the film containing 25% glycerol was close to this limit, suggesting that a further increase could produce an excessively thick film (Japanese Standards Association, 2019).

The increase in thickness can be explained by two related mechanisms. First, adding glycerol increased the total dissolved solids in the same volume of film-forming solution and casting area, resulting in a greater amount of dry material per unit surface. Second, glycerol molecules entered between gelatin chains, weakened protein-protein interactions, increased chain mobility, and expanded the free volume of the matrix. Similar increases in thickness have been reported in glycerol-plasticized starch- and gelatin-based films (Aydin & Ilberg, 2016; Rahmi et al., 2022; Ardi et al., 2023; Sardiansyah et al., 2025). The absence of a significant difference between 5% and 10% glycerol suggests that, at low concentrations, glycerol occupied available spaces in the gelatin network before causing a substantial increase in matrix dimensions.

Thickness influences film handling, dimensional stability, gas and water-vapor transfer, and the visibility of a natural indicator. A thicker layer may provide a longer diffusion pathway for external compounds, but excessive thickness can increase material use, cause uneven drying, and alter flexibility. Based on dimensional control and material efficiency, the 5% and 10% treatments were preferable; however, mechanical testing is still required to determine whether the thinnest formulation has sufficient tensile strength and elongation for practical handling.

Water Solubility

Water solubility increased from 86.66% at 5% glycerol to 93.58% at 25% glycerol. P1 had the lowest solubility and differed significantly from all higher concentrations. P2 was higher than P1 but shared a statistical grouping with P3, whereas P3 and P4 overlapped with adjacent treatments and P5 produced the highest mean (Table 2). The strong increase between 5% and 10%, followed by smaller differences at 15%-25%, suggests that glycerol initially caused a rapid loosening of the gelatin network before the response became more gradual at higher concentrations.

Glycerol is a low-molecular-weight compound with three hydrophilic hydroxyl groups. Its incorporation between gelatin chains reduces internal hydrogen bonding, enlarges intermolecular spaces, and facilitates the penetration of water into the matrix. Water uptake can first cause swelling and subsequently dissolve gelatin, glycerol, and other water-compatible components. Therefore, the increase in solubility reflected both the hydrophilic nature of glycerol and the inherent water affinity of the gelatin matrix (Aydin & Ilberg, 2016; Farahnaky et al., 2013; Rusli et al., 2017; Sardiansyah et al., 2025).

Purple sweet potato solution may further influence this response because anthocyanins and other polar constituents contain hydroxyl groups capable of forming hydrogen bonds with gelatin, glycerol, and water. These interactions can support pigment incorporation but can also increase the overall affinity of the film for an aqueous environment (Sitanggang et al., 2020; Etxabide et al., 2021). At the higher glycerol levels, the partial plateau in solubility may indicate that the gelatin network had already reached a relatively open structure, while additional glycerol increasingly interacted with other glycerol molecules rather than producing a proportional increase in structural expansion (Jouki et al., 2014).

High solubility can be advantageous for an edible film intended to dissolve during consumption and is consistent with rapid environmental degradation. In contrast, a freshness-indicator film designed for contact with high-moisture foods must retain sufficient integrity to maintain its shape and limit premature migration of the anthocyanin indicator. Accordingly, the 5% glycerol treatment provided the most favorable water-resistance profile within the tested range. This interpretation remains application-specific because the required solubility differs between an edible coating, a dissolvable portion, and a film intended for prolonged contact with moist food (Sulistyo et al., 2018; Sitanggang et al., 2020).

Moisture content

Moisture content increased from 13.13% at 5% glycerol to 21.79% at 25% glycerol. P1 and P2 were statistically similar, whereas P3, P4, and P5 differed from one another and showed a successive increase (Table 2). The low and similar values at 5% and 10% suggest that most glycerol molecules were still strongly associated with polar sites in the gelatin network. As glycerol concentration increased, the network approached saturation, more hydroxyl groups remained available for water binding, and the expanded matrix permitted a greater amount of environmental moisture to enter and remain retained.

This trend is consistent with the hygroscopic and humectant character of glycerol. Glycerol binds water through hydrogen bonding, while its plasticizing effect increases free volume and chain mobility within the polymer network. Consequently,

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higher glycerol concentrations provide more water-binding sites and a less compact matrix. Comparable behavior has been reported in gelatin and other hydrophilic edible-film systems (Sothornvit & Krochta, 2005; Farahnaky et al., 2013; Rahmi et al., 2022).

The polar groups of gelatin and purple sweet potato anthocyanins may also contribute to moisture retention through hydrogen-bond interactions. Although these interactions are useful for incorporating a natural pH-sensitive component, excessive moisture can reduce dimensional and storage stability, increase surface tackiness, promote pigment mobility, and increase susceptibility to microbial deterioration under unfavorable storage conditions. The substantially higher moisture content of the 20% and 25% treatments therefore limits their suitability as a stable indicator-film matrix unless the formulation or barrier system is further improved (Sitanggang et al., 2020).

The 5% glycerol treatment, with a moisture content of 13.13%, was closest to the 13% reference value discussed for film stability, while all higher concentrations moved progressively further above this level (Japanese Standards Association, 2019). Lower moisture supports film stability, but a sufficient amount of plasticizer is still necessary to prevent excessive rigidity and brittleness. Within the variables measured in this study, 5% glycerol therefore produced the most favorable moisture profile.

Integrated interpretation and study limitations

The three response variables changed in the same direction: increasing glycerol increased thickness, solubility, and moisture content. This integrated pattern reflects the dual role of glycerol as a plasticizer and a hydrophilic humectant. Considering only the physical variables evaluated, 5% glycerol produced the most favorable formulation because it had the lowest thickness, the lowest water solubility, and the lowest moisture content. This conclusion should not be interpreted as evidence that 5% glycerol is universally optimal for every performance criterion.

Glycerol is principally added to improve flexibility, yet tensile strength, elongation at break, puncture resistance, folding endurance, water-vapor permeability, oxygen permeability, anthocyanin retention, pH-dependent color response, migration, sensory acceptance, and performance on an actual food product were not evaluated in this experiment. Gelatin-based films may also respond differently to environmental humidity, refrigerated storage, and direct contact with protein-rich foods. Thus, 5% glycerol should be regarded as the preferred concentration within the measured properties and as a starting formulation for subsequent mechanical, barrier, color-response, storage-stability, and food-application studies (Gomez-Estaca et al., 2009; Putri et al., 2025; Luo et al., 2022).

Further development should validate the film's visual response across relevant pH conditions and determine whether the indicator remains stable during storage and contact with perishable food. Testing on meat or other high-moisture products is required before the formulation can be recommended as a complete smart packaging system. The utilization of residual materials generated during rabbit skin gelatin processing should also be considered to improve the environmental efficiency of the overall production chain.

CONCLUSION

Glycerol concentration significantly affected the thickness, water solubility, and moisture content of rabbit skin gelatin smart edible film containing purple sweet potato solution. Increasing glycerol from 5% to 25% increased thickness from 0.15 to 0.24 mm, solubility from 86.66% to 93.58%, and moisture content from 13.13% to 21.79%. Among the tested concentrations, 5% glycerol produced the most favorable physical profile, with the lowest thickness, solubility, and moisture content. This formulation may serve as a basis for developing environmentally friendly smart food packaging, subject to further validation in food application and indicator-performance studies.

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