Journal of Food Security and Agroindustry, Vol. 4 No. 3, October 2026, pages 211–230
Available online at: https://pakisjournal.com/index.php/jfsa
e-ISSN: 2986-0563

RESEARCH ARTICLE

Characteristics of Gelatin–Alginate Films with Different Glycerol Concentrations and Cinnamon Essential Oil–Tween 80 Emulsion Systems for Active Food Packaging

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Abstract. The development of biodegradable active packaging from renewable biopolymers offers an alternative to conventional plastics. This study descriptively compared gelatin–alginate films formulated with different glycerol concentrations and cinnamon essential oil (CEO)–Tween 80 emulsions. One-way ANOVA assessed overall differences among formulations. Films were prepared by solution casting with glycerol at 1, 2, and 3% (w/v) and CEO at 0.3, 0.5, and 0.7% (v/v). Film thickness, tensile strength, elongation at break, swelling degree, and antibacterial activity against *Staphylococcus aureus* ATCC 25923 were evaluated. Higher glycerol concentrations generally increased flexibility but reduced tensile strength. The CEO–Tween 80 emulsion affected mechanical properties and water uptake depending on formulation. Based on a composite *weighted effectiveness index*, G1C1 (1% glycerol, 0.3% CEO) ranked highest, driven by its maximum tensile-strength subscore. G1C1 showed a tensile strength of 15.97 MPa, elongation at break of 130.67%, and swelling degree of 528.30%. Its mean inhibition zone against *S. aureus* was 12.07 ± 0.42 mm (mean ± standard deviation, n = 2 independent plates). Each plate value represented the mean of two perpendicular diameter measurements. These findings suggest that gelatin–alginate films containing low CEO concentrations may be promising biodegradable functional packaging materials, although further evaluation is needed before application in active food packaging.
Keywords: active packaging, antibacterial activity, cinnamon essential oil, gelatin–alginate film, glycerol.

Cite this article (APA Style):
Susanti, A., Hermawan, P. K., & Suharti, P. H. (2026). Characteristics of Gelatin–Alginate Films with Different Glycerol Concentrations and Cinnamon Essential Oil–Tween 80 Emulsion Systems for Active Food Packaging. *Journal of Food Security and Agroindustry*, *4*(3), 211–230. https://doi.org/10.58184/jfsa.v4i3.1131

Submitted: 21 Aug 2026; Accepted: 12 Oct 2026; Published: 31 Oct 2026

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1. INTRODUCTION

The increasing environmental burden associated with conventional petroleum-based plastic packaging has encouraged the development of sustainable packaging materials derived from renewable biopolymers (Samuel *et al*., 2024; Sinha, 2024). Protein- and polysaccharide-based films have attracted considerable research interest because their compositions can be modified to achieve specific mechanical, barrier, and functional properties for food packaging applications (Chen *et al*., 2025; Thivya *et al*., 2024). However, replacing conventional plastics remains challenging because biopolymer films often exhibit limitations in mechanical stability, moisture resistance, and processing performance. Consequently, blending complementary biopolymers and incorporating plasticizers or functional compounds have become important approaches to improving film performance (Dutta & Sit, 2024).

Gelatin and sodium alginate are promising film-forming biopolymers with complementary properties. Gelatin can form cohesive polymer networks and continuous films, although its hydrophilic nature limits moisture resistance. Sodium alginate is an anionic polysaccharide with favorable film-forming properties, but alginate-based films are also sensitive to water, and their mechanical performance depends on formulation and intermolecular interactions. Combining gelatin and sodium alginate may modify polymer network organization and provide a suitable matrix for incorporating functional compounds. Previous studies have demonstrated the potential of gelatin–sodium alginate films for food packaging and reported improvements in their physicochemical and mechanical characteristics through cross-linking and the incorporation of bioactive compounds (Chaari *et al*., 2022; Karami *et al*., 2022; Li *et al*., 2022). In the present study, a fixed sodium alginate-to-gelatin proportion of 3% (w/w) was adopted based on Jafari *et al*. (2022). This proportion was intended to maintain a gelatin-dominated matrix while incorporating alginate as a complementary biopolymer. However, alternative gelatin–alginate proportions were not evaluated.

Plasticizers are commonly incorporated into biopolymer films to reduce brittleness and improve flexibility. Glycerol is widely used because its hydroxyl groups can form hydrogen bonds with polymer chains, increasing molecular mobility and facilitating chain movement (Wang *et al*., 2023; Yan *et al*., 2024). Increasing glycerol concentration generally enhances film extensibility but may reduce tensile strength because of weakened intermolecular interactions within the polymer matrix. This behavior creates a trade-off between flexibility and mechanical strength. Similar effects have been reported in starch-, alginate-, protein-, and gelatin-based films, suggesting that glycerol concentration should be optimized for each polymer system rather than directly extrapolated from other formulations (Putri *et al*., 2025; Syarifuddin *et al*., 2025).

In addition to providing a physical barrier, biopolymer films can function as carriers of antimicrobial compounds for active food packaging applications. Cinnamon essential oil (CEO) is a potential antimicrobial agent because its naturally occurring constituents, particularly cinnamaldehyde, have been associated with antibacterial activity (Casalino *et al*., 2023; Ju *et al*., 2023). Previous studies have incorporated CEO into different polymer matrices, including carboxymethyl cellulose, sodium alginate, whey protein, and biodegradable synthetic polymers, to develop films with antimicrobial functionality (Fattahi *et al*., 2020; Lee *et al*., 2023; Mohammadi *et al*., 2020). Nevertheless, essential oil incorporation can also affect film

microstructure and mechanical performance. Higher oil concentrations may disrupt interactions within the polymer matrix, increase structural heterogeneity, and reduce tensile strength. Furthermore, changes in aroma and appearance may limit the suitability of essential-oil-containing films for certain food packaging applications (Moghadas *et al*., 2024; Gupta *et al*., 2024).

The incorporation of hydrophobic CEO into a hydrophilic gelatin–alginate matrix requires adequate dispersion of the oil phase. Tween 80 is a nonionic surfactant that facilitates oil-in-water emulsification by reducing interfacial tension and promoting the distribution of essential oil droplets throughout the film-forming solution. Previous research has demonstrated that Tween 80 can improve essential oil dispersion in gelatin-based films and influence their physical and antimicrobial properties (Li *et al*., 2020). Consequently, the characteristics of films containing CEO–Tween 80 emulsions should be interpreted as the combined effects of the emulsified system rather than those of CEO alone. Although previous studies have investigated gelatin–sodium alginate films containing essential oils and CEO-containing films prepared from different polymer matrices (Karami *et al*., 2022; Lee *et al*., 2023), the formulation-dependent effects of simultaneously varying low glycerol and CEO concentrations in gelatin–alginate films remain insufficiently characterized. In particular, further investigation is needed to understand the balance between tensile strength, extensibility, and water-related properties within these concentration ranges.

Accordingly, this study aimed to evaluate gelatin–alginate films containing different combinations of glycerol concentrations (1–3%, w/v) and CEO concentrations (0.3–0.7%, v/v) incorporated through a CEO–Tween 80 emulsion system. The films were characterized in terms of thickness, tensile strength, elongation at break, and swelling degree to determine how formulation variations affected their physical and mechanical properties. A composite *weighted effectiveness index* was then used to identify the best-performing formulation based on the evaluated selection criteria. The selected formulation was subsequently tested for antibacterial activity against *Staphylococcus aureus*. By examining these formulation combinations, this study seeks to identify a suitable balance between mechanical strength, flexibility, and water-related characteristics while providing preliminary evidence of antibacterial functionality in the selected film. The findings are expected to support further development and evaluation of gelatin–alginate-based materials for potential active food packaging applications.

2. METHODS

This study was conducted as an initial laboratory-scale formulation screening experiment. Technical-grade glycerol and Tween 80 were used to assess the mechanical and functional characteristics of the film system. The use of food-grade or food-contact-compliant materials is recommended in subsequent development stages before the films can be considered suitable for active food packaging applications. The materials included Type B gelatin and sodium alginate of technical food-grade quality, while glycerol, Tween 80, cinnamon essential oil (CEO), Mueller–Hinton Agar (MHA), NaCl, gentamicin, and alcohol were of technical grade. All materials were purchased from several retail chemical suppliers in Malang, East Java, Indonesia, rather than directly from manufacturers. Consequently, information regarding manufacturers, countries of origin, and batch or catalogue numbers was unavailable and is not reported. Distilled water was used throughout film preparation.

For antibacterial testing, *Staphylococcus aureus* ATCC 25923, obtained from the Bioprocess Laboratory of Politeknik Negeri Malang, was used as the test microorganism. Mueller–Hinton Agar (MHA), 0.9% NaCl solution, and gentamicin discs (10 µg) were used for microbiological analysis. The main instruments included a Universal Testing Machine (UTM) equipped with a 500 N load cell and MSATL-Lite software, operated at a testing speed of 50 mm/min; a micrometer screw gauge with a resolution of 0.01 mm; a shearing hotplate for homogenization and emulsification; an autoclave operated at 121 °C; and an incubator maintained at 37 °C. Instrument brands and model numbers were not recorded. Film preparation and characterization were conducted at the Applied Chemistry Laboratory, Department of Chemical Engineering, Politeknik Negeri Malang, Indonesia, in 2026. Antibacterial testing was performed at the Integrated Laboratory and Production Unit, Politeknik Kesehatan Putra Indonesia Malang, Indonesia, in 2026, as documented in test report SKHP:345/LAB.PIM/VII/2026.

A laboratory-scale quantitative experimental design was employed to evaluate 13 gelatin–alginate film formulations, consisting of one matrix control (G0C0), three glycerol-only formulations (G1C0–G3C0), and nine glycerol–CEO combinations. Glycerol concentrations were varied at 1, 2, and 3% (w/v), while CEO concentrations were varied at 0.3, 0.5, and 0.7% (v/v). Tween 80 was maintained at 0.5% (v/v) in all CEO-containing formulations (Table 1). Glycerol concentration was calculated relative to 100 mL of the gelatin–alginate base solution. In contrast, CEO and Tween 80 concentrations were calculated relative to 100 mL of the base–glycerol mixture before the addition of each respective component, rather than relative to the final volume of the film-forming solution. For each formulation, three films were separately cast and characterized (n = 3), resulting in a total of 39 films. Each cast film was treated as one experimental replicate. However, whether separate film-forming solution batches were independently prepared for each replicate was not documented.

Table 1. Experimental Formulation of Gelatin-Alginate Films

Code	Glycerol (% w/v)	CEO (% v/v)	Tween 80 (% v/v)
G0C0	0	0	0
G1C0	1	0	0
G2C0	2	0	0
G3C0	3	0	0
G1C1	1	0.3	0.5
G2C1	2	0.3	0.5
G3C1	3	0.3	0.5
G1C2	1	0.5	0.5
G2C2	2	0.5	0.5
G3C2	3	0.5	0.5
G1C3	1	0.7	0.5
G2C3	2	0.7	0.5
G3C3	3	0.7	0.5

G0, G1, G2, and G3 represent glycerol concentrations of 0, 1, 2, and 3% (w/v), respectively, whereas C0, C1, C2, and C3 represent cinnamon essential oil (CEO) concentrations of 0, 0.3, 0.5, and 0.7% (v/v), respectively. Tween 80 was incorporated at 0.5% (v/v) into all

CEO-containing formulations (C1–C3), while C0 formulations contained neither CEO nor Tween 80. Because no formulation containing Tween 80 without CEO was prepared, the individual effects of CEO and Tween 80 could not be distinguished. Therefore, differences between C0 and C1–C3 formulations were interpreted as the combined effects of the CEO–Tween 80 emulsion system rather than those of CEO alone.

Gelatin–alginate films were prepared using the solution casting method adapted from Jafari *et al.* (2022). Type B gelatin (4 g) was dispersed in 100 mL of distilled water preheated to 65 °C and stirred until no visible aggregates remained. After cooling to room temperature, sodium alginate (0.12 g, equivalent to 3% w/w relative to gelatin) was added. This proportion was adopted from Jafari *et al.* (2022) without further optimization. The mixture was homogenized for 20 min using a shearing hotplate to obtain a uniform base solution. Subsequently, glycerol was added at concentrations of 1, 2, or 3 g per 100 mL of the base solution, and the mixture was stirred at 45 °C for 15 min. For CEO-containing formulations, CEO (0.3, 0.5, or 0.7 mL) and Tween 80 (0.5 mL) were added per 100 mL of the base–glycerol mixture. The resulting mixtures were homogenized for 30 min using a shearing hotplate to disperse the oil phase and form an emulsion (Li *et al.*, 2020). Stirring speed, impeller type, pH, emulsification temperature, and emulsion droplet size and stability were not measured or documented.

The resulting film-forming solutions, with nominal volumes of approximately 100–104 mL per formulation, were poured into molds measuring 20 × 20 cm (400 cm^2), corresponding to an approximate casting volume of 0.25–0.26 mL/cm^2. The films were dried in open air at room temperature for 72 h (3 × 24 h). Ambient temperature and relative humidity were not monitored during drying. Therefore, partial volatilization of CEO constituents during prolonged open-air drying could not be excluded. After drying, the films were carefully removed from the molds and subjected to physical, mechanical, and water-uptake characterization.

Film thickness was measured using a micrometer screw gauge with a resolution of 0.01 mm. Measurements were performed at five positions on each film, comprising the four corners (upper left, upper right, lower left, and lower right) and the center, to account for potential variations in film thickness. Measurements near the film edges were included because edge regions were not specifically excluded. The mean of the five measurements was calculated and used as the thickness value for each film replicate. However, the micrometer resolution of 0.01 mm was relatively coarse compared with the measured film thickness range of 0.067–0.157 mm. In addition, the applied contact pressure and use of the micrometer ratchet mechanism were not documented. These limitations may have affected measurement precision. Future studies should consider using a micrometer with a resolution of 0.001 mm and documenting the measurement contact pressure.

Tensile strength (TS) and elongation at break (EB) were determined using a Universal Testing Machine (UTM) equipped with a 500 N load cell. Tensile testing was performed using a procedure adapted from ASTM D882-18 for thin plastic sheeting (ASTM, 2018). Film specimens were cut into rectangular strips measuring 90 × 20 mm. The thickness of each specimen was measured before testing to determine its initial cross-sectional area. Each specimen was mounted between the UTM grips with an initial gauge length of 50 mm and stretched at a crosshead speed of 50 mm/min until rupture.

The testing procedure was adapted from ASTM D882-18 for comparative evaluation and did not constitute full compliance with the standard. Although the specimens were conditioned before testing, the conditioning temperature, relative humidity, and duration were not documented. Measures taken to prevent specimen slippage between the grips were also not recorded. Furthermore, the selected specimen dimensions and crosshead speed may not correspond to all testing conditions prescribed by the standard for highly extensible films. Accordingly, the tensile strength and elongation at break results were primarily interpreted as comparative measurements among formulations within the present study rather than as directly comparable absolute values across different studies.

Tensile strength was calculated using Equation (1):

$$TS = F_{max} / A$$

where:
TS : tensile strength (MPa)
F_{max} : the maximum force at rupture (N)
A : the initial cross-sectional area of the specimen (mm^2)
because 1 N mm^{-2} is equivalent to 1 MPa, TS is directly expressed in MPa.

The cross-sectional area was obtained from Equation (2):

$$A = w \times t$$

where:
W : specimen width (mm)
t : specimen thickness (mm).

Elongation at break was determined from the change in specimen length at the point of rupture and calculated using Equation (3):

$$EB\ (\%) = (L_1 - L_0) / L_0 \times 100$$

where:
EB : elongation at break (%)
L_0 : the initial gauge length (mm)
L_1 : the specimen length at rupture (mm).

Swelling degree was determined to evaluate the water absorption capacity of the gelatin–alginate films. Film specimens were cut into 30 × 30 mm pieces and weighed before immersion to determine their initial mass (W_0). Each specimen was immersed in 30 mL of distilled water in a 100 mL beaker at room temperature for 4 h. Following immersion, the specimen was removed from the water and weighed to determine its final mass (W_1). The procedure was adapted from ASTM D570-98 for determining water absorption in plastics (ASTM, 1998). However, the method used to remove residual surface water before weighing

was not standardized, including the type of absorbent paper, blotting duration, and applied pressure. Consequently, residual surface water may have resulted in an overestimation of W_1 and contributed to variability among replicates. Therefore, swelling degree was interpreted as an indicator of water uptake under the specific experimental conditions rather than as a standardized measure directly comparable across studies. Measurements were performed in triplicate for each formulation (n = 3). Swelling degree was calculated using Equation (4):

$$\text{Swelling degree (\%)} = (W_1 - W_0) / W_0 \times 100$$

where:
W_0 : film mass before immersion (g)
W_1 : film mass after immersion (g).

A preliminary formulation screening was conducted to identify the most suitable film for subsequent antibacterial evaluation. Tensile strength (TS), elongation at break (EB), and swelling degree of the nine CEO-containing formulations were integrated using a *weighted effectiveness index*. Film thickness, which ranged from 0.067 to 0.157 mm across all formulations, was reported separately and excluded from the selection criteria. Higher TS and EB values and lower swelling degrees were considered desirable characteristics. Weights of 40% were assigned to TS and swelling degree, while EB was assigned a weight of 20%. These weights reflected the relative importance of mechanical strength and resistance to water uptake as primary functional requirements of packaging films, with extensibility serving as a supporting indicator of flexibility. The weights were determined based on functional considerations rather than an established weighting standard. Their influence on formulation rankings was subsequently examined through sensitivity analysis, as described in Section 3.5.

Each response was normalized using the min–max normalization method across the nine CEO-containing formulations. For TS and EB, the weighted normalized score was calculated as, $w \times (x - x_{min})/(x_{max} - x_{min})$. For swelling degree, where lower values were considered preferable, the weighted normalized score was calculated as, $w \times (x - x_{min})/(x_{max} - x_{min})$ where x represents the mean response value of a given formulation, x_{min} and x_{max} represent the minimum and maximum mean values among the nine formulations, respectively, and w represents the assigned weighting factor (0.4 for TS, 0.2 for EB, and 0.4 for swelling degree). The total effectiveness score was calculated by summing the three weighted normalized scores, with a theoretical maximum of 1.0. The formulations were then ranked according to their total scores (Table 4). The resulting rankings were considered alongside the amount of CEO incorporated. Formulation G1C1 (1% w/v glycerol and 0.3% v/v CEO) was selected for antibacterial evaluation because it achieved the highest overall effectiveness score while containing the lowest CEO concentration investigated.

2.1 Antibacterial Activity

The antibacterial activity of the selected gelatin–alginate film was evaluated against *Staphylococcus aureus* ATCC 25923 using an adapted agar diffusion method. The procedure was modified from the disc diffusion method described by Husen and Ratnaningtyas (2022), primarily by replacing conventional paper discs with film specimens. Consequently, inhibition

zone measurements could be influenced by film dimensions, thickness, and the diffusion characteristics of the incorporated compounds, which were not strictly controlled. The bacterial culture was grown on Mueller–Hinton Agar (MHA) at 37 °C for 24 h. A bacterial suspension was subsequently prepared and adjusted to a 0.5 McFarland turbidity standard, corresponding to approximately 1.5×10^8 colony-forming units (CFU)/mL (Husen & Ratnaningtyas, 2022).

An aliquot of 100 µL of the standardized bacterial suspension was uniformly spread over the MHA surface. The selected G1C1 film was then aseptically placed onto the inoculated medium. Formulations G0C0 and G1C0 served as comparison films, while a gentamicin disc (10 µg) was used as the positive control. Additional sterilization of the film specimens was not documented, and their dimensions were not recorded.

Neither G0C0 nor G1C0 contained Tween 80. Therefore, these comparison films could not distinguish the individual contribution of CEO from that of Tween 80 to the observed antibacterial activity. Furthermore, a formulation containing 1% glycerol and 0.5% Tween 80 without CEO was not included. Accordingly, the observed antibacterial activity was interpreted as the response of the combined CEO–Tween 80-containing film system rather than as the independent effect of CEO.

The inoculated plates were incubated at 37 °C for 24 h. Antibacterial activity was assessed by measuring the clear inhibition zones surrounding the samples. Inhibition zone diameters were measured in two perpendicular directions (horizontal and vertical) using a vernier caliper and expressed in millimeters. Each sample was evaluated using two independent replicate plates (n = 2). The horizontal and vertical measurements from each plate were averaged to obtain one replicate value. The resulting replicate values were then summarized as the mean ± standard deviation (SD).

2.2 Statistical Analysis

Physical, mechanical, and water-uptake characteristics were evaluated using three experimental replicates per formulation (n = 3). Replicate-level raw data were used for statistical analysis, and the results were expressed as the mean ± sample standard deviation (SD). Differences among the 13 gelatin–alginate film formulations were evaluated using one-way analysis of variance (ANOVA) at a significance level of 5% ($\alpha = 0.05$). Post hoc multiple comparison tests and statistical assumption tests were not performed. Therefore, ANOVA results were interpreted solely as evidence of overall differences among formulations, without establishing statistically significant pairwise differences or superiority of individual formulations. Antibacterial activity data obtained from two independent replicate plates (n = 2) were analyzed descriptively and presented as the mean ± SD. No inferential statistical analysis was performed on the antibacterial results because of the limited number of replicates.

3. RESULTS AND DISCUSSION

3.1 Film Thickness

All formulations successfully produced continuous gelatin–alginate films suitable for physical, mechanical, and water-uptake characterization. Film thickness ranged from 0.067 to 0.157 mm, as presented in Table 2. The control formulation without glycerol or cinnamon essential oil (G0C0) exhibited the lowest mean thickness (0.067 ± 0.006 mm). Among the CEO-

free formulations, an increase in glycerol concentration from 1% to 3% was accompanied by an increase in mean film thickness from 0.071 ± 0.006 mm to 0.125 ± 0.056 mm.

Table 2. Thickness Of Gelatin-Alginate Films at Different Glycerol and Cinnamon Essential Oil Concentrations

Formulation	Thickness (mm)	CV (%)
G0C0	0.067 ± 0.006	9.1
G1C0	0.071 ± 0.006	9.0
G2C0	0.080 ± 0.007	8.7
G3C0	0.125 ± 0.056	44.9
G1C1	0.115 ± 0.024	21.3
G2C1	0.113 ± 0.046	40.9
G3C1	0.095 ± 0.029	31.1
G1C2	0.101 ± 0.018	17.9
G2C2	0.101 ± 0.018	17.9
G3C2	0.083 ± 0.008	9.7
G1C3	0.097 ± 0.027	27.9
G2C3	0.157 ± 0.046	29.6
G3C3	0.141 ± 0.013	9.0

Note: Values are presented as mean ± sample standard deviation (SD), based on three replicates per formulation (n = 3). CV = coefficient of variation.

The coefficient of variation (CV) for film thickness in each formulation is presented in Table 2, while replicate-level tensile strength and elongation at break data are provided in Table 3. Considerable variation in film thickness was observed among replicates in several formulations, particularly G3C0 (CV = 44.9%) and G2C1 (CV = 40.9%). One-way ANOVA indicated a statistically significant overall difference in film thickness among formulations ($F(12, 26) = 2.55$, $p = 0.022$). Substantial inter-replicate variability was also observed in tensile strength, particularly for G3C3 (CV = 104.3%) and G0C0 (CV = 65.6%), as well as in elongation at break for G3C0 (CV = 62.6%) and G1C0 (CV = 46.6%). These variations may be associated with manual film casting and uncontrolled ambient drying conditions, which could have affected film uniformity and reproducibility. Given the considerable variability among replicates, the observed trends were interpreted descriptively based on differences in mean values rather than as evidence of statistically significant pairwise differences.

The observed increase in film thickness with glycerol addition may be associated with a greater amount of nonvolatile material remaining after solvent evaporation and changes in intermolecular spacing within the plasticized polymer matrix. However, although the casting volume was intended to remain consistent across formulations, the exact volume and total solid content deposited in each mold were not recorded. Consequently, variations in the casting process may also have contributed to the observed differences, and the increase in thickness cannot be attributed exclusively to glycerol addition. A similar trend was reported by Putri *et al.* (2025), who observed an increase in gelatin film thickness with increasing glycerol concentration. In contrast, CEO-containing films did not exhibit a consistent concentration-dependent trend in thickness.

Table 3. Replicate-Level Data and Coefficient of Variation (CV) of Tensile Strength (TS) and Elongation at Break (EB).

Formulation	TS, individual values (MPa)	TS CV (%)	EB, individual values (%)	EB CV (%)
G0C0	23.25; 16.75; 4.21	65.6	15.07; 9.35; 9.53	28.7
G1C0	18.27; 6.05; 11.40	51.4	36.82; 102.30; 99.87	46.6
G2C0	5.67; 4.66; 3.33	25.8	224.83; 80.09; 232.68	47.9
G3C0	3.65; 0.78; 2.44	62.9	327.28; 80.78; 406.51	62.6
G1C1	16.13; 20.80; 11.00	30.7	66.63; 129.17; 196.21	49.6
G2C1	24.11; 7.05; 10.38	65.3	136.22; 232.11; 145.42	30.9
G3C1	7.43; 10.97; 7.51	23.5	180.75; 84.40; 150.82	35.6
G1C2	4.73; 5.41; 6.59	16.9	412.71; 339.00; 445.03	13.6
G2C2	3.94; 8.25; 5.33	37.7	337.21; 371.75; 425.29	11.7
G3C2	3.58; 3.30; 3.99	9.6	447.25; 310.24; 173.35	44.1
G1C3	4.21; 2.24; 2.80	32.9	390.90; 507.94; 387.61	16.0
G2C3	3.72; 3.10; 3.19	10.0	430.30; 611.23; 618.29	19.3
G3C3	9.08; 2.29; 1.06	104.3	345.80; 388.16; 371.36	5.8

Note: Individual values represent measurements obtained from three replicate films for each formulation (n = 3). CV = coefficient of variation, calculated as (sample standard deviation / mean) × 100%.

The mean thickness of these films ranged from 0.083 ± 0.008 mm for G3C2 to 0.157 ± 0.046 mm for G2C3. This variability suggests that film thickness may have been influenced by several factors, including formulation composition, casting uniformity, drying conditions, and the distribution of the dispersed oil phase. Zhou *et al.* (2021) similarly reported changes in film thickness associated with the incorporation and dispersion of essential oils within polymer matrices. Furthermore, Bao *et al.* (2026) reported thickness variability in gelatin–sodium alginate composite films containing benzyl isothiocyanate and eugenol essential oils, highlighting the potential contribution of oil-phase dispersion to structural heterogeneity. Nevertheless, because emulsion droplet distribution and film microstructure were not directly characterized in the present study, these mechanisms remain plausible explanations rather than experimentally confirmed causes of the observed thickness variations.

3.2 Tensile Strength

The tensile strength of gelatin–alginate films varied with glycerol concentration and the incorporation of the CEO–Tween 80 emulsion system, as illustrated in Figure 1. Among CEO-free formulations, increasing glycerol concentration from 1% to 3% was accompanied by a decrease in mean tensile strength from 11.91 ± 6.12 MPa to 2.29 ± 1.44 MPa, indicating reduced resistance to tensile loading. This trend is consistent with the plasticizing effect of glycerol, which can increase polymer chain mobility by modifying intermolecular interactions within the film matrix, thereby reducing its resistance to applied tensile forces. Similar reductions in tensile strength with increasing glycerol concentration have been reported in other biopolymer-based films (Putri *et al.*, 2025; Tarique *et al.*, 2021). However, given the considerable variability among experimental replicates, the observed reduction should be interpreted as a descriptive trend rather than evidence of statistically significant differences between individual formulations.

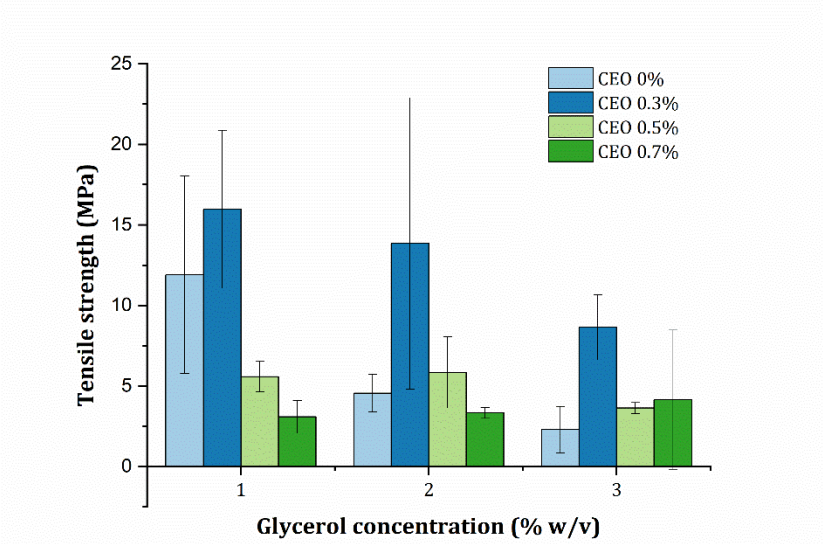


Figure 1. Tensile Strength of Gelatin-Alginate Films at Different Glycerol and Cinnamon Essential Oil Concentrations

Among the formulations containing the CEO–Tween 80 emulsion system, G1C1 exhibited the highest mean tensile strength (15.97 ± 4.90 MPa). However, this finding represents a descriptive comparison of mean values rather than evidence of statistical superiority. Formulations containing 0.5% and 0.7% CEO exhibited formulation-dependent variations in mechanical properties, with tensile strength generally tending to be lower than that observed at 0.3% CEO. This trend suggests that increasing the proportion of the dispersed oil phase may influence the mechanical integrity of the gelatin–alginate polymer network. One-way ANOVA revealed a statistically significant overall difference in tensile strength among formulations ($F(12, 26) = 3.50$, $p = 0.004$). Nevertheless, because post hoc multiple comparisons were not performed, the statistical superiority of G1C1 over any individual formulation could not be established. Similar reductions in tensile strength following CEO incorporation have been reported in other biopolymer-based films (Fattahi *et al.*, 2020; Zhou *et al.*, 2021). Furthermore, Bhatia *et al.* (2023) reported a comparable trade-off between essential oil incorporation and mechanical strength, accompanied by increased flexibility, in sodium alginate–acacia gum hydrogel films containing cinnamon essential oil. However, because Tween 80 was incorporated simultaneously with CEO, the observed mechanical changes cannot be attributed exclusively to CEO and should instead be interpreted as the combined response of the CEO–Tween 80 emulsion system. In addition, the relatively large standard deviations observed in several formulations indicate substantial inter-replicate variability, suggesting that small differences in mean tensile strength should be interpreted cautiously.

3.3 Elongation at Break

Elongation at break generally increased with glycerol concentration and was further influenced by the incorporation of the CEO–Tween 80 emulsion system, as illustrated in Figure 2. Among CEO-free formulations, mean elongation at break increased from 79.66 ± 37.12% at 1% glycerol to 179.20 ± 85.92% and 271.52 ± 169.87% at 2% and 3% glycerol, respectively. This trend is consistent with the plasticizing effect of glycerol, which enhances polymer chain

mobility and facilitates greater deformation before film rupture (Putri *et al.*, 2025; Tarique *et al.*, 2021).

Several CEO-containing formulations exhibited higher elongation at break, particularly those containing 0.5% and 0.7% CEO. The highest mean elongation was observed in G2C3, reaching 553.27 ± 106.56%. This increased extensibility, accompanied by the generally lower tensile strength observed at higher CEO concentrations, suggests a shift toward greater film flexibility at the expense of mechanical strength. Similar increases in elongation following CEO incorporation have been reported in other biopolymer-based films (Fattahi *et al.*, 2020; Zhou *et al.*, 2021). However, because Tween 80 was incorporated simultaneously with CEO, the observed changes cannot be attributed exclusively to CEO and should instead be interpreted as the combined response of the CEO–Tween 80 emulsion system. Furthermore, the non-monotonic trends observed across several formulations suggest that film extensibility was influenced by the overall formulation rather than by glycerol or CEO concentration independently. One-way ANOVA indicated a statistically significant overall difference in elongation at break among formulations ($F(12, 26) = 11.26$, $p < 0.001$). Nevertheless, because post hoc comparisons were not performed, statistically significant differences between specific formulations could not be established.

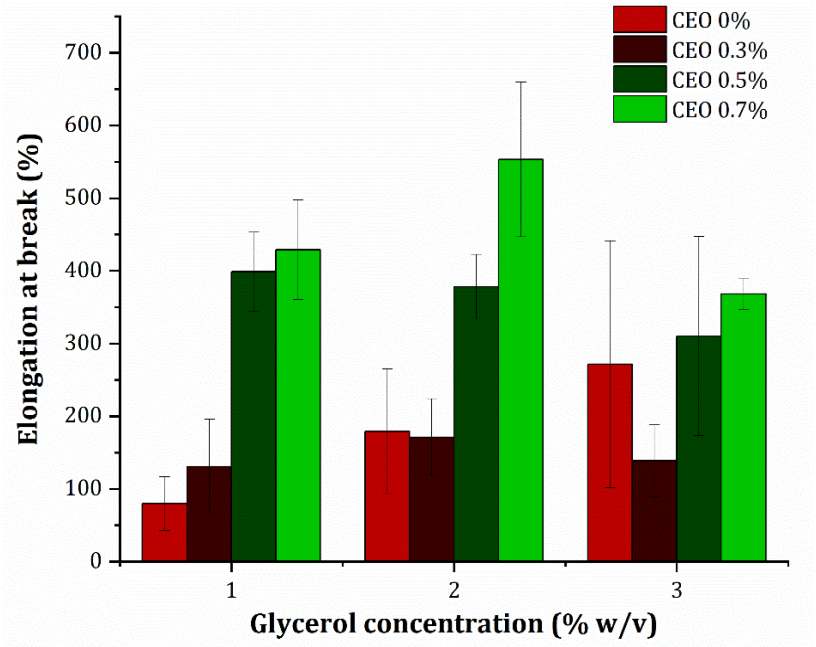


Figure 2. Elongation at Break of Gelatin–Alginate Films at Different Glycerol and Cinnamon Essential Oil Concentrations

Considerable inter-replicate variability in elongation at break was observed in several formulations, particularly G3C0 (CV = 62.6%) and G3C2 (CV = 44.1%), as presented in Table 3. This variability may be partly associated with differences in film thickness among replicates (Table 2), manual casting procedures, and potential heterogeneity within the film matrix. Additional factors, including specimen slippage at the grips, outliers, and localized structural irregularities, were not systematically evaluated. Furthermore, representative stress–strain curves were not recorded, limiting further assessment of the deformation behavior of the films. Accordingly, the elongation results should be interpreted as descriptive indicators of

formulation-dependent differences in extensibility rather than precise estimates of mechanical performance.

3.4 Swelling Degree

The swelling behavior of gelatin–alginate films varied with glycerol concentration and the incorporation of the CEO–Tween 80 emulsion system, as illustrated in Figure 3. The CEO-free formulations exhibited relatively high water uptake, with swelling degrees of 2128.52 ± 1524.76%, 1308.80 ± 579.71%, and 1296.76 ± 512.14% for G1C0, G2C0, and G3C0, respectively. These high swelling values are consistent with the hydrophilic nature of gelatin and alginate, which facilitates water penetration and expansion of the polymer network (Ji *et al*., 2022).

The incorporation of the CEO–Tween 80 emulsion system was generally associated with lower swelling degrees, although no consistent concentration-dependent trend was observed. Formulation G1C1 exhibited a relatively low swelling degree of 528.30 ± 99.06%, while G3C3 showed the lowest mean swelling degree among the CEO-containing formulations at 465.24 ± 345.04%. The observed reduction in water uptake may be associated with the presence of hydrophobic oil domains within the predominantly hydrophilic polymer matrix. Similar changes in water-related properties following CEO incorporation have been reported in other biopolymer films (Zhou *et al*., 2021). Furthermore, Upadhye *et al.* (2025) reported reduced water uptake in sodium alginate–gelatin films containing clove essential oil. However, the non-monotonic response and considerable inter-replicate variability observed in several formulations suggest that swelling behavior may also depend on overall formulation composition, emulsion distribution, and potential leaching of water-soluble components during immersion. Because these mechanisms were not directly investigated, they remain possible explanations rather than experimentally established causes of the observed differences.

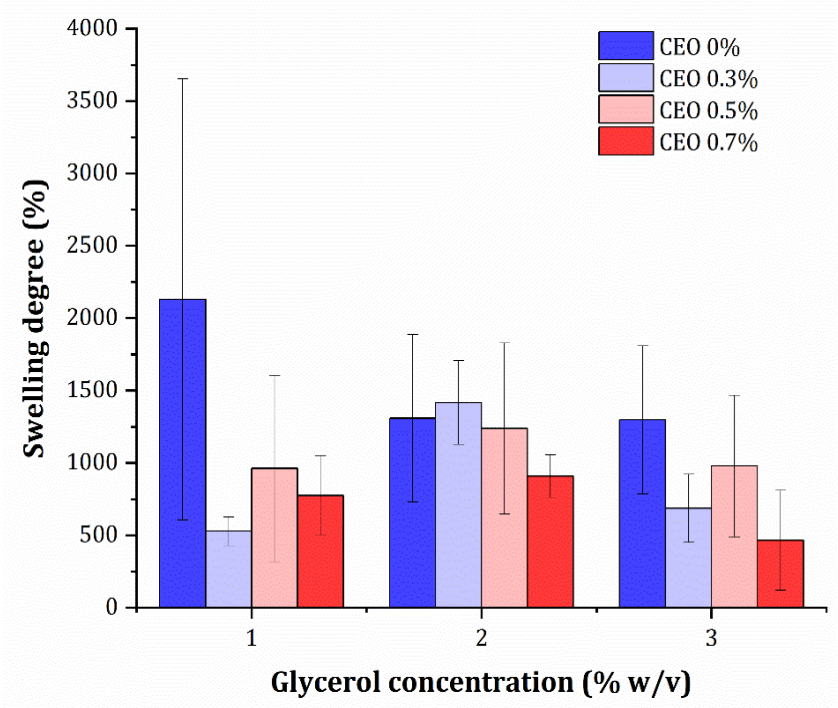


Figure 3. Swelling Degree of Gelatin-Alginate Films at Different Glycerol and Cinnamon Essential Oil Concentrations

One-way ANOVA did not reveal a statistically significant overall difference in swelling degree among formulations ($F(12, 26) = 1.69$, $p = 0.128$). Accordingly, the observed differences were interpreted descriptively rather than as evidence of statistically significant effects between formulations. Furthermore, because the removal of residual surface water before weighing was not standardized, the swelling values may have been affected by measurement variability. Therefore, swelling degree should be regarded as an indicator of water uptake under the specific immersion conditions employed in this study rather than as a direct measure of water vapor barrier performance. Future studies should include water vapor permeability (WVP) measurements to provide a more comprehensive assessment of the films' suitability for food packaging applications.

3.5 Selection of Film Formulation

A preliminary screening was conducted to identify the most suitable formulation for subsequent antibacterial evaluation using the *weighted effectiveness index* based on tensile strength (TS), elongation at break (EB), and swelling degree, as described in the Research Method section. The normalized subscores, total effectiveness scores, and rankings of the nine CEO-containing formulations are presented in Table 4. Among the evaluated formulations, G1C1, containing 1% (w/v) glycerol and 0.3% (v/v) CEO, achieved the highest total effectiveness score of 0.77 out of a theoretical maximum of 1.0. This result was primarily attributed to its maximum tensile strength subscore (0.40) and relatively high swelling subscore (0.37), whereas its elongation at break subscore was the lowest (0.00). Formulation G1C1 exhibited a tensile strength of 15.97 ± 4.90 MPa, elongation at break of 130.67 ± 64.80%, and swelling degree of 528.30 ± 99.06%.

Table 4. Weighted Effectiveness-Index Scores of the Nine CEO-Containing Formulations

Formulation	TS (MPa)	TS score (0.4)	EB (%)	EB score (0.2)	Swelling (%)	Swelling score (0.4)	Total score	Rank
G1C1	15.97	0.400	130.67	0.000	528.30	0.374	0.774	1
G3C3	4.14	0.033	368.44	0.113	465.24	0.400	0.545	2
G3C1	8.64	0.172	138.66	0.004	686.28	0.307	0.483	3
G2C3	3.34	0.008	553.27	0.200	908.20	0.214	0.422	4
G1C3	3.08	0.000	428.82	0.141	774.50	0.270	0.411	5
G1C2	5.58	0.077	398.91	0.127	961.81	0.191	0.396	6
G2C1	13.85	0.334	171.25	0.019	1417.86	0.000	0.353	7
G3C2	3.62	0.017	310.28	0.085	978.02	0.185	0.286	8
G2C2	5.84	0.085	378.08	0.117	1237.00	0.076	0.279	9

Note: Scores were calculated using the mean values of three replicates per formulation (n = 3) and min–max normalization, with weighting factors of 0.40 for tensile strength (TS), 0.20 for elongation at break (EB), and 0.40 for swelling degree. The maximum possible total score was 1.00. The selected formulation (G1C1) is highlighted.

Sensitivity analysis was performed using seven alternative weighting schemes for TS, EB, and swelling degree: equal weights (1/3 each), 0.34/0.33/0.33, 0.50/0.25/0.25, 0.30/0.20/0.50, 0.50/0.20/0.30, 0.40/0.40/0.20, and 0.60/0.10/0.30. G1C1 consistently maintained the highest ranking across all seven weighting schemes, with total effectiveness

scores ranging from 0.59 to 0.88. However, its ranking advantage was primarily driven by its relatively favorable tensile strength and swelling characteristics rather than elongation at break. Accordingly, G1C1 was selected for subsequent antibacterial evaluation because it achieved the highest composite effectiveness score, maintained relatively high tensile strength, exhibited comparatively low water uptake, and contained the lowest CEO concentration investigated. This selection reflects the predefined multicriteria assessment and does not establish statistically significant superiority over the other formulations.

3.6 Antibacterial Activity

Antibacterial activity was evaluated to determine whether the selected G1C1 formulation exhibited inhibitory effects against *Staphylococcus aureus* ATCC 25923. The G1C1 formulation produced a measurable inhibition zone with a mean diameter of 12.07 ± 0.42 mm, while the gentamicin positive control exhibited a mean inhibition zone diameter of 15.43 ± 0.75 mm. Values were expressed as mean ± standard deviation (SD) based on two independent replicate plates (n = 2), with each replicate value calculated as the average of two perpendicular diameter measurements. In contrast, neither the matrix control (G0C0) nor the glycerol-only formulation (G1C0) produced a visible inhibition zone. These findings provide preliminary evidence of antibacterial activity in the selected CEO–Tween 80-containing film. However, the limited number of independent replicates precluded reliable statistical inference, and no inferential statistical tests were performed. Furthermore, differences in the physical characteristics and diffusion behavior of the film specimens and gentamicin discs limit direct comparisons of their antibacterial performance. Future studies should include at least three independent replicate plates and appropriate additional controls to strengthen the reliability and interpretation of the antibacterial findings. Detailed inhibition zone measurements are presented in Table 5.

The absence of visible inhibition zones in the CEO-free formulations suggests that the antibacterial activity observed for G1C1 was associated with the incorporation of the CEO–Tween 80 emulsion system. However, because neither CEO-free control formulation contained Tween 80, the individual contributions of CEO and Tween 80 to the observed antibacterial response could not be distinguished. Cinnamon essential oil has been reported to contain bioactive constituents, particularly cinnamaldehyde, which may compromise bacterial membrane integrity and interfere with essential cellular functions (Shu *et al.*, 2024). Accordingly, cinnamaldehyde and other constituents may have contributed to the antibacterial activity observed in the present study. Nevertheless, because the chemical composition of the CEO used was not characterized, the involvement of specific compounds and their underlying antibacterial mechanisms could not be confirmed. Previous studies have demonstrated antibacterial activity against *Staphylococcus aureus* in films containing cinnamon essential oil, supporting their potential application in active packaging systems (Rachmawati *et al.*, 2023). Similarly, Yan *et al.* (2025) reported antibacterial activity against food-relevant bacteria in gelatin-based active packaging films incorporating encapsulated essential oils, further supporting the potential of essential-oil-loaded gelatin films as antimicrobial packaging materials.

However, the agar diffusion assay provides evidence of bacterial growth inhibition only under the experimental conditions employed. The observed inhibition zone depends on the

release and diffusion of antibacterial constituents from the film into the surrounding agar medium. Therefore, inhibition zone measurements cannot establish the minimum inhibitory concentration, bactericidal activity, or antimicrobial effectiveness of the film under actual food storage conditions. Further investigations under relevant food-contact and storage conditions are necessary to evaluate its practical antimicrobial performance. In addition, the amount of CEO retained in the film after 72 h of open-air drying remains unknown because partial volatilization of its constituents may have occurred during film formation. Consequently, future studies should quantify CEO loading and retention, investigate the release kinetics of active compounds, and assess antibacterial activity under realistic food-contact and storage conditions. These evaluations are essential for determining the suitability of the developed film for potential active food packaging applications.

Table 5. Antibacterial Activity of Selected Gelatin-Alginate Films Against *Staphylococcus Aureus* ATCC 25923

Sample	Replicate	Horizontal diameter (mm)	Vertical diameter (mm)	Average diameter (mm)	Mean ± SD (mm)
Gentamicin (10 µg)	1	15.70	14.10	14.90	15.43 ± 0.75
	2	15.88	16.04	15.96	
G0C0	1, 2	–	–	–	–
G1C0	1, 2	–	–	–	–
G1C1	1	12.30	11.24	11.77	12.07 ± 0.42
	2	12.44	12.28	12.36	

Note: Each replicate represents an independent assay plate (n = 2). The average inhibition zone diameter for each replicate was calculated from two perpendicular measurements (horizontal and vertical). Mean ± SD represents the mean and sample standard deviation of the two replicate averages. The symbol (–) indicates that no visible inhibition zone was observed.

4. CONCLUSION

Glycerol concentration and the cinnamon essential oil (CEO)–Tween 80 emulsion system were associated with formulation-dependent variations in the mechanical and water-uptake properties of gelatin–alginate films, without a consistent concentration-dependent trend for CEO. Increasing glycerol concentration generally improved film flexibility but reduced tensile strength, indicating a trade-off between extensibility and mechanical strength. Among the evaluated formulations, G1C1 (1% w/v glycerol and 0.3% v/v CEO) achieved the highest *weighted effectiveness index* score, reflecting its favorable combination of tensile strength and swelling characteristics. The selected formulation also exhibited antibacterial activity against *Staphylococcus aureus* ATCC 25923, with a mean inhibition zone diameter of 12.07 ± 0.42 mm based on two independent replicate plates (n = 2). However, because Tween 80 was incorporated simultaneously with CEO and no Tween 80-only control was included, the observed antibacterial activity could not be attributed exclusively to CEO. These findings indicate that the developed gelatin–alginate films have potential as functional biopolymer materials, although their suitability for active food packaging remains unconfirmed. Biodegradability and barrier properties were not evaluated, and antibacterial activity was

assessed against only one Gram-positive bacterial strain. Further studies should investigate water vapor permeability, active compound retention and release kinetics, appropriate Tween 80-only controls, and antibacterial activity against additional Gram-positive and Gram-negative foodborne microorganisms using more independent replicates. Evaluation under realistic food-contact and storage conditions is also necessary before practical applications can be considered.

5. ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Applied Chemistry Laboratory and the Bioprocess Laboratory, Department of Chemical Engineering, Politeknik Negeri Malang, and the Integrated Laboratory and Production Unit, Politeknik Kesehatan Putra Indonesia Malang, for providing the laboratory facilities used for film preparation, characterization, and antibacterial testing.

6. AUTHOR CONTRIBUTION STATEMENT

The authors declare that the contribution of each author to the preparation of this manuscript is as follows: AS (first author, corresponding author, and principal supervisor) was responsible for conceptualization, research design, methodology, supervision of data analysis, validation of data, and manuscript writing, review, and editing. PKH was responsible for conceptualization, methodology, data collection (investigation), and data analysis. PHS (second supervisor) was responsible for methodology, supervision, and validation of the data and analysis results. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

7. CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to the publication of this article.

8. FUNDING

This research was self-funded by the authors and did not receive any specific funding from public, commercial, or non-profit funding agencies.

9. DECLARATION OF GENERATIVE AI USE

During the preparation of this manuscript, the authors used Claude (Anthropic) to assist with language editing and to improve the clarity of the text. The authors reviewed and edited the output and take full responsibility for the content of the published article.

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