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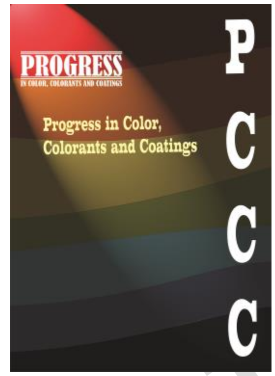

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Innovation in Adding Natural Antimicrobial Agents to Improve the Physicochemical Performance of Konjac-Glucomann-Based Edible Films

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Abstract

Edible Film made from natural and edible ingredients has emerged as a promising alternative to conventional packaging. This study aims to investigate the physicochemical properties of edible films consisting of konjac glucomannan, natural antimicrobial ingredients, and glycerol. Konjac-glucomann-based edible film (KEF) has emerged as an alternative to eco-friendly packaging. However, challenges related to mechanical strength, elasticity, and microbial resistance still need to be addressed. The edible film was prepared by combining konjac glucomannan flour (0.3%) with 2% thyme, cinnamon, and clove oils, along with 0.5% chitosan, in an acidic solution. The edible konjac glucomannan film was tested for its characteristics in treatment without glycerol and with glycerol 1%. Analysis of its physicochemical properties includes measurements of thickness, color, structure, moisture content, elongation, tensile strength, surface hydrophobicity, solubility in water, water vapor transmission rate, water vapor permeability, Fourier transform infrared spectroscopy, thermogravimetric analysis, and transmission. The results of the study show that KEF, a natural antimicrobial ingredient, and glycerol have good physicochemical properties. The addition of glycerol increases the flexibility and elasticity of KEF, reducing its tensile strength and mechanical properties. The addition of essential oils such as thyme, cloves, and cinnamon increases the antimicrobial activity of KEF, making it effective for food applications. The novelty of this study lies in the incorporating of natural antimicrobial agents to enhance the physicochemical performance of KEF. Research to develop edible films that do not affect food sensory is a challenge for the future.

Keywords: edible film, konjac-glucomannan, antimicrobial agents, glycerol,

physicochemical characteristics

1. Introduction

The use of eco-friendly packaging has considerably increased in recent years due to the awareness of the negative impact of plastic waste on the environment. Konjac-glucomannan-based edible film (KEF), which is made from natural and edible ingredients, is emerging as a promising alternative to conventional packaging [1]. One of the interesting materials for the development of KEF is glucomannan, which is known for its biopolymeric, biodegradable, and gel-forming properties [2]. However, the main challenge in the utilisation of this edible film is to increase mechanical strength, elasticity, and resistance to microbes. KEF is a thin layer of edible food and is formed to coat food ingredients and/or between food ingredients to facilitate food handling [3]. In addition, edible film is also made from hydrocolloid raw materials, lipids, proteins, or a combination of the three [4, 5]. The main challenges of edible films is maintaining their physical and chemical stability during storage. They are often more susceptible to moisture and environmental conditions, which can affect their quality [6]. Many edible films made from natural materials have poor mechanical properties compared to conventional plastics [7]. Edible films should have a taste and aroma that does not interfere with the packaged food product. More research is needed to develop films that do not affect food sensory [8]. Producing edible film on a large scale with quality consistency is a challenge that must be overcome to compete with traditional packaging [1].

One of the innovations in this field is KEF, which is increasingly gaining attention as an alternative to conventional plastic packaging [8, 9]. KEF is made from glucomannan derived from the tubers of the konjac plant (*Amorphophallus muelleri*), a plant native to Southeast Asia that is known to have a high glucomannan content. Konjac plants, classified as tuber plants in the Araceae family, have a relatively high glucomannan content (15-64% dry basis) and are widely used in various industrial sectors, including food, non-food, and health products [10, 11]. Glucomannan is a compound that has high levels of water-soluble fibre, along with low calories and distinctive hydrocolloid properties [12, 13]. KEF has several superior characteristics that make it an attractive choice, both for food packaging and other industrial applications [1, 14]. Its natural and environmentally friendly base material makes the film a more sustainable solution than plastic or other packaging materials that are difficult to decompose [15, 16]. In addition, this film has a high-water absorption ability, provides good moisture for food, and extends the shelf life of the packaged product [17, 18]. Another characteristic that is no less important is the capacity of the film to coat food products well, providing attractive transparency without altering the taste or aroma of the food [19, 20]. The KEF also has good mechanical strength and flexibility, allowing it to be used in various forms of food products [14]. KEF shows great potential as a beneficial packaging solution for the food and health industry [21, 22]. However, the challenges in the use of this edible film include mechanical strength, elasticity, and resistance to microbial contamination [4, 9].

Essential oils from plants such as thyme, clove, and cinnamon hold significant potential as additives in the development of KEF [23-25]. These oils are renowned for their antimicrobial properties and have been traditionally used in food preservation. The addition of essential oils not only enhances microbial resistance but also influences the physicochemical characteristics of the resulting film [26, 27]. Thyme oil, rich in active compounds such as thymol and carvacrol, shows effective antimicrobial properties against various pathogens [23, 28]. Research has shown that incorporating thyme oil enhances the film's microbial resistance while imparting a refreshing aroma to the product [29]. Clove essential oil, containing eugenol, a compound with antimicrobial, anti-inflammatory, and antioxidant properties, not only increases microbial resistance but also extends the shelf life of KEF through its curing effects [30].

Meanwhile, cinnamon essential oil provides strong antimicrobial properties and enhances the taste and aroma of food products [31]. The active compounds of the oil, known for their antioxidant benefits, further enhance the film's added value [24, 32]. Given this background, investigating the influence of thyme, clove, and cinnamon essential oils on the physicochemical characteristics of KEF is critical for advancing sustainable and effective packaging solutions.

Glycerol, a natural plasticiser, plays a crucial role in the development of KEF. Its addition not only improves the film's flexibility and elasticity but also affects various other physicochemical properties [33]. Key contributions of glycerol to enhancing the quality of KEF include the following. 1) Glycerol helps reduce the stiffness of the film, thereby increasing flexibility and elasticity. 2) The addition of a natural plasticiser can increase the tensile strength of KEF. 3) Glycerol has hygroscopic properties, which means it can attract and retain moisture. 4) This natural plasticiser may contribute to the development of hydrophobic properties in KEF. 5) The addition of glycerol may affect the thermal stability of the film. 6) The natural plasticiser can also increase the interaction between konjac-glucomannan and other additives, such as essential oils. 7) Glycerol can affect the transparency and colour of the film [34]. Therefore, glycerol is important in enhancing the physicochemical properties of KEF. Chitosan also plays an important role in the development of edible films due to its biodegradable, biocompatible properties, and its ability to increase mechanical strength and resistance to moisture and oxidation in food packaging [31]. Continued research and development into the use of glycerol can significantly improve the quality and effectiveness of the film as a packaging solution.

The characteristics of a good edible film include: 1) Strong enough to withstand pressure and friction during use and storage. 2) Transparent and allow consumers to see the packaged products, improving visual appeal. 3) It can inhibit moisture penetration to maintain the freshness of food. 4) Non-toxic and safe to consume, and does not affect the taste or aroma of food. 5) Decomposes naturally, so it is environmentally friendly. 6) Able to control the exchange rate of gases (such as oxygen and carbon dioxide) to extend the shelf life of food. 7) Efficient and economical manufacturing process. These characteristics are important to ensure that edible films are effective in maintaining food quality and safety[35, 36]. Based on the above description, this research aims to evaluate various physicochemical characteristics of KEF made from glucomannan konjac. The analysis involved parameters such as thickness, colour, surface morphology (using Scanning Electron Microscopy, SEM), moisture content, elongation, tensile strength, surface hydrophobicity, water solubility, water vapour transmission rate (WVTR), and water vapour permeability (WVP). Additionally, Fourier Transform Infrared Spectroscopy (FTIR)and thermogravimetric analysis (TGA) were adopted to assess the chemical structure and thermal stability of KEF. This research aimed to investigate the physicochemical properties of KEF enhanced with natural antimicrobial agents, including thyme, clove, and cinnamon oils, alongside glycerol as a plasticiser. The addition of antimicrobial agents was anticipated to improve the film's resistance to microbial contamination, while glycerol was expected to enhance its flexibility and elasticity. The results showed that incorporating glycerol and natural antimicrobial agents into KEF not only improved its physicochemical properties but also enhanced its resistance to microbial growth. This research significantly contributes to advancing environmentally friendly KEF and highlights its potential as a sustainable packaging solution, while opening new avenues for sustainable packaging technology.

2. Eperimental

2.1. Tools and materials

The materials used to produce KEF included 1 gram of konjac glucomannan (derived from *Amorphophallus muelleri* tubers, Ikarie Organic), chitosan as much as 4 grams (Chitosan Shrimp Pharmaceutical Grade, Phy Edumedia), and essential oil as much as 0.02, which is a mixture of thyme oil (Thymus vulgaris with the brand Essence Drops), eugenol (cloves, Syzygium aromaticum with the brand Essence Drops), and sinamaldehyde (cinnamon, Cinnamomum verum with the brand Drops Atsiri). A 1% acetic acid solution is prepared using glacial acetic acid (purity ≥99%, Merck, Germany) and aqueducts (PT Brataco, Indonesia). The tools used during the preparation and analysis of the film were a beaker, a measuring flask, a spatula, a stirrer, a scale, an oven (Memmert, Germany), micrometer-707 (Mitutoyo Corporation, Japan), Color difference meter (CR-20, Konica Minolta Inc., Japan), SEM (SU3500, Hitachi, Japan), Motorized force test stand (FGS-50E-L, Nidec-Shimpo Co., Japan), and digital force gauges

(FGPX-05, Nidec-Shimpo Co., Japan). These tools also included Smart Contact Mobile Entry M411 device (Exima Yokohama Lab Co., Ltd., Japan), oven (WFO-520, EYELA Tokyo Rika Kikai Co. Ltd., Japan), Incubator (IN 802 Yamato, Japan), Temperature and humidity chamber (TPAV-120-20, ISUZU CAP, Isuzu Seisakusho. Co., Ltd. Japan), ATR-FTIR (Jasco, FT/IR-4700, Japan), TG/DTA7300 (Hitachi Hi-Tech Science Co., Ltd. Tokyo, Japan) and UV-visible spectrophotometer (Jasco, V-530, Japan).

2.2 Formulation of KEF solution

F1 (KEF without glycerol): This formulation consisted of 22 mL of KEF solution per sheet. The preparation involved mixing 200 mL of distilled water, 2 mL of acetic acid, 1 g of konjac-glucomannan flour (derived from the tuber *Amorphophallus muelleri*), 4 g of chitosan, and 0.02 g of essential oils (a blend of thyme, cinnamon, and clove).

F2 (KEF with glycerol): Similar to F1, this formulation also used 22 mL of KEF solution per sheet. The mixture included 200 mL of distilled water, 2 mL of acetic acid, 1 g of konjac-glucomannan flour, 4 g of chitosan, 0.02 g of essential oils (from thyme, cinnamon, and clove), and an additional 1 mL of glycerol to enhance the film's flexibility and elasticity, each KEF treatment was made as many as 8 replications.

2.3. Stages of making KEF

The process to make KEF from konjac-glucomannan has three stages, namely: 1) The manufacture of a 1% acetic acid solution (v/v) is made by dripping a certain amount of glacial acetic acid into a 100 mL measuring flask that has partially contained water. The presence of heavy metals in raw materials can affect the physical and chemical properties of edible films. By removing lead metals, properties such as mechanical strength, elasticity, and moisture resistance can be improved. In acidic solutions, high concentrations of H^+ ions can affect the solubility of lead metals. Under certain conditions, this can help reduce lead solubility, facilitating the adsorption process. After that, the solution was diluted with water until it reaches the tera mark and homogenized until it forms a stable 1% acetic acid solution. 2) The procedure for making a film-forming solution: 4 g of chitosan and a mixture of 0.02 g of essential oil are dissolved in 200 mL of pre-prepared 1% acetic acid solution. This solution was stirred using a magnetic stirrer at a speed of 12 for 30 minutes, utilizing a homogenizer-type magnetic stirrer, specifically a Thermo SP131320-33Q model. Stirring speed 1200 rpm for 2 hours at room temperature until homogeneous. After that, 1 g of glucomannan flour is gradually added to the solution, and the mixture is stirred using a hot plate stirrer at 70 °C for 2 hours until a thick, homogeneous solution is obtained. 3) Film printing and drying, as much as 22 mL of homogeneous solution is poured into a circular mold (18 cm diameter), then dried in the oven at 83 °C for 14 hours. Once dry, the formed film sheets are removed from the mold and stored in a desiccant to prevent moisture contamination before further characterization is performed.

2.5. Physicochemical characteristics analysis of KEF

Physicochemical analysis of KEF, including test Thickness. Colour, SEM, Moisture content, Elongation, Tensile strength, Surface hydrophobicity, Water solubility, WVTR and WVP, FT-IR, Thermogravimetric analysis (TGA), and transmittance UV. The sample analysis method is as follows:

1. Thickness

Film thickness was evaluated with a precision of 0.001 mm using an auto digital micrometre (micrometre-707, Mitutoyo Corporation, Japan) at random positions on each film for each treatment. This measurement was done on 5 different films [19].

2. Colour

A colour difference meter (CR-20, Konica Minolta Inc., Japan) was used to measure the colour of the film at ambient temperature. Before the measurement, a white standard colour plate (L^* = 94.2) was used for calibration. Subsequently, the colour parameters L^*, a^*, and b^* were recorded by taking multiple measurements at various locations on the film. This measurement was done on 5 different films [19].

3. Scanning Electron Microscope (SEM)

The samples were mounted on double-sided conductive adhesive tabs and sputter-coated with a thin layer of gold. The ternary film surface and cross-sectional morphology were observed using an SEM (SU3500, Hitachi, Japan) with an acceleration voltage of 15 kV at 1000 × magnification [19].

4. Moisture content

The moisture content evaluation method used is described by [20]. Separate weighing of small stainless-steel plates with and without (2 × 2 cm) film samples was performed. Samples were then dried at 105 °C in an oven for 24 h, with moisture content calculated as follows, (Eq. 1):

$$\text{Moisture Content (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

Where W_1 represents pre-dry sample weight (g), W_2 represents after-dry sample weight (g)

5. Elongation

The films were cut into 1 cm × 5 cm rectangles to measure elongation. The films were clamped by the grip pair of a motorised force test stand (FGS-50E-L, Nidec-Shimpo Co., Japan) equipped with digital force gauges (FGPX-05, Nidec-Shimpo Co., Japan). The initial gap separation was set to 3 cm, then stretched by moving the grip with a speed of 60 mm s^{-1} until breaking (Eq. 2) [20]

$$\text{Per cent elongation at breaking} = \frac{l_f - l_0}{l_0} \times 100 \qquad (2)$$

where l_0 and l_f are the initial and final lengths of the gap.

6. Tensile strength

The tensile properties of the films were characterised using the tensile strength, per cent elongation at breaking, and Young's modulus. The tensile properties were calculated using the following equations (Eqs. 3, 4):

$$\text{Tensile strength} = \frac{F_{max}}{t \times w} \qquad (3)$$

$$\text{Young's modulus} = \frac{\text{Stress}}{\text{Strain}} = \frac{F / A}{\Delta l - l_0} \qquad (4)$$

Where F *max* is the maximum tensile force, t is the film thickness, and w is the width of the sample. F is equal to the force applied to the structure, A is the cross-sectional area of the film, and Δl is the change in length of the film when the force is applied to it [20].

7. Surface hydrophobicity

Surface hydrophobicity on films was measured by the contact angle, which was determined by using the Smart Contact Mobile Entry M411 device (Exima Yokohama Lab Co., Ltd., Japan). The film sample (10 ×20 mm) was placed on a metal specimen holder, and then a droplet of distilled water (5 µL) was dropped on the film surface in three replicates using a micropipette. Images of droplets were captured using Smart Contact software that was provided with the device [15].

8. Water solubility

The water solubility of the films (2 cm × 2 cm) was measured. The initial dry weight (*W*i) of the film was determined by drying at 80 °C for 48 h using a laboratory oven (WFO-520, EYELA Tokyo Rika Kikai Co. Ltd., Japan). Each film sample was then immersed in 25 mL of distilled water in an incubator (IN 802 Yamato, Japan) set at 25 °C for 24 h. After 24 h of immersion, the remaining film pieces were filtered and weighed (*W*f) and then dried at 80 °C for 48 h to a constant weight. The water solubility (*W*s) of the film was determined by (Eq. 5) [15].

$$W_S\ (\%) = \frac{W_i - W_f}{W_i} \times 100\% \quad (5)$$

9. Water Vapour Transmission Rate (WVTR) and Water Vapour Permeability (WVP)

The WVP of the films was measured with three replicates for each film type following the method of JIS Z 0208. A circular test cup (diameter 28 cm^2) was used to determine the water vapour permeability of the films. The film was cut into a circle larger than the inner diameter of the cup. Approximately 15 g of anhydrous calcium chloride was placed in the cups as a desiccant at 0% relative humidity (RH). The edible film was then placed on the top of the permeable cup. The cup was then covered with a lid and sealed with a screw. After measuring the weight of the cup, it was left in a constant temperature and humidity chamber (TPAV-120-20, ISUZU CAP, Isuzu Seisakusho Co., Ltd., Japan) set at 25 °C and 85% RH. The weight of the cup was measured every hour for 7 hours. WVTR and WVP were determined using the following equations (Eqs. 6 and 7).

$$WVTR = (\Delta m/\Delta t)\ A^{-1} \quad (6)$$

$$WVP = WVTR \times L\ \Delta p^{-1} \quad (7)$$

10. Fourier Transform Infrared Spectroscopy (FT-IR)

The molecular interactions of the films were analysed using ATR-FTIR. Each film was cut and placed on the sample holder of the ATR-FTIR (Jasco, FT/IR-4700, Japan). The infrared spectra were recorded at wavenumbers ranging from 4000 to 500 cm^{-1} with a resolution of 4.0 cm^{-1}. The analysis was conducted using the Spectra Manager software provided with the FTIR machine [16].

11. Thermogravimetric analysis (TGA)

TGA was determined using TG/DTA7300 (Hitachi Hi-Tech Science Co., Ltd., Tokyo, Japan). The measurement was carried out by weighing a 4 mg sample into an open aluminium pan and heating 30–600°C, at a heating rate of 10 °C/min under a nitrogen flow rate of 300 mL/min [4].

12. The transmittance of the films (UV)

The transmittance of the films was measured in the range of 300 to 800 nm using a UV-visible spectrophotometer (Jasco, V-530, Japan)[27].

2.5. Statistical analysis

The effects of the treatment administered were tested using ANOVA to determine whether there was a significant difference between the average quantity of responses from the groups compared. ANOVA results in a p-value of less than 0.05 ($\alpha = 0.05$), which indicates that at least one group is significantly different from the others. The analysis was conducted using IBM SPSS Statistics Version 29, which is a widely used statistical software for data analysis. If the ANOVA results show significance, further analysis may be required to find out which groups are different using the Tukey post hoc test.

3. Results and Discussion

KEF is made using materials that can break down naturally, like glucomannan from konjac tubers. Unlike regular plastic, which stays in the environment for many years, KEF decomposes in a shorter time, reducing pollution. This research tested KEF both with and without the addition of glycerol. The result of each parameter and significance (sig) KEF without glycerol and with glycerol can be seen in Table 1. Figure 1a shows KEF without glycerol, and Figure 1b shows KEF with glycerol.


(a)

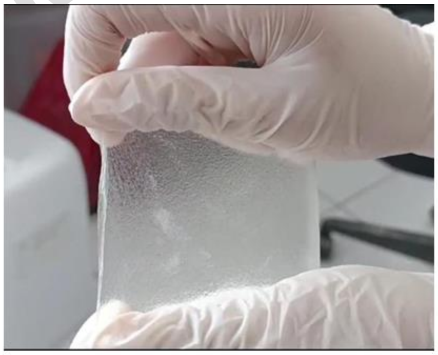
(b)

Figure 1. (a) KEF without glycerol (F1), (b) KEF with added glycerol (F2)

Table 1. Parameter and Significance (Sig) of KEF without glycerol and with glycerol.

Parameter	Sig	KEF without glycerol(F1)	KEF with glycerol (F2)
Thickness (mm)	0.051	0.074 ± 0.009	0.085 ± 0.004
Colour L	0.188	96.820 ± 0.230	96.600 ± 0.240
Colour a*	0.120	0.240 ± 0.050	0.340 ± 0.050
Colour b*	0.056	4.940 ± 0.160	5.740 ± 0.150
Moisture content (%)	0.397	17.000 ± 1.710	18.880 ± 2.970
Elongation (%)	0.003	3.810[a] ± 0.630	9.410[b] ± 1.410
Tensile strength (MPa)	0.007	243.360[b] ± 18.330	96.930[a] ± 46.640

Surface hydrophobicity (θ)	0.570	47.210 ± 14.810	54.390 ± 13.560
Water solubility (%)	0.096	1.671 ± 0.903	3.844 ± 0.486
Water vapour transmission rate (WVTR) $(g\ h^{-1}m^{-2})$	0.058	0.170 ± 0.016	0.201 ± 0.012
Water vapour permeability (WVP) $(g\ mm\ h^{-1}m^{-2}\ kPa)$	0.005	0.394 [a] ± 0.037	0.563 [b] ± 0.035
Roughness (nm)	0.001	2.705[a] ± 0.109	9.070[b] ± 0.525

3.1. Thickness of KEF

The KEF without glycerol was thinner, measuring 0.074 ± 0.009 mm. Meanwhile, the KEF with glycerol was thicker, measuring 0.085 ± 0.004 mm. Adding glycerol made the film thicker, but there was a little difference. Glycerol works as a plasticiser, helping to make the film's matrix thicker [37].

3.2. Colour of KEF

Adding glycerol to the KEF significantly increases its flexibility, strength, and resistance to moisture, making it more suitable for a wide range of applications in food packaging. Glycerol to KEF is not directly related to the "colour" part; it can only affect the transparency or colour stability. These improvements contribute to better performance, stability, and consumer acceptance, making glycerol a valuable additive in the development of edible films. The brightness (L*) values of KEF with and without glycerol were almost the same (Table 1). This showed that glycerol did not significantly affect the film brightness. Glycerol, being transparent and colourless, helped make the film more flexible and elastic while keeping its original colour[7, 38].

3.3. Structure of KEF

From using the SEM tool with three sample replications, as shown in Figure 3, the KEF that did not have glycerol was smoother and more transparent compared to the one with glycerol. The SEM results revealed that the KEF without glycerol exhibited better qualities, such as greater smoothness and transparency, with minimal roughness. This smooth surface meant the konjac-glucomannan dissolved evenly, resulting in a transparent and smooth KEF[2]. On the other hand, the SEM showed that the film with glycerol had bubbles and indentations. The surface of this KEF was rougher and had more irregularities than the one without glycerol [1]. This roughness resulted from the interaction of glycerol molecules with the film matrix, causing uneven distribution of materials within the film [39, 40]. KEF on SEM as seen in Figure 2.

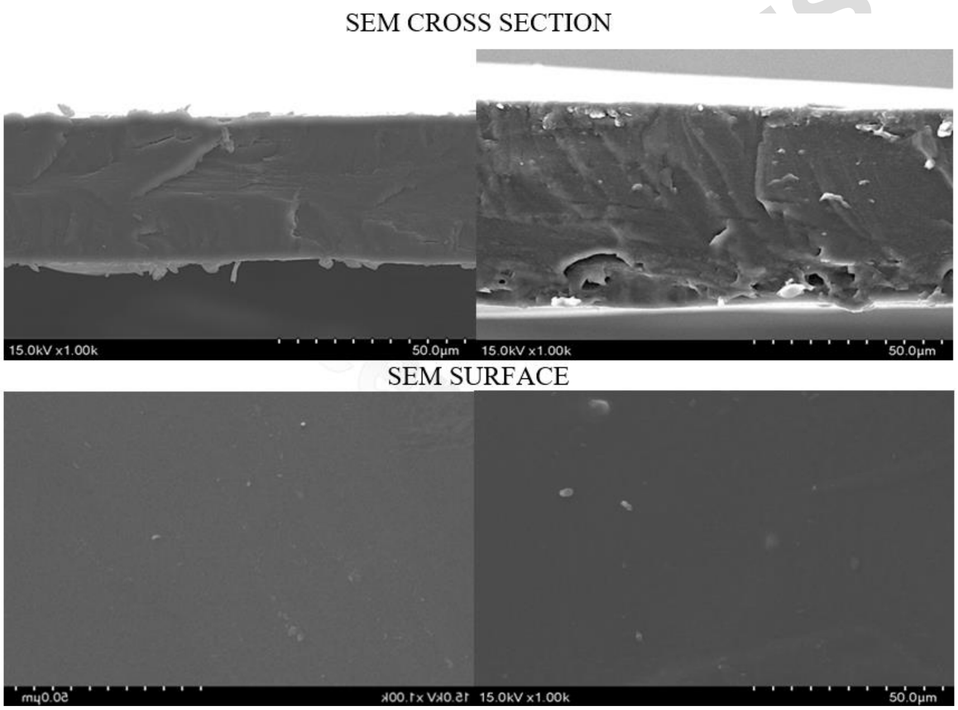

Figure 2.KEF on Scanning Electron Microscope (SEM) observations

3.4. Moisture content of KEF

KEF without and with the addition of glycerol produced water that did not differ significantly, measuring 17.00 ± 1.71% and 18.88 ± 2.97%, respectively. Meanwhile, the film with glycerol had slightly higher moisture. This was because when there was more glycerol, it absorbed more water. Glycerol is hydrophilic, which helps retain water in the film. Additionally, as the glycerol content increased, the hydroxyl group (-OH) became more active, leading to higher water retention in the film [41, 42].

3.5. Elongation of KEF

Statistical analysis showed that there was a real difference in the elongation variable. The addition of glycerol helped in producing film with high elongation characteristics. Also, it affects the permeability of water vapour. Glycerol has a low molecular weight and can easily fit into the polymer matrix of the film made from materials such as glucomannan or starch [24]. By entering the intermolecular space, glycerol reduces intermolecular forces, particularly hydrogen bonds, which normally bind polymer chains together. This decrease in force allows the polymer chain to move more freely, increasing the flexibility and elongation of KEF. Glycerol contains a (-OH) that can interact with functional groups on polymers. This interaction helps in creating a more elastic and flexible structure in the film [40].

3.6. Tensile strength of KEF

Statistical analysis showed a significant difference in the tensile strength of the film. KEF without glycerol had a higher tensile strength of 243.360 b ± 18.330 MPa, whereas the addition of glycerol resulted in a decrease, yielding a tensile strength of 96.93 ± 46.64 MPa. Glycerol functioned as a plasticiser, reducing the intermolecular forces between polymer chains in the film matrix [38]. This reduction in interactions increased the flexibility of the polymer chains but simultaneously decreased the total tensile strength of

KEF. Based on this, KEF became more stretchable but less resistant to weight or pressure. Glycerol's hydrophilic properties enabled it to attract and retain water, further contributing to the changes in film characteristics (Ben et al., 2022). The effects of glycerol concentration on tensile strength (TS) and percentage elongation at break (%) of composite edible film based on konjac glucomannan (KGM) were investigated. Glycerol was found to be a suitable plasticizer in terms of mechanical properties and TS [43].

3.7. Surface hydrophobicity of KEF

Surface hydrophobicity referred to the ability of KEF to repel water, which could be measured by the contact angle between a water droplet and the film surface[17][44]. A higher contact angle showed greater resistance to water, and film with contact angles above 90° was considered to have good hydrophobic property [45]. In this research, KEF without glycerol had a contact angle of 64.34°, which was not significantly different from the film containing glycerol, with a contact angle of 63.22°. These results showed that both types of film possessed good hydrophobic properties. A contact angle of less than 90° usually indicates a hydrophilic surface; a variety of factors, including surface structure, material composition, and specific application, can cause the surface to be considered "good" hydrophobic. Surface hydrophobicity (ϴ) (Highest value) of KEF as seen in Figure 3.

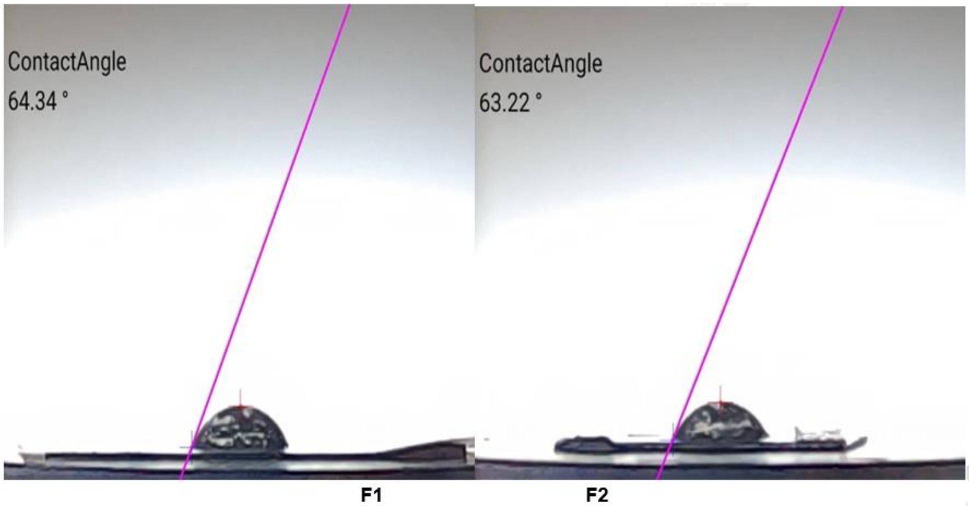

Figure 3. Surface hydrophobicity (Ө) (Highest value)

3.8. Water solubility of KEF

KEF without glycerol had lower water solubility, recorded at 1.671 ± 0.903%, indicating higher resistance to solvents. This reduced solubility was attributed to the dense and robust structure of glucomannan polymers, which resist decomposition or dissolution when exposed to water [46]. On the other hand, KEF with higher glycerol concentrations showed reduced permeability, helping to retain the moisture of packaged products [1, 47].

3.9. WVTR and WVP of KEF

Statistical analysis showed significant differences in WVP and surface roughness variables. KEF without glycerol addition had lower WVP and WVTR, showing its superior barrier properties. Research has shown that the film without glycerol possessed a denser and stronger structure, which effectively reduced water vapour transmission and helped retain moisture in packaged products [48]. The lower WVTR of glycerol-free

KEF suggested it was more effective at preventing water vapour transmission compared to the film containing glycerol. KEF containing glycerol typically exhibits higher WVTR due to the hydrophilic properties of glycerol [49]. A lower WVTR enhanced the ability of the film to preserve the moisture content of packaged products, thereby preventing drying and maintaining food quality during storage [50].

In contrast, glycerol-free film had higher resistance to solvents, enabling it to maintain structural integrity when exposed to moisture. The incorporation of glycerol increased the moisture content and WVTR of KEF [51]. Glycerol's hydrophilic nature allowed it to attract and bind water within the film matrix, thereby increasing solubility and reducing solvent resistance [7]. The denser and more stable structure of KEF without glycerol contributed to its lower WVP. In the absence of glycerol, molecular bonds within the film remained strong, reducing the penetration of water vapour [41]. Conversely, glycerol enhanced moisture retention by binding water molecules within the matrix, which increased WVP. Lower WVP in glycerol-free film was crucial for preserving the moisture of packaged products, preventing quality degradation, and maintaining freshness during storage. The difference between WVTR and WVP significantly affects the choice of food packaging [52]. Considering these properties carefully, the product manufacturers ensure integrity, extend shelf life, and meet regulatory requirements and standards Effectively managed WVTR and WVP ultimately result in the best quality products and customer satisfaction [20].

3.10. FTIR of KEF

The KEF containing glycerol had an elongation ability of 9.41 ± 1.41%, showing increased flexibility. The FTIR spectrum analysis showed the presence of key functional groups, including -OH, -CH, and -C=C. Specifically, the -OH group in glycerol-containing KEF was quantified at 3301.54. The presence of this group suggested a hydrogen interaction between glucomannan and glycerol within the film matrix (Kurt, 2019), which contributed to the hydrophilic properties of the resulting film. While the addition of glycerol positively affected characteristics of KEF, it did not significantly alter the FTIR spectrum, indicating that glycerol primarily functioned as a plasticizer. This enhanced the flexibility and mechanical properties of the film without changing the fundamental chemical structure of the other components. Moreover, FTIR analysis showed an increase in absorption within the -OH region, particularly in the range of 3200–3500 cm−1. Fourier Transform Infrared Spectroscopy of KEF, as seen in Figure 4.

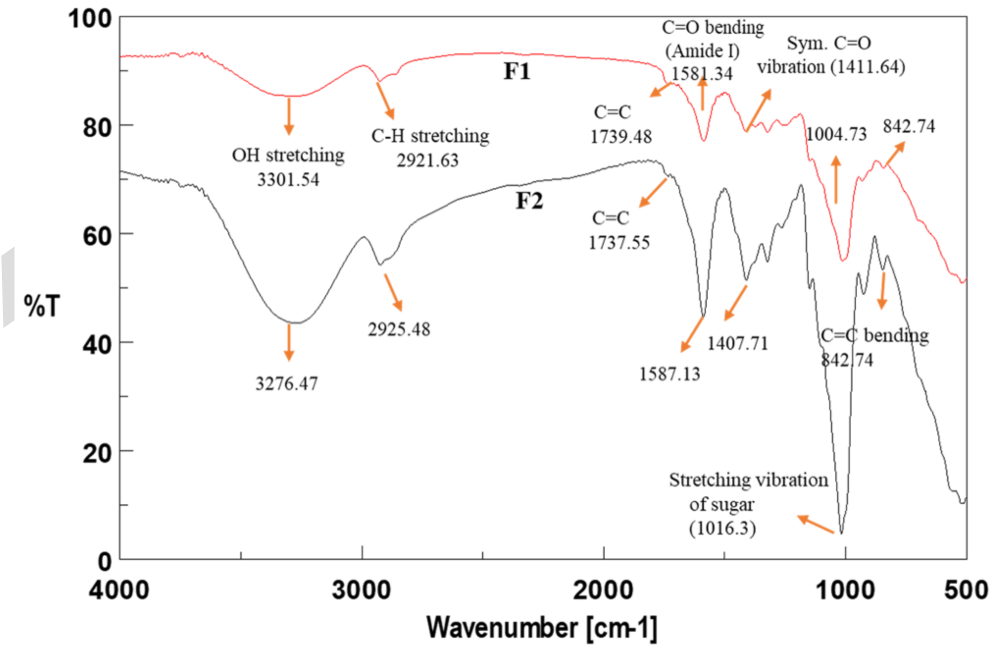

Figure 4. Fourier Transform Infrared Spectroscopy of KEF

3.11. TGA of KEF

TGA was used to assess the thermal properties, including the glass transition temperature (Tg), of KEF. These properties were crucial for understanding how the film behaved under temperature variations. Key factors included the glass transition and melting temperatures, which define the thermal and mechanical stability of KEF. The Tg showed the transformation of the film from a glassy to a more relaxed state [45]. The results showed that the addition of glycerol raised the transition temperature of KEF. As a plasticiser, glycerol reduced the intermolecular forces between polymer chains by weakening hydrogen bonds. This reduction in bonding allowed for increased mobility of the polymer chains, thereby raising the Tg. Due to its small molecular size, glycerol could penetrate the polymer network, further enhancing molecular mobility. As a result, glycerol concentration influenced the film's mechanical properties, particularly by increasing the Tg when optimally applied [40, 53]. Thermogravimetric analysis of KEF as seen in Figure 5

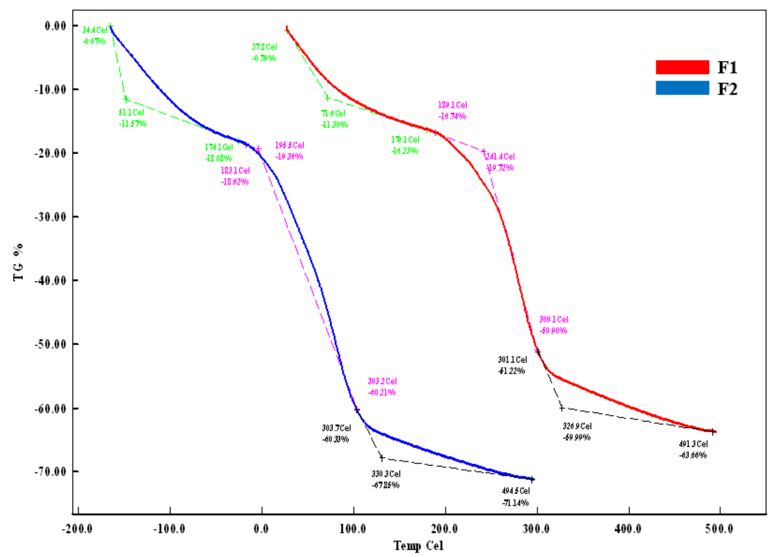

Figure 5. Thermogravimetric analysis of KEF

3.12. The transmittance of KEF

The KEF without glycerol shows a higher transmittance value, which means the film is more transparent. KEF, with the addition of glycerol, produces the characteristics of a film that is less transparent and has a bubbly, rough texture. This causes the transmission to be lower than that of films without glycerol. Films with glycerol tend to have lower transparency, enabling them to block more light [41]. The higher transmittance levels of glycerol-free edible film indicate potential use in food packaging applications, where transparency can be an important factor in attracting consumers' attention and displaying products inside the packaging. The results of this study are supported by the results of the Roughness test, where the KEF formula with glycerol has a higher level of roughness, which is 9.070b ± 0.525 (Table 1). Edible film with higher levels of roughness will have a harder time transmitting light, resulting in a lower transmittance [33]. The UV-vis spectrophotometer analysis of KEF is shown in Figure 6

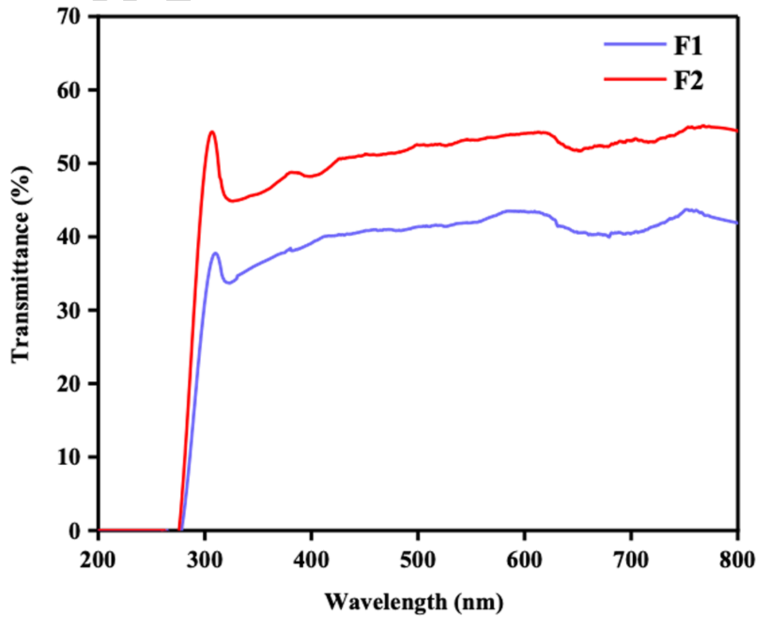

Figure 6. Observation of KEF on a UV-vis spectrophotometer.

3.13. Antibacterial KEF against S. aureus

KEF, with and without glycerol, exhibits antibacterial activity against *Staphylococcus aureus*. KEF without glycerol (F1) has a bacterial inhibition zone diameter of 18.517±2.455 mm, and KEF with glycerol (F2) has a bacterial inhibition zone diameter of 17.710±2.230 mm; the differences between the two was not statistically significant. The addition of glycerol does not affect the antibacterial activity of the edible film, as glycerol function solely as a plasticizer. Glucomannan porang acts as a matrix that supports antibacterial activity, and studies have also shown that proteins and other compounds in porang tubers can function as antimicrobial agents [54]. The antibacterial activity of glucomannan is believed to be related to the content of proteins and other bioactive compounds that can damage the bacterial cell wall, thereby interfering with bacterial metabolism [55]. Glucomannan can inhibit the synthesis of bacterial proteins through bioactive compounds such as alkaloids and protein lectins. In addition, glucomannan can damage the cell wall integrity of Gram-positive bacteria (S. aureus) more effectively than Gram-negative bacteria (E. coli) due to differences in membrane structure [32, 56]. The diameter of the KEF inhibition zone (Zoi) and the antibacterial activity of KEF against S. aureus can be seen in Table 2 and Figure 7.

Table 2. Diameter of KEF Inhibition Zone (Zoi).

Treatment	Zoi (mm)	Sig
Positive Control (Chloramphenicol Antibiotic 10 µg/L)	31.290 ± 1.185^{a}	0.034
KEF without glycerol (F1)	18.517 ± 2.455^{b}	
KEF with glycerol (F2)	17.710 ± 2.230^{b}	

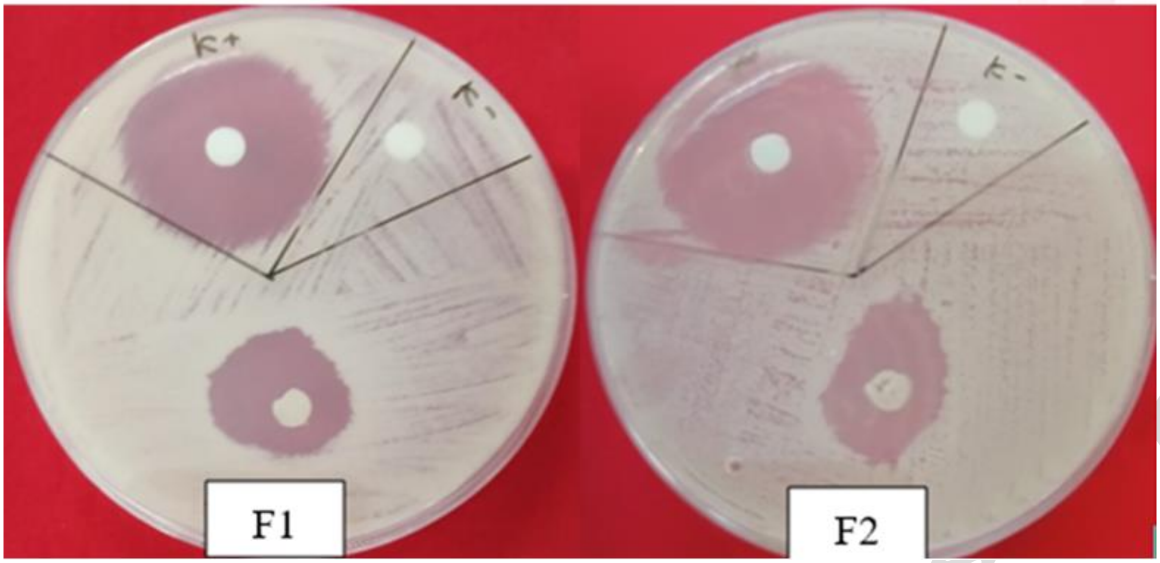

Figure 7. Antibacterial EFK against S. aureus

4. Conclusion

The study concludes that KEF, containing natural antimicrobial ingredient and glycerol, exhibits favorable physicochemical properties. Glycerol, added as a plasticizer, increases the flexibility and elasticity of KEF, while reducing its tensile strength and mechanical properties. The addition of essential oils such as thyme, cloves, and cinnamon increases the antimicrobial activity of KEF, making it more effective for food applications. The novelty of this study lies in the innovative use of natural antimicrobial agents to improve the physicochemical performance of KEF. Research to develop edible films that do not affect food sensory is a challenge for the future.

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