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Antioxidant Film Packaging from Red Tilapia Fish Scale Gelatin with Kesum (*Persicaria Odorata*) Powder

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ABSTRACT

This study examines the feasibility of using scale, an organic waste product from processing red tilapia, as a source of gelatin for packaging materials. The antioxidant activity of gelatin-based films is increased by adding kesum leaf powder, thereby enhancing the films' functionality as active packaging materials. However, the particular effect of kesum leaf powder on these films hasn't been fully investigated. The study aims to close this gap by methodically examining a number of factors, such as moisture content, optical characteristics, mechanical strength, scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and antioxidant activity in gelatin-glycerol films combined with kesum leaf powder (Gel/Gly/Kes). The analysis findings show that the Gel/Gly/Kes films retain their transparency while adding a deeper colour with green and yellow undertones. Additionally, adding the kesum powder lowers the moisture content and modifies mechanical characteristics, most notably by increasing elongation at break (28.2%). Furthermore, the Gel/Gly/Kes films show a significant increase in antioxidant activity, with 73.5% inhibition, indicating potential use in active food-preservation packaging. The successful incorporation of kesum leaf powder results in an uneven surface with white areas visible under scanning electron microscopy (SEM). In conclusion, the Gel/Gly/Kes films exhibit favourable characteristics, establishing them as a successful active packaging material with the potential to preserve the quality and prolong food products' shelf lives.

Keywords: Antioxidant packaging, gelatin-based film, kesum leaf powder, red tilapia fish scale

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INTRODUCTION

Malaysia has established aquaculture facilities supporting the cultivation of at least 49 freshwater and marine fish species. The most commonly farmed marine fish species include asian seabass, snapper, and grouper. In freshwater aquaculture, catfish, tilapia, and riverine catfish dominate, with a combined production of 121,553.75 metric tons in 2020 (Ridzuan et al., 2022). The Department of Fisheries (n.d.) data showed that up to 20,000 tonnes of red tilapia were produced, making it the second-largest cultured species in 2025. However, as fish production increases in Malaysia, so does the generation of fish waste, including bones, scales, skin, and shells. Additional waste also arises from aquaculture-related activities such as canning, filleting, salting, and smoking. An estimated 7.2 to 12 million metric tons of fish waste are thrown away globally each year (Qin et al., 2022). The dumping of fish waste, especially fish scales, in landfills can seriously damage the environment and waste resources (Qin et al., 2022). As a result, transforming fish scales into useful products like gelatin might generate additional revenue streams and contribute to a more sustainable and environmentally friendly aquaculture industry. Red tilapia fish scales have a high protein content of up to 47%, showing their suitability as a gelatin source (Pertiwi et al., 2025). According to Luo et al. (2022), the food industry uses gelatin extensively because of its many useful properties.

Moreover, gelatin has garnered significant attention as a promising material for edible films and food packaging (Hanani et al., 2014; Hoque et al., 2011; Nagarajan et al., 2012; Prodpran et al., 2017; Tongnuanchan et al., 2016). It has been fascinating due to its ability to form films and its potential to produce active packaging with practical applications in the food industry (Kumnerdsiri et al., 2024). Polymer films, which are thin and continuous structures, are commonly used in packaging to protect products from the external environment. The addition of plasticisers to gelatin films enhances their viscoelasticity, workability, elongation, and uniformity. Azmi et al. (2020) demonstrated that different types and concentrations of plasticisers used in gelatin films significantly affect the functionality of tilapia fish-scale gelatin films. Furthermore, gelatin-based films function not only as barriers against oxygen, carbon dioxide, and volatile compounds but also as platforms for integrating diverse additives. These additives include antimicrobials, antioxidants, antifungal agents, colorants, and other nutrients, as demonstrated by previous studies (Ahmad et al., 2012; Rhim & Ng, 2007). This feature allows for the design of active bio-based packaging materials with antioxidant capabilities.

Research into active packaging has seen recent growth due to its potential to prevent food degradation and extend shelf life. Previously, gelatin-based packaging films have been approved for their ability to protect, preserve, and demonstrate strong film-forming capabilities (Said & Sarbon, 2022). The use of gelatin-based packaging film with antioxidants has been shown to prolong shelf life, as antioxidants inhibit the growth of microorganisms

(Ratna et al., 2023). This is supported by a study by Sinlapapanya et al. (2024), which concluded that antioxidant activities can significantly inhibit lipid oxidation and microbial growth, thereby extending the shelf life of the product. The incorporation of antioxidant capabilities from natural extracts, as highlighted by Najwa et al. (2020), is a key aspect in the development of active packaging solutions. According to Christapher et al. (2015), kesum powder has shown promising antibacterial, antifungal, anti-inflammatory, and antioxidant properties. As stated by Ahmad et al. (2014) and Salbiah et al. (2015), kesum has created an inhibitory zone against a variety of foodborne pathogens. To prolong the shelf life of food by reducing oxidation rates, antioxidant-active food packaging has been proposed as a solution. Although the bioactive qualities of kesum-derived extracts have been investigated, there is still a lack of research on the use of kesum leaf powder in fish scale gelatin films. The effects of kesum leaf powder towards the physicochemical, structural, and functional characteristics of fish scale gelatin film remain insufficiently explored. Therefore, the purpose of this study was to investigate the effects of incorporating kesum leaf powder into gelatin-based films, especially on their antioxidant packaging properties.

MATERIALS AND METHODS

Extraction and Proximate Analysis of Gelatin from Red Tilapia Fish Scale

The procedure adhered to the methods outlined by Limpisophon et al. (2009) and Weng & Wu (2015), with a minor adjustment. The red tilapia scales were collected from a wet market in Pahang and washed thoroughly to remove dirt and blood. Subsequently, the scales went through alkaline treatment by soaking in a solution of 0.05 M sodium hydroxide (NaOH) and 25% methanol with a scale-to-solution ratio of 1:4 at 10 °C for 24 hours to remove the lipids, pigments, and non-collagenous proteins. Following the alkaline treatment, the scales were soaked for 2 hours in 0.05 M hydrochloric acid (HCl) at room temperature. The scales were thoroughly cleaned and rinsed before the gelatin was extracted. The gelatin was then heated in distilled water to 80 °C for an hour. Then, the mixture was centrifuged (Universal 320, Hettich GmbH & Co., Canada) at 9000 rpm for 33 minutes. The gelatin extract supernatant was freeze-dried using a freeze-dryer (Scanvac, Labogene, Denmark) and stored at -20 °C until further use.

The Association of Official Analytical Chemists (AOAC) International (2016) methodologies were used to determine the protein, fat, ash, and moisture contents of fish scales and gelatin powder.

Preparation and Proximate Analysis of Kesum Leaf Powder

Polygonum minus (kesum) was obtained from a nearby wet market located in Kajang, Selangor. The botanical sample was cleaned with flowing tap water and then rinsed with

distilled water to eliminate dirt and impurities. The procedure for preparing kesum powder followed Abu Samah et al. (2019), with a minor modification. The prepared plant material was subsequently placed in an oven at 50 °C and dried for 3 days until a stable weight was reached. The dried plant sample was then weighed, blended with a high-speed mixer, and kept at room temperature for additional analysis.

The proximate analysis of the powdered leaves was conducted to determine the protein, fat, ash, and moisture content following the standard procedures outlined by the Association of Official Analytical Chemists (AOAC) (AOAC International, 2016).

Preparation and Characterisation of Kesum/Gelatin-Based Film

The gelatin films were developed using the same method for solution casting method as in González et al. (2016), with some modifications. The film-forming solution was created by dissolving 2% (w/v) of freeze-dried gelatin, along with 30% (w/w of the gelatin) of plasticisers, in distilled water at 60 °C for 30 minutes with continuous stirring. Glycerol served as the plasticiser. Kesum powder was incorporated at varying concentrations of 0.05%, 0.1%, and 0.2% to produce Kesum-incorporated films of Gel/Gly/Kes (C0.5), Gel/Gly/Kes (C1.0) and Gel/Gly/Kes (C2.0), respectively. The concentrations was chosen based on a study by Sabbah et al. (2023) for the incorporation of olive and guava leaves with pectin films.

The solution underwent sonication for 10 minutes (Chang et al., 2010) to eliminate air bubbles. Then, the film-forming solution was cast into 14-cm-diameter Petri dishes placed on a levelled surface and allowed to dry at room temperature for 48 hours. Upon complete drying, the film was carefully peeled off and stored in sealed plastic film at 57% humidity (Xu et al., 2005).

Optical Properties of Films

The colour was determined using a colourimeter (ColourFlex EZ, HunterLab, Virginia, USA). The colour profile was described using the colour parameters L* (lightness component), a* (green-red), and b* (blue-yellow). An average of at least three replications is shown by the colour values. The formula, where ΔL*, Δa*, and Δb* represent the differences between the standard white tile and film samples, respectively, was used to get the total colour difference (ΔE). Furthermore, film opacity was calculated using the formula opacity = A600/L, where L is the film thickness (mM), and A600 is the absorbance value at 600 nm (Nordin et al., 2020).

Moisture Content

The weight loss of the films during drying was used to assess their moisture content. The film was dried in a ventilated oven C (UF110, Memmert, Germany) at 105 °C for 24 hours.

The film's weight was recorded at the beginning and end, and the moisture content was assessed by calculating the weight change.

Fourier-Transform Infrared Spectroscopy (FTIR)

A Thermonicolet (Model Nicolet 6700, United States) spectrometer was used to record FTIR spectra, and thin, transparent potassium bromide (KBr) pellets were used to acquire transmission spectra (Saikia et al., 2010).

Scanning Electron Microscopy (SEM)

This analysis will result in an image taken at 15 kV as the accelerating voltage. The Scanning Electron Microscope (SEM), specifically a JEOL Model JSM-6400 from the USA, was used to assess the film's morphology. To eliminate charging effects, a thin layer of gold was applied to coat the specimens.

Film Mechanical Performance Analysis

The measurements of tensile strength (TS) and percentage elongation at break (EAB) are crucial for linking the mechanical characteristics of films to their chemical composition. The Texture Analyser (TA.XT2, Stable Micro Systems, UK) was used to determine these properties following the guidelines outlined in ASTM D882-02 (ASTM, 2002). Sample strips measuring 100 × 15 mM from each film type were cut for testing. Prior to testing, the film thickness was measured using a Digital Vernier Caliper (MIT-500-196-20, Japan). The averages of these data were used to calculate the cross-sectional area of each film.

Antioxidant Activity

The antioxidant activity was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay method (Hsu et al., 2008, 2011). In particular, 20 mL of a 55 mM DPPH solution in methanol was applied to the 20 film sections (1 × 1 cm^2). Following a 24-hour incubation period in the incubator, the absorbance of the supernatant was measured at 516 nm using a UV-Vis Spectrophotometer (Ultrospec 3100, Amersham Biosciences, Germany). A control was established using a blank DPPH solution without any sample. The free radical scavenging effect of all kesum powders on DPPH radical was evaluated to assess their antioxidant activities, using a method by Akowuah et al. (2005) with minor adjustments. Two milliliters of 0.1 mM DPPH methanolic solution were combined with 200 microliters of sample extracts and 0.8 millilitres of methanol. The blend was completely stirred and stored in the dark for one hour. The control was made by blending 2 mL of DPPH with 1 mL of methanol. Absorbance was recorded at 517 nm using a UV-Vis spectrophotometer, and the samples were assessed in triplicate.

Statistical Analysis

The statistical analysis of the data obtained from the physicochemical, morphological and antioxidant properties of the films was performed by ANOVA using SPSS to determine significant differences among treatment means at a significance level of $p < 0.05$. In film properties analysis, Gel/Gly film was used as a control. Triplicates of each of the films were used in the analysis, and the values were presented as means ± standard deviation.

RESULTS AND DISCUSSIONS

Gelatin Extraction



Figure 1. The gelatin extracted from red tilapia's fish scales

The successful extraction of gelatin from raw red tilapia fish scales was achieved, with approximately 6.8 wt% of gelatin obtained from a dry weight of 100 g of fish scales. Based on the visual appearance represented in Figure 1, the gelatin extracted from the red tilapia fish scales displayed a snowy white, crystal-like appearance and a light texture after freeze-drying. Additionally, the extracted gelatin exhibits a nearly unnoticeable fishy odour.

The freeze-dried gelatin exhibited a porous structure. The flank of the freeze-dried gelatin was bright white and shiny. On the wall side, the sample gelatin had a rough, porous surface. The scaffold's bottom surface is smooth and features a radial design. The gelatin scaffold is white, while gelatin film, capsules, or foam are typically light yellow (Wu et al., 2010).

The approximate composition of the fish scales and the extracted gelatin is presented in Table 1, which include the amounts of protein, fat, ash, and moisture content. The red tilapia fish scales contained approximately 24.6% protein, 0.6% total fat, 15.8% ash, and 59% moisture. Protein and moisture comprised the majority of the scales, with smaller amounts of ash and fat. The protein content figures are generally consistent with the findings of Sockalingam ane Abdullah (2015), but there are differences in the moisture and ash contents, possibly due to variations in tilapia species.

Table 1
Percentage of proximate composition of red tilapia's fish scale and extracted gelatin

Composition	Yield (%)	
	Fish Scale	Extracted Gelatin
Protein	24.6	85.8
Total fat	0.6	0.4
Ash	15.8	2.5
Moisture	59.0	11.0

Kesum Leaf Powder

Proximate analysis involves the identification and quantification of the primary components present in feed and other raw plant materials. Its primary purpose is to evaluate whether the feed adheres to its typical compositional parameters or if any adulteration has occurred. In the present study, proximate parameters were assessed for the kesum leaf powder. Based on the results in Table 2 showed that kesum leaf powder has total ash (23.0%), moisture content (7.5%), total fat (0.6%), carbohydrate (57.2%), and protein (11.7%).

Table 2
Proximate analysis of the kesum leaves powder

Parameters	%
Total ash	23.0
Moisture content	7.5
Total fat	0.6
Carbohydrate	57.2
Protein	11.7
Energy	281 kcal/100g

Film Properties

Optical Properties of Film

The colour of films is an important attribute that consequently affects their appearances, marketability, and suitability for various applications. According to Table 3, the L* value of gelatin-based film decreases significantly ($p < 0.05$) from 86.78 to 64.74 with increasing incorporated concentration of kesum leaf powder. This is due to the kesum powder, which is opaque in nature, directly affecting the overall luminosity of the film. Hence, decreased the film's lightness.

Table 3
Optical properties of different concentrations of kesum incorporated into the kesum/gelatin-based films

Sample	L*	a*	b*	Opacity	ΔE
Gel	85.83 ± 0.26	0.73 ± 0.02	0.43 ± 0.29	3.80 ± 2.04	11.24 ± 0.25
Gel/Gly	86.78 ± 0.56	-1.38 ± 0.12	3.22 ± 0.51	7.02 ± 1.45	10.33 ± 0.30
Gel/Gly/Kes(C0.5)	80.79 ± 0.44	-0.57 ± 0.16	-12.76 ± 1.38	5.19 ± 2.42	21.97 ± 1.05
Gel/Gly/Kes(C1.0)	68.90 ± 2.66	-1.38 ± 0.60	-37.45 ± 2.96	4.17 ± 1.24	48.53 ± 3.61
Gel/Gly/Kes(C2.0)	64.74 ± 4.62	-3.25 ± 0.97	-37.39 ± 2.88	3.78 ± 2.61	74.82 ± 5.30

Note. ΔE: Total colour different; Gel: Gelatin; Gly: Glycerol; Kes: Kesum powder; C: Concentration of kesum powder incorporated into the film

Colour analysis revealed that the Gel/Gly film exhibited a light yellowish tint. However, the values of a* and b* significantly increased ($p < 0.05$) as the concentration

of kesum powder in the film increased. This can be attributed to the dark green colour of the kesum powder, which dominated the film's overall colour, resulting in stronger green (a-) and blue (b-) hues. The total colour difference (ΔE) showed that the inclusion of kesum powder in gelatin film can affect the overall colour, with an increase as the kesum powder concentration increases. According to Kuan et al. (2020), the edible film produced becomes darker, greener, and yellower as the amount of mulberry leaf extract increases. In accordance with the findings of Kuan et al. (2020), the result demonstrates that the kesum powder colour gives a greener colour to the gelatin-based film, as shown in Figure 2. The increase in the yellowish and greenish colour of the film was attributed to the natural colour of the kesum leaf. In the Gel/Gly film, the film appeared to be nearly transparent, with no visible green or yellow colouration due to the absence of kesum powder. The Gel/Gly/Kes (0.5) film exhibited a slight yellowish-green tint, while the Gel/Gly/Kes (1.0) film showed a more noticeable yellowish-green colouration. Meanwhile, the Gel/Gly/Kes (2.0) film exhibited the strongest yellowish-green coloration, giving the film a distinctly darker yellow-green appearance due to higher kesum powder content.

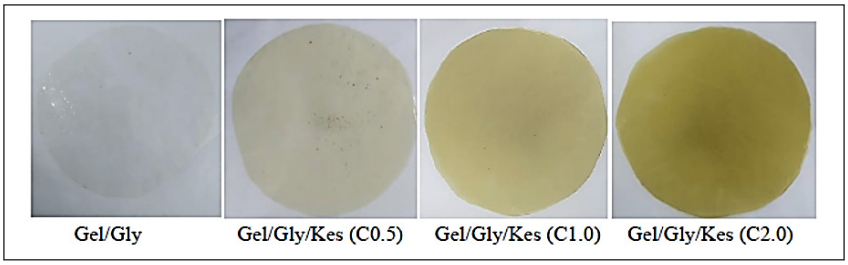


Figure 2. Gelatin-based film with and without incorporation of kesum powder

Moisture Content Analysis

The gelatin film without plasticiser had the greatest moisture content, at 27.05%. The incorporation of kesum leaf powder, as shown in Table 4, significantly ($p < 0.05$) decreased the moisture content of the gelatin composite films from 15.48% to 6.77%. The reduced moisture level of the film with kesum leaf powder may suggest the establishment of an inclusion complex between the gelatin matrix and the polyphenolic compounds via hydrogen

Table 4
Moisture content of different concentrations of kesum powder incorporated into the kesum/gelatin-based films and moisture content of kesum powder

Sample	Moisture Content (%)
Gel	27.05 ± 5.84
Gel/Gly	17.29 ± 5.63
Gel/Gly/Kes (C0.5)	15.48 ± 2.89
Gel/Gly/Kes (C1.0)	12.39 ± 6.43
Gel/Gly/Kes (C2.0)	6.77 ± 0.85
Kesum Powder	8.83 ± 0.61

Note. Gel: Gelatin; Gly: Glycerol; Kes: Kesum powder; C: Concentration of kesum powder incorporated into the film

bonds. Weak intermolecular interactions between the gelatin matrix and polyphenols competed with water molecules, reducing the matrix's capacity to interact with water and leading to a decrease in the moisture content of the developed film. The results align with findings by Rambabu and Bharath (2019), who explain that moisture content decreased as the concentration of mango leaf extract increased due to the hydrophobic nature of moisture, which reduces the capacity for water absorption in films.

The moisture content of kesum powder was measured at 8.83 ± 0.61%. Typically, during the drying process, fresh kesum leaves are dehydrated to reduce their moisture content and increase their shelf stability. It is important to note that the moisture content of kesum powder can be influenced by storage conditions. If stored improperly in a humid environment, the powder can absorb moisture from the surroundings, increasing its moisture content over time.

Fourier Transform Infrared Spectroscopy (FTIR)

Data for Fourier transform infrared spectroscopy of the film were based on Figure 3 and Table 5. The Amide A band in the Gel/Gly/Kes films were mainly attributed to the presence of N-H stretching vibrations, which were observed in the range of 3307.48 cm^{-1} - 3312.64 cm^{-1}. As shown in Table 5, N-H bond stretching is detected across all kesum concentration loadings in the films, indicating the presence of the amide group.

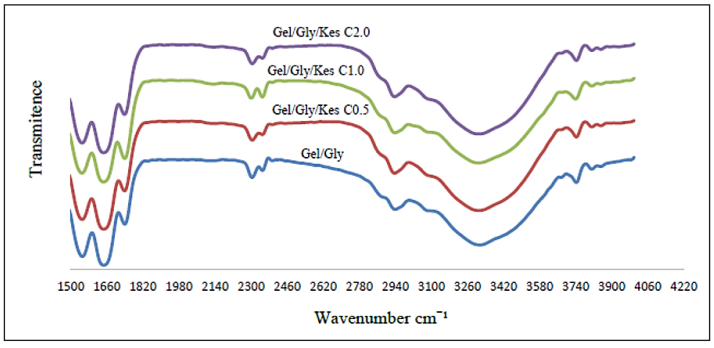


Figure 3. FTIR result for the gelatin-based film incorporation with kesum powder

Next, the Amide I band was mainly associated with the stretching vibrations of the carbonyl group along the polypeptide backbone of the gelatin-based films, which is the major structure of gelatin. With the addition of kesum powder, the peaks in the amide A-III region showed no obvious shift, indicating no conformational changes in the higher structure of gelatin. The absorption bands in the film with a concentration of 1.0% kesum powder, amide band III at 1226.58 cm^{-1}, are slightly different from those in films with 0.5% and 2.0% kesum powder, with bands at 1236.34 cm^{-1} and 1231.33 cm^{-1}, respectively.

This showed that the kesum-gelatin films correspond to C—O—C stretching vibrations and a composite of C—O—C stretching vibrations and C—C skeleton vibrations, which are typical functional groups of gelatin (Wang et al., 2017). For kesum powder, common functional groups found in plant materials, including kesum, include hydroxyl (OH), carbonyl (C=O), carboxyl (COOH), aromatic (C=C), and alkyl (C-H) groups (Xiao et al., 2021). The presence of amide bands, particularly the amide I (C=O stretching) and amide II (N-H bending) bands, can provide information about the proteinaceous components in the sample. Kesum powder, like many plant materials, may contain cellulosic components. Peaks in the 1600 - 1800 cm^{-1} range often indicate the presence of cellulose and hemicellulose, while peaks in the 1500-1600 cm^{-1} range may be associated with lignin.

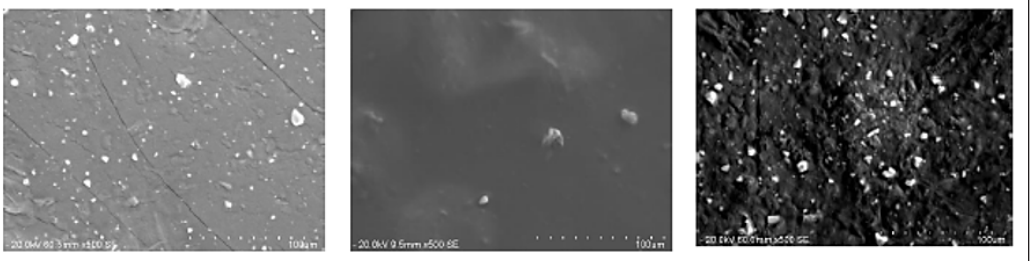
Table 5
Amide band wavenumber for different concentrations of kesum powder incorporated with gelatin-based film and kesum powder

Amide Band	Wavenumber (cm^{-1})				
	Gel/Gly	Gel/Gly/Kes (C0.5)	Gel/Gly/Kes (C1.0)	Gel/Gly/Kes (C2.0)	Kesum Powder
A	33742.48	3312.64	3307.48	3310.20	3371.12
I	1739.51	1739.04	1739.01	1738.40	1737.16
II	1646.12	1646.06	1645.23	1645.79	1632.59
III	1233.95	1236.34	1226.58	1231.33	1221.34

Note. Gel: Gelatin; Gly: Glycerol; Kes: Kesum powder; C: Concentration of kesum powder incorporated into the film

Morphological Analysis of the Film

As shown in Figure 4, the surface structures were observed in SEM at 100 μm. Based on the SEM micrograph, the surface was not smooth; white patches were observed, likely due to kesum powder. White patches in both samples seemed uneven. This indicated that supplementation of kesum powder might affect the active ingredients. Kesum powder contains polar organic compounds, which are only soluble in polar solvents. The extraction effectiveness of a particular compound depends on its solubility in the solvent, which in turn reduces the film matrix density and affects the film porosity. Smaller particles have a higher surface area, which facilitates their dispersion and interaction with the solvent or polymer matrix. This can lead to better uniformity and homogeneity of the powder distribution within the film.



(a) Gel/Gly/Kes (C0.5) (b) Gel/Gly/Kes (C1.0) (c) Gel/Gly/Kes (C2.0)

Figure 4. SEM micrograph of the kesum/gelatin-based film with different concentrations

Mechanical Properties of Kesum/Gelatin-Based Film

In finding stretchability and flexibility, elongation plays a vital role. The tensile strength required for the versatility of packaging films depends on their intended use and the subsequent transportation, handling, and storage of packaged foods. Table 6 clearly showed the effect of kesum concentration at 0.5%, 1.0%. and 2.0% had an inverse relationship between the elongation and the corresponding tensile strength. As expected, the increase in kesum concentration significantly enhanced film elongation from 14.67 ± 3.42% to 28.23%. On the other hand, the elongation of the control film without plasticiser and of the gelatin with plasticiser showed values of 1.57% and 8.37%, respectively. This is because active ingredients such as polyphenols can alter the membrane's physicochemical characteristics, causing the polymeric network to break. (Nguyen et al., 2022).

Table 6
Mechanical properties of different concentrations of kesum powder incorporated into the film

Sample	TS (MPa)	EAB (%)
Gel	26.32 ± 14.96	1.57 ± 0.94
Gel/Gly	21.09 ± 0.95	8.37 ± 4.37
Gel/Gly/Kes (C0.5)	23.65 ± 8.44	14.67 ± 3.42
Gel/Gly/Kes (C1.0)	15.89 ± 4.86	17.06 ± 1.36
Gel/Gly/Kes (C2.0)	9.47 ± 1.16	28.23 ± 2.68

Note. TS: Tensile strength; EAB: Elongation at break; Gel: Gelatin; Gly: Glycerol; Kes: Kesum powder; C: Concentration of kesum powder incorporated into the film

Generally, incorporating kesum powder into a gelatin film can reduced tensile strength. The reduction in tensile strength observed in the films can be attributed to the intrinsic properties of the gelatin matrix and the overall structural composition of the film. Similar results were reported by Kuan et al. (2020), who found that adding 4% mulberry leaf

extract decreased the tensile strength of alginate films. Nguyen et al. (2022) explained that incorporating leaf extracts potentially filled the spaces between the film matrix, thereby reducing biopolymer interactions and increasing the free volume and mobility of polymer chains within the matrix. In contrast, films with a more open or flexible structure typically exhibit increased elongation at break, albeit with reduced tensile strength. Arfat et al. (2014) reported similar findings, as tensile strength decreases while elongation at break increases in films incorporated with basil essential oils. This suggested that incorporating essential oil promotes a heterogeneous film structure that disrupts the continuity of the film network, thereby reducing the rigidity and mechanical strength of the film matrix.

Antioxidant Activity

Similar to other plant-based antioxidants, kesum leaf powder can scavenge the DPPH radical, thereby reducing oxidative activity due to the presence of active compounds such as phenolics and flavonoids. Compared with ginger and turmeric, kesum exhibited antioxidant activity and contained total phenolic compounds (Maizura et al., 2011). The antioxidant activity of kesum leaf powder is influenced by several factors, including the herb's quality, cultivation conditions, processing techniques and storage conditions.

Figure 5 illustrates the percentage of antioxidant inhibition in the films. The results revealed a significant ($p < 0.05$) increase in the antioxidant activity of the gelatin-based film after incorporating kesum leaf powder. Even a low loading of kesum is sufficient for the radical-scavenging performance of the film, as kesum has reversible redox properties and can be readily oxidised and reduced by species in contact with the film. This efficiency in scavenging the free radical DPPH can be attributed to kesum's redox-active property, which enhances the antioxidant activity of the material. The concentration-dependent increase in antioxidant activity corresponds to the increasing active compounds in the films. This observation aligns with other research by Nurdiani et al. (2022) that showed gelatin-based films incorporated with *B. gymnorhiza* and *S. alba* leaves exhibited higher antioxidant

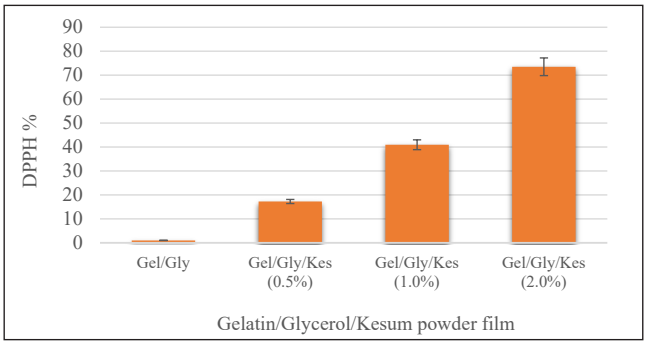


Figure 5. Antioxidant activity of gelatin-based film with and without incorporation of kesum powder

activity with the increase in plant material extracts, demonstrating the enhanced antioxidant activity in active packaging. This shows that the antioxidant agents in kesum leaf powder enhanced the antioxidant functionality of the developed Gel/Gly/Kes films.

CONCLUSION

Gelatin with good film-forming properties has been extracted successfully from red tilapia's fish scales and further developed as a film incorporated with kesum leaf powder. The incorporation of kesum leaf powder into gelatin-based films modified their physicochemical and functional properties, including visual appearance, mechanical properties, moisture content, and antioxidant activity. The gelatin-based film incorporated with the highest kesum leaf powder concentration exhibited a yellowish-green colouration with lower moisture content (6.77%) and increased elongation at break (28.2%). The functional properties of antioxidant activity of the gelatin-based film increased with the addition of kesum leaf powder, as the Gel/Gly/Kes (2.0) exhibited 73.5% antioxidant inhibition. This demonstrated that incorporating kesum leaf powder enhanced the antioxidant functionality of the gelatin-based film. Overall, this study implemented sustainable approaches through the valorisation of fish by-products and the incorporation of natural plant-derived bioactive compounds. The demonstration showed of the potential of kesum leaf powder to be incorporated into fish scale gelatin-based films as active packaging materials with antioxidant functionality, thus providing a foundation for their application in food packaging systems. Future research on food applications and storage analysis can be conducted to establish the functionality of these films in preserving food quality and extending shelf life.

IMPLICATION OF STUDY

This study demonstrated the potential of biological resources as the raw materials for the development of value-added functional packaging materials. The incorporation of kesum leaf powder into red tilapia fish scale gelatin films demonstrates the potential to valorise fish by-products as biopolymers and to combine them with natural, plant-based bioactive materials to impart additional functionality to gelatin packaging materials. In particular, this research revealed enhanced antioxidant activity in gelatin films as kesum leaf powder concentration increased. This indicated that kesum leaf powder provides bioactive components that reduce the oxidation processes in the films. Overall, this study provides a foundation for the green development of sustainable antioxidant packaging materials by valorising fish scale gelatin and incorporating plant-derived bioactive compounds from kesum leaves.

LIMITATION AND RECOMMENDATION FOR FUTURE RESEARCH

Future research on the performance of the antioxidant films on actual food products under controlled storage conditions should be done to determine the functionality of the developed films. Physicochemical, microbial, and antioxidant analyses of the food products can be conducted to evaluate the effectiveness of the antioxidant films in maintaining quality and extending shelf life. These analyses would provide a comprehensive assessment to determine the suitability of the films as antioxidant film materials for food packaging applications.

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