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Antioxidant Activity, Morphological Structure, Barrier and Mechanical Properties of Tuna Bone Gelatin-*Thalassia hemprichii* Leaf Extract-Based Bio-packaging

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ABSTRACT

The development of bio-packaging materials derived from environmentally friendly biopolymers stands for a significant advancement in addressing the growing concern over plastic waste and its detrimental impact on the environment. Gelatin derived from fish bones and bioactive components of seagrass leaf extract can be raw materials to produce bio-packaging as an alternative to plastic. The objective of this research was to obtain a gelatin derived from tuna fish bones and *Thalassia hemprichii* leaf extracts-based bio-packaging, which exhibits antioxidant activity, favorable mechanical and barrier properties. In addition to the 5% and 7,5% gelatin concentrations and 0,5% and 1% nonpolar seagrass extracts, the heating temperature was also varied at 50, 65 and 80°C. This resulted in a completely randomized factorial design for the experiment. The parameters examined included antioxidant activity, evaluated using the DPPH method; morphological structure, assessed via scanning electron microscopy; and mechanical properties of the films, including tensile strength, elongation, thickness, solubility, and water vapor transmission rate, which were determined using the ASTM method. The results showed that the highest antioxidant activity, with an IC_{50} value of 67.73ppm, was observed in the bio-packaging formulation comprising 5% gelatin and 0.5% seagrass extract, processed at 65°C. The highest tensile strength (8.42 MPa) was observed in the 7.5% gelatin formulation without extract, processed at 65°C, and with a low water vapor transmission rate when using 0.5% seagrass extract. The highest solubility was seen in the formulation containing 7.5% gelatin and 1% seagrass extract, with a value of 74.52%. The greatest elongation (196%) was observed in the formulation containing 7.5% gelatin and 0.5% seagrass extract, processed at 80°C. Additionally, the thickness of the bio-packaging increased with the addition of seagrass extract. Edible films containing 7.5% gelatin and 0.5% seagrass extract processed at 80°C appear smoother and flatter than the other treatments.

INTRODUCTION

Packaging represents a significant human endeavor aimed at extending the shelf life of products through the utilization of technology, thereby enabling the products in question to be enjoyed for a longer period of time. Plastic packaging is the most prevalent form of packaging utilized for a multitude of products. However, due to its potential to contaminate the items it contains and its inability to degrade in the environment, there has been a growing interest in developing packaging alternatives derived from natural materials, commonly referred to as bio-packaging. The packaging composed of protein and carbohydrate hydrocolloids, combined with fatty components **(Prasetyaningrum *et al.*, 2010)**. They posited that edible packaging can be used to extend the shelf life and maintain product quality. However, **Kumar and Yaakob (2011)** postulated that protein-based packaging has low permeability to oxygen and oil, yet is sensitive to water vapor. This high sensitivity to water can be mitigated through the combination with other nonpolar ingredients, such as nonpolar extracts of natural ingredient components. Natural ingredients can be extracted from aquatic biota, including the seagrass plant *Thalassiahemprichii,* which is found in the waters of Tomini Bay in Bone Bolango Regency, Gorontalo Province. The nonpolar natural ingredients of seagrass extracts have the potential to function as antioxidant biological compounds in packaging. The findings of **Ulfa *et al*. (2014)** indicated that *T. hemprichii* from Karimunjawa and Panjang Island contains steroidal, triterpenoid, alkaloid, and phenolic compounds with antioxidant potential. As stated by **Kannan *et al.* (2013)**, the N-hexane extract of *T. hemprichii* exhibits a pronounced inhibitory effect against *S. aureus* and *E. coli* bacteria. However, it should be noted that the extract's efficacy is subject to considerable environmental temperature-dependent fluctuations.

This research was conducted to address the shortcomings of existing studies in the field of bio-packaging. In a study conducted by **Naiu and Yusuf (2018)**, gelatin was extracted from fish bones using palm vinegar, resulting in the production of gelatin with a high fat content of 20%. The research findings of **Naiu *et al*. (2023)** demonstrated the potential to reduce the fat content of gelatin to 3.23% through modifications to the gelatin manufacturing process, specifically the drying of fish bones prior to extraction. **Kalaka *et al.* (2022)** observed that gelatin-chitosan-ginger bio-packaging retained a sour aroma. **Nilsuwan *et al*. (2016)** reported that gelatin-based bio-packaging demonstrated excellent oxygen barrier properties despite exhibiting susceptibility to moisture. **Naiu *et al.* (2021a)** observed that the combination of tuna bone gelatin edible film with chitosan and nanocitin exhibited a water vapor permeation rate of 23.03gm^{-2} over a 24-hour period. Similarly, the study conducted by **Naiu *et al*. (2021)**, which combined tuna bone gelatin with carboxy methyl cellulose, resulted in a permeation rate of 18.6gm^{-2} over the same time frame. Conversely, the utilization of natural active compounds to enhance the functional attributes of packaging materials is gaining attraction, driven by the growing public consciousness regarding health and wellbeing. It is anticipated that the incorporation of non-polar seagrass extract will enhance the bio-packaging characteristics of the results. **Pranoto *et al*. (2005)** reported that the addition of garlic essential oil to alginate-based packaging resulted in a reduction in microbial growth and a decrease in the water transmission rate. However, the biological activity of natural material components is contingent upon the prevailing temperature of the surrounding environment. **Komala and Husni (2021)** observed that the antioxidant activity of natural materials was diminished when subjected to elevated temperatures during the extraction process. As stated by **Soehendro *et al.* (2015)**, the elevated temperatures can cause structural damage to cells, leading to increased solubility of phenols. This, in turn, results in a reduction in the biological activity of the material components. Conversely, the formation of bio-packaging films is dependent on temperature-induced gelation. **Bourtoom (2008)** posited that the strength of the film is contingent upon the chemical structure of the polymer and the environmental conditions that prevail during the formation of the bio-packaging.

It is of paramount importance to conduct research on the temperature optimization of the manufacturing process, particularly since compounds of a biological nature are susceptible to high temperatures. To ascertain the efficacy of the method employed, the parameters evaluated in the context of packaging include (a) mechanical properties, encompassing tensile strength, percent elongation, solubility, and thickness; (b) barrier properties, specifically water vapor transmission rate; (c) antioxidant activity of bio-packaging; and (d) microstructure of bio-packaging. To the best of our knowledge, research on bio-packaging synthesized from tuna bone gelatin (*Thunnus* sp.) and seagrass leaf extract (*T. hemprichii*) processed at different temperatures has never been reported. Therefore, this research has novelty properties. Building on these considerations, this study aimed to determine the optimal conditions for developing bio-packaging using tuna bone gelatin and *T. hemprichii* leaf extract.

MATERIALS AND METHODS

1. Materials

Materials and equipment included an extractor, evaporator, oven, incubator, magnetic stirrer, and glass bottles for the extraction of seagrass leaves, gelatin production, and the fabrication of bio-packaging. The test equipment comprises an analytical balance, burette, thermometer, porcelain cup, spectrophotometer, filter paper, shaker, KLT, SEM, Soxhlet, homogenizer, and other glassware. The materials employed for the extraction of seagrass leaves, the production of gelatin, and the fabrication of bio-packaging films included seagrass leaves, tuna fish bones, palm vinegar, aquadestillate, N-hexanes, glycerol, and NaOH. The materials employed for the assessment of the antioxidant activity and mechanical properties of the film included methanol, DPPH, distilled water, silica gel and sodium azide (0.02%).

2. Research procedures

The seagrass leaves were extracted using the maceration method with an N-hexane solvent at a ratio of 1:11 for two days. The extract solution was then evaporated at 50°C until thickened and then dried at room temperature. The extraction of gelatin from tuna fish bones is in accordance with the method proposed by **Naiu *et al*. (2023)**. This involves washing and drying the bones following the demineralization stage, before proceeding with the extraction process. The resulting gelatin solution was then filtered and dried in an oven.

The preparation of bio-packaging film was conducted using gelatin at concentrations of 5% (b/v) and 7.5% (b/v) (GL1 and GL2), in conjunction with non-polar seagrass leaf extract at concentrations of 0.5% (b/v) and 1% (b/v) (SE1 and SE2). Additionally, the heating temperature was optimized at this stage. The temperature treatments were conducted at 50, 65, and 80°C (T1, T2, and T3). Gelatin solution was prepared and heated at the temperature according to the treatment for 15 minutes. 1.3% (b/v) tapioca starch was added to the gelatine solution and stirred for 10 minutes. Then 1.3% (v/v) glycerol was added and stirred for 10 minutes at the same temperature. *T. hemprichii* leaf extract (according to treatment) was dissolved with a small amount of heated gelatin solution and then added back to the gelatin solution mixture and stirred for 10 minutes. The mixture was then poured into a mould and dried in a 54°C dryer for 8 to 9 hours to form a film. The film was then removed from the mould.

The antioxidant activity of the bio-packaging film was evaluated via the DPPH method. The mechanical properties of the bio-packaging, as outlined in the **ASTM (1997)**, were also assessed. These properties include tensile strength, elongation (percent elongation), solubility, and thickness. Furthermore, the film's barrier properties, specifically its water vapor transmission rate **(ASTM, 1997)**, were also calculated and the surface morphological structure was analyzed using the SEM method.

3. Data analysis

The microstructure data of the bio-packaging were subjected to descriptive analysis, and the data from the optimization tests on gelatin concentration, seagrass leaf nonpolar extract concentration, and heating temperature were examined to observe the response in terms of antioxidant activity, barrier, and mechanical properties of the bio-packaging. The experimental design was a completely randomized factorial design, and the data were analyzed by ANOVA and Duncan's further test at the 95% confidence level. All observational data were tabulated and subjected to statistical processing using the SPSS 26 software.

RESULTS

1. Antioxidant activity

The antioxidant activity of the bio-packaging film was determined by calculating the inhibition value against DPPH free radicals at 50% (IC_{50}) in all experimental units, as presented in Table (1).

Table 1. The IC_{50} value of gelatin-based bio-packaging incorporated *T. hemprichii* leaf extract at varying processing temperatures

Heating Temp.	Gelatin 5% (ppm)		Gelatin 7,5% (ppm)	
	T. hemprichii leaf extract 0.5%	*T. hemprichii* leaf extract 1%	*T. hemprichii* leaf extract 0.5%	*T. hemprichii* leaf extract 1%
Temp. 50°C	71.677 ± 0.33 d	69.49 ± 1.49 c	63.717 ± 1.85 b	95.197 ± 0.16 g
Temp. 65°C	61.87 ± 0.33 a	63.953 ± 1.17 b	63.857 ±1.75 b	95.303 ± 0.67 g
Temp. 80°C	84.097 ± 0.20 e	69.747 ± 0.75 c	95.863 ± 0.25 g	86.647 ± 0.33 f

Table (1) illustrates that in all gelatin and seagrass extract treatments, the application of a higher process temperature resulted in a notable increase in the IC_{50} value. The highest temperature employed (80°C) exhibited a statistically significant reduction ($P < 0.05$) in the antioxidant activity of gelatin-based bio-packaging incorporated with N-hexane extract of seagrass leaves. The results of the statistical tests indicated that the primary treatments and the interaction between the three treatments had a statistically significant impact on the inhibition of DPPH free radicals. The optimal process temperature to produce bio-packaging with an enhanced antioxidant activity was determined to be 65°C. The use of gelatin in isolation revealed that an elevated gelatin concentration within the formula led to a notable elevation in the IC_{50} value, thereby indicating a reduction in antioxidant activity. Similarly, the incorporation of a greater quantity of seagrass extract resulted in an elevated IC_{50} value. The combination of heating temperature and treatment yielded the most favorable results in terms of antioxidant activity. The best treatment, in which 5% gelatin and 0,5% seagrass extract were processed at 65°C, demonstrated the strongest antioxidant activity.

2. Tensile strength

Tensile strength is one of the mechanical properties of a film that describes the maximum tensile force that a biofilm can withstand until it is cut off. The tensile strength of the bio-packaging samples in this study can be seen in Table (2).

Table 2. Tensile strength of gelatin-based bio-packaging incorporated *T. hemprichii*leaf extract at varying processing temperatures

Heating Temp.	Gelatin 5% (MPa)			Gelatin 7,5% (MPa)		
	T. hemprichii leaf extract 0%	*T. hemprichii* leaf extract 0.5%	*T. hemprichii* leaf extract 1%	*T. hemprichii* leaf extract 0%	*T. hemprichii* leaf extract 0.5%	*T. hemprichii* leaf extract 1%
Temp. 50°C	5.973 ± 0.78 gh	2.623 ± 0.24 ab	1.603 ± 0.04 a	6.571 ± 1.23 h	4.553 ± 0.14 de	6.665 ± 0.21 h
Temp. 65°C	8.131 ± 0.74 i	1.973 ± 0.08 ab	5.103 ± 0.51 e	8.418 ± 0.82 i	5.110 ± 0.57 ef	2.771 ± 0.53 ab
Temp. 80°C	3.725 ± 0.77 bc	6.531 ± 0.86 h	4.465 ± 0.62 d	3.835 ± 1.09 cd	3.940 ± 0.00 d	5.150 ± 0.27 fg

Table (2) shows that tensile strength tends to decrease with the incorporation of seagrass extract into the formula yet appears to increase with the addition of gelatin. The results of the statistical tests demonstrated that both the main effects and the interaction between treatments had a statistically significant effect ($P< 0.05$) on the tensile strength of the bio-packaging film. The results of the Duncan test indicated that there was no significant difference between the temperatures of 50 and 80°C. However, both temperatures differed significantly from the temperature of 65°C, which was identified as the optimum temperature for achieving the greatest tensile strength, with a maximum value of 8.418 MPa.

3. Elongation

Elongation, or percent elongation, is defined as the ratio of the maximum length of a plastic film until it breaks to its initial length **(Krochta *et al.*, 1994)**. Table (3) presents the elongation of bio-packaging samples produced from the interaction of temperature treatment, gelatin and seagrass extract.

Table 3. Percent elongation of gelatin-based bio-packaging incorporated *T. hemprichii*leaf extract at varying processing temperatures

Heating Temp.	Gelatin 5% (%)			Gelatin 7,5% (%)		
	T. hemprichii leaf extract 0%	*T. hemprichii* leaf extract 0.5%	*T. hemprichii* leaf extract 1%	*T. hemprichii* leaf extract 0%	*T. hemprichii* leaf extract 0.5%	*T. hemprichii* leaf extract 1%
Temp. 50°C	96.993 ± 5.77 e	28.373 ± 0.68 ab	67.158 ± 20.47 d	20.882 ± 8.15 a	91.654 ± 17.45 e	77.708 ± 5.21 de
Temp. 65°C	58.369 ± 8.53 cd	117.398 ± 23.51 f	152.842 ± 20.46	58.043 ± 3.61 c	44.390 ± 13.18 bc	169.787 ± 0.28 h
Temp. 80°C	99.634 ± 15.70 e	28.333 ± 6.75 ab	73.912 ± 12.55 de	134.598 ± 9.49 gh	196.707 ± 20.56 i	129.601 ± 1.49 g

The results of the statistical analysis demonstrate that the primary treatments, namely temperature, gelatin and seagrass extract, and the interaction between the three treatments, have a statistically significant effect ($P < 0.05$) on the percent elongation of bio-packaging. The results of the Duncan test demonstrate significant differences between the various treatment levels. It was observed that an increase in temperature, gelatin and seagrass extract resulted in a notable rise in percent elongation.

4. Solubility

The solubility of a film is a critical factor that determines the biodegradability of the film when it is utilized as a packaging material. The data pertaining to the solubility of the bio-packaging are presented in Table (4).

Table 4. Solubility of gelatin-based bio-packaging incorporated *T. hemprichii*leaf extract at varying processing temperatures

Heating Temp.	Gelatin 5% (%)			Gelatin 7,5% (%)		
	T. hemprichii leaf extract 0%	*T. hemprichii* leaf extract 0.5%	*T. hemprichii* leaf extract 1%	*T. hemprichii* leaf extract 0%	*T. hemprichii* leaf extract 0.5%	*T. hemprichii* leaf extract 1%
Temp. 50°C	81.993 ± 0.29	75.554 ± 3.19	68.206 ± 13.28	88.386 ± 8.11	80.7934 ± 1.87	68.854 ± 3.37
Temp. 65°C	79.673 ± 8.56	64.091 ± 4.09	59.608 ± 0.32	83.029 ± 4.21	77.848 ± 12.03	65.588 ± 7.94
Temp. 80°C	67.554 ± 10.41	59.499 ± 2.04	53.633 ± 0.57	82.188 ± 2.19	67.483 ± 2.44	56.519 ± 2.08

The results of statistical analysis showed that each treatment singly had a significant effect ($P<0.05$) on the solubility of bio-packaging, but the interaction between treatments gave the opposite result ($P> 0.05$). Duncan's test results showed that there were significant differences ($P< 0.05$) between treatment levels in all single treatments.

5. Thickness

Thickness is one of the film properties that exert an influence on the packaged product. It can be reasonably deduced that an increase in the thickness of the packaging film will result in a corresponding increase in the safety of the packaged product. The thickness data for the bio-packaging produced in this study can be found in Table (5).

Table 5. Thickness of gelatin-based bio-packaging incorporated *T. hemprichii*leaf extract at varying processing temperatures

Heating Temperature	Gelatin 5% (%) (mm)			Gelatin 7,5% (%) (mm)		
	T. hemprichii leaf extract 0%	*T. hemprichii* leaf extract 0.5%	*T. hemprichii* leaf extract 1%	*T. hemprichii* leaf extract 0%	*T. hemprichii* leaf extract 0.5%	*T. hemprichii* leaf extract 1%
Temp. 50°C	0.152 ± 0.01	0.17 ± 0.03	0.176 ± 0.01	0.14 ± 0.01	0.158 ± 0.01	0.178 ± 0.01
Temp. 65°C	0.136 ± 0.00	0.158 ± 0.02	0.18 ± 0.02	0.144 ± 0.01	0.166 ± 0.03	0.184 ± 0.03
Temp. 80°C	0.158 ± 0.02	0.178 ± 0.02	0.172 ± 0.02	0.196 ± 0.02	0.21 ± 0.02	0.224 ± 0.03

The results of the statistical analysis demonstrate that each treatment had a statistically significant effect ($P < 0.05$) on the thickness of the bio-packaging. However, only the interaction between temperature and gelatin exhibited a statistically significant effect, while the interaction between the other treatments did not ($P>0,05$). The results of the Duncan test demonstrate that the treatments at 50 and 65°C, which result in a thinner thickness than the temperature of 80°C, are not statistically different from one another. Conversely, the incorporation of greater quantities of gelatin and seagrass extract resulted in a notable increase in the thickness of the bio-packaging.

6. Water vapor transmission rate

The water vapor transmission rate is expressed as a unit of measurement per unit area of material with a flat surface of a certain thickness, resulting from a unit difference in vapor pressure between two specific surfaces under defined conditions and temperatures **(Krochta, 1992)**. The smaller the water vapor migration that occurs in the packaged product, the more effective the bio-packaging properties in maintaining the shelf life of the packaged product. The water vapor transmission rate of bio-packaging results can be seen in Table (6).

Table 6. Water vapor transmission rate of gelatin-based bio-packaging incorporated *T. hemprichii* leaf extract at varying processing temperatures

Heating Temperature	Gelatin 5% (gm^{-2}d^{-1})			Gelatin 7,5% (gm^{-2}d^{-1})		
	T. hemprichii leaf extract 0%	*T. hemprichii* leaf extract 0.5%	*T. hemprichii* leaf extract 1%	*T. hemprichii* leaf extract 0%	*T. hemprichii* leaf extract 0.5%	*T. hemprichii* leaf extract 1%
Temp. 50°C	15.977 ± 0.27 e	15.284 ± 1.12 de	10.941 ± 0.86 bc	24.924 ± 1.16 i	9.546 ± 0.31 a	13.108 ± 1.07 d
Temp. 65°C	20.234 ± 0.41 g	9.125 ± 1 a	9.437 ± 0.55 a	21.701 ± 1.58 h	10.551 ± 0.39 b	9.783 ± 0.11 b
Temp. 80°C	13.360 ± 1.22 d	13.901 ± 0.88 d	8.973 ± 1.07 a	17.975 ± 0.57 f	14.009 ± 0.55 d	11.878 ± 0.31 c

Statistical analysis showed that both the main treatment and the interaction between treatments had a significant effect ($P < 0.05$) on the water vapor transmission rate of bio-packaging. Observations on the effect of a single treatment showed that higher process temperature and more seagrass extract significantly decreased the water vapor transmission rate, but on the contrary, more gelatine would increase the water vapor transmission rate of bio-packaging.

7. Morphological structure

The morphological structure of a packaging films processed at a heating temperature of 50, 65, and 80°C are presented in Figs. (1, 2, 3), respectively.

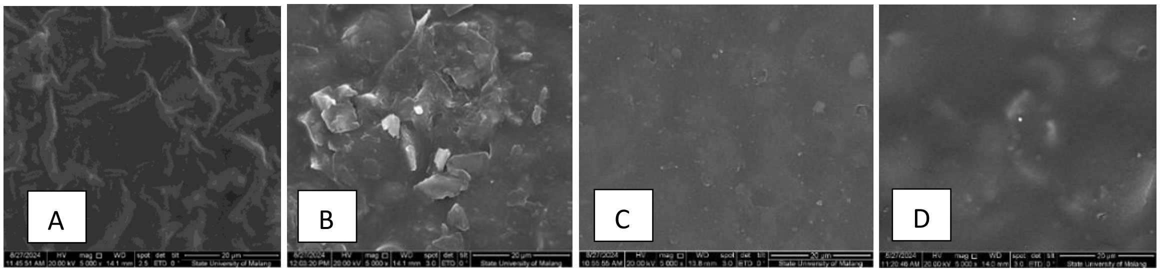

Fig. 1. Microstructure of the film surface heated at 50°C: A. GL1, SE1; B. GL1, SE2; C. GL2, SE1; D. GL2, SE2

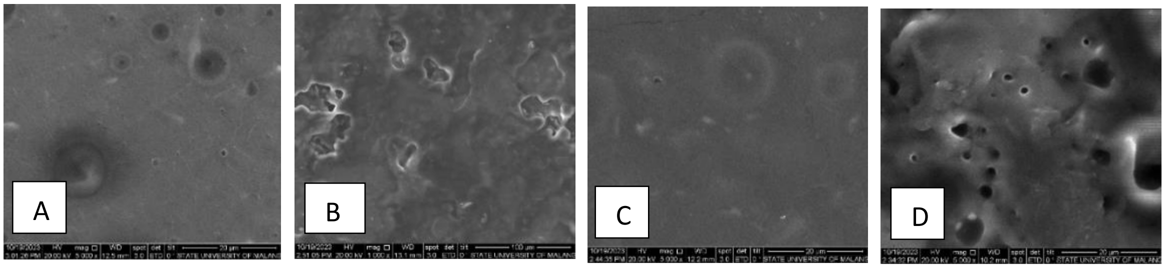

Fig. 2. Microstructure of the film surface heated at 65°C: A. GL1, SE1; B. GL1, SE2; C. GL2, SE1; D. GL2, SE2

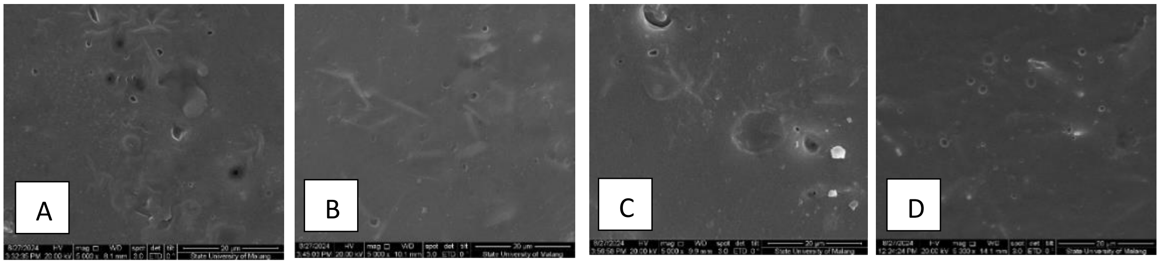

Fig. 3. Microstructure of the film surface heated at 80°C: A. GL1, SE1; B. GL1, SE2; C. GL2, SE1; D. GL2, SE2

The results demonstrate that the bio-packaging film incorporating 0.5% seagrass hexane extract with a 5% and 7.5% gelatin composition, respectively, exhibits a more homogeneous appearance than the film containing 1% seagrass hexane extract. This appearance is discernible at 5000x magnification. The heating process temperature of 80^{o}C exhibits a more uniform edible film microstructure than at other temperatures in this experiment. Furthermore, the distinct seagrass extract treatments resulted in notable differences in appearance, particularly in edible films formulated with 7.5% gelatin.

DISCUSSION

The optimal process temperature for the production of bio-packaging with enhanced antioxidant activity is 65°C. The incorporation of gelatin in isolation led to a notable elevation in the IC_{50} value, suggesting a decline in antioxidant efficacy. Similarly, the incorporation of a greater quantity of seagrass extract resulted in an elevated IC_{50} value. The combination of 5% gelatin and 0.5% seagrass extract, processed at 65°C, was found to be the most effective treatment for enhancing antioxidant activity. The observation of temperature on antioxidant activity is consistent with the findings of **Soehendro *et al.* (2015)** and **Kurniati *et al.* (2019)**, namely that high temperatures can destroy bioactive components that are antioxidant. This is indicated by the observation that as the temperature applied increases, the IC_{50} value also increases.

The incorporation of gelatin resulted in an enhancement of tensile strength, yet this was found to be inversely proportional to the utilization of non-polar seagrass extract. This finding is consistent with that reported by **Fera and Nurkholik (2018)**, who produced edible films with the greatest tensile strength when gelatin was added at a concentration of 75%. According to **Han and Aristippos (2005)**, tensile strengths of 1-10 MPa are classified as marginal. Higher tensile strengths indicate greater resistance to damage due to stretching and pressure, which improves the physical quality of the film. On the other hand, **Jimenez *et al*. (2010)** mentioned that fatty acids added to hydroxypropyl methylcellulose (HPMC) edible film caused the tensile strength value of the film to decrease.

Observations of each treatment showed that the higher the process temperature, the more gelatin and seagrass extract significantly increased the percent elongation. Many studies have found that activated gelatin composite films with the incorporation of essential oils or natural extracts such as bergamot, lemongrass, basil, cinnamon, peppermint, citronella and palm oil produce higher elongation values because these compounds can exert plasticizing effects in the resulting films, increasing the free volume between the gelatin molecules and promoting greater mobility. This is also consistent with the findings of **Warkoyo *et al*. (2014)**, who found that the addition of the active ingredient potassium sorbate also increased the elongation of the film. However, the elongation of the bio-packaging film from this study was reduced when combined with the temperature treatment in the excess seagrass extract treatment, although it was still in the excellent category because it was above 50%. **JSA (2019)** mentioned that JIS categorizes the percent elongation of films into three groups, namely not good, good, and incredibly good, with a value range of <10%, 10-50%, and >50%, respectively.

The solubility of the film increased with the addition of gelatin but was observed to decrease with increasing process temperature and seagrass extract. This may be due to the fact that gelatin is a water-soluble material, while the seagrass extract used is a non-polar material. **Nugroho *et al*. (2013)** stated that the addition of hydrophilic components to the film formulation increases the percentage of film solubility. The increase in the solubility of the edible film with the addition of nanocarageenan particles is consistent with the findings of **Zhang *et al*. (2019)**, who found that the water vapor absorption by the edible film increased with the addition of nanosilica particles in the edible film formulation, because a large number of hydroxy (OH) groups on the nanosilica bind to water to form hydrogen bonds, but is inconsistent with the solubility characteristics of the nanocomposite film they obtained.

The water vapor transmission rate of the bio-packaging film was found to be lower in the increasingly gelatin treatment with both of 0.5 and 1% seagrass extract incorporation. Conversely, the treatment without seagrass extract recorded a higher water vapor transmission rate. At elevated temperatures, the water vapor transmission rate increased, likely due to diminished tensile strength, particularly in films containing 7.5%

gelatin. This finding is consistent with the results reported by **Liu *et al.* (2019)**, who demonstrated that films produced through a high-temperature heating process (75-80°C) can disrupt intermolecular interactions, leading to a reduction in tensile strength. Similarly, **Qin *et al.* (2023)** have demonstrated that a low temperature of 40-60ºC increases the binding of hydrogen bonds between konjac glucomannan and agar in the film, thereby reducing the water vapor transmission rate. Other research conducted by **Pereda *et al.* (2011)** on protein-based films has shown that films made from casein protein incorporated with nanocellulose are unable to resist water vapor transmission.

Process temperature can alter molecular interactions, phase separation and crystal structure. The results of this study are in line with those of **Bagheri *et al.* (2019)**, who found that higher drying temperatures resulted in a smoother surface structure. In this study, the surface structure of the film is not only influenced by other ingredients such as tapioca starch and glycerol, but also by the presence of gelatin, which can act as an emulsifier capable of emulsifying polar and non-polar components to form a good film structure. Edible films containing 7.5% gelatin and 0.5% seagrass extract appear smoother and flatter than edible films made from breadfruit starch and PEG-400 plasticizer, as studied by **Marpongahtun (2016)**. This difference is thought to be due to differences in molecular size.

Research data on the antioxidant activity, mechanical and barrier properties and surface microstructure of bio-packaging films made from tuna bone gelatine and *T. hemprichii* leaf extract has the potential to be applied as primary packaging for food ingredients. For this reason, further research is needed on shelf life and degradation in nature.

CONCLUSION

The bio-packaging film, formulated with 5% gelatin and 0.5% *T. hemprichii* leaf extract and processed at 65°C, exhibited robust antioxidant properties, along with commendable mechanical strength and barrier functionality. The film provides a sustainable alternative to conventional plastic packaging. The research has yielded a packaging film formula that contributes to the advancement of Sustainable Development Goals (SDGs) 12 (Responsible Consumption and Production) and SDGs 14 (Ocean Ecosystem).

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