



## Chemical-Physical Characteristics of Edible Film Incorporated Bali Cattle Hide Gelatin and Encapsulated Gaharu Leaf Extract

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### A B S T R A C T

The objectives of this study were to analyze the potential of incorporating Bali cattle hide gelatin and gaharu leaf extract encapsulated as an edible film with antioxidant potential. The research method used Completely Randomized Design, ie concentration of gaharu leaf extract encapsulated (G0 = 0%, G1 = 5%, G2 = 10%, G3 = 15% and G4 = 20%). The results showed that the characteristics of edible films were significantly different (P < 0.05), such as moisture content (7.37%) in G4, protein (86.25%) in G0, phenol (10.84 mg / 100 g GAE) in G4 and antioxidant (150.36 mg / L. GAEAC) in G4, and the effect was not significant on ash content of the film. The film thickness, tensile strength, elongation, and water vapour transmission rate of the edible film were significant (P < 0.05) influence with results for 0.04 mm (G0); 1.64 Mpa (G0); 54.86% (G1) and 11.20 g.mm/m<sup>2</sup>.day in G4, respectively. The research concludes that the edible film of incorporation of Bali cattle skin Bali gelatin and gaharu leaf extract encapsulated with 20% concentration yielding functional characteristics (potency antioxidant) with the highest value. Indicators of chemical characteristics include moisture content (7.37%); ash content (0.49%); protein content (71,98%); phenol (10.84 mg / 100 g GAE), and antioxidant (150.36 mg / L GAEAC). At the same time, physical indicators include film thickness (0.02 mm), tensile strength (1.54 MPa), elongation (71.48%) and water vapor transmission rate (11.20 g.mm/m<sup>2</sup>.day).

## 1. INTRODUCTION

### 1.1. Research Background

Advances in packaging technology currently encourage various innovations to produce effective and environmentally friendly packaging. Active packaging is the incorporation (incorporation) of particular materials into packaging. It can interact with the main ingredients to maintain the organoleptic quality during storage and provide new added value [1]. One added value in question is providing functional properties, such as packaging with antioxidant potential. The edible film is one of the active packagings that can insert antioxidants. Utilization of gaharu leaves as a source of antioxidants and incorporating gelatin from bali cattle skin is an exciting potential to be developed. Gaharu leaves (*Gyrinops versteegii*) are part of traditional medicinal plants that have potential as a source of antioxidants [2]. It was further stated that the results of phytochemical screening and free anti-radical activity test of gaharu leaf methanol extract contained secondary metabolites of flavonoids, terpenoids and phenolic compounds. The potential for the incorporation of encapsulated gaharu leaf extract and gelatin from bali cattle skin produces a multi-beneficial edible film. This study aimed to analyze the potential for incorporation of Bali cattle hide gelatin and encapsulated agarwood leaf extract as edible films with antioxidant potential.

### 1.2. Literature Review

Gelatin is a product of partial hydrolysis of cattle hide collagen, for example Bali cattle hide [3]. Collagen is one of the main components in leather and cattle hide in the presence of a high hydroxyproline content causing a dense structure of collagen. This is associated with gel viscosity and gelatin temperature stability [4]. One of the uses of gelatin is its processing into edible film, a biodegradable packaging product, a thin layer that can be eaten, formed to coat food components (coating). In food products, this thin layer serves to inhibit the transfer of moisture [5] and gas exchange [6], prevent loss of aroma and transfer of fat [7], improve physical characteristics, and as a carrier for additives. One of the raw materials for edible film from the protein class of animal origin that has good properties and has the potential to be used as raw material is protein in bali cattle hide. Meanwhile, the gaharu plant, especially the leaves, is a source of antioxidants and the results of phytochemical screening and free anti-radical activity tests showed that the methanol extract of gaharu leaves contains secondary metabolites of flavonoids, tannins and phenolic compounds. This potency indicates that this gaharu leaf extract contains antioxidants [2]. This phenolic compound has an active group that functions as a catcher and inhibitor for free radical reactions, namely in the presence of a –OH group and a double bond >C=C<. This hydroxy group is substituted at the ortho and para positions for the –OH and –OR groups [8]. The potential of this extract is very potential to be corporation with bali cattle hide gelatin into edible films.

### 1.3. Research Objective

This study aims to analyze the potential of incorporating bali cattle skin gelatin and gaharu leaf extract encapsulated as an edible film with antioxidant potential.

## 2. MATERIALS AND METHODS

### 2.1. Materials

The main research material is gelatin from Balinese cow skin protein extraction and encapsulated agarwood leaf extract. Other supporting materials, namely the plasticizer used, are glycerol (Brataco chemika), aluminum foil, clear plastic, and silica gel. Other supporting materials are buffer pH 4.00, buffer pH 7.00, buffer pH 9.00, phenolphthalein (pp), distilled water, deionized water, ordinary filter paper, and Whatman 42 filter paper. The main research equipments include: analytical balance, hot plate, magnetic stirrer, water bath, pH meter, thermometer, digital oven, Teflon, and universal Testing Machine (Test Zwick).

### 2.2. Research Methods

#### 2.2.1. Procedure of Research

The research procedure begins with the manufacture of gelatin from bali cattle skin using the method of [9] and [3] and the manufacture of gaharu leaf extract using the [10]. The process of encapsulation of gaharu leaf extract was carried out, namely 10 ml concentrated extract of gaharu leaf was added with a mixture of maltodextrin 13% of the total amount of concentrated gaharu leaf extract (w/v). The next process is homogenization until evenly mixed. Then dried using an oven at a temperature of 50oC for 24 hours. The next stage is making an edible film. The encapsulated gaharu leaf extract was then combined with gelatin products from bali cattle skin and made into edible film. The concentration of encapsulated gaharu leaves (0%; 5%; 10%; 15% and 20%) and gelatin from bali cattle skin was 10 g each. The plasticizer of the glycerol type (1 ml) and a total of 500 ml of edible film solution were added. The process of making edible films is done by casting according to the method of [11] and [12] with slight modifications. The film solution made is then heated

on a hot plate at a temperature of 70°C for 45 minutes while stirring (1000 rpm) until the gelatin and glycerol particles are completely mixed (clear). treatment and homogenized on a hot plate at 1000 rpm for 1 hour. Furthermore, it is poured into a Teflon mold as thin as possible (30 ml) in a hot state and then placed in the oven in a flat position. The Teflon containing the film solution was dried at 55oC for 20 hours to form a thin layer. The Teflon was removed from the oven and conditioned at room temperature for approximately 10 minutes. The thin film formed is gradually peeled off with a blunt knife tip until the entire film is removed. The film is then wrapped with aluminum foil and put in a plastic container previously coated with silica gel to prevent damage to the film by moisture and then the film is ready to be tested. The research variables included water content, ash, protein, phenol, antioxidant, film thickness, tensile strength, elongation and water vapor transmission rate.

#### 2.2.2. Analytical Methods

Research on the incorporation of bali cattle skin gelatin with encapsulated gaharu leaf extract was carried out using a completely randomized design (CRD) with 5 (five) treatment concentrations of encapsulated gaharu leaf extract (G), namely G1 (0%); G2 (5%); G3 (10%); G4 (15%) and G5 (20%) and each treatment were repeated 3 times. The data obtained were analyzed for variance with the help of the statistical program SPSS Version 20. The treatments that showed a significant effect were then tested for significant differences with Duncan's Multiple Range Test (DMRT) at the 5% level [13].

## 3. RESULTS AND DISCUSSION

Production of gelatin-based edible films from bali cattle skin and antioxidant potential from gaharu leaf extract was studied and the results of complete statistical analysis are presented in Table 1. The protein binding of Bali cattle skin gelatin with encapsulated agarwood leaf extract statistically gave a significant effect ( $P<0.05$ ) in reducing the water content of the edible film (Table 1).

**Table 1.** Chemical Characteristics of Edible Film Resulting from the Incorporation of Bali Cowhide Gelatin with Encapsulated Gaharu Leaf Extract

Characteristics	Addition of Encapsulated Gaharu Leaf Extract				
	G0	G1	G2	G3	G4
Moisture Content(%)	9.32±0.02 <sup>d</sup>	8.12±0.03 <sup>c</sup>	7.77±0.32 <sup>b</sup>	7.87±0.12 <sup>bc</sup>	7.37±0.01 <sup>a</sup>
Ash content (%)	0.47±0.02	0.47±0.01	0.48±0.00	0.48±0.01	0.49±0.01
Protein Content (%)	86.25±0.10 <sup>d</sup>	86.12±0.02 <sup>d</sup>	79.03±0.06 <sup>c</sup>	75.34±0.06 <sup>b</sup>	71.98±0.33 <sup>a</sup>
Phenol (mg/100g GAE)	9.41±0.06 <sup>a</sup>	10.23±0.08 <sup>b</sup>	10.79±0.14 <sup>c</sup>	10.77±0.02 <sup>c</sup>	10.84±0.09 <sup>c</sup>
Antioxidants (mg/L GAEAC)	105.83±0.88 <sup>a</sup>	125.23±0.48 <sup>b</sup>	140.05±0.10 <sup>c</sup>	145.87±0.12 <sup>d</sup>	150.36±0.11 <sup>e</sup>

Noted:

G0 : concentration of encapsulated gaharu leaf extract 0%

G1 : concentration of encapsulated gaharu leaf extract 5%

G2 : concentration of encapsulated gaharu leaf extract 10%

G3 : concentration of encapsulated gaharu leaf extract 15%

G4 : concentration of encapsulated gaharu leaf extract 20%

a,b,c,d,e : different superscripts in the same line show significantly different ( $P<0.05$ )

This was thought to be due to structural reformulation between gelatin and leaf extract proteins. gaharu is encapsulated

so that it gives a narrow space in its bond with hydrogen and impacts the low water content of this edible film. The moisture content of the obtained edible film ranged from 7.37% to 9.32%. A good water content of the edible film is that it has a low water content so that in its application as a primary packaging, it does not cause new problems. The low water content of the edible film will have a good impact as a packager by not contributing water to the packaged product. Because the high water content of edible films causes the packaged product to be damaged quickly and reduce the shelf life [14]. The statistical analysis results in Table 1 show that the increase in the use of encapsulated gaharu leaf extract has a good impact on the characteristics of edible films with low water content.

The results of the statistical analysis (Table 1) showed that the edible film from the gelatin formulation and encapsulated gaharu leaf extract had no significant impact on the ash content of the edible film. Protein binding of Bali cattle skin gelatin with increasing amylose percentage of encapsulated gaharu leaf extract did not change the ash content of the edible film. The ash content as an inorganic residue from the combustion of organic materials that compose the edible film in this study is very low so that it becomes an indicator of the high level of purity of the organic materials that make up the edible film. Inorganic content that is counted as ash is usually in the form of sodium, chlorine, calcium, phosphorus, magnesium, and sulfur [15]. If the gelatin standard approach is used, the ash content of the edible film produced in this study is still lower than that standardized in [16], namely the maximum allowed ash content of 3.25%

The protein content of edible film incorporation of Bali cattle skin gelatin significantly decreased ( $P < 0.05$ ) if the percentage of gaharu leaf extract was added to increase the percentage (Table 1). The increase in the use of gaharu leaf extract reduces the protein content of the edible film. This may be due to the antioxidant properties in the encapsulated gaharu leaf extract causing degradation of the gelatin protein of Bali cattle skin as a result of inhibition of oxidation by the presence of phenol in the gaharu leaf extract. The aqueous extract of gaharu leaves produced high phenolic compounds, namely 14,980 (mg GAE/100 gr) with an inhibition concentration value of 50% (IC<sub>50</sub>) of 3.03 mg/L (60 minutes) which means a concentration of 3 0.03 mg/L of gaharu leaf extract was able to inhibit 50% of the oxidation reaction [17]. Therefore, the incorporation of the two resulted in a decrease in the protein content of the edible film and an increase in the concentration of gaharu leaf extract. The mechanism of decreasing the protein content of edible films by increasing the addition of gaharu leaf extract can be explained that the protein binding of gelatin with the tannin component in gaharu leaves is stronger if the proportion increases so that the total N calculated is low.

The concentration of 10% - 20% of gaharu leaf extract encapsulated in its incorporation with Bali cattle skin gelatin had no statistically significant effect on the phenol content of the edible film (Table 1). However, the effect was significant ( $P < 0.05$ ) compared to the addition of 5% and the control (without addition). The increase in encapsulated gaharu leaf extract gave high characteristics of the phenol content in the edible film. This active compound is believed to be a natural antioxidant compound. The importance of this active compound in the product as well as in the body is to prevent or inhibit oxidation reactions involving free radicals. The mechanism of inhibition is

through the initiation or propagation of reactions in the oxidation of fats or other molecules in the body by absorbing or neutralizing free radicals or decomposing peroxides [18].

The results of the statistical analysis study showed that the increase in the use of encapsulated gaharu leaf extract significantly ( $P < 0.05$ ) increased the antioxidant content of the edible film (Table 1). Therefore, this edible film made from gelatin from Balinese cow skin extract becomes functional, in addition to being a natural packaging, it also contains natural antioxidant potential from the source of compounds in the gaharu leaf extract. The potential compounds in gaharu leaves that give antioxidant properties to the product are flavonoid and phenolic compounds [17]. The increase in the antioxidant properties of this edible film is expected to help in its application as a natural packaging for sausages to inhibit fat oxidation reactions through neutralizing free radicals and decomposing peroxides [18].

Meanwhile, the physical characteristics test of the edible film is presented in Table 2. The thickness of the edible film (in mm) is an essential parameter in using edible film as product packaging. The results of statistical analysis in Table 2 show that the increase in the concentration of encapsulated gaharu leaf extract tends to decrease significantly ( $P < 0.05$ ) the thickness of edible film with Balinese beef skin gelatin as the base material. The antioxidant properties and characteristics of encapsulated gaharu leaf extract with increasing additions tend to decrease the thickness of the edible film. This may be due to the high content of phenolic compounds in the encapsulated gaharu leaf extract, thereby increasing the molecular density as a result of which the free space formed in the film matrix is smaller and the film formed is thinner. The thickness of the edible film is influenced by the nature and composition of the material [19]. The aqueous extract of gaharu leaves contains about 14,980 phenolic compounds (mg GAE/100 g) and this phenolic compound as a source of antioxidants, is thought to have the ability to inhibit oxidation [17]. In addition, it is suspected that this gaharu leaf extract has a weak hydrogen bond, resulting in a large absorption and an impact on the reduced thickness of the edible film.

Tensile strength is a mechanical indicator in testing the quality of edible films. Tensile strength indicates the maximum force required to break the edible film. The results of statistical analysis showed that increasing the concentration of encapsulated gaharu leaf extract significantly decreased ( $P < 0.05$ ) the tensile strength of the edible film (Table 2). This decrease is thought to be due to the weak hydrogen bonding structure in Bali cattle skin gelatin with the amylose structure in gaharu leaf extract. The tensile strength of edible films is determined by the strength of the structural bonds of the constituents [20]. The potential antioxidant content in the encapsulated gaharu leaf extract is suspected to weaken the bond, therefore it may be necessary to study the use of this gaharu leaf extract with the formulation of adding plasticizers with a higher concentration so as to provide a higher tensile strength function in the resulting edible film. The tensile strength of edible films will be high by adjusting the addition of plasticizer formulations, such as glycerol, sorbitol and PEG (Poly ethylene glycol) [21]. The tensile strength of edible film is related to its strength to withstand physical damage during food packaging. This is following the opinion of the edible films with high tensile strength will be able to become good food packaging [22].

**Table 2.** Physical Characteristics of Edible Film Result of Incorporation of Bali Cattle Skin Gelatin with Encapsulated Gaharu Leaf Extract

Characteristics	Addition of Encapsulated Gaharu Leaf Extract				
	G0	G1	G2	G3	G4
Thickness (mm)	0.04±0.01 <sup>b</sup>	0.01±0.01 <sup>a</sup>	0.03±0.01 <sup>ab</sup>	0.02±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>
Tensile strength Mpa)	1.64±0.04 <sup>c</sup>	1.52±0.07 <sup>c</sup>	0.91±0.24 <sup>a</sup>	1.25±0.05 <sup>b</sup>	1.54±0.04 <sup>c</sup>
Elongation (%)	61.30±0.15 <sup>c</sup>	54.86±0.41 <sup>a</sup>	64.19±0.09 <sup>d</sup>	60.15±0.05 <sup>b</sup>	71.48±0.13 <sup>e</sup>
Water Vapor Transmission Rate (g.mm/m <sup>2</sup> .day)	9.36±0.01 <sup>a</sup>	14.59±0.01 <sup>d</sup>	14.54±0.05 <sup>d</sup>	12.59±0.01 <sup>c</sup>	11.20±0.03 <sup>b</sup>

Noted:

G0 : concentration of encapsulated gaharu leaf extract 0%

G1 : concentration of encapsulated gaharu leaf extract 5%

G2 : concentration of encapsulated gaharu leaf extract 10%

G3 : concentration of encapsulated gaharu leaf extract 15%

G4 : concentration of encapsulated gaharu leaf extract 20%

a,b,c,d,e : different superscripts in the same line show significantly different ( $P<0.05$ )

Elongation at break is the change in maximum length at the time of stretching until the edible film sample is cut off. The average elongation value is presented in Table 2. Increasing the concentration of the addition of encapsulated gaharu leaf extract in its formulation with gelatin from Bali cattle skin protein extraction resulted in significantly higher elongation of the edible film ( $P<0.05$ ). This is inversely proportional to the tensile strength of the edible film produced, as in Table 2. This result follows the study that reported that the elongation of the skin has an inverse relationship with the tensile strength of the skin [23]. The analysis of variance showed that the increase in encapsulated gaharu leaf extract ( $P<0.05$ ) significantly increased the edible film's elongation. The edible film's high elongation indicates the edible film's quality with a good level of flexibility (softer). That elongation is said to be good if the value is above 50% and bad if the value is below 10% [24]. Gaharu leaf extract provides antioxidant potential (in the presence of phenol) and has an active hydroxyl group OH-. The more this gaharu leaf extract is added, the more OH- groups in the edible film matrix. The OH group plays a role in increasing the mobility of the edible film matrix polymer chain, and this can cause the elasticity or per cent elongation of the edible film to increase [25]. Meanwhile, [26] stated that the addition of soluble solids in the manufacture of edibles affects the performance of the polymer chain so that it affects the percent elongation of the edible film.

The results of statistical analysis (Table 2) showed that the addition of encapsulated gaharu leaf extract in the manufacture of an edible film made from gelatin from Bali cattle skin significantly increased ( $P<0.05$ ) the water vapor transmission rate (WVTR) compared to the control (Table 2). without the addition of gaharu leaf extract). The rate of water vapor transmission indicates the rate of penetration of water vapor (g.mm/m<sup>2</sup>.day) per unit area of edible film or the ability of the film to inhibit water transmission, so its permeability to water vapor should be as low as possible [27]. The increased rate of water vapor transmission from the edibles produced is thought to be due to the weak hydrogen bonding of gelatin with amylose molecules from gaharu leaf extract, resulting in a non-compact structure. Meanwhile, [28] and [29] stated that a compact structure would produce an edible film that inhibits the diffusion rate of water vapour. The addition of encapsulated gaharu leaf extract found a weakness, namely the high rate of water diffusion compared to the control. Therefore, adding encapsulated gaharu leaf extract is possible to make other modifications to reduce the rate of water

diffusion. The alleged weak hydrogen bond with amylose from gaharu leaf extract to produce the edible film with a weak structure needs to be added with the addition of higher plasticizers and surfactants so that edible films with the addition of encapsulated gaharu leaf extract with high antioxidant potential will still have good mechanical properties. These good mechanical properties include having a low water vapor transmission rate. The water vapour transmission rate in edible films can be reduced by adding surfactants [30] and [31].

#### 4. Conclusion

The production of the edible film from incorporating Bali cattle skin gelatin and encapsulated gaharu leaf extract with a concentration of 20% produced functional characteristics, namely the antioxidant potential with the highest value. Chemical characteristic indicators include water content (7.37%); ash content (0.49%); protein content (71.98%); phenol (10.84 mg/100 g GAE), and antioxidants (150.36 mg/L GAEAC). Meanwhile, physical indicators include film thickness (0.02 mm); tensile strength (1.54 MPa); elongation (71.48%) and water vapor transmission rate (11.20 g.mm/m<sup>2</sup>.day).

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