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Effect of Ginger (*Zingiber officinale*) Extracts on Mechanical and Antimicrobial Properties of Ganyong Starch Edible Films as Primary Packaging of Crabstick

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Abstract

Research has been conducted on the effect of ginger (*Zingiber officinale*) extract addition in the combination of an edible film made from Ganyong starch, sorbitol and chitosan. This study aims to analyze whether this formulation can be used as a substitute packaging for crabsticks that have been using conventional plastic packaging. By using a variation of ginger extract concentrations of 0, 3%, 6% and 9% into 3 % Ganyong starch, 4 % sorbitol and 0.3 % chitosan (w/v). Mechanical characteristics were carried out in the form of tensile strength, elongation, young modulus, swelling, moisture content, thickness, and solubility test. Antimicrobial ability and DSC (Differential scanning calorimetry) for the thermal properties of the casted film were also conducted. Data was analyzed using One-way Anova SPSS version 29 with Turkey's test. The addition of ginger extract to edible films based on Ganyong starch, sorbitol and chitosan can be used as primary packaging for crabsticks. The best formulation was found in the 6 % ginger extract variation with the results of swelling (95 %), moisture content (13 %), elongation (76 %), and antimicrobial properties of *Escherichia coli* (7.5 mm) and *Bacillus cereus* (6.9 mm). In addition, the melting temperature (T_m) of 6 % ginger extract concentration was also higher (134 °C), yet was not significantly different from the other variations. Therefore, this formulation has the potential as an alternative primary crabstick packaging.

Keywords: Crabstick, Ganyong starch, Ginger extracts, Mechanical properties, Primary packaging

Introduction

The use of edible film as a food packaging material that is safe for human consumption has been widely researched by food experts, especially in the field of packaging technology [1-3]. One of the biopolymer edible film materials that are abundant in Indonesia is Ganyong starch. According to Anggarini *et al.* [2] the existence of Ganyong tubers in the East Java region is quite abundant, reaching ±700 tons/year and spread in several regions such as Trenggalek, Bojonegoro, Ngawi, Ngan-juk, Banyuwangi and Malang. The high carbohydrate content of Ganyong has excellent prospects to be developed into edible films. According to Santoso *et al.* [4], Ganyong starch contains amylose and amylopectin of 21.14 - 24.44 % and 75.56 - 78.86 %, respectively. Starch with a high amylose content produces a strong edible film, since the linear structure of amylose allows the formation of hydrogen bonds between glucose molecules during constituent heating and is able to form a three-dimensional network that can simultaneously bind water to produce a gel-like solution [5]. However, edible films made from starch are brittle, hence it is necessary for an addition of plasticizers to form a more flexible film [4]. According to Anggarini *et al.* [2] edible coatings of 1 % Ganyong starch and 6 % glycerol can increase the shelf life of apples with better physical and chemical properties than other variations. In its utilization as edible film, Ganyong starch needs to be added with an additive such as antimicrobial compounds to improve the quality and ability of edible film to prevent damage to food ingredients. Syaichurrozi *et al.* [6] has conducted edible film research by adding 1 % sorbitol, turmeric and garlic extracts to 2 % Ganyong starch. The results obtained showed that the edible film had an inhibition zone for *Escherichia coli* and a decrease in moisture content, percentage elongation and tensile strength of turmeric powder. Whilst, garlic powder caused a decrease in moisture content, percentage elongation and tensile strength yet an increase in film thickness. Other antimicrobial compounds added to Ganyong starch edibles include ginger extract [7] . The optimal results of turmeric extract were edible film with the addition of ginger extract, glycerol, starch as much as 0.75 % (w/w), namely the tensile strength value of 5.17 MPa, elongation of 9.74 % Ganyong, properties and WVTR (Water Vapor Transmission Rate) value of 8.92 g/m^2/24 hour. The addition of ginger extract into edible mechanical film able to maintain the quality and shelf life of tomato fruit when viewed based on 50 % weight shrinkage and 63 % texture damage.

According to Sulistyowati *et al.* [7], the addition of red ginger extract and glycerol to edible films made from cassava starch showed significant differences in thickness, transparency, tensile strength and antimicrobial inhibition zone at 1.5 % red ginger concentration. Therefore, the current research was conducted by making edible films from the basic ingredients of 3 % w/v Ganyong starch, 4 % sorbitol, 0.3 % chitosan and the addition of ginger extract variations with concentrations of 0, 3, 6 and 9 %, respectively as an antimicrobial agent that will be applied to crabstick. This study aims to analyze the physical, chemical and antimicrobial properties of this edible film as primary packaging for crabstick. By testing tensile strength, elongation, young modulus, moisture content, thickness, solubility, stiffness, antimicrobial test (cell metabolism inhibitor test and protein damage) and thermal properties of edible films.

Materials and methods

Materials used in the manufacture of edibles are Ganyong starch (E-Commerce Genki Plant), ginger (*Zingiber officinale*) powder (Koepoe-Koepoe), sorbitol (Citra Sari Kimia), chitosan (CV. ChiMultiguna), distilled water and crab stick (Cedea).

Sample preparation

The casting of edible film was carried out by experimenting with the basic ingredients of Ganyong starch with the addition of varied ginger extracts. Preparation of edible film by mixing all ingredients, namely 3% w/v Ganyong starch, 4 % sorbitol, 0.3 % chitosan and the addition of ginger extract with varying concentrations of 0, 3, 6 and 9 %, respectively and all ingredients were suspended using 100 mL distilled water. The suspense solution was then poured into a 20 cm diameter petri dish and cooled for 10 min at room temperature. Then the edible film solution was dried in an oven at 70 - 80 °C for 8 - 12 h. Prior to peel, the edible film is allowed to stand for 1 - 2 days at room temperature to facilitate the film release.

Physicochemical characterization of edible film

Thickness

The thickness of the edible film was measured using a thickness gauge with a precession of 0.01 mm and based on 5 different points with 4 end sides and one center on the edible film with a size of 20 cm diameter [8]. According to JIS (Japanese Industrial Standard), the average edible film has a thickness of < 0.25 mm [9]. The thickness test value was based from the following equation:

$$Thickness\ (mm) = \frac{A+B+C+D+E}{5} \quad (1)$$

The swelling degree and water resistance test methods were carried out following the modified method [10] . The swelling degree test was carried out by cutting the edible film with a square size of 2×2 cm^2, then weighing the initial weight of the edible film before soaking (Wo), and inserting it into a Petri dish containing 10 mL of distilled water for 5 min. Then the water on the surface of the edible film is removed using a tissue, after which the final weight of the edible film is weighed (W). The swelling test value was based from the following equation:

$$\text{Water absorbed (\%)} = (W-Wo)/Wo\times 100\ \% \quad (2)$$

Mechanical properties (Tensile Strength, Elongation, Young's Modulus)

The tensile strength, elongation, and young's modulus tests refer to ASTM-D882 using the Hounsfield universal testing machine. Moisture content testing refers to AOAC (1995).

Solubility

Solubility refers to the Gontard procedure, using the percentage of film solubility dissolved in water after immersion for 24 h. The edible film was cut with a square size of 3×3 cm^2, placed in an aluminum cup that had previously been dried and weighed. The cut samples were put into an oven (100 °C) for 30 min. The dried edible was weighed as the initial dry weight (W_o). Then the sample was soaked for 24 h in an aluminum cup containing 30 mL of distilled water, then the sample that was not dissolved for 24 h was removed and dried again in the oven (100 °C) for 2 h, after which it was stored in a desiccator for 10 min. Then, the dry sample after soaking (W1) was weighed. The percentage of solubility can be calculated with the following equation:

$$S\,(\%) = \frac{w_0 - w_1}{w_0} \times 100\% \quad (3)$$

Antimicrobial activity of bioactive film

In vitro antimicrobial activity of the bioactive film was conducted by agar disc diffusion method [11,12]. Two strains of spoilage and pathogenic bacteria of *Escherichia coli* and *Bacillus cereus* were assessed in the study. The 2 strains were cultured in a 50 mL Nutrient broth (HiMedia, Mumbai, India) in a 250 mL Erlenmeyer flask for 12 h at 30 °C with 250 rpm *via* an incubator shaker. Then, 100 µL of each bacterium broth was equally smeared over Mueller Hunton agar with a sterile hockey stick. All 4 variants (Variation 0, Variation 3, Variation 6, and Variation 9) of the films were punched into 6 mm diameter discs and placed on the streaked plates. Streptomycin disc was utilized as a control. The treatments were incubated at 37 °C for 8 h to examine the inhibition zone. The diameter of the inhibitory zone was measured with a digital micrometer [13,14].

Differential scanning calorimetry

Differential Scanning Calorimetry (DSC; Perkin Elmer, USA) was used to investigate the thermodynamic parameters of the bioactive films. The approach was based on the ASTM D3418-15 standard (ASTM, 2015). The samples were made in a standard aluminium pan with approximately 10 mg of film and a blank pan was used as a reference. The samples were scanned at a heating rate of 5 °C/min, a cooling rate of 10 °C/min, and a holding duration of 3 min. The purge gas was nitrogen at a rate of 50 mL/min with a temperature range of 25 - 300 °C [14,15].

Statistical analysis

The characteristics of edible film and the data were analyzed using SPSS 26 one-way ANOVA with triplicate at α = 0.05 significance level.

Results and discussion

Figure 1 shows the result of edible film from Ganyong starch, sorbitol, chitosan and variation of ginger extract. The thickness of edible film is an important parameter that affects the packaging of the packaged product. **Table 1** is the standard value of edible film as food packaging according to Japan industrial standards.

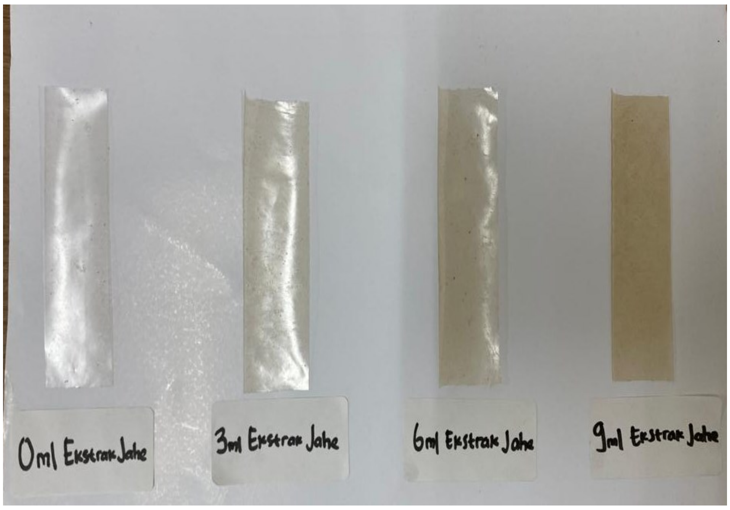

Figure 1 Edible Film with Variation of Ginger Extract.

Table 1 Standard of edible film according Japan Industrial Standard.

Characteristics	Value
Thickness (mm)	<0.25
Tensile Strength (MPa)	>3.92266
Elongation at break (%)	Bad <10
	Good >50
Modulus Young (MPa)	>0.35

Source from Sulistyowati *et al.* [7]

Table 2 shows result of physical characteristics of edible film (Tensile strength, elongation at break, modulus Young, swelling properties and moisture content). The tensile strength value without ginger extract is greater than that with ginger extract added, but it is not significantly difference. The tensile strength of edible film is one of the important mechanical properties to determine the quality of edible film. Tensile strength is the maximum tensile strength that can be achieved until the film remains before breaking [16] .

Table 2 Physical characteristics of edible film.

Physical Characteristics	Variation 0	Variation 3	Variation 6	Variation 9
Tensile Strength (MPa)	1.536±1.126[a]	1.011±0.45[a]	0.692±0.212[a]	0.921±0.163[a]
Elongation at break (%)	44.270 ±8.94[b]	19.290±1.250[a]	76.350±6.551[c]	59.713±13.278[bc]
Modulus Young (MPa)	3.569±3.879[a]	4.554±3.714[a]	0.895±0.211[a]	1.638±0.646[a]
Swelling properties (%)	68.55±10.827[a]	87.233±8.003[ab]	95.013±3.025[b]	81.027±6.351[ab]
Moisture Content (%)	13.553±0.206[a]	12.72±1.883[a]	12.667±1.472[a]	13.833±0.687[a]

*Different alphabets indicate significant difference.
*Variation 0; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan; Variation 3; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan, 3 mL ginger extract; Variation 6; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan, 6 mL ginger extract; Variation 9; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan, 9 mL ginger extract

The tensile strength of edible film determines the ability of the edible film to withstand the load as primary or secondary packaging. Based on the Japan Industrial Standard in **Table 1**, the tensile strength value obtained is still less than the specified value. However, this edible film is applied to crabstick as primary packaging with a fairly light load. So that this edible is still feasible to use as crabstick packaging. The results showed that the tensile strength ranged from 0.921 - 1.536 MPa. The highest tensile strength value on edible film at a concentration of 0 mL ginger extract was 1.536 MPa and the lowest tensile strength value on edible film at a concentration of 6 mL ginger extract was 0.692 MPa. This value is almost the same as the tensile strength value obtained by Agusman *et al.* [17] on edible films added with chitosan. It is also supported by the findings of [18-20] which stated that chitosan with the addition of other ingredients such as sorbitol, banana peel starch, and sago starch produce bioplastics with tensile strength ranging from 0.60 - 1.55 MPa. There was a consecutive decrease at concentrations of 3 and 6 % and continued to increase the tensile strength at a concentration of 9 %. According to Sariningsih *et al.* [21] , the tensile strength may be influenced by chitosan which interferes with the formation of the film matrix by the ginger polymer.

The highest elongation value on edible film at a concentration of 6 mL of ginger extract was 76.35 % and the lowest elongation value on edible film at a concentration of 3 mL of ginger extract was 19.29 % (**Table 2**). There was a decrease in the concentration of 3 mL and 9 mL and an increase in the concentration of 6 mL. The process of mixing the solution is less homogeneous so that the insertion of plasticizers into the composite edible film matrix has not taken place perfectly and the resulting elongation percent is not optimal [22]. In ginger extract, two bioactive compounds were found including shogaol and gingerol. Both of these bioactive compounds contain hydroxyl (O-H) groups. Gingerol is a bioactive extract obtained in fresh ginger while shogaol is obtained by drying the ginger prior to cooking. O-H group composition is more in gingerol. The ginger extract obtained in this study is dried ginger. According to Santoso *et al.* [23], the greater the number of O-H groups in the edible film matrix may contribute to the elasticity or elongation percentage of the edible film to increase. The O-H group plays a role in increasing the mobility of the polymer chain of the edible film matrix.

Young Modulus is the level of rigidity in edible film to determine the flexibility of the film by calculating the division of the tensile strength value by elongation. The highest value of Young's modulus in edible film at a concentration of 3 mL ginger extract was 4.554 MPa and the lowest value of Young's modulus in edible film at a concentration of 6 mL ginger extract was 0.895 MPa (**Table 2**). Although the data obtained is not stable, the difference is not significantly different. According to Paula *et al.* [24] the Young's modulus graph is directly proportional to tensile strength, but inversely proportional to percent elongation. The factor that affects the mechanical properties of polymer-based films is the interaction of the components that make up the film solution.

Water resistance in edible film is the ability of the film to absorb water and swell. The data shows that the swelling results range from 68.55 - 95.01 %. The swelling graph can be seen in **Table 2**. The highest value in the swelling test was at a concentration of 6 mL of ginger extract at 95.01 % and the lowest was at a concentration of 0 mL of ginger extract at 68.5%. The higher the swelling value, the more water absorbed by the edible film, while the lower the swelling value, the less water absorbed by the film [25]. Factors that cause swelling results to increase are improper drying time and unstable drying temperature [26]. The swelling test results that fluctuating are caused by pouring the edible film solution on an uneven cup which results in uneven edible film thickness [27].

Moisture content is a test that maintains edible stability in product coating. Water contained in edible film can affect the texture, flavor, flexibility, and durability of the product inside. The results of the water content can be seen in **Table 2**. The table shows that highest value of water content in edible film at concentrations of 0 and 9 mL of ginger extract was 14 % and the lowest value of water content in edible film at concentrations of 3 mL and 6 mL of ginger extract was 13 %. At concentrations of 3 and 6 %, there was a slope in the graph and an increase in the graph at a concentration of 9 %. However, the data were not significantly different, proving that ginger extract has no effect on moisture content.

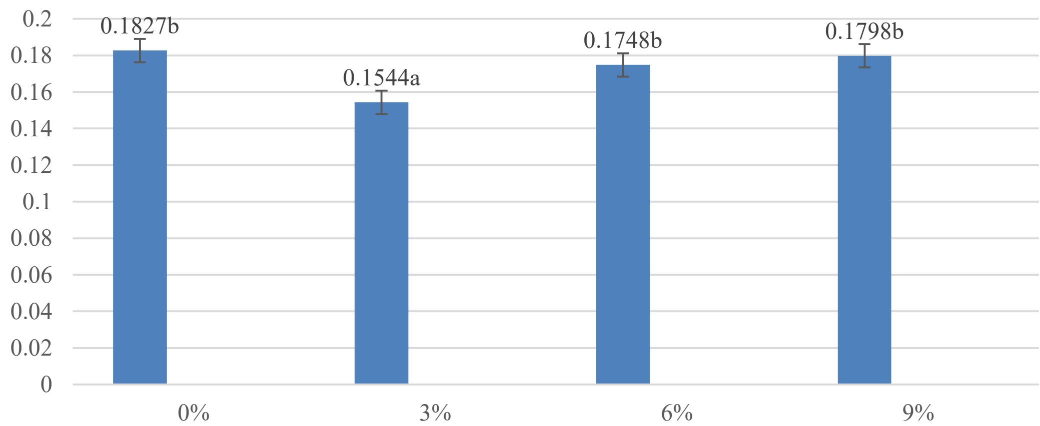

Figure 2 Thickness of Edible Film with variation of ginger extracts.

*Different alphabets indicate significant difference. *Variation 0; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan; Variation 3; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan, 3 mL ginger extract; Variation 6; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan, 6 mL ginger extract; Variation 9; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan, 9 mL ginger extract

Based from **Figure 2**, it shows that the results of the highest edible film thickness value produced at 0 % ginger extract (without using ginger extract) was 0.1827 mm. The lowest edible film thickness value was produced at the addition of 3 mL ginger extract which was 0.1544 mm. The Japanese Industrial Standard (JIS) (**Table 1**) shows that the standard edible film categorized for food packaging has a thickness of less than 0.25 mm. In this study, the thickness of edible film reached 0.1544 - 0.1827 mm which it meets the thickness standard in this study, which there is no significant difference in thickness. Thickness is influenced by drying time, drying temperature and air humidity [26] .

Having important properties in edible films, solubility is one of the properties so that edible films dissolve easily when consumed [28]. A high solubility value indicates that the edible film is easier to consume [29]. The solubility results can be seen in **Figure 3**. The results of water content in **Figure 3**, shows the data of solubility results ranging from 57.18 - 80.49 %. The highest solubility value results in edible film at a concentration of 0 mL ginger extract of 80.49 % and the lowest solubility value in edible film at a concentration of 6 mL ginger extract of 57.18 %. There was no significant difference from the addition of ginger extract concentration to the edible film produced.

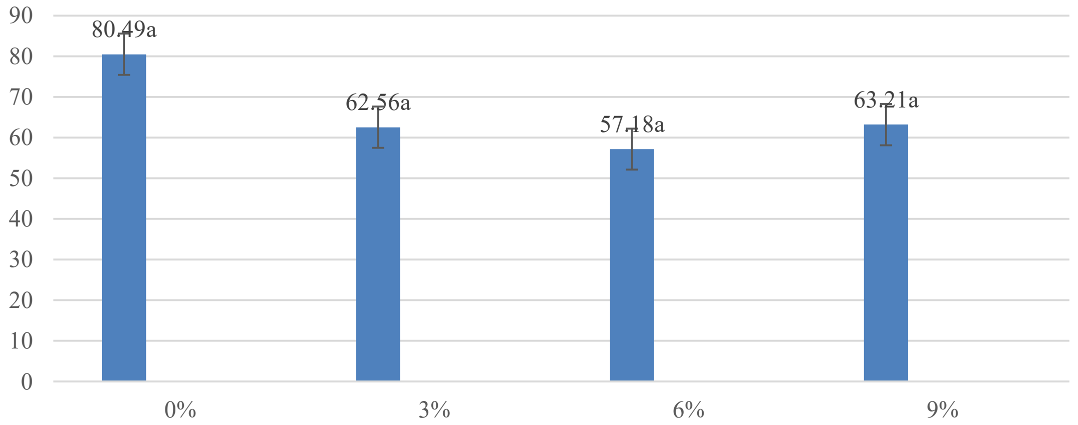

Figure 3 Solubility of edible film with variation of ginger extracts.

*Different alphabets indicate significant difference. *Variation 0; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan; Variation 3; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan, 3 mL ginger extract; Variation 6; 3 % Ganyong starch,4 % sorbitol, 0.3 % chitosan, 6 mL ginger extract; Variation 9; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan, 9 mL ginger extract

Table 3 Antimicrobial properties of edible film from Ganyong starch.

Inhibition Zone (mm)					
Sample strain	**Streptomycin**	**Variation 0**	**Variation 3**	**Variation 6**	**Variation 9**
E. coli	14.8 ± 0.3^{a}	7.6 ± 0.1^{b}	7.3 ± 0.5^{bc}	7.5 ± 0.7^{b}	7.1 ± 0.6^{bc}
B. cereus	14.2 ± 0.4^{a}	7.2 ± 0.1^{bc}	7.4 ± 0.1^{bc}	6.9 ± 0.2^{c}	6.8 ± 0.1^{c}

*Different alphabets indicate significant difference. *Variation 0; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan; Variation 3; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan, 3 mL ginger extract; Variation 6; 3 % Ganyong starch,4 % sorbitol, 0.3 % chitosan, 6 mL ginger extract; Variation 9; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan, 9 mL ginger extract

Table 3 depicted the assessment of the antimicrobial properties of the bioactive films. *E. coli* and *B. cereus* are gram-negative and gram-positive bacteria, respectively and the results shown that the inhibition zone of all the variation were within range of 7 - 7.6 mm for *E. coli*, whilst 6.8 - 7 .4 mm for *B. cereus*, respectively (**Figure 4**). Chitosan possesses antibacterial action, with the mechanism being dependent on positive charges (NH^{3+}). The NH^{3+} disrupts the electronegatively charged of microbial cell membrane. This causes the release of proteinaceous, ionic, and other intracellular components of a microbe, resulting in cell death [30-32]. Ginger extract is also known to have antimicrobial characteristics due to the presence of antibacterial terpenoids such as zingerone, shogaol, nerolidol, and other phenolic compounds. Ginger extract is also known to have antimicrobial characteristics due to the presence of antibacterial terpenoids such as zingerone, shogaol, nerolidol, and other phenolic compounds. Ginger extract has been shown to have a high antibacterial impact on *E. coli* and *B. cereus* [33]. As a result, the researched bioactive film generates synergism. The efficacy of an antibiotic impact, however, is regulated by changes in cell membrane permeability, inhibition of intracellular metabolic pathways, and disruption of enzyme systems [14,34]. Similar outcome was also obtained by Haroun *et al.* [11], Haroun *et al* [12] and Galal *et al.* [35].

The *E. coli* and *B. cereus* were inhibited by the presence of copper nanoparticles and montmorillonite in these studies.

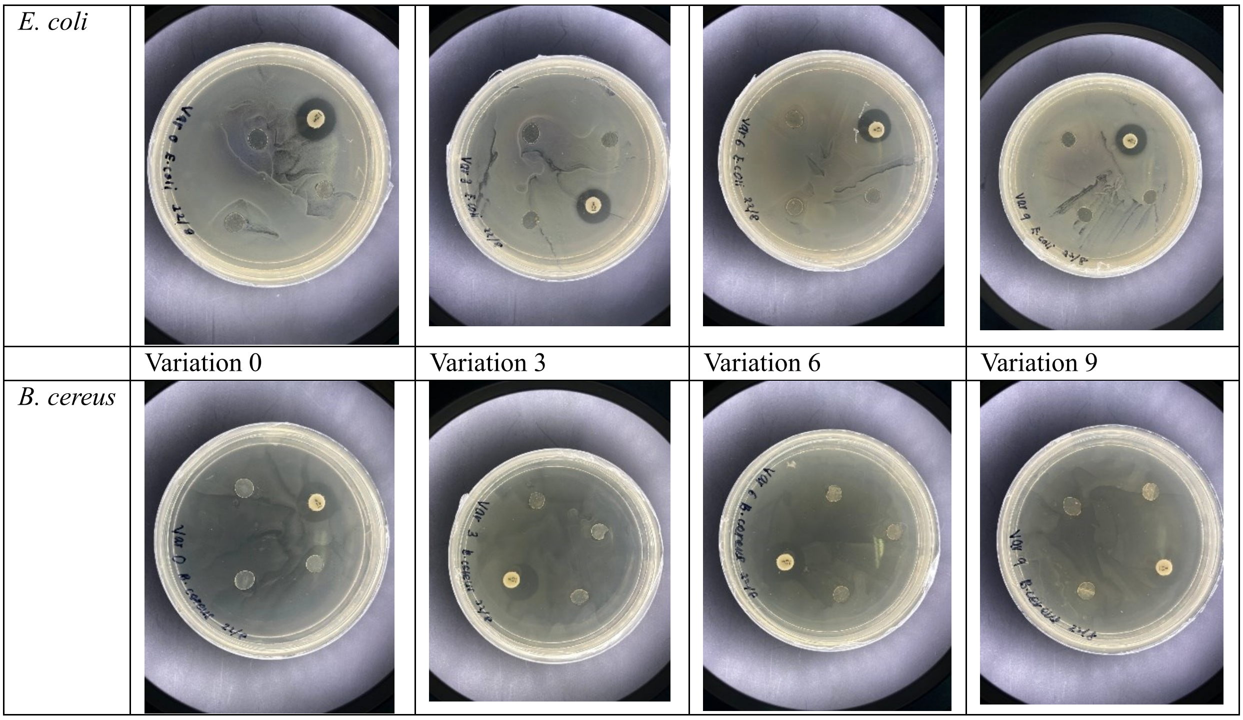

Figure 4 The antimicrobial properties of the bioactive films based of inhibition zones of *Escherichia coli* and *Bacillus cereus.*

* Variation 0; 3 % Ganyong starch, 4% sorbitol, 0.3% chitosan; Variation 3; 3% Ganyong starch, 4% sorbitol, 0.3% chitosan, 3 mL ginger extract; Variation 6; 3% Ganyong starch, 4% sorbitol, 0.3% chitosan, 6 mL ginger extract; Variation 9; 3% Ganyong starch, 4% sorbitol, 0.3% chitosan, 9 mL ginger extract; S; Streptomycin

Table 4 DSC of edible film from Ganyong starch.

Sample	T_m (°C)
Variation 0	118
Variation 3	104
Variation 6	134
Variation 9	133

*Variation 0; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan; Variation 3; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan, 3 mL ginger extract; Variation 6; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan, 6 mL ginger extract; Variation 9; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan, 9 mL ginger extract

DSC was used to assess the endurance and thermal stability of the bioactive films, as shown in **Table 4** and **Figure 5**. The table shows that variation 6 has the greatest melting temperature (134 °C) when compared to other samples, and it is followed by variations 9 (133 °C), variation 0 (118 °C), and variation 3 (104 °C). Variation 3 shows the lowest melting temperature, which might be related to moisture loss linked with the hydrophilic groups in the bioactive layer and lead to polymer disintegration. The reduction in melting temperature for variation 3 also showed the possible plasticization of polymeric chains in the films by the addition of ginger extract. Contrarily, the variation 6 and variation 9 showed greater melting temperature, even higher than the variation 0. This rise in the thermal stability as of increased melting temperature corroborates that the addition of ginger extract raised the melting temperature, perhaps due to increased interactions of hydroxyl and -NH^3 with the ginger extract. Al-hilifi *et al*. [14] also reported an increased addition of ginger essential oil raised the thermal stability of chitosan films and similar was reported by Amalraj *et al.* [36] for chitosan/gum Arabic/ polyvinyl alcohol films. However, the melting temperature of variation 0 is comparable to that of Daza *et al.* [37], where 2 % Ulluco starch addition with 1.5 % chitosan and 1 % glycerol and 0.1% Tween-80 as plasticizers, and a melting temperature of 119 °C was achieved [14,37].

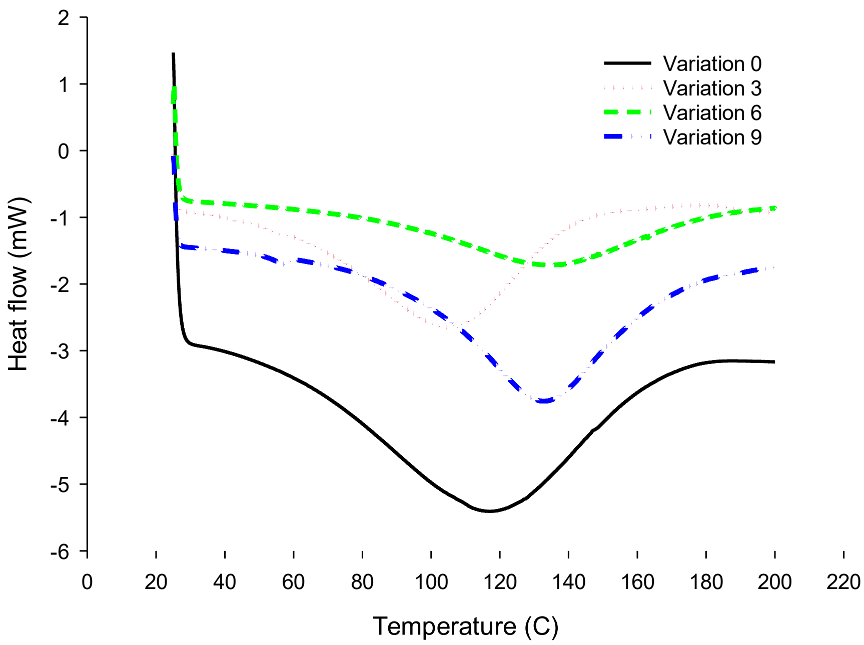

Figure 5 Result of DSC on edible film.

*Variation 0; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan; Variation 3; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan, 3 mL ginger extract; Variation 6; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan, 6 mL ginger extract; Variation 9; 3 % Ganyong starch, 4 % sorbitol, 0.3 % chitosan, 9 mL ginger extract

Conclusions

The addition of ginger extract to edible films based on Ganyong starch, sorbitol and chitosan can be used as primary packaging for crabsticks. The best formulation was found in the 6% ginger extract variation with the results of swelling (95%), moisture content (13%), elongation at break (76%), and antimicrobial activity *E. coli* (7.5 mm) and *B. cereus* (6.9 mm). In addition, the thermal properties result from DSC at 6% concentration was also higher than the others (134 °C). However, the difference was not significantly different from the other variations. So that this formulation has the potential as an alternative primary crabstick packaging.

Acknowledgments

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