Cassava Starch-Based Edible Film with Durian Seed Flour Addition and Green Tea Extract Enriched Microemulsion

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Abstract. Increasing hydrophobicity and antioxidant activity was an effort to improve the quality of edible film. Adding durian seed flour (DSF) and microemulsion was expected to increase cassava starch (CS)-based edible film properties. The objective of this research was learned about the effect of DSF and microemulsion on edible film's physical, mechanical, and antioxidant proper-ties. Coconut oil was the material for oil in the water microemulsion, where-as green tea extract was a substitute for the water phase. The DSF was mixed with CS with various percentages, which were 0%, 20% and 40%. Water as solvent was combined with microemulsion; the variation was 0%, 10%, 20% and 30%. The result showed that adding DSF and microemulsion had significantly decreased tensile strength and elongation but had increased thickness and solubility. Microstructure analysed by SEM showed a less dense and compact structure when the DSF and microemulsion had added. Although there were decreases in physical and mechanical properties, the green tea extract enrichment in microemulsion had significantly increased antioxidant activity. The range of antioxidant activity was 17.58 ± 0.48 % to 35.47± 0.18 %.

Keywords: Solubility, Hydrophobic Film, Microstructure, Elongation, Tensile Strength, Antioxidant Activity.

1 Introduction

There is an increasing interest in the study and utilization of edible film because it is renewable and biodegradable (1). The film comprises bio-polymeric materials such as polysaccharides, lipids, or proteins. Therefore, edible film is considered a “food safety” and “eco-friendly” packaging system. Among edible film materials, polysaccharides are excellent, fully biodegradable, usually inexpensive, and have good film-forming abilities (2).

Cassava starch (CS) is a polysaccharide obtained from cassava root (*Manihot esculenta*), an abundant and economical source. The preferred properties of CS include: high transparency and resistance to acidity also can produce a viscous gel. The CS-based edible film has good physical and mechanical properties and has been extensively

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The CS-based edible film has good physical and mechanical properties and has been extensively studied (3–5). The physical and mechanical properties of edible film from CS generally need modification for further application.

Durian (*Durio zibenthinus* Murr.) is a tropical fruit that grows in Southeast Asia, including Indonesia (6). During harvest season, there is abundant durian production and consumption where the consumable part is only 30–35 % w/w, and the rest are seed 20–25 % w/w and skin 45–50 % w/w (7). According to reference durian seed flour has 6.26 ± 0.10 % crude protein, 0.57 ± 0.05 % crude fat, 2.86 ± 0.09 % ash, 9.70 ± 0.07 % water, 80.61 ± 0.24 % carbohydrate, 0.85 ± 0.01 % crude fiber and 41.42 ± 3.56 % starch content (8). The main component of durian seed flour is polysaccharide, whereas according to (9), a significant fraction of durian seed polysaccharide is gum (50% or more) along with starch. Meanwhile, according to reference, durian seed has an 18% gum content (10).

The edible film properties can be improved through the multi-component mixture of different types of edible polysaccharides. Combining CS and durian seed flour can improve film characters more than a single component. Due to adding gum, several studies have shown increased physical and mechanical properties of edible film from CS. Adding xanthan gum into the CS matrix improved the young modulus, stress at break, and solubility in water (11). Studied in CS film-forming solutions, showed evidence there were improvements in the apparent viscosity, storage modulus, and loss modulus after adding Hsian-Tsao leaf gum (12). Moreover, the combination of CS-gum film showed more resistance to humidity than a pure commercial pullulan film (13).

Easily soluble in water is one of the edible packaging weaknesses that limit its application, mainly when applied to foods with high water content. Therefore, various studies have been carried out to increase hydrophobicity by adding hydrophobic materials such as oil (14–16) or fatty acids (17–19). The main obstacle to increasing the hydrophobicity of edible packaging is the formation of oil-in-water emulsions. Therefore, it is necessary to develop technology for its manufacture.

Microemulsions are emulsion systems on a Nano-scale which are thermodynamically stable and transparent with particle sizes ranging from 5 to 10 nm (20). Several studies have carried out the addition of microemulsions to the edible film matrix ((21–23). The results showed significant increases in the film's physical and mechanical characteristics, especially in water/moisture resistance.

The newest researches trend in edible film has focused on incorporating bioactive plant extract that is rich in antioxidant and antimicrobial to create active package materials. For example, green tea extract had extensively used in edible film making (24–27). The previous study showed improvements in the mechanical properties and bioactivities of the edible film after adding green tea extract (28). Green tea leaves are one of the richest sources of antioxidants: catechin, theaflavin, thearubigin, oxy aromatic acid, flavonol, flavone and tannin (29). Meanwhile, incorporating tea polyphenols into microemulsion can control the release of bioactive compounds and increase bioavailability. There are few studies on developing microemulsion-enriched green tea extract CS-based edible film. Thus, this research aimed to study the effect of adding durian seed flour and microemulsion enriched with green tea extract on edible film characters. The microemulsion was an oil-in-water system where coconut oil was hydrophobic and green tea extract was a hydrophilic phase.

2 Materials and Methods

2.1 Materials

Materials performing edible film were cassava starch (CS) (79.56 ± 3.63 % carbohydrate, 6.72 ± 0.47 % protein, 1.07 ± 0.23 % lipid, 10.01 ± 1.18% water and 2.64 ± 0.58 % ash), durian seed flour (DSF) and coconut oil microemulsion. DSF was produced by steam blanched (100ºC) the chip of durian seed (0.2 - 0.5 mm thick) for 5 min before drying. After drying, the chip was crushed (Basic Analytical Mill IKA 2900000 A11) and screened for an 80-mesh Taylor Sieve. The proximate analysis of flour showed that the water content was 9.36 ± 1.04 %, the lipid content was 1.02 ± 0.45 %, the protein content was 8.26 ± 0.77 %, the ash content was 3.68 ± 0.47 %, and the carbohydrate was 77.68 ± 0.73 %. The other materials for edible film preparation were glycerol as a plasticizer, Isolate Soy Protein (SPI) (8.81 ± 0.32 % carbohydrate, 81.06 ± 1.47 % protein, 0.28 ± 0.03 % lipid, 6.42 ± 1.18% water and 5.41 ± 0.62 % ash) and silica gel to control the atmosphere's relative humidity (RH). The microemulsion was prepared from coconut oil (“Barco “), tween 20 (Merck) lecithin (“NaturesPlus”) and green tea (“Kepala Jenggot”). All chemicals for analysis were obtained from a local distributor (Merck) with pro analysis grade.

2.2. Green tea extract preparation

Green tea extract preparation began with heating 500 mL of distilled water until 100ºC. Two g of green tea (a sachet) were put in distilled water and stirred for 10 min to maximize the extraction process. The solution stood at room temperature for 15 min and was filtered (Whatman Filter Paper with 2.5 µm pore size) to obtain the green tea extract, which was kept in the refrigerator at 4ºC before being applied for edible film preparation.

2.3. Microemulsion preparation

The preparation of microemulsion referred to the research of reference with modification (30), began by mixing 1.35 g of coconut oil with 0.34 g of lecithin and 7.31 g of the tween 20 at room temperature, then continuing with heating at 50ºC and stirring for 5 min. The mixing process used a hot plate magnetic stirrer (Thermo Scientific Cimarec) at a speed of 250 rpm. Then add green tea extract, little by little, as much as 72 g, while heating for 12 min at 50ºC. The emulsion was kept at room temperature for edible film preparation.

2.4. Edible film preparation

The wet process was applied and thus consisted of solution forming, casting, drying and peeling processes (1). The solution forming began with mixing CS and DSF where the total flour weight was 5 g. The variation of DSF added was 0%, 14.3% and 28.6%. Then, the mixed flour was added to water where the water volume variation

was 100 mL, 90 mL, 80 mL, 70 mL. The slurry was mixed using a spatula and heated using a hot plate magnetic stirrer to a temperature of 70ºC for ± 15 min. Then, 2 g of SPI (Soy Protein Isolate) was added and stirred for 10 min until homogeneous. The microemulsion was added according to the predetermined variations that were 0, 10 mL, 20 mL and 30 mL. For the last, 2 g of glycerol was added and heated while stirred again for 15 min until homogeneous. The final gel was poured into the mold where 10 g for each 10 cm^2. Before dried the gel was tempered at room temperature for 15 min. They were then dried using an oven cabinet at 50ºC for ± 12 h.

2.5. Films thickness measurement

This measurement used a micrometer (Mitutoyo Corp., Code No. 543-551-1, Model ID-F125, Japan) with an accuracy of 0.001 mm. Measuring thickness was at three positions on the cut specimen, and then the average value was taken.

2.6. Mechanical properties

Mechanical properties measured in this research were elongation and tensile strength. The specimen size was a width of 1 cm and a length of 8 cm. Before measurement, the film was for 24 hr placed in a desiccator containing silica gel. Then the mechanical properties were measured using the Universal Testing Machine (Shimadzu). The tensile strength was calculated with this formula: $\sigma = A/F$ where σ = tensile strength (N / mm^2); F = tensile force (N); A = area of work (mm^2). Following the tensile strength procedure, elongation was measured with this formula: $\varepsilon = \Delta l/l_0$; where Δl = length addition (mm); l_0 = Initial length (mm). Tensile strength and elongation measurements were repeated five times for each sample.

2.7. Solubility

The method to measure solubility referred to reference (31) which calculated according to the following formula:

$$S = (Ws/W1) \times 100\%$$

Where S = solubility; Ws = W2 – W1; Ws = weight of soluble film; W2 = weight of dried insoluble film; W1 = weight of the dried film. Square shape (2 cm^2) samples cut from edible plastic sheet and dried at 105 °C for 24 h and weighted (W1). The dried samples were dipped in 100 mL water for 24 h, and then the insoluble film was dried for 24 h and weighed (W2). The solubility measurement was repeated three times for every sample.

2.8. Microstructure Observation

Before observation, the film was cut 1.5 cm wide and 3 cm long and was folded like a straight chair using double-sided tape. The microstructure of the cross-section area was recorded by Scanning Electron Microscope (SEM, TableTop Hitachi TM3000).

2.9. Antioxidant Activity

Antioxidant activity was presented as a percentage of DPPH inhibition referred to (32). For each sample, the measurement was repeated three times. Four mg of edible

One ml of extract mixed with 2.5 ml ethanol and 400 µM DPPH reagent and then received ethanol that was added until the final volume was 5 ml. Before lying in the dark room for 20 min, the sample solution was stirred virgiously (vortex). The absorbance of sample and blank was read at 517 nm. The capability to scavenge DPPH radical was calculated by the following formula.

$$\% \text{ Inhibition} = (A0 - A1)/A0 \times 100\%$$

A0 = Absorbency of solution without a sample (blank); A1 = Absorbency of solution with a sample.

2.10. Data Analysis

This study used three repetitions of the experiment. The data result of the experiment was subjected to a two-way analysis of variance. In contrast, the least significant difference (LSD) at a significance level of 0.05 was calculated using the Tukey test. All results were expressed as the average of all the repetitions. The statistical software package (Minitab) was used in data calculation.

3. Results and Discussion

Table 1 is the result of the physical and mechanical properties measurement. The physical properties are thickness and solubility, while the mechanical properties are tensile strength and elongation.

Table 1. The physical and mechanical properties of edible film with variations of microemulsion and DSF percentage.

Samples		Thickness (mm)	Tensile strength (MPa)	Elongation (%)	Solubility (%)
Microemulsion (%)	DSF (%)				
0	0	0.16 ± 0.01^{d}	1.13 ± 0.05^{a}	70.89 ± 0.71^{a}	17.58 ± 0.40^{d}
	14.3	0.21 ± 0.01^{c}	0.80 ± 0.03^{b}	64.33 ± 0.75^{b}	19.36 ± 0.20^{cd}
	28.6	0.22 ± 0.03^{c}	0.76 ± 0.05^{b}	42.21 ± 0.98^{e}	20.28 ± 0.27^{cd}
10	0	0.19 ± 0.01^{cd}	0.87 ± 0.05^{b}	46.79 ± 0.56^{cd}	21.04 ± 0.40^{c}
	14.3	0.22 ± 0.02^{bc}	0.82 ± 0.05^{b}	43.79 ± 0.58^{de}	24.08 ± 0.43^{c}
	28.6	0.21 ± 0.01^{c}	0.78 ± 0.04^{b}	31.38 ± 0.62^{e}	25.30 ± 0.08^{bc}
20	0	0.19 ± 0.03^{cd}	0.77 ± 0.02^{b}	47.47 ± 0.52^{c}	25.24 ± 2.66^{bc}
	14.3	0.22 ± 0.02^{c}	0.45 ± 0.04^{c}	45.45 ± 0.61^{d}	30.84 ± 1.07^{b}

	28.6	0.26 ± 0.01^{ab}	0.22 ± 0.03^{de}	21.57 ± 0.42^{g}	30.59 ± 1.17^{b}
30	0	0.22 ± 0.00^{c}	0.34 ± 0.05^{cd}	27.82 ± 0.32^{f}	35.17 ± 0.42^{a}
	14.3	$0.25 \pm 0{,}00^{b}$	0.29 ± 0.03^{d}	20.91 ± 0.81^{gh}	35.47 ± 2.18^{a}
	28.6	0.28 ± 0.00^{a}	0.19 ± 0.04^{e}	15.69 ± 0.31^{h}	33.65 ± 1.84^{ab}

Note: The same letters in the same column indicated no significantly different ($p < 0.05$).

3.1. Thickness

The result of the ANOVA test showed that DSF and microemulsion significantly affected the thickness of the edible film. Table 1 shows that the edible film with the highest thickness value was the highest DSF and microemulsion proportion (30% microemulsion and 28.6% DSF). That thickness value was not significantly different from 20% microemulsion and 28.6% DSF edible film. The lowest thickness was edible films without DSF and microemulsion addition. That was not significantly different from the same level of DSF with 10% and 20% of microemulsion.

Increasing the DSF percentage resulted in thicker edible film because of the high fiber content in DSF. Previous studies (33,34) showed the same result where the addition of tobacco stem and baggage powder on cassava peel and CS edible films increased thickness because of the high fiber content of tobacco stem and baggage powder. The same trend was also shown in the addition of microemulsions. There was an increase in thickness as more microemulsion was added to the edible film solution. Each component in the microemulsion has a role in increasing the thickness. The component is tween 20 and lecithin (15) and the main is oil (16). Adding more material increased the total mass of edible film related with the increase of thickness.

3.2. Tensile strength

DSF and microemulsion significantly lowered the tensile strength ($P<0.05$). The interaction of those two parameters had a significant effect on tensile strength (Table 1). The interaction treatment of DSF and microemulsion that produced the highest tensile strength was film without DSF and microemulsion; on the contrary, the lowest was film with the highest amount of DSF and microemulsion. The highest value of tensile strength was 1.13 ± 0.05 MPa, whereas the research of reference showed, the tensile strength of DSF film was 2.14 ± 0.20 MPa (35). The difference in flour's particle size was the reason for lowering tensile strength. The particle size of DSF in our research was 80 mesh, and reference was -100/+140 mesh. Reducing particle size probably resulted in increasing molecular interaction and producing a film with better tensile strength (36). The microstructural cross-sectional by SEM showed that increased DSF and microemulsion made a more porous and less compact structure. These phenomena caused a decrease in tensile strength. The less compact structure decreased tensile strength (37,38). The mechanical properties of starch-based edible film depended on the physical properties of starch–water–plasticizer gel formed by molecular ordering and subsequent chain aggregation/crystallization. DSF and

microemulsion probably disturbed the gel formation and interaction of starch–water–plasticizer, which resulted in the less tight edible film matrix.

3.3. Elongation

Table 1 shows that increasing DSF and microemulsion decreased the elongation, and the interaction of those two parameters significantly affected elongation ($P<0.05$). The film without DSF and microemulsion had the highest elongation value, 70.89 ± 0.71 %. The lowest was obtained from the highest addition of DSF and microemulsion, where the value was 15.69 ± 0.31%. The DSF and microemulsion did not support the edible film structure, whereas the result of SEM showed a more porous and less compact structure along with the addition of DSF and microemulsion Fig 1 and Fig 2.

The high fiber concentration may interfere with the starch gelatinization process. Starch gel is one of the important components of the edible film matrix. A less viscous gel results in a less compact and porous film. In conjunction with the addition of microemulsion, the presence of lipids caused the intervention of hydro-gen bonds between molecules in the matrix of edible film. The hydrophobicity of lipids disrupted the gelatinization process. In addition, the content of tween 20 in micro-emulsion can reduce the surface tension, which interferes with the formation of the edible film matrix (39).

The measurement of mechanical properties (tensile strength and elongation) showed that DSF had a negative effect in the form of a decrease in mechanical properties. Although it is expected that DSF will have a positive effect due to its gum content. This may be due to the DSF preparation process, which involves high temperatures and mechanical forces. The gum extraction process is usually obtained from fresh fruit (10,40). Extraction of gum from DSF required a defeated process using petroleum ether solvent (10). According to the reference, the use of high temperatures should be avoided in the gum extraction process (9). Therefore, the DSF in this study is thought to have a very low gum content.

3.4. Solubility

Solubility is an edible film's property essential to evaluate, mainly if the film is purposed to coat/wrap high-moisture food. The low-solubility edible film is suitable for application in high-moisture food or high relative humidity room. As shown in Table 1, the solubility of edible film ranged from 17.58 ± 0.40 % to 35.47 ± 0.42 %. The increase of DSF significantly increased solubility ($P<0.05$), but the microemulsion did not significantly influence the solubility ($P>0.05$). The result showed that the higher solubility followed the lower tensile strength and elongation. The increase in solubility generally indicates the more brittle edible film's structure, which causes a decrease in mechanical properties. Previous studies showed that the reinforcement of fiber in the edible film matrix increased solubility and decreased tensile strength value (33,34). The solubility of CS film in the research of reference was 22%, which increased to around 31% after addition of sorbate (41). The result also indicated that increasing solubility decreased mechanical properties.

3.5. Microstructure Analyzes

SEM can be used to observe the microstructure arrangement, one of which is the structural arrangement of the different components in the composite film. Fig. 1 shows cross-sectional arrangement micrograph of different samples. As can be seen, the CS film without DSF and microemulsion exhibited a compact and continuous structure. It increased DSF, and microemulsion caused a less dense and porous arrangement. The smooth and compact cross-section without any crack or separation indicates good compatibility.

The addition of DSF and microemulsion caused significant changes in the film's morphology, which became uneven. The roughness increased the cross-sectional micrographs of the films with increasing DSF and microemulsion. However, there was no evidence of microemulsion droplets separated from the biopolymer blend. These showed that microemulsion could disperse in the film matrix. A similar result in structure of biopolymer-blend film incorporated with nano-emulsified oils had been previously reported by (42).

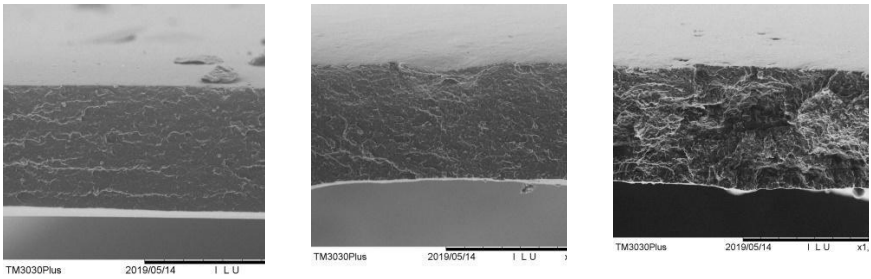

Fig 1.a. **Fig 1.b.** **Fig 1.c.**

Figure 1. Cross-sectional image of edible films with the addition of durian seed flour (a = 0% microemulsion and 0% DSF; b = 0% microemulsion and 14.3% DSF; c = 0% microemulsion and 28.6% DSF

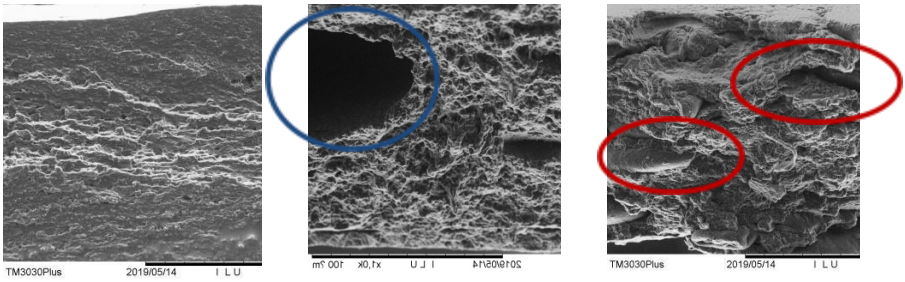

Fig 2.a **Fig 2.b** **Fig 2.c**

Figure 2. Cross-sectional image of edible films with the addition of microemulsion (a = 10% microemulsion and 0% DSF; b = 30% microemulsion and 0% DSF; c = 30% microemulsion and 28.6% DSF)

Fig 2.a and Fig 2.b was film microstructure at the same level in DSF addition and differed in microemulsion percentage. Fig 2.b showed a big pore (blue circle) in higher microemulsion content, probably because tween 20 and lecithin disturbed the

gelatinization process of CS. The research of reference showed a decrease in tensile strength and elongation along with tween 20 and lecithin addition (15). The role of surfactant in the stabilized emulsion was lowering surface tension between two different phases, which probably caused lowering gel consistency and produced a porous matrix.

Comparing Fig 1.c and Fig 2.c, both was edible CS film which had 28.6% DSF but different level of microemulsion. Fig 2.c shows the based material's insoluble part (the red circle). It is probably DSF fiber. It lowered the surface tension because the presence of tween 20 and lecithin in microemulsion lowered the ability of tapioca gel to bind the fiber and produce an inconsistency matrix.

3.6. Antioxidant activity

Fig 3 shows that adding microemulsion into the edible film matrix significantly ($p<0.05$) increased antioxidant activity; otherwise, when DSF was added, there was no significant effect. The green tea extract content in microemulsion can increase the antioxidant activity of edible film. The study of reference showed the antioxidant activity of edible films were around 50% after incorporating 20% green tea extract in edible films solution (27). The range of antioxidant activity in this research was from 17.58 ± 0.48 % to 35.47± 0.18 %, which was lower than the result of reference (27) because the green tea extract was incorporated in microemulsion before being added in edible films matrix.

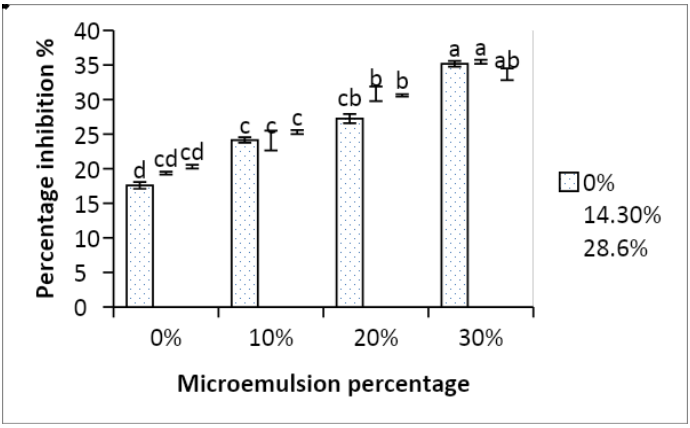

Figure 3. The antioxidant activity of edible films with varied addition of DSF and microemulsion.

4. Conclusion

In conclusion, durian seed flour and microemulsion significantly affected decreasing tensile strength and elongation, increasing thickness and solubility. The microstructure analyzed by Scanning Electron Micrograph showed within DSF, and microemulsion addition film structure got less dense and compact, which caused deterioration of mechanical properties. Otherwise, the microemulsion significantly

improved the antioxidant activity of the film. The improvement was because of the microemulsion enriched with green tea extract. The range of antioxidant activity was 17.58 ± 0.48 % to 35.47 ± 0.18 %.

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Authors' Contributions

The first author was the corresponding author and the one who was responsible with the all-research process and output. The second, third and fourth authors were coauthors. The fifth and sixth authors

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