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Antimicrobial Potency of Jackfruit Straw Films with Enriched Red Ginger Extract for *Galamai* Packaging in Storage.

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ABSTRACT

Galamai is one of traditional foods from Indonesia (particularly from Lima Puluh Kota Regency and Payakumbuh City- West Sumatera) but it has a short life (2 weeks). Unpleasant smell and white powder-like surface indicated deterioration in *galamai*. This present study was aimed determining antimicrobial potency of jackfruit straw films with enriched red ginger (*Zingiber officinale,* var. Rubra) extract for *galamai* packaging in fourth week storage. Enriched red ginger extract are; 2%, 4%, 6%, 8%, 10% and control was no extract ginger, with three replication. Data was analyzed by using SPSS 6.5 for windows. This study showed that a bigger clear zone was achieved along with increasing concentration of red ginger in jackfruit straw films (significant 5%). Further, based on TBA and FFA number, it was safe to be consumed *galamai* with enriched jackfruit straw film after four weeks storage.

Keywords: Antimicrobial potency; edible film; red ginger extract; *galamai*; storage

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INTRODUCTION

Galamai is one of traditional foods from West Sumatera, Indonesia (particularly from Lima Puluh Kota Regency and Payakumbuh City). This intermediate moisture food was made from rice flour, palm sugar, and coconut milk which were mixed and cooked on a high temperature until dark brown dough is formed. It is suitable for serving as snack with sweet taste and chewy texture but it has a short life (2 week) in plastic wrapping . Unpleasant smell (rancidity) and white powder-like surface indicated degradation of *galamai* as result of spoilage on food with high fat content [1-3]. Spoilage, off flavor, rancidity, deterioration because of lipid oxidation, and undesirable growth of microorganisms are unacceptable for consumption [4]. Preventing odd taste of *galamai* and its limited shelf life are interesting challenge in packaging innovation such as antimicrobial edible films or edible coatings [1, 3].

Edible films are used to enhance the quality of food products by giving protection from physical, chemical and biological [2, 5]. Edible films are produced from biopolymer, such as: proteins, polysaccharides, lipid and its combinations [6]. Polysaccharides film is made of materials such as starch, non-starch, carbohydrates, gums, and fibers [7]. Jackfruit straw has been developed as a raw material in the manufacture of edible films, its contain carbohydrates that consist such as; glucose, fructose, sucrose, starch, pectin, and cellulose in quite high amount [2].

Edible film can be used as carriers of a variety of additives to extend product shelf life and reduce possibility of microbial growth on food surfaces. Antimicrobial agents can be added into edible film to provide microbiological stability or to reduce undesirable microorganisms during ready-to-eat product storage [8-9]. Antimicrobial agents are added to inhibit spoilage and pathogenic microorganisms [10]. Several research studied natural antimicrobials activity from plants (including fruits, vegetables, grains, herbs, and spices), animals, bacteria, algae and mushroom [2, 8]. A number spices showed antimicrobial activity [11] such as rhizome of ginger (*Zingiber officinale*, Roscoe.) is effective as antioxidant and antimicrobial activities [12-16] while ginger has antimicrobial activities against various bacteria, fungi, and nematodes [14].

Major compound in spices such as phenolics, phenolic acids, tannins, quinones, flavonoids, essential oils, steroids, alkaloids, glycosides, coumarines [3, 10-11, 17]. The distinct yellow, pungent, aromatic rhizome is the plant's organ that confers its value to the spice and the source of oleoresin and essential oil. It was detected that ginger had high antioxidant activity of oleoresin component while its essential oil is effective for anti-microbe [13]. Red ginger (*Z. Officinale,* var. Rubra) cultivated in Indonesia and Malaysia contains essential oil (3.9%), starch (52.9%), and diluted extract in alcohol (9.93%) which is higher than those of two other species of ginger. It was noted by [17] that main components of atsiri in ginger is zingiberene while other components are curcumene, farnesene and several amount of bisabolene and β-sesquiphelandrene [17].

This research aimed to obtain information related to antimicrobial potency from red ginger extract in jackfruit straw films during four weeks storage of *galamai*. Anti-microbial edible film from this study could lengthen shelf life of *galamai*.

MATERIALS AND METHODS

Materials used in this study were jackfruit straws obtained after removing arils seeds, and rind, red ginger (7-8 months), *galamai* for applying edible film, distilled water, glycerol, and CMC. Stainless steel knife, bucket, analytical balance, stainless steel spoon, blender, juicer, filter cloth, glass plates (20 cm x 20 cm x 20 cm), beaker glass, measuring glass, thermometer, stopwatch, magnetic stirrer, and hot plate.

Materials Preparation

Jackfruit straw is washed clean then crushed by adding water 1:1 using blender to obtain its filtrate to make edible film. Fresh rhizome was peeled and washed with distilled water. Red ginger extract then was obtained by juicer extracting.

Edible Film Preparation

Jackfruit straw was crushed and its 100 ml filtrate was separated then added glycerol (5% of jackfruit straw filtrate) as plasticizer and mixed thoroughly. Next, CMC 1% in 50 ml distilled water was added to filtrate and heated. This mixture was heated on hot plate with magnetic stirrer at temperature 70°C. Then, red ginger extract (2%, 4%, 6%, 8%, and 10%) was added to mixture after it was cooled down at temperature 45°C. Film solution was molded on thin layer glass and dried using oven at temperature 60°C for 24 hours. Film was removed from glass molding after cooled down for 10 minutes at room temperature.

Edible Film Application in *Galamai* Packaging

Edible film was cut in certain size then applied on *galamai*. *Galamai* with film packaging was put in storage at room temperature for observation.

Analytical Method

Thiobarbituric-Acid (TBA)

A 3-g sample was measured and added to Waring blender. Sample was crushed for 2 minutes after adding 50 ml distilled water. Sample was added successively to distillation flask 1000 ml then washed with 48.5 ml distilled water. Then, 1.5 g chloride acid 4 N (one part of chloride acid with two parts of water) was added. Boiling stones and few of anti-foam agents were added and mixed in distillation flask for distillation. Distillation was conducted at high temperature for 10 minutes to obtain 50 ml distilled sample. Next, sample was screened then 5 ml of distilled sample was removed to erlenmeyer 50 ml with lid and added 5 ml TBA reagent. TBA reagent consisted of 0.02 M of thiobarbituric-acid in 90% glacial acetic acid. This mixture was heated in boiled water for 35 minutes then cooled down in cool water. Sample was observed its optical density while a blank sample was prepared following the same procedure without a test sample. Result was explained as optical density or absorbancy (A) to compare rancidity level [19].

Free Fatty Acid (FFA)

Sample was measured about 0.2-10 gram and put in erlenmeyer 50 ml with 50 ml of alcohol. Erlenmeyer with sample was covered with aluminium foil and heated for 10 minutes in water bath. Next, 2 ml phenolphtalein was added to sample and titrated by adding NaOH 0.1 N until pink color stays for 30 seconds [19].

Antimicrobial Activity Test

Agar plate diffusion method was used to observe anti-microbe activity. Firstly, mold from *galamai* was isolated in liquid medium potato dextrose agar and incubated at temperature 27°C for 24-48 hours. Sample was inoculated by placing one loopful sample in sterile distilled water then 0.6-1 ml was removed to petri dish. Then, 15 ml of PDA were poured into petri dish and let it solid. About 6 mm diameter of film was dried and placed on the surface inoculated PDA. This petri dish was incubated for 24 h at 30 ^{0}C prior examination. Observation was on width of growth zone indicated by halos around (clear zone) [20].

Total Plate Count

Total plate count was utilized to obtain microbial colonies number. There was 5 g sample of dough was serially diluted six times to obtain a 1:10^{6} dilution then spread on plate and incubated for 24 hours [21].

Experimental Design

Complete randomized design was conducted with 5 treatments and 3 replications. Observation data was analyzed using SPSS 6.5 for Windows and while the differences among samples were determined by Duncan's New Multiple Range Test (DNMRT) for antimicrobial test. There were five treatments of red ginger extract additions which were 2%, 4%, 6%, 8%, and 10%. There was also film without red ginger extract addition as control. *Galamai* with edible film were kept for 4 weeks in storage

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RESULT AND DISCUSSION

Antimicrobial Activity

Microorganism growth on food surfaces indicated degradation since it caused physical and chemical changes on food. Based on analysis of variance, it showed that red ginger extract in edible film had a significant different in antimicrobial activity of jackfruit straw edible film. DNMRT test results are showed on Table 1.

Table 1: Antimicrobial Activity

Treatments	Antimicrobial Activity (mm)
A= 2% red ginger extract	8.25 ± 1.0606 a
B= 4% red ginger extract	11.50 ± 2.8284 ab
C= 6% red ginger extract	11.75 ± 2.4748 b
D= 8% red ginger extract	16.25 ± 0.3535 bc
E= 10% red ginger extract	19.25 ± 1.7677 c
KK = 14.35%	

Means without sharing common letter were significantly different at 5% of DNMRT.

It can be analyzed from Table 1 that antimicrobial activities of edible film obtained from this study were about 8.25-19.25 mm. Increasing red ginger extract showed significant different at 5%. It was indicated an increasing microbial inhibition zone edible film averagely increased with increasing red ginger extract in film. This condition is in agreed with [22] that the higher concentration of material containing antimicrobial compound means the higher antimicrobial compound and its inhibition activity. Further, it was noted by [15] that the larger diameter of microbial growth inhibition zones obtained implied the higher concentration of antibacterial agents of red ginger extract.

Generally, the main component of red ginger from Indonesia is gingerol which are converted into shogaol and zingeron during edible film production. These components are effective as antimicrobial as explained in [18] while active compounds such as gingerol can inhibit bacterial growth [23]. Moreover, there are [6]-gingerol and 3R,5S-[6]-gingerdiol which are characteristic and active compounds of red ginger. Comparing to other ginger, red ginger is rich in gingerol and shogaols as explained in [16-17].

Another study explained that gingerol and shagaol are giving strong pungent smell while zingeberen is pre-dominant component in oil. Some volatile components with antimicrobial properties are α-pinene, borneol, camphene, and linalool [24]. It was explained by [13] that phenolic compounds in ginger, such as; shogaols, zingerone, gingerdiols and gingerols are responsible for the observed antimicrobial potency. On the other hand, smells and aroma characteristics of red ginger comes from mixed of zingeron, shogaol, and atsiri which are about 1-3% of fresh ginger. However, zingeron gives less pungent smell as explained by [18].

Analysis of *Galamai* during Storage

Thiobarbituric Acid (TBA) Analysis

Table 2: Thiobarbituric Acid (TBA) *Galamai*

Treatment	TBA value (mg malondialdehyde/kg sample)			
	Week 1	Week 2	Week 3	Week 4
Control	0.14	0.17	0.22	0.36
2%	0.13	0.12	0.18	0.23
4%	0.13	0.12	0.18	0.20
6%	0.13	0.11	0.17	0.21
8%	0.12	0.11	0.15	0.19
10%	0.11	0.10	0.15	0.19

Since classified as intermediate moisture food, *galamai* is easily to spoil. Spoiled *galamai* can be identified by off flavor and mold existence on its surface. Fat degradation was counted as thiobarbituric-acid. Thiobarbituric-acid test was conducted to analyze rancidity on fat contained product. Average TBA values during one month storage are displayed on Table 2 as followed.

It can be observed an increasing TBA value for one month storage *galamai* with edible film with a slight drop on 2 weeks of storage. Average value of TBA was about 0.10-0.36 mg malondialdehyde/kg sample. On the first week, TBA value was about 0.11-0.14 mg malondialdehyde/kg sample then became 0.10-0.17 mg malondialdehyde/kg sample on 2 weeks of storage. There were increasing values of TBA during 3 weeks (0.15-0.22 mg malondialdehyde/kg sample) and 4 weeks of storage (0.19-0.36 mg malondialdehyde/kg sample). These changes of TBA value of all treatments including control were still on accepted limit which were below 0.5 mg malondialdehyde/kg sample [25].

Increasing TBA value of sample during storage indicated oxidation still occurred along with oxidation of unsaturated acid in *galamai* during storage. A slight decreasing of TBA value on 2 weeks storage might be related to aldehyde reaction on other components of *galamai*. Low TBA value is not only because oxidation on fat but also reaction on aldehyde accumulation with other components or it was evaporated during storage [25]. TBA test value acquired from condensation reaction between two TBA molecules with one molecule of malondialdehyde indicated by red pigment appeared on sample. Theoretically, malondialdehyde can be obtained from forming of diperoxide on pentadiene complex followed by molecule chain or oxidase more than 2-enol from mono- hydro peroxide [26]. Even there were increasing TBA values during storage, increasing TBA value of *galamai* with red ginger extract in edible film was lower than those without red ginger extract. This condition indicated that red ginger extract addition can reduce fat rancidity. Like other herbs, red ginger can maintain food quality as anti-microbe and anti-oxidant [27].

It was noted by [28] that anti-oxidant can be added to fat-containing food in order to lengthen shelf life, inhibit lipid oxidation and growth of pathogenic microorganism [4]. Essential oil and oleoresins from herb and spices contain phenolic compounds such as eugenol, shogaols, zingerone, gingerdiols, gingerols in ginger as anti-microbe and anti-oxidant [13].

Further, [29] analyzed proximate components in ginger which are alkaloids, flavonoids, polyphenol, saponin, steroid, tannin, fiber, carbohydrate, vitamins, carotenoid and minerals. Polyphenols and flavonoids are recognized as anti-oxidants [29]. Most of gingerol and shogaol can be found in rhizome as 6-gingerol and 6-shogaol with another active component in ginger such as curcumin. These components were observed to have a good anti-oxidant activity [13].

Free Fatty Acid Analysis

Increasing free fatty acid value of galamai with edible film in various concentration of red ginger extract was observed during storage. Average value of FFA during one month storage showed in Table 3.

Table 3: Free Fatty Acid of Galamai with Edible Film in Average

Treatment	Free Fatty Acid (%)			
	Week 1	Week 2	Week 3	Week 4
Control	1.62	2.41	2.74	3.20
2%	1.60	2.32	2.62	3.20
4%	1.54	2.28	2.60	3.14
6%	1.56	2.11	2.38	2.93
8%	1.50	2.03	2.42	2.56
10%	1.52	2.00	2.46	2.55

It can be noted from Table 3 that FFA value during one month storage was about 1.50-3.20% with increasing trend on every week. For one week storage, FFA value was about 1.50-1.62% then increasing in two weeks storage about 2.00-2.41%. Trend was continuing in three weeks and four weeks storage with FFA value were 2.46-2.74% and 2.55-3.20% on average.

Increasing FFA value is related to fat contents. Fat content from animal or plants contain hydrolyzing fat enzyme. This enzyme including lipase can hydrolyze neutral fat (tri-glycerides) become free fatty acid and glycerol. The longer storage of fat-containing food means the higher free fatty acid content since hydrolyzing happened continuously [26].

Moreover, higher FFA contents indicated higher rancidity happened during storage. Although there was an increasing value of FFA, galamai with red ginger extract film had lower FFA value compared to galamai without red ginger extract film. It indicates that red ginger extract can limit rancidity.

It was explained by [26] that free fatty acid was analyzed based on milligrams of KOH to neutralize free fatty acid in one gram oil or fat. Acidity number is related to number of free fatty acid in fat or oil. Free fatty acid in fat or oil is result of oxidation or enzyme hydrolyzation during process and fat or oil during storage. A higher free fatty acid is related to a lower quality of oil or fat.

Fat hydrolysis into free fatty acid and glycerol could be conducted by microorganism with producing lipase enzyme indicated by unpleasant smell. Beside oxygen, microorganism can produce oxidase enzyme that oxidizing unsaturated fatty acid then producing components with lower molecular weight such as acid, aldehyde, ketones, and peroxide [30]. Hence, anti-microbe and anti-oxidant components in ginger can limit rancidity reaction in *galamai* during storage.

Total Plate Count

Total plate count of *galamai* with edible film in various concentration of red ginger extract was observed during four weeks storage as shown in Table 4.

Table 4: Total Plate Count of *Galamai* with Edible Film (CFU/g)

Observation	Treatments					
	Control	2%	4%	6%	8%	10%
Week 1	9.7×10^2	8.3×10^2	6.1×10^2	5.6×10^2	4.4×10^2	3.5×10^2
Week 2	8.1×10^3	9.0×10^3	8.6×10^2	7.3×10^3	9.8×10^2	5.8×10^2
Week 3	4.2×10^4	8.4×10^3	8.2×10^3	8.8×10^3	7.9×10^3	5.3×10^3
Week 4	1.3×10^5	1.1×10^5	1.5×10^5	6.2×10^4	5.9×10^4	5.1×10^4

It can be analyzed that there was an increasing number of microbe during *galamai* storage for one week until four weeks. On the other hand, decreasing number of microbe was obtained by increasing concentration of red ginger extract. But, number of microbe was increasing on week 2 at concentration 2% and 6%. This condition could be found also in week 3 at concentration 6% and week 4 at concentration 4% compared to those at lower concentration.

It was indicated no spoilage on this intermediate moisture food with number of microbe about 10^3-10^5, while number of microbe is about 10^6-10^7, mucus and unpleasant smell. Changing food structure such as deforming or watery can be fund when number of microbe is about 10^8-10^{10} [31]. Based on this study, *galamai* in four weeks storage showed no spoilage since number of microbe in week-1 until week 4 were still about 10^2-10^5 (CFU/g). It was indicated that there was no unpleasant smell and mold from *galamai* appearance.

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