



Formulation of Edible Film Ethanol Extract of Kaffir Lime (*Citrus hystrix*) Leaves Using Corn Starter and Cassava Starter Variations

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Abstract

Introduction: Kaffir lime leaves are plants that have antibacterial activity against *Streptococcus mutans*. *Streptococcus mutans* bacteria are bacteria located in the oral cavity and can cause dental caries disease. **Objective:** This research will develop edible film preparation of the extract of kaffir lime leaves ethanol using cassava starch and corn starch as gelling material. **Methods:** Edible film of kaffir lime leaf extracts is then evaluated for physical properties that include fragility, shrinkage drying, pH and thickness. The data obtained were analyzed by qualitative and quantitative analysis. **Result:** Kaffir lime leaf extract with 25% extract concentration showed that its physical properties qualified physical properties in accordance with comparative products, but for pH test only formula with the use of cassava starch as a hydrocolloid binder that meets the pH of mouth. **Conclusion:** Panelists prefer an edible film with cassava starch composition as a hydrocolloid former.

Keywords: Leaves, Starch, Edible Film, Physical Properties

Introduction

Halitosis (bad breath) is the most complained problem among mouth and teeth health (Arifin *et. al.*, 2010). Bacteria cause halitosis (bad breath) in the oral cavity, producing volatile sulfur compounds (VSCs). Halitosis can also be caused by food debris that accumulates in the mouth, smoking habits, alcoholic beverages, and consuming foods that have a sharp taste, such as garlic or onions (Mokeem S. A. 2014). Kaffir lime (*Citrus hystrix* DC) is a type of plant from the Rutaceae tribe used as an antibacterial. Plants are native to Indonesia, which contain flavonoids, tannins, latex, alkaloids, and essential oils (Maimunah *et. al.* 2020).

Research conducted by Warsito *et. al.* (2017) stated that kaffir lime leaf essential oil has the main components of citronellal (85.07%), linalool (3.46%), and sabinen (2.79%). Kaffir lime leaves are one of the natural ingredients that have antibacterial activity. The study results (Miftahendarwati, 2014) showed that kaffir lime leaves extracted by the maceration method had antibacterial activity against *Streptococcus mutant* bacteria with a concentration of 25%. Research (Astriani *et. al.* 2021) showed that orange leaf extract has antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* bacteria. Yuliani *et al.* (2011) stated that the essential oil content in kaffir lime leaves could inhibit the growth and kill *Staphylococcus aureus* and *E. coli*. Essential oils with concentrations of 2% and 1% were able to inhibit the growth of *Staphylococcus aureus*. The concentration of 1% essential oil is the

minor concentration capable of inhibiting the growth of *Staphylococcus aureus* so that the concentration is expressed as MIC.

Development Preparations commonly used for mouth fresheners include toothpaste, mouthwash, chewable tablets, lozenges, mouth freshener spray, and candy. To make it easier and provide comfort when used, it is necessary to develop a preparation that can function as a mouth freshener, eliminate bad breath, and as an antibacterial on the teeth. Therefore, the preparation was developed in the form of an edible film. This mouth freshener in the edible film is already on the market; the product uses menthol as an active substance that provides freshness in the mouth (Harmely et al. 2015).

Edible film or Oral dissolving film (ODF) is a thin transparent film cut at a certain length and width which dissolves immediately after contact with a small amount of water (saliva) (Dewi et al. 2019). Based on these things, in this study, the development of kaffir lime leaf extract in edible film preparations will be carried out.

Methodology

Tools

The tools used in this study were ruler, scissors, stirring rod, watch glass, porcelain dish, spray bottle, funnel, beaker, measuring cup, parchment paper, digital scale, refrigerator, maceration container, filter paper, dropper, electric stoves, ovens, tweezers, spatels, edible film printing equipment using a flat surface box pan, film thickness tester (caliper), pH meter and friabilator.

Materials

kaffir lime leaves, ethanol 96%, corn starch, cassava starch, Hydroxypropyl cellulose, sorbitol, sodium saccharin, oleum menthae piperatae, menthol, nipasol, melon essence, and aquadest.

Volunteer

As many as 20 volunteers were asked to consume the edible film preparation of kaffir lime leaf extract and give their opinion about the organoleptic and antihalitosis activity to freshen the mouth from the edible film preparation; the results were obtained in the form of a score.

Methods

The method used in this research is the solvent casting method. The solvent casting method is a method that dissolves the active ingredients and additives in a volatile solvent, such as water or ethanol. The mixed solution is combined, then the solution is formed into a film and dried in an oven at a temperature of 45-50oC and cut into pieces for further packaging and sealing (Suput et al. 2015).

Table 1: Edible Film Formula of Kaffir lime leaf extract

Materials	Formula		
	I	II	III
Kaffir lime leaf ethanol extract (g)	2.5	2.5	2.5
HPC (g)	4	4	4
Sorbitol (g)	4	4	4
Na. saccharin (g)	0.10	0.10	0.10
Menthol (g)	0.3	0.3	0.3
Candy oil (g)	1	1	1
Nipasol (g)	0.02	0.02	0.02
Melon essence (g)	0.25	0.25	0.25
Corn starch (g)	3	6	-
Cassava starch (g)	3	-	6
distilled water ad	100	100	100

Edible film ethanol extract of kaffir lime leaves (*Citrus hystrix*) was made in 3 (three) formulas with variations in the composition of corn starch and cassava starch as a carbohydrate hydrocolloid-forming material. Made in 3 (three) formulas, a formula I with a ratio of corn starch and cassava starch 1: 1, formula II with a ratio of corn starch 1: 0, and formula III with a ratio of 0 cassava starch; 1, can be seen in table 1.

Starch is dispersed in some aquadest then heated at a temperature of $\pm 60^{\circ}\text{C}$, stirred until a gel is formed. HPC was dissolved in hot water plus sorbitol, stirred at a temperature maintained at $\pm 60^{\circ}\text{C}$. The two gels were mixed at a temperature of $\pm 60^{\circ}\text{C}$, added with other ingredients (sodium saccharin solution, melon essence, kaffir lime leaf extract, nipasol, menthol, mint oil, and remaining water). Homogeneously stirred and then poured evenly into the mold (mold with a flat surface) at room temperature. Drying is carried out in the oven at a temperature of $45\text{-}50^{\circ}\text{C}$ for 24 hours, then removed from the mold and then cut into pieces with a size of 2 x 3 cm (Harmely *et. al.* 2015).

Edible Film Evaluation

Organoleptic Examination

The organoleptic examination includes observing the shape, color, smell, and taste of the edible film produced. The investigation was carried out at room temperature ($15\text{-}30^{\circ}\text{C}$) every week of the study (Harmely *et. al.* 2015).

Friability Examination and Edible Film

The fragility of the edible film was carried out according to the table fragility test using a Friabilator. Twenty sheets of edible film free of dust were weighed together (W_1), then put into the Friabilator, run the tool for 4 minutes with a rotation speed of 25 rpm. Clean the 20 sheets of edible film from dust and weigh again (W_2). The fragility of edible films can be calculated by the formula: (Harmely *et. al.* 2015).

$$\text{Friability} = 1 - \frac{W_2}{W_1} \times 100\%$$

Drying Shrinkage Check

The porcelain dish was dried in an oven at a temperature of 105°C until a constant weight (A) was obtained. Edible film weighed 2 g in a porcelain dish (B) then dried in an oven for 2-5 hours until a constant weight (C) was obtained; the drying loss was determined in percent of the weight of the sample used (Harmely, 2015).

$$\% \text{ Drying Loss} = \frac{\text{Initial weight without cup} - \text{Final weight without a cup}}{\text{Initial weight without a cup}} \times 100\%$$

pH check

This test is carried out using a pH meter. The pH of the edible film of kaffir lime leaf extract was measured by diluting 1 g of an edible film with distilled water up to 10 ml. The pH meter is immersed in the container, and the numbers show the pH meter is the pH value of the kaffir lime leaf extract edible film. pH examination was carried out in 3 weeks, assessment at 1, 2, and 3 weeks during the study.

Edible Film Thickness Check

The thickness of the edible film was checked using a caliper which was measured at five different places. Then add up and find the average thickness.

Panelist's Preference Test

Testing to panelists is divided into several points, namely:

- a. Observation of the smell and taste of edible film

- b. Edible film color and shape
- c. Antihalitosis activity to freshen the mouth (Harmely, 2015).

Result

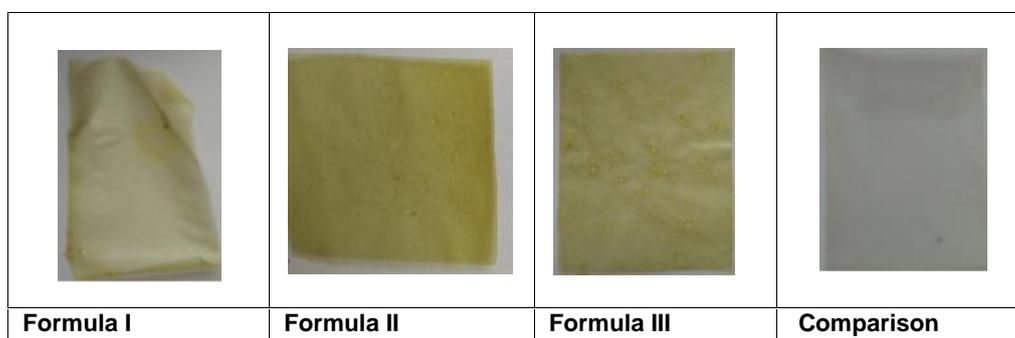


Figure 1: Physical Form of Edible Film Ethanol Extract of Kaffir lime (*Citrus Hystrix*) Leaves Using Variations of Corn Starch and Cassava Starch.

Table 2: Evaluation Results of Edible Film Ethanol Extract of Kaffir lime (*Citrus Hystrix*) Leaves Using Variations of Corn Starch and Cassava Starch

No	Evaluation	Observation			
		FI	FII	FIII	P
1.	a. Shape	Thin	Thin	Thin	Thin
	b. Color	Pale Green	Pale Green	Pale Green	Light Green
	c. Smell	Typical simplicia	Typical simplicia	Typical simplicia	Menthol
	d. flavor	A little sweet	A little sweet	A little sweet	Fresh mint
2.	Fragility %	10%	0%	10%	10%
3.	Drying Loss%	9%	7.5%	9.50%	11.01%
4.	pH	5.3	5.3	5.7	5.5-7.9 (mouth pH)
5.	Thickness (mm)	0.03	0.05	1.8	0.25
	Standard Deviation	0.007	0.016	0.010	0

Discussion

The use of ethanol with a concentration of 96% to separate the essential oil contained in kaffir lime leaves. The solvent liquid in the extract manufacturing process is a suitable solvent. These compounds can be separated from the material and other content compounds and the extract only contains most of the desired content compounds. For the total extract, the solvent selected dissolves almost all the secondary metabolites contained (Citra, 2016).

The polymer materials that make up edible films are divided into three categories, namely hydrocolloids, fats, and both composites (Yulianti, 2015). One of the edible film materials from the hydrocolloid group is a polysaccharide with several advantages, including being selected for oxygen and carbon dioxide, non-greasy appearance, and low-calorie content. Among the types of polysaccharides, starch is a potential raw material for the manufacture of edible films with physical characteristics similar to plastic, which is colorless, odorless, and tasteless.

The process of making edible films uses a film-forming base made in 3 formulas, namely corn starch, cassava starch, and a combination of the two starches. A starch gel is made by mixing hot water and starch and then stirring until it forms a gel on an electric stove. According to (Winarno, 1984), when starch is heated, it will undergo two separation reactions. The soluble reaction is called amylose, and the insoluble is called amylopectin. When heated in water, amylopectin will form a transparent layer, namely a solution with high viscosity and in the form of layers (Herawati, 2016).

Starch is one of the binders often used in tablet preparations but with different levels of amylopectin, so additional binders are used so that all ingredients do not separate in the formula-making process (Sugiyono, 2011). The binder used is Hydroxypropyl Cellulose/(HPC) by first dissolving it in hot water and then heating it to form a gel. After the HPC started a gel, all the ingredients, including the starch that had created a gel were put into the HPC mixture, then aquadest was added to the calibration limit and stirred while heated until all homogeneous mixtures.

Organoleptic

Based on the table on the organoleptic evaluation of the ethanol extract of kaffir lime leaf edible films carried out for four weeks at room temperature (15-30°C), the results obtained all have the same organoleptic properties. Table 2. Uniform organoleptic due to the concentration of all non-comparative materials used in the same amount. The comparison material in question is starch with different comparison variations. The thing that affects the edible film produced is the comparison material's physical properties. The starch content in corn starch is 25% in the research of Kusumawati and Putri (2013), while the cassava starch is 90%, in the study of Saleh *et al.* (2017). According to Junaedi (2012), corn starch contains amylose as much as 28% and amylopectin 72%. Cassava starch has 17% amylose and 83% amylopectin. According to Sugiono (2011), amylopectin is more concentrated than amylose. This shows that corn starch and cassava starch have more or less the same content. The thing in| This causes the organoleptic properties of the edible film produced to be the same, even though the composition of corn starch and cassava starch is different in each formula.

Edible Film's Fragile

Evaluation of the fragility of edible films is used to compare products sold in the market with the brittleness of 10%. The friability test got the results in table 3. Formula II with the composition of corn starch only as a carbohydrate hydrocolloid former got 0% results, which means it was not brittle at all. This is because the amylose content in corn starch is greater than that of cassava starch (Wulan *et al.* 2006). Starch with high amylose content produces a flexible and edible solid film (Yulianti, 2015).

Drying Loss

Measurement of drying shrinkage by the oven method at a temperature of 105°C can only measure the amount of free water in the material because bound water is difficult to remove at this heating temperature. The drying shrinkage level will affect the existing water content and storage because if the water content is too high, it will facilitate microorganisms. To grow (Paradita, V. 2013). The results that experienced the highest drying loss were formula III compared to formulas I and II, and all formulas met the requirements because they did not exceed 11.01% (BSN, 2008).

pH

Examination of the pH value is critical because if the resulting pH is not following the existing range, it can irritate the oral mucosa (Wahyuni *et al.* 2021). Evaluation of pH on the edible film of kaffir lime leaf extract was observed for four weeks. The pH test is carried out every week, and the results are always stable every week; there is no change in pH, which means this formula is stable in room temperature storage. (Asridiana & Thioritz, 2019). The standard salivary pH value in the mouth is 7 (Tanabe *et al.* 2013). The results of the pH checks consecutively every week, namely Formula I and Formula II, have the same pH value of 5.3 while the highest pH is in Formula III 5.7. Formulas that are suitable for oral pH are only in formula III.

Thickness

The edible film printing process using a mold with a flat surface produces different thicknesses for each formula. The build-up of layers causes the consistency with a more significant value during repeated printing. The thickness results affect the value of the standard deviation or the value of the distribution of the formula in each measurement, and the number of varying thicknesses causes different contents in each formula. Based on the study results, it was shown that only formulas I and II met the requirements for the thickness of the edible film, namely a thickness of less than 0.25 mm

(Harmely, 2015). The thickness of the edible film is influenced by the area of the mold, the volume of the solution, and the total amount of solids in the solution (Putera, 2017). The higher the concentration of corn starch causes the thickness of the edible film increase. The more corn starch used, the more polymer structure makes up the film to produce a thicker film. Making edible films using polysaccharides, the higher the concentration of the constituent polymers at a specific limit can increase the thickness and stability of the edible film (Amaliya & Putri 2014).

Panelist's Preference Test

All formulas stated moderate organoleptic, and formula III's preferred freshness result. This is influenced by the thickness of formula III, which can be seen in table 2, which has a different value of 1.8 mm. According to Siregar and Saleh (2010), thickness affects the substance content of each formula. In addition to the thickness, the panelists considered that the organoleptic formula III gave a good taste.

Conclusion

Kaffir lime leaf extract with an extract concentration of 25% in each formula can be made edible film preparations using corn starch and cassava starch as hydrocolloid formers.

The results of the test of the physical properties of the edible film of kaffir lime leaf extract showed that its physical properties met the physical properties requirements according to the comparison product, but for the pH test, only the formula with the use of cassava starch as a hydrocolloid former that met the oral pH. The preference test showed that the panelists preferred the edible film with the composition of cassava starch as a hydrocolloid former.

Conflict of Interest

There was no conflict of interest.

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