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by Suryanto_Andi

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Utilization of Kalamasi Orange Peel Extract as a Formulation Edible Film Mouth Freshening

Andi Suryanto¹, Novy Saputri², Fhika Wulandari³, Mustafiah⁴

^{1,2,3,4}Department of Chemical Engineering, Faculty of Industrial Technology Universitas Muslim Indonesia Makassar, 90231



ABSTRACT: An accumulation of bacteria leads to an unbalanced level of oral acids, which results in non-fresh breath. It is anticipated that these issues will be solved by the flavonoids in Kalamansi orange peel acting as an antibacterial in edible films. This study was carried out to ascertain the composition of Kalamansi orange peel extract as an inventive mouth freshener edible film and to ascertain edible film formulation that satisfied the characteristics evaluation test and organoleptic test as a mouth freshener. Kalamansi orange peel was extracted by maceration with 70% ethanol which was then analyzed for its content. The edible film formulation was made into 5 formulation (0-4%). Each of which was tested for characteristics and organoleptic evaluation. The results showed that the content of Kalamansi orange peel extract met the requirements to be formulated in edible films, ethanol content test, yield, moisture content and qualitative tests of flavonoid compounds. The characteristic evaluation test, in each formulation of F0-F4 has reached the corresponding requirements. As for the evaluation test of thickness characteristics of 0.22-0.32 mm, pH of 6.72-7.25, disintegration time of 11-35 seconds and uniformity weight of 100-105 mg. In organoleptic tests, the panelists preferred formulation of edible film was 0% (without Kalamansi orange peel extract). In addition, there are significant differences between edible film (0-4%) and control (K+) in the parameters of color, aroma, texture, taste and refreshing activity.

KEYWORDS: Edible Film, Ethanol, Flavonoid, Kalamansi Orange Peel, Maseration

I. INTRODUCTION

Oral condition is something that requires special attention. Protecting a healthy oral cavity is the same as protecting a healthy body. Protecting oral condition can reduce the risk of infectious disease [1]. Oral condition related to unhealthy breath that can arise because of various factors, such as amass of *Streptococcus* *airus* bacteria [2]. Bacteria will produce volatile sulfur compounds (VSCs) which will disrupt the acid balance in the mouth that triggers unhealthy breath [3]. Non-fresh breather can be reduced by using mouthwash. However, long-term use of mouthwash has side effects such as dry mouth, decreased saliva production that affects bad breather and increases the risk of tooth decay [4].

WHO (World Health Organization) recommends traditional medicine in the national health care program because it is free from harmful side effects, easy to get, cheap and safe [1]. Therefore, it is necessary to innovate to overcome affordability, increase practicality, and create innovations that can be planned as edible films.

Edible film is a thin layer that can dissolve in the mouth when exposed to saliva. Edible film they have proven it to be safer to be consumed and does not cause side effects because it comes from natural ingredients, unlike mouthwashes [5].

A natural ingredient that can be applied is the peel of citrus crops. Citrus crops are one fruit that is in great demand by the people of Indonesia, which continues to develop by 70-80%. However, this growth also has a negative impact to utilizing orange peels that have not been used to the fullest. Citrus peels have many efficacious ingredients, such as flavonoids, that have antibacterial activity. One type of citrus crop peel that can be used is Kalamansi orange peel, which is expected to be an alternative as an edible mouth freshening film [6].

Analysis on flavonoid compounds on edible film to avoid non-fresh breath has been carried out. As research directed by [3], the benefit of celery leaves containing flavonoid compounds with a concentration of 2.5% and a research directed by [7], using kasturi bark with a concentration of 0.94% in edible film which also contains flavonoid compounds can reduce the problem of not fresh breath. However, there are no research on the benefit of Kalamansi orange peel. Flavonoid compounds in Kalamansi orange peel amounted to 10,958 mg/ml [8]. So, it is able to avoid the growth of bacteria that cause breather is not fresh. This

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research was conducted to measure the content of Kalamansi orange peel extract as an innovation of edible oral freshening film and to measure the formulation of edible film Kalamansi orange peel extract that meets the characteristic evaluation and organoleptic test as a mouth freshener.

II. EXPERIMENTAL SECTION

Materials

The materials used in this research were Kalamansi orange peel extract, 70% ethanol, gelatin, glycerol, sorbitol, glucose, peppermint oil, menthol, and aquadest.

Instrumentation

The instruments used were a sieve, petri dish, analytical balance, maceration bottle, oven, desiccator, simple distillation device, breaker glass, alcohol meter, magnetic stirrer, hot plate, edible film mold, micrometer scrup and pH meter.

Procedure

Sample preparation and Kalamansi orange peel extract

The analysis method started with the preparation of the Kalamansi orange peel samples, which were removed from the impurities and then dried. After drying, the Kalamansi orange peel is first cut into pieces and then mashed using a blender and it carried the process out with a 100 mesh sieve. Next, the maceration extraction process was carried out by taking as much as 500 grams of the sample powder and then putting it into a maceration bottle using 1 liter of 70% ethanol for 3×24 hours. It then evaporated the maceration results that have been obtained with the solvent composition through a simple distillation process, with the temperature being maintained at 70-78°C. Then again, the remaining solvent was evaporated using an oven at a temperature of 70-78°C.

Content Analysis of Kalamansi Orange Peel Extract

Ethanol Content Test

Testing ethanol standards to determine ethanol standards in the resulting extract. Performed using an alcohol meter. Require ethanol content is not more than 0.5% [9].

Amendment Results

The extract yield can be calculated by comparing the substance of the Kalamansi orange peel extract got with the initial sample substance. The assignment is used to get the yield of the amendment [10],

$$x = \frac{W1 \text{ (gr)}}{W2 \text{ (gr)}} \times 100\% \dots\dots\dots(1)$$

where x is the initial of amendment, W1 is the extract weight and W2 is the original sample weight.

Extract Moisture Content Test

Testing the moisture content of the extract is carried out using the oven method. 1 gram of Kalamansi orange peel extract was weighed. Then the oven extracts at a temperature of 105°C for 3 hours after which it is cooled with a desiccator for 15 minutes. Do this step until a constant weight is obtained on the extract sample. Determination [10] is used to get moisture content,

$$\text{Moisture Content (\%)} = \frac{(W1-W2)}{(W1-W0)} \times 100 \% \dots\dots(2)$$

where W0 is the empty cup weight, W1 is the cup weight + extract and W2 is the cup weight + extract (constant)

Qualitative Test of Flavonoid Compounds

Testing contain flavonoid compounds in Kalamansi orange peel extract was carried out by taking as much as 0.2 grams of extract samples into petri dish, then adding Mg powder as much as 0.1 grams and a few drops of HCl 2N. The existence of an orange color change in the sample indicates flavonoid compounds [11], [12].

Edible Film Kalamansi Orange Peel Extract

In making edible films, it dissolve the first step beef gelatin using aquadest and stirred on a hot plate with a temperature of ±60°C for 15 minutes. In the second step, add a mixture containing glycerol, sorbitol, glucose, menthol, Kalamansi orange peel extract and the remaining aquadest into a thick gelatin polymer solution and fuss until a homogeneous edible solution is formed. In the third step, add peppermint oil into the edible solution and stir until homogeneous. In the fourth stage, let the edible solution stand at a temperature of space to remove air bubbles to facilitate the edible film printing process. In the fifth stage, pour the edible solution into the cavity to be dried using an oven at room temperature for 24 hours. The last step, after the edible film is dry, it is removed from the cavity and cut to a size of 2 × 3 cm. Edible film formulation (Table 1).

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Table 1. Edible Film Formulation of Kalamansi Orange Peel Extract

Materials	Formula (% b/b)				
	F0	F1	F2	F3	F4
Kalamansi Orange Peel Extract (gr)	0	1	2	3	4
Gelatin (gr)	5	5	5	5	5
Glycerol (gr)	2	2	2	2	2
Sorbitol (gr)	2	2	2	2	2
Glucose (gr)	3	3	3	3	3
Menthol (gr)	0.2	0.2	0.2	0.2	0.2
Peppermint Oil	q.s	q.s	q.s	q.s	q.s
Aquades Ad	100	100	100	100	100

It carried examination of additional materials contained in the formulation of edible film out according to Farmakope Indonesia 4th Ed and Handbook of Pharmaceutical Exipientas 2nd Ed.

Characteristics Evaluation of Edible Film

Determine the quality of edible film as a mouth freshener has not been determined so that the quality test of SNI (Indonesian National Standard) confectionery and the results of measuring edible film on the market are used as a comparison or control and previous studies.

Film Thickness

Thickness evaluations are carried out using a micrometer scrup. This measurement is carried out by taking one random piece of edible film on each formulation, measurements are made on the left, middle and right sides of the edible film. Thereafter, record each edible thickness of the measured film. Do three repetitions of thickness measurements to get the average value for each formulation. The requirement for the thickness of edible film is a maximum of 0.41 mm [13].

Film pH

pH evaluation is carried out to determine the resulting pH does not cause irritation in the oral cavity. This evaluation is carried out by placing pieces of edible film on a petri dish, then dissolved using an aquades of 10 mL and allowed to stand for 5 minutes and then restrained using a pH meter. Subsequently, record each restrained pH edible film. Perform three repetitions of pH measures to obtain the average value of each formulation. The requirement for edible film pH is pH 5.5-7.9 [14].

Film Disintegration Time

Measurements of the shattering disintegration time are carried out to indicate the process of disintegration of edible film inside the mouth. This measure was carried out by placing pieces of edible film on a petri dish and then dissolved using a phosphate of pH 6.8 as much as 10 mL. Use the stopwatch tool to see the time it takes for the edible film to crumble. Perform three repetitions of the crushed time measure to obtain the average value on each formulation. As for the time requirement for the destroyed edible film, it is only 1 minutes [15].

Film Weights Uniformity

Measurement of weight uniformity is carried out to determine if there is a weight imbalance in each edible film. This measurement is made by taking three pieces of edible film at random in each formulation, then weighing each weight of edible film. Thereafter, record each edible weight of the measured film. Do three repetitions of weight measurements to get the average value for each formulation. The requirement for uniformity of weight of edible film is a maximum of 132 mg [16].

Organoleptic Tests

Organoleptic testing was carried out practicing 15 panelists to assess edible film extract of Kalamansi orange peel on five parameters, color, aroma, texture, taste and refreshing activity. The organoleptic data obtained were then processed applying the non-parametric test of the Kruskal-Wallis test and continued with the Mann-Whitney test.

III. RESULTS AND DISCUSSION

Content Analysis of Kalamansi Orange Peel Extract

Ethanol Content Test

The ethanol content test results is 0.3%. This content in the extract has met the standards set by the fatwa MUI (Majelis Ulama Indonesia), food or beverage products that contain ethanol levels of at least less than 0.5% meaning they

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can be consumed. Kalamansi orange peel extract can be added to edible film because it is safe for consumption and does not harm health [9].

Amendment Results

The yield results in the extract produces an amendment of 12.15%. The resulting amendment results have met the requirements for the optimal extract to be consumed, >10% [10]. This has been under the research conducted by [17], the use of 70% ethanol solvent causes the extract to be extracted because the water content in the solvent can activate the cells in the pattern so that the solvent can penetrate faster and the extraction process can take place more efficiently. In addition, particles of dry Kalamansi orange peel with a size of 100 mesh cause the surface state of the substance to increase more. This is under research [7], that the finer and smaller the particle size, the more pores formed in the sample will increase in the area of contact between solids and solvents in the extraction process so that the extract results can be optimal.

Extract Moisture Content Test

The results of the extract moisture content test is 9.30%. This is under the theory established by [10], that the determination of a good water content is <10%. The determination of water content aims to prevent the growth of fungi on the extract. Mushrooms can grow on extracts that exceed the standard. The determination of the water content used is a general specification for extracts because the specification for determining the moisture content for Kalamansi orange peels does not yet have a determination.

Qualitative Test of Flavonoid Compounds

The qualitative test results of flavonoid compounds show that Kalamansi orange peel extract contains flavonoid compounds. This is because of the sign of the change in the extract's color to orange [12]. The presence of flavonoid content as antibacterial activity in the extract can be interpreted that the extract has flavonoid compounds has the ability as an antibacterial compound. This is under the research conducted by [11].

Characteristics Evaluation of Edible Film

Table 2 shows the characteristics evaluation for each formulation in this research. Where F0, F1, F2, F3 and F4 is the initial for each formulation and K+ is the initial for edible film control.

Table 2. Characteristic Evaluation Edible Film of Kalamansi Orange Peel Extract

Characteristic Evaluation	Formula					
	F0	F1	F2	F3	F4	K+
Thickness (mm)	0.22 ± 0.02	0.25 ± 0.02	0.29 ± 0.02	0.31 ± 0.02	0.32 ± 0.03	0.01 ± 0
	7.25 ± 0.03	7.16 ± 0.05	7.11 ± 0.06	6.91 ± 0.04	6.72 ± 0.03	6.94 ± 0.01
pH	20 ± 2.00	24 ± 1.53	27 ± 1.53	32 ± 2.08	35 ± 1.15	11 ± 1.53
	100.53	101.33	101.94	103.60	105.29	96.51
Destroyed Time (second)	± 0.03	± 0.18	± 0.44	± 0.61	± 0.08	± 0.00
	Weight Uniformity (mg)					

Film Thickness

Based on table 2, it shows that the results of evaluate thickness characteristics on experimental and control film edibles have met the thickness requirement of no more than 0.41 mm. Each formula has a variation in thickness because of the difference in the concentration of extracts in each formula. This is under the research conducted by [18]. The higher concentration of the extract, the thicker the edible film produced. In addition, although the concentration of gelatin polymer for each formulation is the same, the printing process that is carried out (solvent casting) results in several sides of the edible film having differences. The edible film control uses advanced tools and methods so that the resulting thickness does not be different on each side.

Film pH

Based on table 2, it shows that the results of the evaluation of pH characteristics in edible film have met the set pH requirements, pH 5.5-7.9. It is also not much different from edible film control so that the edible film of each formulation is safe for consumption and does not irritate the oral cavity. Edible film that has a pH that is too acidic or does not meet the requirements can facilitate the growth of bacteria. The difference pH in each formulation can be influenced by the level of

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concentration of Kalamansi orange peel extract. This is under research conducted by [5], that the higher the concentration of extracts, the lower the pH produced. Meanwhile, there are differences in Standard Deviation (SD) caused by the sensitivity of the pH meter used and the edible film storage process.

Film Disintegration Time

Based on table 2, it shows that the results of evaluate the characteristics of crushed time on experimental and control film edibles have met the requirements. The crushing time may vary depending on the formulation and printing method of edible film, the requirement of edible film crushing time is a maximum of 1 minute. The crushing time test is expected to indicate the time at which the edible film undergoes disintegration in the oral cavity. In addition, the difference in crushing time is because of the thickness of each edible film. This is under research conducted by [3], that the smaller the thickness of the edible film, the faster the destruction time. This also has an influence on the resulting Standard Deviation (SD).

Film Weights Uniformity

Based on table 2, it shows that the results of the evaluation of crushing time characteristics in experimental and control film edibles have met the weight uniformity requirement of no more than 132 mg. There is a difference in weight in the comparison edible film because of its thinner texture when compared to the experimental edible film so that it affects the weighing process. As for the Standard Deviation (SD) value, it has different results. This is because when the cutting process is done, the edible film has a different weight This is under the research conducted by [3].

Organoleptic Tests

Table 3 shows the results of organoleptic with Mann-Whitney test. This test for compare edible film on this research (F0-F4) and edible film control (K+).

Table 3. Results of organoleptic processing using the Mann-Whitney test

Assessment	K+				
Parameters	F0	F1	F2	F3	F4
Color	0.02	0.00	0.00	0.00	0.00
Aroma	0.00	0.00	0.00	0.00	0.00
Consistency	0.01	0.01	0.00	0.00	0.00
Taste	0.00	0.00	0.00	0.00	0.00
Refreshing Activity	0.00	0.00	0.00	0.00	0.00

Asymp Sig.
or p value

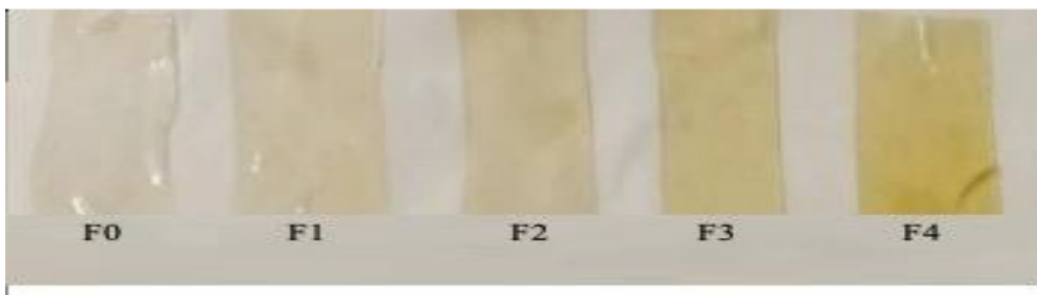


Fig 1. Edible film on any formulations

Based on table 3 shows the data of the Mann-Whitney test results on the Asymp Sig. or p value < 0.05. This means that each edible film (F0, F1, F2, F3, F4) and edible film control (K+) on the market has a significant difference. Research assumptions regarding the significant differences between edible films on the market and experimental edible films:

The difference influences the difference in color intensity in edible film add Kalamansi orange peel extract concentration in each variable. The higher the concentration of the extract, the color on each edible will also be darker brownish. Edible control film is most preferred because it has more attractive colors. So, the research assumption is the need to add coloring agents to the experimental film edible.

The high temperature influences the difference in aroma in edible film in making edible film. Although concentrate mint aroma is the same, the mint addition process carried out at a temperature of $\pm 60^{\circ}\text{C}$ can remove the aroma of menthol when it

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has formed into edible film. The research assumption is a change in the method by which it carried the addition of mint in the edible film solution out at room temperature.

The consistency of the material influences the difference in texture in experimental edible film and edible control film. The texture of the material may change because of the material storage process and the temperature in making edible film. The research assumption is that it only used a temperature of $\pm 60^{\circ}\text{C}$ for gelatin dissolution, while it carried the addition of other materials out at room temperature.

The difference in taste in edible film experiments was influenced by the difference in the concentration's addition of Kalamansi orange peel extract. The higher the addition of extract, the more bitter the edible film will taste, which will reduce the panelist's favorability level. Panelists' tongues are more sensitive and sensitive if they consume bitter foods. The research assumption is that it is necessary to increase the concentration of sweeteners or decrease the concentration of the added Kalamansi orange peel extract.

The difference in refreshing activity has something to do with the edible taste of the film. The difference lies in the time the refreshing taste will last when edible film is consumed. Both have refreshing activities, but on edible experimental movies have refreshing activities that don't last long. The research assumption is that it is necessary to increase the concentration of menthol in edible film.

V. CONCLUSION

The Kalamansi orange peel extract content analysis involves met the specifications to be formulated in edible film including ethanol content test, amendment results, water content and qualitative tests of flavonoid compounds.

The evaluation test of edible film characteristics in each formulation of F0, F1, F2, F3 and F4 has reached the corresponding requirements. As for the evaluation test of thickness characteristics of 0.22-0.32 mm, pH of 6.72-7.25, disintegration time of 11-35 seconds and uniformity weight of 100-105 mg. In organoleptic tests, the panelists preferred formulation of edible film was 0% (without Kalamansi orange peel extract). In addition, there are significant differences between edible film (0%, 1%, 2%, 3% and 4%) and edible film control (K+) in the parameters of color, aroma, texture, taste and refreshing activity.

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