

Functional Characteristics of Marshmallow with *Kinang* (Chew of Betel) Extract Addition

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Abstract The research objective was to determine functional characteristics of marshmallow with *kinang* (chew of betel) extract addition which consisted of 10 treatment levels: E1 (10 %), E2 (20 %), E3 (30 %), E4 (40 %), E5 (50 %), E6 (60 %), E7 (70 %), E8 (80 %), E9 (90 %) and E10 (100 %) dissolved in 100 mL water used for gelatin soaking and 3 best treatments will be taken later. Determination of the best treatment was decided by organoleptic analysis based on the panelist preference. The three best treatments based on panelist preference were E4 (marshmallow + 40% *kinang* extract), E6 (marshmallow + 60% *kinang* extract) and E8 (marshmallow + 80% *kinang* extract). The testing conducted in the research were antibacterial analysis, antioxidant analysis and anti dental caries analysis, respectively. The best treatment was E8 (marshmallow + 80% *kinang* extract) having antibacterial activity of 2.15 mm, antioxidant activity of 2.78 mg/ml and dental caries value of 4.0 and caries inhibition percentage of 83%, respectively.

Keywords: *chew of betel extract, marshmallow, functional, antibacteria, antioxidant*

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1. Introduction

Kinang (chew of betel) is a mixture of ingredients such as betel leaf, areca nut, lime and gambier used for betel chewing activity. The habit of betel chewing had been done since long time ago in Asia regions [4]. Betel chewing activity in Indonesia culture usually can be seen during marriage ceremony and most society members believe that betel chewing activity can strengthen teeth and prevent caries occurrence. According to [15], the longer the panelists conduct betel chewing activity, the lesser the caries occurrence.

Ingredients mixture for betel chewing activity have different benefits. For instance, gambier has benefit as bacterial growth inhibitor of *Streptococcus mutans* and antioxidant [15], due to its catechin content with magnitude of 7 to 33% [7]. Areca nut has benefits as antibacteria and antioxidant [8] because it has tannin content with magnitude of 15% [6]. Betel leaf produces specific aroma because it contains essential oil with magnitude of 4,5 %. Betel lime has capability to change acid environment into alkali (bases) environment [2].

The main ingredients in chew of betel formulation are gambier and areca nut that have benefit as antibacteria. Several studies had been conducted in conjunction with gambier as antibacteria. Pambayun et al (2008) had extracted catechin from gambier (*Uncaria gambir* Roxb) in order to observe sensitivity of Gram positive bacteria [15]. Pambayun, et al (2007) had studied phenol content and antibacterial characteristics from several gambier

products extraction (*Uncaria gambir* Roxb) [14]. Several studies had been conducted in conjunction with areca nut as antibacteria. Ryan (2015) had studied selenium from seeds and roots of areca nut (*Areca catechu* L.) which fermented with consortium of *Acetobacter Saccharomyces* as the antiseptic for mouthwash [19].

Antibacterial content of gambier and areca nut are capable to inhibit teeth blighter bacteria, i.e. *Streptococcus mutans*, that can produce teeth caries [13]. Teeth caries is infectious disease that damage teeth structure (enamel) resulting in cavity of teeth [18]. Based on the previous statement, betel chewing activity has beneficial effect on teeth health, but its main drawbacks are impractical and capable to produce environmental pollution. This problem can be solved by producing functional food product, marshmallow added with chew of betel extract. Functional food is fresh or processed food that provides health benefit. *Marshmallow* is kind of soft candy made from mixture of sugar, glucose syrup, gelatin and flavoring ingredient [12]. *Marshmallow* has soft texture, can be bitten and not sticky in teeth when chewed, but it is easily melt in mouth. These characteristics are used as the consideration in producing of marshmallow product added with *kinang* extract.

2. Materials and Methods

2.1. Production of *marshmallow*

Production of *marshmallow* can be described as follows:

- a. Batter 1: gelatin of 30 g is soaked with water at ratio of water and chew of betel extract in accordance to the treatment with total volume of 100 mL for 10 minutes.
- b. Batter 2: 30 mL of sugar syrup and 150 g of granulated sugar are added with water up to 100 mL.
- c. Batter 2 is heated for ± 7 minutes at temperature of 105°C.
- d. Mixing of batter 1 and batter 2 by using *mixer at speed 5 until* homogenous for 10 minutes.
- e. Homogenous batter is put into moulding and it is left over for ± 4 jam.
- f. Cutting of *marshmallow* with dimension of 2 x 2 cm.
- g. *Marshmallow* is sprinkled with fine granulated sugar and maizena flour (1:1)
- c. The dilution sample was taken with magnitudes of 1 mL, 2 mL, 3 mL, 4 mL and 5 mL followed by addition of methanol solution;
- d. Sample of 2 mL was taken according to its dilution;
- e. The dilution sample is added with 2 ml of DPPH solution;
- f. Sample was vortexed until homogenous;
- g. Sampel was incubated at temperature of 37°C for 30 minutes;
- h. Absorbancy of sample was determined by using *spectrophotometer* at $\lambda=517$ nm;

$$\text{Percent of inhibition (\%)} = \frac{A_0 - A_s}{A_0} \times 100\%$$

where:

A_0 : Results of spectrophotometer at 0 minute.

A_s : Results of spectrophotometer at 30 minutes.

- i. After inhibition percentage value obtained, IC_{50} value could be calculated through straight line equation which relate dilutionconcentration and inhibition percentage. IC_{50} value was obtained from X value in the straightlineequation with Y value of 50. Straight line equation with dilutionconcentration as an axis andinhibition percentage as an ordinate is given in the following equation:

$$y = ax + b.$$

2.2. Organoleptic Test

Evaluation for aroma, texture, color, and taste of *marshmallow* was done organoleptically by using hedonic test. Testing was done to 30 somewhat trained panelists. Sampel was put on plate which is randomly given 3 digits code. Panelists were asked to give total preference assesment in term of color, taste, aroma, and texture by giving scores in 1-6 scale (1 = highly dislike, 2 = very dislike, 3 = dislike, 4 = like, 5 = very like, 6 = highly like). Results of organoleptical test were analyzed by using non-parametric statistics with *Friedman-Conover* test.

2.3. Test of Antibacterial Activity [5]

Bacterium of *Streptococcus mutans* was previously rejuvenated by taking bacterial inoculum on agar miring with magnitude of 1 Ose, put it into test tube containing nutrient broth of 9 mL and incubated for 24 hours at temperature of 37°C. Soft media production with ratio of 1.3 g nutrien broth and 1 g bacteriological agar was then added with 100 mL aquadest and heated at temperature of 100°C. Hard media production with ratio of 1.3 g nutrien broth and 1.5 g bacteriological agar was then added with 100 mL aquadest and heated at temperature of 100°C.

Soft media, hard media and equipments used for inoculation were sterilized at temperature of 121°C for 15 minutes. Equipments used for bacteria implantationis put within *laminar flow*. Soft media preparation, addition of 0.1 mL *Streptococcus mutans* bacterium at concentration of 10^9 sel/mL was put into soft media and followed by pouring into petri dish. After cold, 15 mL hard media was poured into petri dish and was awaited until harden. Well is made with 6 mm in diameter, 4 mm in depth and added *kinang* extract of 5 μ l. Petri dish was subsequently incubated within incubator at temperature of 37°C for 24 hours. Observation of antibacterial activity was shown by area of clear zone diameter developed in surrounding of *kinang* extract.

2.4. Antioxidant Analysis

Antioxidant activity was determined by using DPPH method as follows:

- a. Sample of 1 mL was taken and put into test tube;
- b. Methanol of 9 ml was added into test tube containing sample and the mixture solution was vortexed;

2.5. Test of Teeth Anticaries

The test for additional effect of *kinang* (chew of betel) formulation in marshmallow on teeth anticaries was done with in vivo method according [3]. Preparation of Exprimental Materials.

Kinang extract *marshmallowis* used according to specifid treatments. Samples in this research were white mice of Wistar strain (*Rattus norvegicus* L.). Inclusion criteria are male white mice, age in the range of 10 to 12 weeks, weight in the range of 100 to 150 grams and in healthy condition. Exclusion criteria are mice in physical and mentally disabilities condition. Sample numbers are 12 mice according to treatment. Marshmallow is given to mice by mixing samples into pellete with size of 1:1 (30 g: 30 g) per day and tap water is also given to mice.

a. Preparation of Experimental Animal

Preparation of experimental animal is started with acclimatization. Acclimatization is adaptation process to the change of environmental condition. Mice are acclimatited within research room for ± 1 week at room's temperature. Mice are put into 12 square cages made of plastics and wire. Each cage contains 1 mouse.

b. Inoculation of Sterptococcus mutans

Sterptococcus mutans prepared with *Colony Forming Unit* (CFU) of 10^9 cells/mL. After acclimatization phase, *Sterptococcus mutans* with magnitude of 0.2 ml is given orally to mouse's mouth by using pipette for three days. This treatment taken place for four months.

c. Euthanasia, preservation, jaw staining and sectioning

After 4 months, mice are given euthanasia treatment by putting them into airtight box and chloroform is given to mice for 5 minutes. Subsequently, two jaws of mice are taken by using *scalpel* and *blade*. These jaws are cleaned with 1-2 % ammonium hydroxide solution for about 30

minutes. Preservation is done by soaking the jaws in formalin for 24 hours in order to prevent decaying. Staining phase is done by soaking jaws in 1% *methylene blue* solution for about 4 hours. After the end of staining phase, jaws are cleaned and dried followed by *sectioning* of mice jaws by using *carborundum disc* attached on *straight handpiece*.

d. Caries Evaluation on Mice

Caries evaluation on 12 molar teeth of white mice of Wistar strain is done by using Keyes method. This method evaluates caries by observing caries lesi based on its depth with the aid of *USB Digital Microscope* 300 magnification from smoothsurface (buccal, lingual) and sulkal surface (pit and fisur). Keyes had classified caries depth into 4 parts as follows:

1) Email (E): Lesions of caries only reach email area.

2) *Slight Dentinal* (Ds): Lesions of caries already reach *dentino email junction* (DEJ) or limited dentin.

3) *Moderate Dentinal* (Dm): Lesions of caries already reach $\frac{1}{4}$ to $\frac{3}{4}$ dentin depth.

4) *Extensive Dentinal* (Dx): Lesions of caries already reach more than $\frac{3}{4}$ of dentin depth.

Caries numbers occurred in each of mouse teeth are noted according to caries depth.

3. Results

3.1. Organoleptical Test

3.1.1. Color

Data analysis of hedonic test in term of *marshmallow* color showed that critical value was less than F-table value so that no further test was conducted. Organoleptical test results for *marshmallow* color of kinang extract showed that the highest preference level score was 4.3 (like) and the lowest one was 3.0 (dislike).

3.1.2. Taste

Data analysis of hedonic test in term of *marshmallow* taste showed that critical value was less than F-table value so that no further test was conducted. Organoleptical test results for *marshmallow* taste were in the range of 4.2 (like) to 3.4 (dislike).

3.1.3. Aroma

Data analysis of hedonic test in term of *marshmallow* aroma showed that critical value was higher than F-table value so that further test was conducted. Panelists assessment to *marshmallow* aroma were in the range of 3.92 to 3.52. *Marshmallow* aroma with the highest score was found on E1 treatment (*marshmallow* with 10% chew of betel extract) and the lowest one was found on E10 (*marshmallow* with 100% chew of betel extract). Results of *Friedman-Conover* test can be seen in [Table 1](#).

3.1.4. Texture

Data analysis of hedonic test in term of *marshmallow* texture showed that critical value was higher than F-table value so that further test was conducted. Results of *Friedman-Conover* test can be seen in [Table 2](#).

Table 1. Results of Friedman-Conover test for marshmallow aroma

Treatments	Rank numbers	X=36
E10(100%boce)	627.6	a
E6 (90% boce)	760.3	b
E8 (80% boce)	769.5	b
E7 (70% boce)	788.3	b
E9 (60% boce)	912.8	c
E4 (50% boce)	996.5	d
E5 (40% boce)	105.5	e
E3 (30% boce)	1,055.5	e
E2 (20% boce)	1,097.3	e
E1 (10% boce)	1,164.5	f

*) boce = kinang extract.

Remarks: Numbers followed by the same letter in the same column are not significantly different.

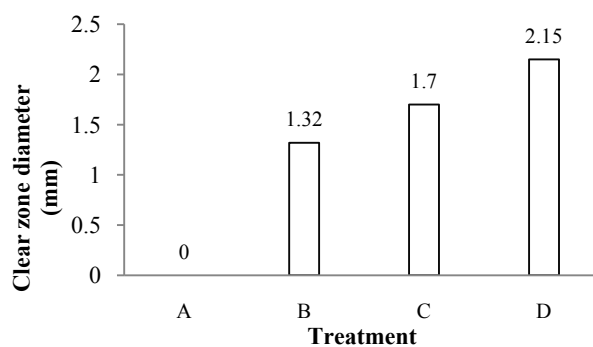
Table 2. Results of Friedman-Conover test for marshmallow texture

Treatments	Rank numbers	X=11,55
E10 (100 %boce)	102	a
E 6 (90 %boce)	668	b
E 8 (80 % boce)	794.3	c
E 7 (70 % boce)	842.8	d
E 9 (60 % boce)	872	e
E 4 (50 % boce)	1,001	f
E 5 (40 % boce)	1,030	g
E 3 (30 % boce)	1,119	h
E 2 (20 % boce)	1,166	i
E 1 (10 % boce)	1,193	j

Remarks: Numbers followed by the same letter in the same column are not significantly different/

3.2. Analysis of Antibacterial Activity

Results of antibacterial activity measurement can be seen in [Figure 1](#).



Remarks:

A: *marshmallow* with 0 % boce

B: *marshmallow* with 40 % boce

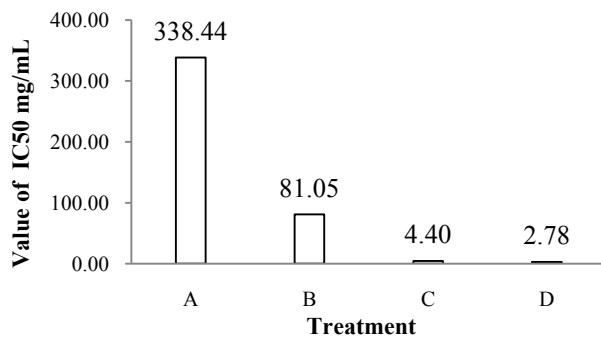
C: *marshmallow* with 60 % boce

D: *marshmallow* with 80 % boce

Figure 1. Antibacterial activity test

3.3. Antioxidant Analysis

[Figure 2](#) showed that the higher the addition of kinang extract to *marshmallow*, the lower the IC_{50} value which means high antioxidant activity. The strength of antioxidant activity on *marshmallow* can be classified as very weak because it had IC_{50} values in the range of 81.05 mg/ml to 2.78 mg/ml. Results of antioxidant test can be seen in [Figure 2](#).



Remarks:

A : marshmallow with 0 % boce

C : marshmallow with 60 % boce

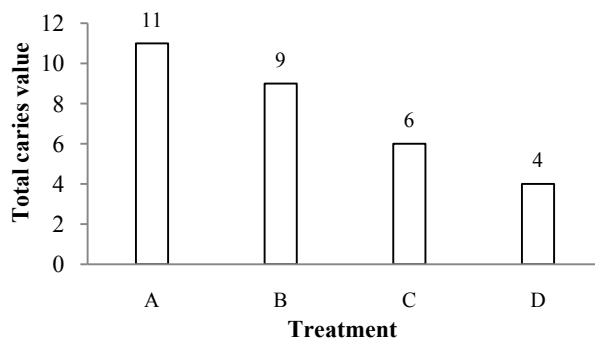
B : marshmallow with 40 % boce

D : marshmallow with 80 % boce

Figure 2. Analysis of antioxidant activity

3.4. Test of Teeth Anticaries

The objective of teeth anti-caries test is to determine marshmallow capability as teeth anti-caries. Results of teeth anti-caries test can be seen in Figure 3.



Remarks:

A : marshmallow with 0 % boce

C : marshmallow with 60 % boce

B : marshmallow with 40 % boce

D : marshmallow with 80 % boce

Figure 3. Test of teeth caries value in teeth enamel

The percentage of caries development prevention can be seen in Figure 4.

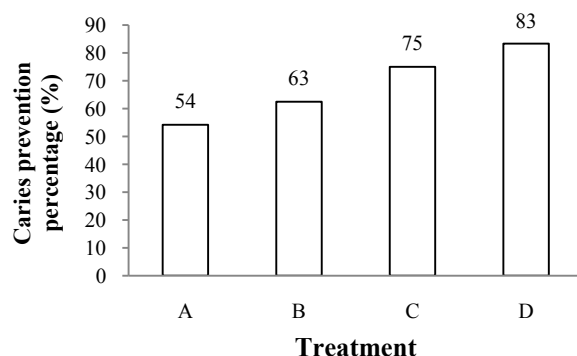


Figure 4. Percentage of caries development prevention

4. Discussion

Organoleptical test result basats of marshmallow with addition of chew of betel extract had effect on organoleptical values of color, taste, aroma and texture. Higher addition kinang extract results in lower preference

of color because the produced marshmallow had brownish red color. The brownish red color in kinang extract is affected by availability of gambier and areca nut which contain catechin and tannin. Kinang processing involved addition of betel lime that has pH higher than 7.0 or in bases condition [11]. Atechin has golden yellow color and it can be degraded at netral or basa condition into tearubigin compound [1]. Tearubigin is compound that produce brownish red color [22]. Catechin and tannin also have effect on taste. Catechin and tannin compounds can produce bitter and splinting tastes. Marshmallow produced in this research had specific taste due to addition of betel leaves in kinang. Distinctive sharp aroma of betel leaf is due to cavicol and betlephenol content in essential oil [20]. The addition of kinang extract on marshmallow production had effect on texture, i.e. the higher the addition results in more supple texture of marshmallow. Textural value of marshmallow can be due to ingredients formulation and production method. The low quantity of air trapped in mixing process results in hard texture of marshmallow.

Marshmallow with kinang extract addition can affect antibacterial activity and antioxidant activity. Catechin compound in gambier and tannin compound in areca nut are capable to inhibit *Streptococcus mutans* bacterium (Figure 2). According to the studies from [1,14] catechin compound in gambier and tannin compound in areca nut are capable to inhibit Gram-positive bacteria. The inhibition mechanisms of Gram-positive bacteria is occurred through bonding between catechin compound in gambier and tannin compound in areca nut with peptide units within peptidoglycan component of cells wall.

Peptidoglycan is component of bacteria cell wall that capable to shape strong bacteria cell wall in which bacteria capable to survive at different osmosis pressure condition than that of within cells. The formation of peptidoglycan is dibantu by transpeptidase enzyme [10]. Transpeptidase enzyme has function to combine one unit of peptidoglycan with other unit of peptidoglycan resulting in strong bacteria cell wall. Catechin and tannin are capable to inhibit activity of transpeptidase enzyme [9] that cause bacteria cell wall become weak which disrupt integrity of bacteria cell wall and subsequently cause leakage of bacteria cell wall. Leakage of bacteria cell wall causes disturbance of metabolisms process resulting in death of cells.

Catechin and tannin have capability as antioxidant (Figure 2) because both compounds are polyphenol group that have more than one hydroxyl group. The compound which contain hydroxyl group (-OH) will donate hydrogen atom (H) to radical compound or will change radical compound into more stable compound [15].

The F5 treatment (8 g betel leaf, 2 g betel lime, 3.5 g areca nut, 2.5 g gambier) with the highest antioxidant value of 2.78 had weak antioxidant intensity. According to [18], antioxidant intensities can be classified according to IC₅₀ values as follows: very strong (IC₅₀ < 0.05 mg/ml), strong (IC₅₀ = 0.05 - 0.1 mg/ml), medium (IC₅₀ in therange of 0.1 to 0.25 mg/ml) and weak (IC₅₀ in therange of 0.25 to 0.50 mg/ml).

Teeth anticaries test showed that the higher the kinang extract addition on marshmallow, the lower the total caries value on mouse's teeth (Figure 3). Caries is developed at enamel part of experimental mice teeth. Caries is

previously developed at enamel because enamel has higher chewing load than that of dentin part. Caries can be developed because *Streptococcus mutans* bacteria which exist in mouth are capable to ferment glucose into lactate acids, resulting in decrease of pH in mouth and subsequently produce demineralization of teeth enamel.

Demineralization is precipitation process of mineral ions from teeth enamel due to reaction of *hidroxyapatite* (HA) mineral which consisted of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Ion of H^+ at acid condition reacts with phosphate group and produce hydrogen phosphate (HPO_4^{2-}), whereas H^+ ions at the same time experience buffering which cause in balance condition of *hidroxyapatite* mineral followed by dissolution of *hidroxyapatite* mineral.

Marshmallow with *kinang* extract addition had effect as inhibitor anti caries because it contains catechin and tanin from *kinang* extract. Catechin and tanin had effect as inhibitor of teeth caries because catechin compound from gambier and tanin compound from areca nut are effective in inhibiting of *Streptococcus mutans* bacteria [14] (Figure 4).

The results showed that *marshmallow* with *kinang* extract addition can not prevent the caries occurrence, but only capable to inhibit the caries occurrence with the lowest value found on F5 treatment (8 g betel leaf, 2 g betel lime, 3.5 g areca nut, 2.5 g gambier).

5. Conclusion

Organoleptical test was conducted on *marshmallow* with *kinang* extract addition at concentrations of 40%, 60% and 80%. The best *marshmallow* was found on D treatment (*marshmallow* with 80% *kinang* extract) having antibacterial activity of 2.15 mm and antioxidant activity of 2.78 mg/ml, respectively. *Marshmallow* with *kinang* extract had brownish red color. Anticaries activity test on *marshmallow* showed that D treatment (*marshmallow* with 80% *kinang* extract) had high inhibition power with caries value of 4 and caries inhibition percentage of 83%, respectively.

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