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Inhibition effect of Rosella (*Hibiscus sabdariffa L.*) petal extract in Hard-Candy against *Streptococcus mutans*

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Abstract

Hard candy with the addition of roselle flower petal extract is considered an innovative product to improve the functional properties and sensory acceptance of roselle hard candy. The aim of the research was to determine the best concentration of rosella petal extract in hard candy that provided maximal bacterial effect against *Streptococcus mutans* and acceptable sensory. The research was arranged in Randomized Complete Block Design (RCBD) with six treatments and three replications. The treatments were the ratio concentration of rosella petal extract: water in the formulation for hard candy making (P). The Anova was performed and followed by LSD test at 5%. The results showed that different concentrations of rosella petal extract and water had a significant effect on total microbes, inhibition of *S. mutans*, and sensory analysis of rosella petal extract hard candy. The best treatment was formulation of P4 (rosella petal extract: water, 15: 85 mL). It produced rosella petal extract hard candy with total microbial of 1.5 x 10^2 cfu/g and *S.mutans* inhibition of 8.43 mm, and met the SNI 3547.1:2008 requirement standards, except for the reducing sugar content. It was concluded that the hard candy with the additional ingredient of rosella petal extract could potentially help prevent the formation of dental plaque.

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1. Introduction

Today's consumer's demand for candy is not only of their sweetness but also their impact on dental health. World candy consumption statistics reported that the highest candy consumption Per Capita is in Germany and Swiss, with an average of 28.7 and 25 pounds per year, respectively, while Indians and Chinese are much lower. Statist Research Department (2022) reports an increase of 2.8 Kg (+10.11%) of sugar consumption per capita within the next year in Indonesia (1). Free sugars are the essential dietary factor in the development of dental caries since dental caries does not occur in the absence of dietary sugars. Dental caries develops when bacteria in the mouth metabolize sugars to produce acid that demineralizes the hard tissues of the teeth (2). The manufacture of hard candy with the addition of roselle flower petal extract is considered an innovative diversification product to improve the functional properties and sensory acceptance of roselle hard candy. Hard candies are a liquid mixture of sucrose and corn syrup that is kept in an amorphous or glassy state by cooking at high temperature in which the majority of the water has been removed (3). It is a solid and glossy appearance, which it can be transparent or opaque. Hard candy has soluble properties when exposed to saliva, delivers flavors and sweetness. It is a popular food product with delicious, fun and a penchant for children's consumption. Based on data from the Indonesian

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Ministry of Health (4), children are one of the groups with a high DMF-T index, where the DMF-T National Index Result is 4.85. DMF (Decay, Missing, Filling)-T index is an index to see dental caries expressed as a number that shows the total number of teeth decayed (D), missing (M), or filled (F), tooth (T) in an individual or group of people, and is an important indicator in viewing the dental health status of the community (5). World Health Organization (WHO) states that the status of dental health in the community can be known by calculating the DMF-T Index. Preschoolers are one of the groups most vulnerable to dental caries due to a lack of self-behavior or habits that supports dental health while they eat too much sweet and sticky food. Basic Health Research (2018) reported that 61.27% of children aged 3 years consuming food and sugar-sweetened beverages (SSB) 1 time per day (6).

In some cases, hard candies could result in dental plaque. When chewed, sweet foods such as sucrose that remain on the teeth become a suitable substrate for oral bacteria such as *Streptococcus mutans* to grow and produce plaque. Sucrose can be broken down directly by extracellular bacterial inverses to form glucose and fructose molecules to produce extracellular polysaccharides (EPS) and intracellular polysaccharides (IPS) (7). These EPS and IPS have a dual function to form a structural matrix of dental plaque and a reservoir of substrate for plaque microorganisms. The functional structure of the matrix enables the plaque bacteria to adhere to the enamel surface (8). In addition, sucrose is fermentable, whereas cariogenic bacteria, i.e., *S. mutans* are able to ferment sucrose to lactic acid, which triggers the cause of dental caries by demineralization of the tooth structure. One of the factors that contribute to the emergence of dental caries is the excessive consumption of sugar (sucrose).

Additional roselle petal extract to hard candy may provide benefits in maintaining healthy teeth through its antimicrobial effect (9). The roselle petal extract has an antibacterial effect which is attributed to the flavonoids (10). Flavonoid has the ability to form a complex with the bacterial cell walls and permeability of bacterial cell surface to the extract. The mechanism of action involves the inhibition of electron transport protein translocation, phosphorylation steps and other enzyme-dependent reactions followed by increased plasma membrane permeability, resulting in the leakage of an ion from a bacterial cell (11). The antibacterial activity of roselle extract, either aqueous or aqueous-methanolic extract, has been studied over foodborne pathogen bacteria in vitro and in a food model (12). Aqueous extract of roselle petal flower had an inhibition effect on nosocomial infectious bacteria such as methicillin-resistant *S. aureus and Klebsiella pneumonia.* Aqueous-methanolic extract of the roselle calyces was found to exhibit an antibacterial effect against *S. aureus, B. stearothermophilus, Micrococcus luteus, Serratia marcencens, Clostridium sporogenes, E. coli, Klebsiella pneumonia, B.cereus, and Pseudomonas fluorescens* (13). *S. mutans*, a facultative anaerobic, gram-positive coccus, is usually found in the human oral cavity and is the causative agent of dental caries, even though other bacteria may be involved (14). The bacteria adhere and accumulate on the tooth surface by producing extracellular polysaccharides from the sucrose in the oral cavity, forming and developing a biofilm. Generally, mechanical cleaning by brushing and flossing can prevent biofilm formation but may be reluctant to reach by some for several reasons. Minimizing the number of *S.mutan* can be one way to prevent its colonization in the teeth. Therefore, rosella petal extract hard candy could be a suitable functional sweet food to help prevent the growth of *S. mutans* which causes dental caries. The objective of the study was to determine the most suitable level of roselle petal extract in hard candy that provides maximal antibacterial effect against *S.mutans*, as well as the most acceptable sensory characteristics. Production of hard candy in this study was not specifically made for children, but rather focused on the benefits of roselle petal extract added in hard candy to prevent the growth of *S. mutants* that inhabits dental plaque.

2. Materials and Methods

Dried roselle flower petals (*Hibiscus sabdariffa* L.) obtained from Indotama distributor, Malang, sugar, and liquid glucose. The chemicals used were PCA media, Blood Agar media, Lamb Blood, *S.mutans* culture (BPPV, Bandar Lampung, Indonesia), Luff scroll solution, paper discs with a diameter of 0.7 cm, and other analysis-supporting chemicals.

2.1. Extraction of Roselle Petals

Rosella petals extract was prepared with the maceration method using water solvent. Briefly, the petal of the dried roselle was separated and washed thoroughly with running water, air dried at room temperature and followed by coarsely grinding of the cleaned calyces. One hundred grams of coarsely ground petals were extracted by maceration using water (the ratio of water and petals was 6:1) in a beaker glass. The mixture was soaked for 25 min and then heated at 90°C on a hot plate for 15 min. Then, it was allowed to stand at room temperature until cool and filtered with sterile gauze in a measuring flask, forming 10% roselle petal extract.

2.2. Making of Hard Candy

Hard candy was produced by direct gas flame cooking following the work done by (15). The first was the mixing step, where samples were formulated with sucrose (80%, w/v) and added to water according to each formulation treatment (100, 95, 90, 85, 80, 75 mL), followed by the addition of 25% (w/v) of liquid glucose. The formula of six candy samples is specified in Table 1. The second was to heat the mixture of sugar while stirring to dissolve sugar, and when sugar was completely dissolved (sugar candy masses), heat further to cook to a boiling point up to 150° C (in about 15 to 20 minutes). Then let the sugar candy masses cool down until they reached about 100-105°C in order roselle petal extract to be added into the mass according to their respective formulations (0, 5, 10, 15, 20, 25 mL). The roselle petal extract was blended into the mass. After thoroughly mixing in the candy mass, then the hot mass was poured into silicone molds and then rapidly cooled to reach 20°C in the refrigerator to set the hot candy mass into a plastic state, and the hard candies were formed. The hard candies were removed and further cooled at room temperature to make them hard candy glassy. Hard candies were properly packed and stored at 10°C until used for analysis. Figure 1 shows the hard candy.

Table 1. formulation of Roselle petal extract hard candy.

Formulation	Treatment (formulation of water: Roselle extract)					
	P1 (20:0)	P2 (19 : 1)	P3 (18 : 2)	P4 (17 : 3)	P5 (16 : 4)	P6 (15 : 5)
White crystal sugar (g)	80	80	80	80	80	80
Liquid Glucose (g)	25	25	25	25	25	25
Roselle extract (mL)	0	5	10	15	20	25
Water (mL)	100	95	90	85	80	75

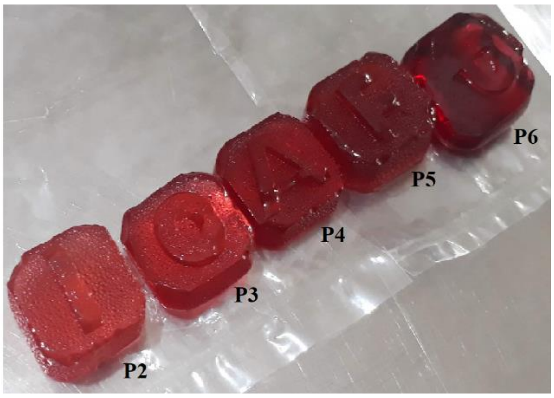

Figure 1. Candy is made based on the formulation.

2.3. Total Aerobic Count

The aerobic counts were enumerated by culturing on plate count agar (PCA, Difco). Weighed 25 g of sample of ground Roselle extract hard candy and put it in an Erlenmeyer which already contains 225 ml of diluent, to obtain a 1:10 dilution (16). After homogenizing, the sample was diluted into the concentration series. One mL of each dilution was planted with the appropriate surface plate calculation method on the media. Incubation continued for 24 hours at 32° C to grow aerobic bacteria.

2.4. Inhibitory Zone Measurement

The inhibitory zone of Roselle petals extract was determined by the agar disc diffusion technique/Kyrbi-Bauer (17). The procedure was as follows: 100 µL of washed and diluted bacterial cultures of *S. mutans* (10^7CFU/mL) were inoculated onto blood agar plates (BA, Difco) and spread onto agar. Sterilized paper discs (Whatman Grade 5, 7-mm diameter) were placed on the surface of the inoculated agar. Roselle extracts hard candy was mashed with a mortar and then put into a sterile Erlenmeyer containing 20 mL of sterile Ringer's solution according to the treatment. Then, 20 µL aliquots containing rosella petals extract hard candy were placed on each paper disk (final concentration of the extracts on disk: 2 mg). A rinses blank disk was used as a control. Treatments were performed in triplicate. Once the extracts and rinses were absorbed by the agar, inoculated plates were incubated at 32°C for 24 h. The diameter (mm) of the inhibitory zone was measured for each treatment and the average was obtained for each one.

2.5. Chemical Analysis

Chemical analysis of the hard candy followed the SNI 3547.1:2008 4 (18) for hard candy. It covers water content, ash, and reducing sugars. Water content (oven method) is weight lost during heating in an oven at 100 °C ± 2°C and is calculated gravimetrically. Ash content is calculated gravimetrically; the ash formed during combustion in a furnace at a temperature of 525°C forms white ash. Reducing sugar was assayed by Luff Schrool method, that reducing sugars such as glucose, fructose, maltose and lactose will be reduced in Luff Schrool solution to CuO_2. The amount of reducing sugar in Luff Schrool solution is determined by titration with sodium thiosulfate solution.

2.6. Sensory Evaluation

Sensory analyses were carried out to measure the degree of liking for the sample, Roselle extracts hard candy by hedonic test on the parameters of taste, aroma, color, texture and overall acceptability. Hedonic test was run by 20 untrained panelists who had or frequently consumed hard candy. Panelists were asked to give an assessment of the level of aroma, color, texture, and overall perception by giving a score according to their respective impressions. The 5-point Hedonic scale was 5 (like very much), 4 (like), 3 (neither like nor dislike), 2 (dislike slightly), and 1 (dislike very much) (19).

2.7. Data Analysis

This research was arranged in a Complete Randomized Block Design (RCBD) with six treatments and four replications. The ratio treatments of the concentration of rosella flower petal extract and water are (P1) 20:0, (P2) 19:1, (P3) 18:2, (P4) 17:3, (P5) 16:4, (P6) 15:5. The data obtained were analyzed for similarity in variance with the Bartlett test and the addition of the data was tested with the Tukey's test, then the data were analyzed for a variance to determine the effect between treatments. If there is a significant difference, the data are further analyzed with the Least Significant Difference Test (LSD) at the 5% level.

3. Result and Discussion

3.1. Total Microorganisms

The analysis of the variance of the total microorganisms of rosella extract hard candy with various treatments is shown in Table 2. The total microorganisms in P1 were significantly different from P6 ($p>0.05$), and P2-P5 were not significantly different. P1, which was hard candy without the addition of roselle petal extract as a control, contained a high number of microorganisms. P6 contained the lowest microbial loads, which could be caused by the high concentration of roselle petal extract added in to the hard candy formulation. This microbial reduction was caused by polyphenols which act as antimicrobial agents. Márquez-Rodríguez (20) reported that beef steak sprayed with rosella extract at increased concentrations between 250 and 1250 mg/L reduced the mesophyll and psychrophilic content due to polyphenol activity. In addition, the effect of Rosella petal extract on microbial inactivation was dose-dependent (21). Al-Hashimi (14) reported that Roselle flower aqueous and ethanol extract contained 77.2 mg/g and 87.7 mg/g of phenolic, respectively. This phenolic functions to inhibit the growth of microorganisms by forming complex compounds with proteins through hydrogen bonds and resulting in cell disruption due to an increase in cell membrane permeability.

In addition, the number of total plate counts (TPC) of Roselle flower petal extract hard candy was in the range of 1.33×10^2 and 2.02×10^2 cfu/g, while the maximum standards set by the hard confectionery SNI are 5×10^2 cfu/g. Therefore, rosella flower petal extracts hard candy is considered safe for consumption and is classified as good according to the standards of SNI 3547.1: 2008.

Table 2. The total numbers of microorganisms in rosella petal extract hard candy in various treatments.

Treatments	Total Number (cfu/g)
P1(water: Roselle extract = 20:0)	2.0×10^{2} a
P2 (water: Roselle extract = 19:1)	1.7×10^{2} ab
P3 (water: Roselle extract = 18: 2)	1.6×10^{2} ab
P4 (water: Roselle extract = 17: 3)	1.5×10^{2} ab
P5 (water: Roselle extract = 16: 4)	1.4×10^{2} ab
P6 (water: Roselle extract =15: 5)	1.2×10^{2} b

Note: The numbers followed by the same letters mean that they are not significantly different in the LSD test level of 5%.

3.2. Inhibition Effect

The analysis of variance showed that the treatments (P) concentration of roselle flower petal extract concentration significantly affected the inhibitory power of *Streptococcus mutans* ($p<0.05$) (Table 3). The inhibition occurred if the rosella flower petal extract was added ≥ 10.04%. The inhibition zone found in the experiment was in the range of 8.358-10.075 mm, which is categorized as a weak inhibition according to Greenwood inhibition zone classification (22). The treatment of P6 was the highest value of the inhibitory zone, which decreased as increasing the use of water in the ratio of water and roselle extract. Nevertheless, there was no significant effect against S. *mutan* among P3-P6, and P1 and P2 were significantly different from P3-P6. The inhibitory activity of the antimicrobial compound was influenced by several factors, including the concentration of the extract, the content of the metabolite compounds in the extract, the diffusion power of the extract, and the type of bacteria is inhibited. It could be that the low concentration of roselle petal extract in the formulation of P1 and P2 (less than 5 mL) did not cause the killing effect. On the other hand, the variation in the concentration of rosella petal extract between treatments at P3-P6 was small, causing an insignificant difference in killing power between treatments. This was in accordance with research conducted by Riwandy et al. (23) that roselle flower petal water extract inhibited the growth of *S.mutans* in vitro, and Elmanama et al. (24) reported that both aqueous and ethanol roselle flower petal extracts produced antibacterial effects against many other bacteria such as *Staphylococcus aureus, Bacillus stearothermophilus, Micrococcus luteus, Serratia mascences, Clostridium sporogenes, Escherichia coli, Klebsiella pneumoniae, Bacillus cereus* and *Pseudomonas fluorescence*. Fullerton et al. (11) explained that the inhibitory effect was caused by the interaction between phenolic compounds and bacterial cell walls, which resulted in increased cell membrane permeability and membrane damage resulting in the release of cell contents. Flavonoids have hydroxyl groups that can cause changes in organic components and nutrient transport that will cause toxic effects on *S. mutans.* Phenol functions as an antibacterial by changing cell proteins and damaging the plasma membrane of *S. mutans*. Tannin works by inhibiting the production of the enzyme *S. mutans*. Saponins have the effect of releasing proteins and enzymes from within *S. mutans* cells (25). In addition to this, the antibacterial mechanism of anthocyanin contained in the rossele petal extract works by entering the inner membrane and decreasing the activity of alkaline phosphatase (AKP), adenosine triphosphate (ATPase) and superoxide dismutase (SOD) in inhibiting the growth of pathogens (26).

Table 3. Inhibition effect of rosella petal extracts hard candy with different formulation treatments against *S. mutans.*

Formulation Treatments	Inhibition Zone (mm)
P6 (water: Roselle extract =15: 5)	10.07[a]
P5 (water: Roselle extract = 16: 4)	8.57[a]
P4 (water: Roselle extract = 17: 3)	8.43[a]
P3 (water: Roselle extract = 18: 2)	8.35[a]
P2 (water: Roselle extract = 19:1)	0[b]
P1(water: Roselle extract = 20:0)	0[b]

Note: The numbers followed by the same letters mean that they are not significantly different in the LSD test level of 5%

3.3. Sensory Analysis

The analysis of variance showed that the concentration of rosella flower petal extract did not significantly affect taste and texture preference but significantly affected the aroma and color ($p>0.05$) (Table 4). The preference score for the taste of hard candy of roselle extract produced in this study ranged from 3.51 (dislike slightly - 3.8 (like). The hedonic test for taste was aimed at determining the level of response of the panelists regarding the preference for hard candy with each concentration of roselle flower petal extract. It showed that the concentration of roselle flower petal extract was still accepted at a concentration of 15 mL, which may produce a candy with a sweet taste and not too sour. In this experiment, the sucrose level used was 80 grams and the liquid glucose was 20 grams for each treatment so that it could help reduce the acidity level of hard candy. The sour taste produced by roselle flower petal extract comes from ascorbic acid, citric acid, acetic acid, and formic acid, which ascorbic acid is quite large in content, about 6.7 mg/100g of dried petals (23). The sweet taste that arises from hard candy comes from sucrose and liquid glucose.

The average value of the panelists' preference level for the parameters of texture ranged from 3.37 and 3.78 (slightly). LSD test showed there were no significant differences ($p>0.05$). However, the increased concentration of roselle petal extract resulted in a lower value of the panelist's preference score for texture. The texture of the P6 has a sticky surface that the panelists gave the smallest score. A sticky surface is an early state of moisture sorption where the surface is sticky, but the bulk may still be in good shape (15). There are some factors that cause the surface sticky of the candy, such as the hygroscopicity of sugar and fructose, either from direct addition or sucrose inversion during cooking, tend to be sticky, and air adsorption effect on the candy surface through hydrogen bonding interaction. Nevertheless, the surface sticky in P6 was probably caused by the ratio of the concentration of roselle petal extract in the formula because the addition of acidic aqueous roselle petal extract to the candy mass could cause sucrose inversion and softening of the product. The soft texture of the hard candy makes it difficult to break when bitten and creates a sticky feeling on the teeth. This lowers consumer preferences. In a similar study on hard nutmeg candies (27), high invert sugar caused by the low pH (3.0) resulted in hard candy with a sticky texture. Hard candy is made based on a combination of sugar, sucrose, and glucose syrups. During the cooking process, water is removed, and the degree of inversion of sucrose increases. The product of inversion, glucose, and fructose, are hygroscopic and absorb water, inducing stickiness (28). On the other hand, when cooking hard candy, the water is removed, and the remaining water is tied to the sugar so that less water is left in the product, which results in a hard texture.

The Least Significant Difference (LSD) test showed that panelists' preference for the parameter of the aroma of P1 was not significantly different from P2 and P3 but that of different from the other treatments (p<0.05). Panelists' preference for aroma ranged between 3.21 and 3.44 (slightly). It was suspected that volatile compounds produced by roselle petal extract were not damaged or lost during processing but contributed to the aroma of hard candy. Dried rosella petals produce volatile compounds when they are soaked in a solution such as water. These compounds include furfural, 5-methyl furfural, and the one with the strongest aroma is 1-octane-3-ol and nonanal with groups of 4 aldehydes and 3 ketones (29).

The ANOVA test showed that the concentration of roselle petals extract in the ratio of water and roselle extract significantly affected the color score of hard candy. P1, P2, and P3 were not significantly different but significantly different, with P4, P5, and P6. The preferred color score ranges from 3.475-4.06 (slightly like to like), and the most preferred color was found in P4, which was hard candy made from a ratio of water and rosella extract (85:15). This produced hard candy with a red color (Fig 1). It is shown that the increased use of rosella petal extract in the hard candy formulation resulted in a dark red color. The color of hard candy can be caused by the presence of anthocyanin compounds in the rosella petal extract. Riaz et al. (10) reported that extracts of roselle petals are rich in anthocyanin, like delphinidin-3-sambubioside and cyanidin-3-siambubioside, which contributes to their red color. The opposite, when the ratio of water in the formulation increased, the color intensity decreased. Olaleye (13) reported that hibiscus anthocyanin, the natural phenolic pigment in dried roselle petals, is water soluble.

Overall acceptance is a combination of preference ratings on sensory attributes that can be used as a basis for the receipt of a product. The overall acceptance score of hard candy from rosella flower petal extract produced in this study ranged from 3.68 to 3.97 (somewhat like/neutral to like). The best overall preference was found in P4 with a score of 3.97 (like), while hard candy without the addition of rosella extract (P1) was the lowest score (3.68 like slightly).

Table 4. Hedonic score and overall reception of rosella petal extract hard candy.

Treatment	Taste	Aroma	Color	Texture	Overall Preference
P1	3.51±0.06^{a*}	3.21± 0.07^{c}	3.48± 0.31^{b}	3.57± 0.10^{a*}	3.68± 0.11^{b}
P2	3.71± 0.21^{a*}	3.23 ±0.14^{bc}	3.59± 0.23^{b}	3.74± 0.12^{a*}	3.91± 0.11^{a*}
P3	3.79± 0.13^{a*}	3.36± 0.12^{bc}	3.77± 0.11^{b}	3.74± 0.13^{a*}	3.93± 0.09^{a*}
P4	3.77± 0.10^{a*}	3.44± 0.06^{a*}	4.06± 0.13^{a*}	3.77± 0.25^{a*}	3.94± 0.11^{a*}
P5	3.8 ±0.03^{a*}	3.44± 0.07^{ab*}	3.95± 0.09^{ab}	3.78± 0.06^{a*}	3.97± 0.06^{a*}
P6	3.71± 0.10^{a*}	3.37± 0.07^{a*}	4.03 ±0.11^{a*}	3.37± 0.11^{a*}	3.97± 0.07^{a*}

Note: The numbers printed in Table were the average of the three replications with standard deviation. The numbers followed by the same letters in the same column mean that they are not significantly different in the LSD test level of 5%.

3.4. Chemical Analysis of the Best Preferred Roselle Extract Hard Candy

Determination of the best treatment or the most preferred product by the panelists based on the assessment of organoleptic parameters. Each assessment parameter organoleptic (color, taste, and texture) are considered to have the same proportions in determining the most preferred hard candy product by the panelists. A recapitulation of the organoleptic assessment of all compositions can be seen in Table 5. The composition of the ratio of water: roselle extract was 85 ml: 15 g (P4) was assessed as the best formulation or the most preferred treatment by the panelist. The color of this composition can be seen in Figure 1.

Table 5. A recapitulation of organoleptic assessment.

Treatment	Assessment of organoleptic parameters				
(water: roselle extract)	Taste	Aroma	Color	Texture	Average
P1 (20:1)	3.51	3.21	3.48	3.57	3.44
P2 (19:1	3.71p	3.23	3.59	3.74	3.57
P3 (18:2)	3.79	3.36	3.77	3.74	3.665
P4 (17:3)	3.77	3.34	4.06	3.77	3.74
P5 (16:4)	3.8	3.34	3.95	3.78	3.71
P6 (15:5)	3.71	3.37	4.03	3.37	3.62

The best sample was then analyzed for its water content, ash, and reduced sugar level (Table 6). It showed that the water content in roselle petal extract hard candy, 1.96%, was in accordance with SNI 3547.1: 2008 standards and European standards on the quality requirements for finished hard candy. This was obtained from hard candy, which was formulated by the addition of 15 mL of rosella flower petal extract. The result was also within the range reported for hard candy, in which water content is approximately 0.20 to 0.30 (3,15). The water content was influenced by the temperature of hard candy making. The sugar solution used in the making of hard candy will harden by heating at high temperatures and reducing the water content. When sucrose is evaporated, the boiling point concentration increases. If this phenomenon continues, all the water evaporates and makes the sucrose melt. Generally, hard candies are transparent to translucent with moisture below 2/100 g and soluble solids between 97 and 98/100 g (15).

Table 6. Chemical analysis of the best-preferred rosella petal extracts hard candy based on SNI.

Chemical Analyzed	Sample	SNI-3547.1: 2008	Recommendation	Finished hard candy (European)*
Water content (%)	1.96	Max 3.5	Satisfy	3.5; (2.1-5.1 range)
Ash content (%)	0.1035	Max 2	Satisfy	
Reducing sugar (%), sucrose	51.04	Max 24	Not satisfy	49.2;(31.7-87.7 range)
Fructose (%)				2.1; (0.2-8.6 range)
Glucose (%)				6.7; (1.1-12.4 range)
Maltose (%)				7.0; (0.7-33.2 range)
Higher saccharides				30.4;(12.9-44.9 range)

*Note: *Smidova et al. (2003)*

Another quality requirement for food products is ash content. Ash is an organic substance, a waste product combustion material. Usually, this component consists of potassium, calcium, sodium, iron, manganese, and magnesium. The ash content of rosella extract hard candy, 0.1035% (db.), was in accordance with SNI 3547.1: 2008 standards on the quality requirements of hard candy. The addition of rosella flower petal extract to the formulation may influence the level of ash in hard candy. The mineral content of rosella flower petals, such as–calcium 160 mg, phosphorus 60 mg, and iron 3.80 mg (10), may contribute to the high ash content in hard candy.

The reducing sugar level of the rosella flower petal extracts hard candy was 51.04%, which did not meet the requirements of reducing sugars either SNI 3547.1: 2008 standards on hard candy quality requirements (Max 24% mass faction) or European Finished hard candy (3). Moisture loss and inversion of sugar were thought as the major reasons for the increase in reducing sugar. Meanwhile, almost all of the content in hard candy was carbohydrates, including sucrose and rosella flower petal extract. Sucrose will be inverted due to the presence of heat into glucose and fructose. Rosella flower petal extract contains pectin, which is a complex carbohydrate or polysaccharide, in which the presence of acid from rosella petal extract and heating causes disaccharide inversion processes. The boundary pH value at which the inversion rate of sucrose grows rapidly is pH = 2.8 (28), and reducing sugar levels are strongly influenced by carbohydrate content in raw materials. Low pH and high temperature accelerate sucrose inversion. Hard candies with high reducing sugar content can be highly hygroscopic and have short shelf life because it melts easily.

4. Conclusions

Rosella petal extract in hard candy has antimicrobial activity against *S. mutan*, so hard candy can help prevent dental plaque formation. Hard candy with the additional ingredient rosella petal extract of 15 mL and 85 mL water (P4) was the most preferred by the panelists. It had a score of texture, aroma, taste, color, and overall acceptance of 3.665 (like), 3.453 (somewhat like), 3.765 (like), 4.06 (like), and 3.970 (like), respectively. The rosella petal extract hard candy had the chemical characteristic of water, ash, and reducing sugar content of 1.95%, 0.1035% (dB), and 51.04%, respectively. In addition, the hard candy had inhibitory potential against *S. mutan*.

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Author Contributions

M.E.K conceived and designed the experiment; S.U.N contributed reagents and analysis tools; D.P.L analyzed data; M.E.K, S.U.N and A.P. performed experiments; M.E.K wrote the paper.

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Not applicable

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Warrel. Candy Consumption Around the World _ The Warrell Corporation. Warrel Creations. Warrel Creations; 2017.
2. Green M, Hadihardjono DN, Pries AM, Izwardy D, Zehner E, Huffman SL. High proportions of children under 3 years of age consume commercially produced snack foods and sugar-sweetened beverages in Bandung City, Indonesia. Matern Child Nutr. 2019;15(S4):e12764.
3. Šmídová I, Čopíková J, Maryška M, Coimbra MA. Crystals in hard candies. Czech J food Sci. 2020;21(5):185–91.
4. Badan Pengawas Obat dan Makanan Republik Indonesia. Peraturan Badan Pengawas Obat dan Makanan Nomor 31 Tahun 2018 tentang Label Pangan Olahan. Badan Pengawas Obat dan Makanan. 2018;53:1689–99.
5. Napitupulu RLY, Adhani R, Erlita I. Hubungan Perilaku Menyikat Gigi, Keasaman Air, Pelayanan Kesehatan Gigi Terhadap Karies Di Man 2 Batola. Dentin. 2019;3(1):17–22.
6. Susilawati MD. Consumption and health impact of sugar beverages in Indonesia based on data SKMI 2014. J Vokasi Kesehat. 2019;5(2):83–8.
7. Anderson CA, Curzon MEJ, Van Loveren C, Tatsi C, Duggal MS. Sucrose and dental caries: a review of the evidence. Obes Rev. 2009;10(1):41–54.
8. Baena-Santillán ES, Piloni-Martini J, Santos-López EM, Gómez-Aldapa CA, Rangel-Vargas E, Castro-Rosas J. Comparison of the antimicrobial activity of Hibiscus sabdariffa calyx extracts, six commercial types of mouthwashes, and chlorhexidine on oral pathogenic bacteria, and the effect of Hibiscus sabdariffa extracts and chlorhexidine on permeability of the bacterial membrane. J Med Food. 2021;24(1):67–76.
9. Indriani L, Dharmautama M. Antimicrobial test of roselle (hibiscus sabdariffa l.) ethanol extract againts porphyromonas gingivalis and streptococcus sanguis using agar method (in vitro study). J Dentomaxillofacial Sci. 2016;1(2):134–8.
10. Riaz G, Chopra R. A review on phytochemistry and therapeutic uses of Hibiscus sabdariffa L. Biomed Pharmacother. 2018;102:575–86.
11. Fullerton M, Khatiwada J, Johnson JU, Davis S, Williams LL. Determination of antimicrobial activity of sorrel (Hibiscus sabdariffa) on Esherichia coli O157: H7 isolated from food, veterinary, and clinical samples. J Med Food. 2011;14(9):950–6.
12. Sutiany A, Dwiyanti RD, Oktiyani N. Inhibition Strength of Rosella (Hibiscus sabdariffa L.) Boiled Water on Salmonella typhi in vitro. Trop Heal Med Res. 2019;1(2):62–7.
13. Olaleye, Mary T. Cytotoxicity and antibacterial activity of methanolic extract of Hibiscus sabdariffa. J Med Plants Res. 2007;1(1):9–13.
14. Alaa GA-H. Antioxidant and antibacterial activities of Hibiscus sabdariffa L. extracts. African J Food Sci. 2012;6(21):506–11.
15. Hartel RW, Elbe JH von, Hofberger R. Hard candy. In: Confectionery Science and Technology. Springer; 2018. p. 211–44.
16. Kustyawati ME, Pratama F, Saputra D, Wijaya A. Viability of molds and bacteria in tempeh processed with supercritical carbon dioxides during storage. Int J Food Sci. 2018;2018(4):1–7.
17. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. Am Soc Microbiol.

2009;15:55–63.
18. Badan Standarisasi Nasional. Syarat Mutu Kembang Gula Keras. Jakarta: Badan Standardisasi Nasional; 2008. p. 2008.
19. Lim J. Hedonic scaling: A review of methods and theory. Food Qual Prefer. 2011;22(8):733–47.
20. Márquez-Rodríguez AS, Nevárez-Baca S, Lerma-Hernández JC, Hernández-Ochoa LR, Nevárez-Moorillon GV, Gutiérrez-Méndez N, et al. In vitro antibacterial activity of Hibiscus sabdariffa L. phenolic extract and its in situ application on shelf-life of beef meat. Foods. 2020;9(1080):1–13.
21. Chao C-Y, Yin M-C. Antibacterial effects of roselle calyx extracts and protocatechuic acid in ground beef and apple juice. Foodborne Pathog Dis. 2009;6(2):201–6.
22. Panjaitan Mikchaell A, Eddy S, Hardoko. Antibacterial compounds activity of mangrove leaf extract Rhizophora mucronata on aeromonas hydrophyla. Russ J Agric Socio-Economic Sci. 2018;73(1):187–93.
23. Mahadevan N, Kamboj P. Hibiscus sabdariffa Linn.–an overview. Nat Prod Radiance. 2022;81(1):77–83.
24. Elmanama AA, Alyazji AA, Abu-Gheneima NA. Antibacterial, antifungal and synergistic effect of Lawsonia inermis, Punica granatum and Hibiscus sabdariffa. Ann Alquds Med. 2011;7:33–41.
25. Akrayi HFS. Antibacterial Potency of Aqueous Plant Extracts against Streptococcus mutans. Med J Islam World Acad Sci. 2014;109(1566):1–5.
26. Ferreira GLS, Bezerra LMD, Ribeiro ILA, Morais Júnior RCD, Castro RD. Susceptibility of cariogenic microorganisms to phytoconstituents. Brazilian J Biol. 2018;78:691–6.
27. Mandei JH, Alim D, Nuryadi M, Riset B, Standardisasi D, Manado I. Effect Of Nutmeg Juice Ph On Reducing Sugar Content And Texture Of Hard Candy. J Penelit Teknol Ind. 2019;11(1):19–30.
28. Nadaletti M, Di Luccio M, Cichoski AJ. Sucrose inversion of hard candies formulated with rework syrup with addition of sodium lactate. J Food Process Eng. 2011;34(2):305–16.
29. Ramírez-Rodrigues MM, Balaban MO, Marshall MR, Rouseff RL. Hot and cold water infusion aroma profiles of Hibiscus sabdariffa: fresh compared with dried. J Food Sci. 2011;76(2):C212–7.