Supplementation of Javanese Long Pepper Extracts into Sucrose-Free Hard Candy for Improving Antibacterial Activity against *Streptococcus mutans*

Supriyanto*, Mojiono Mojiono, and Yulia Ambarwati

Department of Agroindustrial Technology, Faculty of Agriculture, Universitas Trunojoyo Madura, Jl. Raya Telang, Kamal, Indonesia

Corresponding author: supriyanto@trunojoyo.ac.id

Abstract. Hard candy was made from the mixture of sucrose, glucose, and water. However, the excessive intake of sucrose can cause serious dental problems such as dental caries. Sucrose in hard candy formula is replaceable, using xylitol with the calory content of 40% lower than sucrose, while it also supresses the bacterial growth of *Streptococcus mutans* causing dental caries. Hard candy functionality is improved by addition of Javanese long pepper extracts containing various chemicals such as alkaloid, saponin, and piperin found up to 4-6%, and they exert antibacterial activity. This current work characterized the quality (i.e., color, pH, solubility time, sensory) of sucrose-free hard candy supplemented with Javanese long pepper extracts, and evaluated the antibacterial effect against *Streptococcus mutans* causing dental caries. The experiment followed a non-factorial completely randomized design consisting of different proportions of xylitol and glucose: 0:50, 30:80, 35:85, and 20:90, carried out at triplicates. As a result, the higher concentration of xylitol positively affected the characteristics of sucrose-free hard candy, including color, pH, and sensory properties. In addition, the concentration of xylitol showed positive impact to inhibition of *Streptococcus mutans*.

Keywords: Sucrose-Free Hard Candy, Cabya, *Piper retrofractum* Vahl. and *Streptococcus mutans*

1. Introduction

Spices have become one of the precious export commodities from Indonesia, including Javanese long pepper as a popular spice in the country. Oleoresin is extracted from the spices and often applied in a variety of products such as flavour enhancer in food industries, medicine, cosmetics and soaps. To date, the source of oleoresin comes from particular spices, including black pepper (Sulhatun et al., 2013), white pepper (Olalere et al., 2017), cinnamon (Khasanah et al., 2017), and ginger (Sri Hartuti & Supardan, 2014). Javanese long pepper (*Piper retrofractum* Vahl.) is a potential spice for oleoresin, since it is popular material and widely cultivated in many parts of Indonesia such as Wonogiri, Jember, Lamongan, and Madura. In Madura, an island near Java island, Javanese long pepper is frequently added to the coffee, aiming to enhance stimulatory effects. In addition, Javanese long pepper is used in traditional medicine industries for its health-improving effects, while it is also harmless (Haryudin & Rostiana, 2009).

In our previous work, oleoresin was extracted from Javanese long pepper using UAE (ultrasonic-assisted extraction) method. As a result, the extract was evidenced to exert antioxidant and antibacterial effects. This provokes the further use of oleoresin from Javanese long pepper for ingredient of functional foods. In this regard, the oleoresin can be incorporated into hard candy. It has been common to supplement hard candy by spice extracts as reported by former works (Hutami et al., 2021; Indriaty, 2018; Yazakka & Susanto, 2015).

Hard candy is often characterized with its hard texture, glossy appearance, and high clarity, and these properties are formed by essential components such as sucrose, glucose syrup, and other ingredients especially added to modify its taste (Rakhmayanti & Hastuti, 2008). Javanese long pepper extract is added to the formulation of sucrose-free hard candy, aiming to exert functional properties namely antioxidant effect which contributes to scavenge free radicals (Singletary, 2010), while also develop new candy with no sucrose. It is universally understood that the high sugar concentration in candy accounts for dental problems such as caries. Replacing sucrose with alternative sweeteners such as xylitol may a promising attempt. Xylitol is regarded as sugar substitute in sugar-free gum, and it is recognized harmless to human (Rajapaksha *et al*., 2019). It was reported as sweetener in velvet tamarind chewy candy (Sukeaw Samakradhamrongthai & Jannu, 2021). However, the effects of this sugar replacement and supplementation of Javanese long pepper extract on quality and functional properties of hard candy need to be investigated. Therefore, the overarching intent of this current work is to examine the impacts of xylitol and oleoresin from Javanese long extract to the characteristics of sucrose-free hard candy.

2. Material and Method

2.1. Materials

Fresh Javanese long pepper fruits were harvested from local farming in Galis, Pamekasan. Other chemicals were analytical grade. *Streptococcus mutans* were obtained from Indi Laboratory.

2.2. Experimental Design

The experiment followed a non-factorial completely randomized design. Proportion of xylitol to glucose was made different as the treatments. Table 1 represented the formulation of samples.

Table 1. Formulation of sucrose-free hard candy supplemented with Javanese long pepper extract

Ingredients (g)	Sample Code			
	F1	F2	F3	F4
Sucrose	60	0	0	0
Xylitol	0	30	25	20
Glucose	50	80	85	90
Water	15	15	15	15
Javenese long pepper extract	10	10	10	10

F1 is a control samples containing sucrose : glucose of 60 : 50

2.3. Preparation of samples

The fresh fruit of Javanese long pepper was sun-dried to obtain dried fruit. It was then pulverized using a grinder machine and sieved at a 30-mesh. The extraction was carried out by using water infusion at a powder and water ratio of 1 : 4. Briefly, the powder was transferred into boiled water in a beaker glass for 5 min. After cooling for about 10 min, the filtrate was collected through filtration.

For preparation of hard candy, all ingredients (sucrose or xylitol, glucose syrup, water) were heated and consistently stirred in a beaker glass at 145°C for 25 min. Subsequently, the extract of Javanese long pepper was added, and the mixture was constantly heated at 145°C. The heating was terminated after 4 min. The complete mixture was then poured into a round-shape container, then allowed to stand for about 60 min, enabling it to solidify.

2.4. Color test

Color measurement complied with a procedure of Niswah et al. (2019) using ImageJ software, expressed as RGB (Red-Green-Blue). Hard candy samples were photographed by using DSLR camera (canon 1300D) set at food mode and constant distance of ±30 cm above sample. The photographing was conducted in a specially designed box, enabling to remove unwanted external light. The image files were analyzed in the software. RGB color profile between samples was compared.

2.5. Solubility time

Solubility time of the candy was measured by using a method of Hilmy et al. (2015). Sample (5 g) in a beaker glass was added with 25 mL of distilled water, heated using a hotplate at temperature of 30±2°C and constantly stirred at 400 rpm. The time required to completely dissolve the candy was observed, which was expressed as solubility time (in minute).

2.6 Determination of pH

The measurement of pH followed a procedure of Agistia et al. (2015), using a pH meter fomerly callibrated by buffer solution at pH 4 and pH 7. Sample (5 g) was dissolved in 10 mL of distilled water, then the probe was submerged. The level of pH was displayed in pH meter.

2.6. Disk diffusion assay

The antibacteria test against *Streptococcus mutans* followed a method of Anastasia *et al*. (2017) using disk diffusion method. The culture of *Streptococcus mutans* was refreshed in brain heart infusion brain (MHI-B) and nutrient agar. Amoxillin and distilled water were used as positive and negative control, respectively. Briefly, each 5 g of samples and amoxillin was dissolved in distilled water at 1:1. A suspension of the bacteria was spread in the petri dish on Mueller Hinton Agar (MHA). Paper disks were impregnated by solution of samples and amoxillin, and placed on the inoculated petri dishes. The zone of inhibition was measured by calliper after 24 h of incubation at 37°C.

2.7. Sensory Test

Sensory test was carried out according to Rosiani et al. (2015). The sensory test was used to determine the characteristics of the sucrose-free hard candy, including taste, aroma, texture and overall. The panelists (30 untrained panelists) were asked to taste the samples and rate them based on 5-scale scores as follows: extremely like (5), very like (4), neutral (3), dislike (2), extremely dislike (1).

2.8. Statistical Analysis

Data were statistically analyzed by using One-Way ANOVA (Analysis of Variance) in SPSS 25 software using a 95% confidence level. Tukey test was made to verify the difference between means.

3. Results and Discussion

3.1. Color

Color appearances of samples were evaluated using image processing software ImageJ, expressed as RGB color. This color measurement enables to assess sample colors accurately, while it can be operated quickly and easily (Niswah et al., 2019). Our results suggested that candy formulation showed significant effects on RGB (Table 2).

Briefly, as shown in Table 2, RGB values range between 63.92 and 70.82, and control sample shows the highest RGB value despite not significantly different with F4. This suggested that the presence of glucose in the candy formulation was responsible for dark color in candies (Sitepu, 2019). Glucose can easily changed after exposed with high temperature, leading to non-enzymatic browning reactions such as Maillard and caramelization. Maillard involved the interaction between reducing sugar in carbohydrate and particular amino acids, thus producing brown color (Adna Ridhani & Aini, 2021). In this regard, glucose is a reducing sugar that enables to induce Maillard reaction. In addition, the excessive amount of glucose in the formulation could adversely affect the candy quality, including aroma, flavor, pH, and color (Anggraeni, 2017).

Table 2 Comparison of RGB, solubility time, and pH in sucrose-free hard candy samples between treatments

Treatments (xylitol:glucose)	RGB	Solubility time	pH	Inhibitory zone (mm)
F1 (0:50)	70.82±1.11^{b}	22.67±0.57^{a}	4.70±0.04^{c}	2.22±0.25^{a}
F2 (30:80)	63.92±2.07^{a}	20.67± 1.15^{a}	4.38±0.06^{b}	3.29±0.19^{a}
F3 (25:85)	64.72±1.65^{a}	22.33± 2.08^{a}	4.36±0.01^{a}	2.86±0.10^{a}
F4 (20:90)	70.80±1.08^{b}	22.33± 2.08^{a}	4.33±0.01^{a}	2.08±0.30^{a}

F1 is a control samples containing sucrose : glucose of 60 : 50, without xylitol. Different superscripts following means in similar column exhibit significant difference at $p<0.05$.

3.2. Solubility time

The results indicated that formulation of hard candy did not affect the solubility time of samples (Table 2). The solubility of candy was altered by its ingredients, primarily sugar. In this case, xylitol and glucose were added to formula as sweeteners, in which both sugars showed similar solubility.

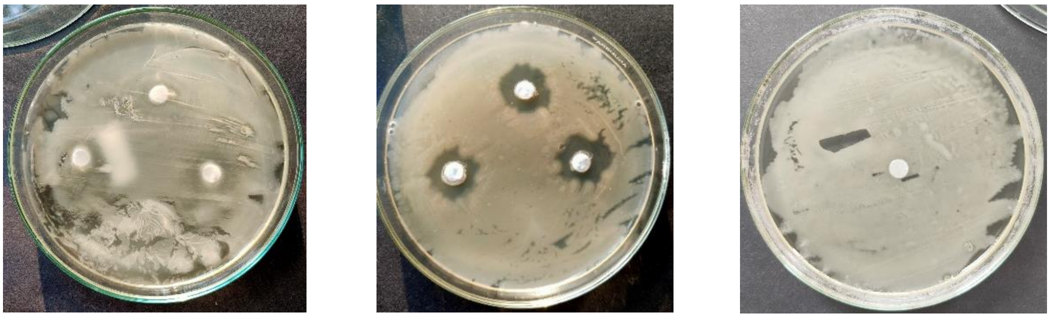
3.3. pH

As shown in Table 2, the formulation of hard candy significantly affected pH. Simply, this value decreased as the higher proportion of glucose and lower level of xylitol. The pH ranged between 4.33 and 4.70, categorized as acidic. The candies with lower pH might be associated with the reduction of dental plaques, while also enhancing the production of saliva. In this regard, xylitol enabled to stabilize pH in the mouth, producing condition in which dental demineralization was reduced (Savita *et al*., 2017). In addition, xylitol-containing candies allowed to raise the concentration of bicarbonate, phosphate, calcium in the mouth. The rising production of salive could triger the dental remineralization due to the formation of calcium-xylitol complex, thus supressing dental decay (Savita *et al*., 2017).

3.4. Antibacterial Activity

The antibacterial activity against *Streptococcus mutans* by sucrose-free hard candy is represented by inhibitory zone formed after 24 h of incubation. The diameter of clear zone is measured by a calliper. The results showed that the treatments indicated significant effects on inhibitory zone (Table 2). Despite no significant difference, the treatments of F1-F4 show a clear zone, ranging 2.08 – 3.29 mm. Briefly, F2 sample containing the highest proportion of xylitol results in the greatest inhibitory zone in comparison with other treatments. This may indicate the antimicrobial effects of xylitol. The plausible mechanism of antibacterial effect against *Streptococcus mutans* by xylitol was explained by a former work, in which xylitol was not metabolized by acid-forming bacteria. Instead, it turned into xylitol-5-phosphate which retarded the metabolic activities of the bacteria (Alshibani *et al*., 2022). Since the cariogenic bacteria growth was suppressed, the plaque pH remained stable, thus inhibiting the enamel demineralization. However, the antibacteria effect was categorized as weak, since the diameter was ≤ 5 mm. Further works can be envisaged, especially using higher proportion of xylitol, enabling to enhance the inhibition.

In this work, we also compared the inhibition zone with positive control (amoxillin) and negative control (distilled water). As a result, amoxillin showed the strongest antibacterial agent, and this confirmed its antimicrobial activity through its highest inhibitory zone of 17.16 mm (data not shown). Conversely, no inhibition was attributed to negative control. Figure 1 exhibits the antibacterial response between treatment, positive control and negative control groups.



(a) (b) (c)

Figure 1. Inhibition zone against *Streptococcus mutans* by treatment groups (a), positive control (b), and negative control (c)

3.5. Sensory Profile

The candies were evaluated in an acceptance test by 30 untrained panelists in relation to taste, aroma, texture, color, and overall impression. The analysis complied with 5-point hedonic scale as follows: 1 (dislike extremely) to 5 (like extremely).

Taste is a crucial attribute for candy. In this work, treatments showed no significant effects on taste (p>0.05). Noticeably, the hedonic score for taste was in range of 3.30 – 3.13, which corresponded to neither like nor dislike. This suggests that the evaluators might not perceive special taste impression. For further experiment, it is suggested to use higher level of sweeteners. As mentioned by Nelwan *et al*. (2015), concentration of ingredients and temperature were found to have considerable impacts to candy taste.

Table 3. Sensory profile of sucrose-free hard candy samples

Treatments (xylitol:glucose)	Hedonic score				
	Taste	Aroma	Texture	Color	Overall
F1 (0:50)	3.30 ±0.34[a]	2.66 ±0.34[a]	1.76±0.28[a]	3.50±0.52[a]	4.10±0.30[c]
F2 (30:80)	3.10±0.26[a]	2.73 ±0.30[a]	2.63±0.56[ab]	3.53±0.15[a]	3.83±0.37[bc]
F3 (25:85)	3.13±0.05[a]	2.73±0.15[a]	3.50±0.34[bc]	3.63±0.15[a]	2.63±0.25[a]
F4 (20:90)	3.13±0.30[a]	2.66±0.25[a]	4.30±0.65[c]	3.43±0.20[a]	3.13±0.41[bc]

F1 is a control samples containing sucrose : glucose of 60 : 50, without xylitol. Different superscripts following means in similar column exhibit significant difference at p<0.05.

In addition to taste, aroma crucially contributes to the consumer acceptance. Similarly, in this work, aroma is also perceived not different between treatments (p>0.05), ranging from 2.66 to 2.73. The candy was added with equal amount of Javanese long pepper extract (10 g), hence this might be a main reason for their similar aroma. It is noteworthy that hedonic score for aroma is <3.0, indicating that the candy aroma is less favorable. The strong spicy aroma, which is produced by Javanese long pepper, can be the reason behind the unacceptability.

The texture of sucrose-free hard candy samples is also meaningful attribute. The results showed that hedonic score for texture ranged from 1.76 to 4.30, and this was significantly different between treatments (p<0.05). As presented in Table 3, samples from F1 have the lowest hedonic score, meanwhile those from F4 exhibit the highest, reaching up to 4.3 which is close to "like extremely" category. The proportion of glucose seemed to remarkably impact the texture, especially hardness (Indriaty, 2018). In addition, it was evidenced able to supress crystallization induced by sucrose (Zalizar *et al*., 2016).

Furthermore, color is essential atribute for candy, since it appears visually. Hence, it strongly determines the acceptance. In this work, the treatments showed no significant effects on color appearance (p>0.05). The use of xylitol and glucose in hard candy formulation leads to formation of dark reddish color. Glucose is a reducing sugar, and it essentially contributes to Maillard reaction, causing the darker color of candy samples (Adna Ridhani & Aini, 2021). As seen in Table 3, the hedonic score for color is 3.43 – 3.63, which is categorized as "neither like nor dislike".

Overall impression of evaluators for the candy sampples was also rated, resulting in 2.63 – 4.10 (p>0.05). In this regard, the highest score is achieved by sample from F1 (4.10), while the lowest is found in sample from F3 (2.62). This suggests that sucrose candy still receives the most acceptance from consumers. The main challenge for manufacture of sucrose-free hard candy is aroma and taste, which inevitably spurs the development of sucrose-free hard candy with better acceptance. The concentration of Javanese long pepper extract and the proportion of glucose and xylitol can be re-adjusted to create more favorable candy.

Conclusion

The research concluded that treatments could significantly altered color and pH of samples, but did not affect solubility time and inhibition diameter. In terms of sensory properties, the attributes of sucrose-free hard candy were significantly affected by the treatments, including taste, texture and overall impression, while the remaining attribiutes were unaltered. It is suggested to develop sucrose-free hard candy with better acceptance through modifying the proportion of Javanese long pepper extract and sweeteners.

References

Adna Ridhani, M., & Aini, N. (2021). Potensi Penambahan Berbagai Jenis Gula Terhadap Sifat Sensori Dan Fisikokimia Roti Manis: Review. *Pasundan Food Technology Journal*, *8*(3), 61–68. https://doi.org/10.23969/pftj.v8i3.4106

Agistia, N., Rahim, F., & Nofiandi, D. (2015). *Formulasi Permen Jeli Ekstrak Daun Ubi Jalar (Ipomoea batatas L .) Sebagai Suplemen Makanan*. *3*(2), 43–48.

Alshibani, N., Shalabi, M., AlMugbel, K., AlSaqer, E., AlFarraj, N., & Allam, E. (2022). Xylitol content and acid production of chewing gums available in the markets of Saudi Arabia. *Saudi Dental Journal*, *34*(2), 121–128. https://doi.org/10.1016/j.sdentj.2021.11.001

Anastasia, A., Yuliet, Y., & Tandah, M. R. (2017). Formulasi Sediaan Mouthwash Pencegah Plak Gigi Ekstrak Biji Kakao (Theobroma cacao L) Dan Uji Efektivitas Pada Bakteri Streptococcus mutans: Mouthwash Formulation of Tooth Plaque Preventing of Kakao (Theobroma cacao L) Seed Extract and Effectivity Test on. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy)*, *3*(1), 84–92.

Anggraeni, M. (2017). Sifat Fisikokimia Roti Yang Dibuat Dengan Bahan Dasar Tepung Terigu Yang Ditambah Berbagai Jenis Gula. *Jurnal Aplikasi Teknologi Pangan*, *6*(1), 52–56. https://doi.org/10.17728/jatp.214

Haryudin, W., & Rostiana, O. (2009). Karakteristik morfologi tanaman cabe jawa (Piper retrofractum Vahl.) di beberapa sentra produksi. *Buletin Penelitian Tanaman Rempah Dan Obat*, *20*(1), 1–10.

Hilmy,N., et al. (2015). Karakteristik Kelarutan Rutin dari Ekstrak Air Daun Singkong (Manihot esculenta Crantz). *Jurnal Mahasiswa Farmasi Fakultas Kedokteran Untan, 3 (1)*(December), 1–6.

Hutami, R., Nur'utami, D. A., & Joana, A. (2021). ANTIOXIDANT ACTIVITY, SENSORY, CHEMICAL, AND MICROBIOLOGY CHARACTERISTICS OF MUNTOK WHITE PEPPER (Piper nigrum Linn.) HARD CANDY. *Indonesian Journal of Applied Research (IJAR)*, *2*(1), 147–27. https://doi.org/10.30997/ijar.v2i1.98

Indriaty, F. (2018). Pengaruh Penambahan Sari Buah Nenas Pada Permen Keras. *Jurnal Penelitian Teknologi Industri*, *8*(2), 159. https://doi.org/10.33749/jpti.v8i2.2223

Khasanah, L. U., Anandhito, B. K., Uyun, Q., Utami, R., & Manuhara, G. J. (2017). Optimization of Two Stages Extraction Process dan Characterization of Cinnamon Leaf Oleoresin (Cinnamomum Burmanii). *Indonesian Journal of Essential Oil*, *2*(1), 20–28. https://doi.org/10.21776/ub.ijeo.2017.002.01.03

Nelwan et al. (2015). Pengaruh Konsentrasu Gelatin dan Sirup Glukosa terhadap Sifat Kimia dan Sensoris Permen Jelly Sari Buah Pala (Myristica fragrans Houtt). *Jurnal Cocos*, *3*(11–2), 1–12.

Niswah, A. A., Bintari, Y. R., & Risandiansyah, R. (2019). Piper betle L . Sebagai Pewarna Bakteri : Uji Akurasi Dan Presisi Warna Pada Staphylococcus aureus Dan Escherichia coli. *Jurnal Kedokteran Komunitas*, *10 (2)*(2), 1–11.

Olalere, O. A., Abdurahman, N. H., Alara, O. R., & Habeeb, O. A. (2017). Parametric optimization of microwave reflux extraction of spice oleoresin from white pepper (Piper nigrum). *Journal of Analytical Science and Technology*, *8*(1), 0–7. https://doi.org/10.1186/s40543-017-0118-9

Rajapaksha, S. M., Gerken, K., Archer, T., Lathan, P., Liyanage, A. S., Mlsna, D., & Mlsna, T. E. (2019). Extraction and analysis of xylitol in sugar-free gum samples by GC-MS with direct aqueous injection. *Journal of Analytical Methods in Chemistry*, *2019*, 1–10. https://doi.org/10.1155/2019/1690153

Rakhmayanti, R. D., & Hastuti, R. T. (2008). *(Caesalpinia sappan L .)*. *3*(3), 1–6.

Rosiani, N., Basito, B., & Widowati, E. (2015). Kajian Karakteristik Sensoris Fisik Dan Kimia

Kerupuk Fortifikasi Daging Lidah Buaya (Aloe vera) Dengan Metode Pemanggangan Menggunakan Microwave. *Jurnal Teknologi Hasil Pertanian*, *8*(2), 84. https://doi.org/10.20961/jthp.v0i0.12896

Savita et al. (2017). Perbandingan Laju Aliran Saliva Sebelum dan Sesudah Mengunyah Permen Karet Nonxylitol dan Xylitol pada Anak Usia 10-12 Tahun. *Journal Caninus Denstistry*, *2*(Mei), 65–70.

Singletary, K. (2010). Ginger Review. *Food Science*, *45*(4), 171–183.

Sitepu, K. M. (2019). Penentuan Konsentrasi Ragi Pada Pembuatan Roti. *Jurnal Penelitian Dan Pengembangan Agrokompleks*, *2*(1), 71–77.

Sri Hartuti, & Supardan, M. D. (2014). Optimasi Ekstraksi Gelombang Ultrasonik untuk Produksi Oleoresin Jahe (Zingiber officinale Roscoe) Menggunakan Response Surface Methodology (RSM). *AgriTECH*, *33*(4), 415–423. https://doi.org/10.22146/agritech.9537

Sukeaw Samakradhamrongthai, R., & Jannu, T. (2021). Effect of stevia, xylitol, and corn syrup in the development of velvet tamarind (Dialium indum L.) chewy candy. *Food Chemistry*, *352*(February), 129353. https://doi.org/10.1016/j.foodchem.2021.129353

Sulhatun, Jalaluddin, & Tisara. (2013). Pemanfaatan Lada Hitam Sebagai Bahan Baku Pembuatan Oleoresin dengan Metode Ekstraksi. *Jurnal Teknologi Kimia Unimal*, *2*(2), 16–30.

Yazakka, I. M., & Susanto, W. H. (2015). (KAJIAN JENIS DAN KONSENTRASI SARI JAHE) Characterization Of Ginger Hard Candy Based On Coconut Sap (Study of Type and Concentration of Ginger Juice). *Jurnal Pangan Dan Agroindustri Vol. 3 No 3 p.1214-1223, Juli 2015*, *3*(3), 1214–1223.

Zalizar, L., Sapitri, E. R., Putri, N. K., Winda, G., & Khoirun, L. (2016). Perbandingan Penambahan Glukosa dan Sukrosa Terhadap Kualitas Permen Susu Kambing Peranakan Etawa Berdasarkan Preferensi Konsumen. *Jurnal Produk Nasional Dan Gelar Produk*, *2*(5), 49–55.