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Optimisation of Sucrose-Free Hard Candy Enriched with *Piper retrofractum* Vahl Extracts Using Response Surface Methodology (RSM)

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ABSTRACT

Javanese long pepper (*Piper retrofractum* Vahl) is a local herbal plant that is rich in bioactive compounds such as flavonoids, polyphenols, and other active components. The main bioactive compound is piperine which is associated with therapeutic potential. Therefore, this research aims to develop an optimum formulation of sucrose-free hard candy with the addition of *Piper retrofractum* Vahl extracts obtained based on the results of previous research. The optimum results were determined using Response Surface Methodology (RSM) with Box-Behnken Design through the four stages in Design Expert 13 software. This was followed by optimisation of the free-sucrose hard candy manufacturing process with the addition of *Piper retrofractum* extract at (5-10) g and xylitol (10-30) g to produce 16 combinations. The parameters observed were percentage of antioxidants, solubility time, pH, and RGB colour. The results showed the optimum condition to be 10 g of extract and 30 g of xylitol which produced a D value of 0.746. Moreover, the responses obtained at the conditions included 55.718% antioxidant, 12.073 min of solubility time, pH 3.228, and RGB colour values of 60.821.

Keywords: Sucrose-free hard candy, Javanese long pepper, Optimisation, Response Surface Methodology

Introduction

Candy is produced from a confectionery which mainly consists of water and sucrose with the addition of syrup, colouring, and flavouring agents.[1] The primary ingredient is sugar which serves as a source of energy and contributes to the attractiveness, sweetness, and crunchiness of the product.[2,3] Candy has a high sugar content derived from glucose, fructose, sucrose, and maltose[3] with the sensory appeal depending on taste and colour. Moreover, the popular candy flavours are caramel, chocolate, peppermint, and vanilla.[2] The use of synthetic and natural flavours enables manufacturers to have a diverse range of products that appeal to different consumer segments. Furthermore, colour enhances the visual appeal of candy, particularly for children.[2]
The development of functional foods is at the centre of innovation in food industry. [4] This is motivated by the demand of increasingly health-conscious consumers in search of foods that provide both basic nutrition and additional health benefits. [5,6] A new trend is the transformation of conventional products to ensure additional health benefits. An example is health-promoting candy products that contain functional ingredients such as probiotics, vitamins, and minerals[7]in addition to others with functional benefits. Sweeteners are generally a primary component with a significant contribution to the quality of candy. This is because sucrose is the main ingredient of candy which contributes 35-45% of the total content

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The content affects hardness, brightness, and storage stability of formulations. [8,9] Candy produced through sucrose has higher levels of hardness and brightness compared to other substitutes. [9,10] Moreover, sucrose is the primary source of bacterial fermentation in the oral cavity, particularly in *Streptococcus mutans*. It is recognised as a cariogenic substrate due to the ability to be converted into acid by oral bacteria such as *S. mutans* and the process can lead to the demineralisation of tooth enamel. The efforts to reduce the risk of caries have led to the replacement of sucrose with non-fermentable sweeteners such as maltitol, sucralose, and xylitol which are effective alternatives. These alternative sweeteners produce less acid and reduce biofilm formation by *S. mutans*.[11,12] For example, maltitol was reported to have the capacity of reducing acid production and the number of *S. mutans* in saliva when used in fermented milk products containing probiotics.[12]
Xylitol is another important low-calorie sweetener with a sweetness level which is equivalent to sucrose. [13] It is an effective and healthy alternative that offers health benefits while providing a sweet taste to food products. [14,15,16] In food applications, xylitol has been used as a substitute for sucrose in different cakes, candies, beverages, and other processed products. Previous research also showed that the substitution of sucrose with xylitol in products led to a softer texture and improved stability during storage. [17,18,19]
Piper retrofractum is a local herbal plant that is rich in bioactive compounds such as flavonoids, polyphenols, and other active components. The main bioactive compound is piperine which has therapeutic potential and is considered an antioxidant, anti-inflammatory, and antimicrobial.[20,21,22,23] *Piper retrofractum* also contains flavonoids and polyphenols which function as antioxidants to neutralise free radicals and have the potential to assist in protecting cells from oxidative damage towards maintaining oral health and preventing disease. [24,25,26] Furthermore, alkaloid compounds are present in the herbal plant in the form of piperlonguminine, methyl piperine, and sylvatine. Previous research showed that *Piper retrofractum* extract exhibited antimicrobial activity against common oral pathogens such as *Staphylococcus* aureus and *Candida albicans*.[21] The antimicrobial properties of the bioactive compounds have the potential to assist in reducing bacterial load and preventing oral infections.

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The development of sucrose-free hard candy with xylitol and *Piper retrofractum* extract potentially offers delicious and healthy products. This combination provides dental health benefits, reduces the risk of chronic diseases, and enhances the quality of life for consumers. An important aspect of developing sucrose-free hard candy is the consideration of physicochemical and sensory characteristics. Research showed that hard candy containing xylitol had better colour, pH, and sensory characteristics compared to sucrose. [27,28] However, xylitol potentially affected the texture of candy which led to a lesser hardness compared to sucrose. [28] This shows the need for optimal formulation to achieve a balance between texture, taste, and health benefits.

Materials and Methods

Materials and Tools

The materials used include dried Javanese long pepper, xylitol, distilled water, water, glucose syrup, sucrose, frying oil, and DPPH (2,2-diphenyl-1-picrylhydrazyl) (MERCK). Meanwhile, the tools used include a set of glass grinder tools (Maksindo, MKS-ML2500), ultrasonic (Elma-LC 60H), rotary evaporator (B- One, RE-1000 VN), thermo gun (SANFIX, IT 380N), pH meter (Senz), colour reader (Konica Minolta CR-10), and vortex (VELP Scientifica). The other tools were an oven (MEMMERT, UNB 400), a hot plate (Cimare Thermo Scientific, SP88857105), scales (HENHERR, BL-H2), UV-VIS spectrophotometers (Shimadzu, UVmini-1240), calipers (Krisbow), and a centrifuge (HEALTH, 80-2).

Experimental Design

Response Surface Methodology (RSM) with Box-Behnken Design (BBD) in four stages was adopted in this research. Moreover, *Piper retrofractum* oleoresin was extracted based on the results of previous research.[27] This was followed by optimisation of the free-sucrose hard candy manufacturing process with the addition of *Piper retrofractum* extract at 5 -10 g and xylitol at 10-30 g to produce 16 treatment combinations. The parameters observed include percentage of antioxidants, solubility, pH, and colour.

The formula was optimised by varying the amount of *Piper retrofractum* extract (factor 1) from 5 to 10 g and the amount of xylitol (factor 2) from 10 to 30 g as shown in Table 1. The minimum and maximum limits were set in the Design-Expert Version 13 (DX 13) program using the D-Optimal design.

Table 1: Formulation of sucrose-free hard candies

Ingredients	Quantity
Extract	5–10 g
Xylitol	10–30 g
Glucose	70 g
Distilled water	10 mL

Extraction of Piper retrofractum Vahl

Piper retrofractum Vahl obtained from local farmers in Pamekasan in July 2023 Madura (GPS coordinates: -7.147845612083923, 113.55404221513955) was sorted, washed, and dried in the sun up to the moment the water content reached approximately 10%. The drying process was followed by crushing and sieving the dried sample through a 10-16 mesh. The extraction was assisted by an ultrasonic bath (Ultrasonic LC 60 H, Elma) at a temperature of 40°C and followed by filtration using a filter paper. The solvent was evaporated using a rotary vacuum evaporator at a temperature of 40°C to obtain the extract.

Preparation of Free-Sucrose Hard Candy

The preparation of hard candy was initiated by mixing xylitol, glucose, and water. The mixture was heated to 140°C followed by the addition of citric acid. The method applied for the preparation was through the modification of the process used in previous research.[10] The process was started with the selection of *Piper retrofractum* Vahl which was subjected to an *Ultrasound Assisted Extraction* to obtain *oleoresin* as a concentrated extract. The oleoresin was prepared in different concentrations of 1%, 2%, and 3% to produce the hard candy formulation. The next step was the mixture of the oleoresin with sweeteners, specifically glucose and a blend of xylitol and isomalt in ratios of 40:20 and 35:15 along with the addition of citric acid. The mixture combined the flavours, sweetening agents, and the natural extract which was subjected to *heating* at temperatures ranging from 140°C to 150°C. This step was important to dissolve the ingredients fully and initiate the hard candy-making reaction processes such as caramelisation or Maillard reaction which were capable of influencing the texture and colour. The provision of sufficient heating was followed by the cooling of the hot candy mass to solidify and be *moulded* into desired shapes. The cooling and moulding steps determined the final hard texture and form of the hard candy produced.

Physical Characteristics Analysis

RGB Colour

Colour of the samples was determined using RGB system and analysed through ImageJ software. The samples were photographed using a digital camera (Canon EOS 1300D KIT 1855MM) in “food” mode. The files were subsequently analysed in the software to generate RGB (red, green, blue) scores and visualised in a histogram.[29]

Solubility Time

Solubility time of the samples was determined using a previous method. [27] The 5 g sample was placed in a glass beaker, added with 25 mL of distilled water, heated on a hot plate at 30 ± 2°C, and constantly stirred at 400 rpm. The time required to completely dissolve the candy was observed and expressed as solubility time in minutes.

pH

The pH test was conducted using a pH meter (Greisinger GPH 014) with a sample of 5 g dissolved in 10 ml of distilled water in a glass beaker and stirred until homogeneous. The pH meter electrode was calibrated using a buffer solution and rinsed with distilled water. Subsequently, the electrode was dipped into the dissolved sample and left for a while up to the moment a stable reading was obtained.[30]

DPPH Assay

Antioxidant activity (AA) was determined by preparing a 0.5 mM DPPH solution through the dissolution of 5 mg DPPH in 25 mL of methanol. Furthermore, a 25-ppm sample solution was prepared through the dissolution in methanol. The first test stage was conducted using a control solution which was a mixture of 0.3 mL 0.5 mM DPPH and 3.5 mL of methanol. Subsequently, the second sample solution was prepared by mixing 0.5 mL of the sample, 0.3 mL of a 0.5 mM DPPH solution, and 3 mL of methanol. The control and sample solutions were incubated for 30 minutes and the absorbance was measured at a wavelength of 517 nm. The antioxidant activity was determined using the following Equation 1 [31]:

$$\%\ antioxidant\ activity = \frac{Blank\ absorbance - sample\ absorbance}{Blank\ absorbance} \times 100\%$$

Data Analysis

Data were processed using RSM in Design Expert 13 software. The results obtained in the form of a formula optimisation were subsequently presented in diagrams and equations.

Results and Discussions

Response Analysis

The responses related to the pH, solubility time, colour, and antioxidant activity are presented in Table 2. The purpose was to determine the hard candy formula with an optimal combination of *Piper retrofractum* Vahl extract and xylitol according to specified criteria for the variables.

The pH values of the sucrose-free hard candy samples remained relatively stable by ranging from 2.9 to 3.5 which shows the acidity level as mild but consistent. This stability was possibly due to the buffering capacity of the ingredients used which was primarily the combination of *Piper retrofractum* Vahl extract and xylitol. The natural

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acidity from the extract contributed to maintaining the low pH while xylitol which was a neutral sweetener did not significantly alter acidity. The maintenance of the pH range was important due to the effect on both the flavour profile and the chemical stability of the candy to prevent spoilage and unwanted reactions during storage.[1] Moreover, the acidic pH level tended to inhibit the growth of decay microbes to extend the shelf life of the sample.[32] The level also facilitates the growth of *S. mutans* bacteria which is capable of causing dental caries.[27]

Table 2: The response of 16 experiments.

Run	Response			
	pH	**Solubility Time (Minutes)**	**Colour**	**Antioxidant Activity (%)**
1	3.4	15.12	46.601	25.5
2	2.9	16.52	70.229	11.8
3	3.3	16.16	56.303	1.1
4	3.4	14.24	42.56	69.8
5	3.3	14.33	40.937	40
6	3.1	19.04	60.822	44.1
7	3.2	8.36	66.245	48.2
8	3	15.21	47.086	58.9
9	3.5	15.88	77.023	75.7
10	3.2	13.28	58.821	33.9
11	3.1	15.28	68.301	4.5
12	3.2	17.02	38.221	34.3
13	3.3	17.16	49.993	53.4
14	3.2	17.56	56.591	7.5
15	3.2	14.19	50.786	61.1
16	3.4	20.05	42.616	70.5

Solubility time showed significant variation from approximately 8.36 to 20.05 minutes which reflected the complex interaction between *Piper retrofractum* Vahl extract and xylitol concentrations in the formulation. The lower times recorded suggest that some formulations dissolved faster in saliva which led to a quicker release of flavour and texture changes probably preferred by certain consumers. [1,32] Meanwhile, higher times showed a slower dissolving candy which could provide a longer-lasting sweetness experience. The variation was possibly due to the interaction of the compound in the extract with the crystalline structure of xylitol which affected how water penetrated and disintegrated candy matrix during dissolution.

The sample colour was expressed in RGB system and the results showed quite varied values which ranged from 38.221 to 77.023. The large range reflected that hard candy colours were becoming increasingly varied. Figure 1 shows quite different colours which appear more dominantly brownish yellow to brownish red. The intensity also ranges broadly due to the influence of the concentration and characteristics of *Piper retrofractum* Vahl extract combined with processing conditions. The extract contained pigments and bioactive compounds that contributed to natural colouration with different concentrations across samples. However, xylitol is colourless and does not have a significant effect on the colour much and this shows the variation observed is mostly due to the extract level and possible Maillard or caramelisation reactions during cooking.[33] The importance of colour to consumer appeal shows the need to control extract concentration and process parameters towards producing visually attractive candies.[34]

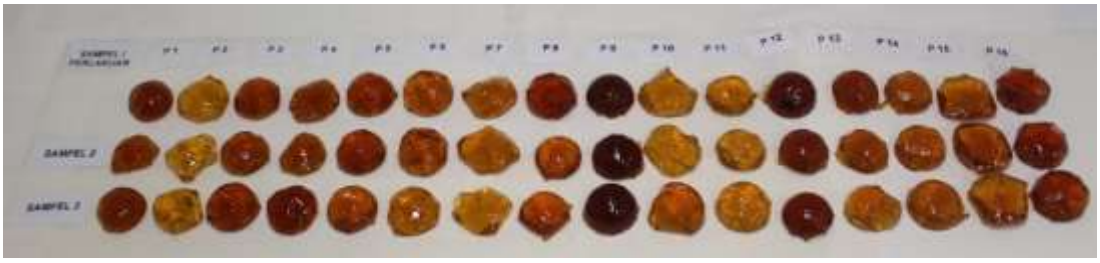

Figure 1: Appearance of 16 experimental samples. Each run was made triplicates.

Antioxidant activity exhibited the widest range from a low value of 1.1% to a high value of 75.7% to show a strong dependency on the amount of *Piper retrofractum* Vahl extract in the formulation. The extract is rich in phytochemicals known for antioxidant properties and the trend explains the significant increase in activity with higher extract concentrations.[25,32] Antioxidant activity tests were conducted to determine the ability of hard candy components to ward off free radicals. Moreover, the comparison with a commercial product produced through ginger showed an antioxidant activity of 1% to 12% which was classified as relatively low. This was because the ginger used as the main ingredient was observed to have lower antioxidants than rosella flowers with a value of 10.35 µg/mL[35] and 43 µg/mL[36,37] respectively. Another research examined hard candy containing basil leaf essential oil with the addition of moringa leaf extract. The results showed a decrease in antioxidant activity with a higher amount of moringa leaf extract. Antioxidant activity was reported to have ranged from 20.91% to 23.91%.[38] Another research showed that the addition of more rosella extract to papaya fruit juice jelly candy [37] increased antioxidant activity specifically from 50% to 60%. It was observed that the range of antioxidant activity from 25% to 27% increased along with a higher concentration of rosella extract. [37]

Response Optimisation

In this stage, the target was initially set by considering both variables to be "in range", solubility time, pH, and colour at "minimum", while antioxidants were at "maximum" as presented in Table 3. Optimisation process was expected to produce the most suitable combination of *Piper retrofractum* Vahl extract and xylitol in manufacturing hard candy. Based on the objectives of each variable, the set value limits, and the weight of importance, the optimal formulation size to have products with the best physical and functional characteristics was determined as

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presented in Table 4. The most desirable option was determined based on desirability (D) value with the highest score (close to 1.0) considered to be favourable. The value was calculated by the software with due consideration for the weight and goal set in optimisation stage.

Table 3: Criteria for Each Variable

Variable	Goal	Lower limit	Upper limit	Weight*
Piper retrofractum Vahl	In the range	5	10	3
Xylitol	In the Range	10	30	3
pH	In the range	2.9	3.5	4
Solubility	Minimal	8.3	20.05	3
Colour	Minimal	38.22	177.02	3
Antioxidant	Maximum	1.17	75.68	3

*A weight (1-5) is assigned to each response or variable to reflect its importance in the overall optimisation goal.

Table 4: Optimal Combination of Extract and Xylitol Based on Desirability Value

	Xylitol	*Piper retrofractum* Vahl	Antioxidant activity	Solubility time	pH	RGB Colour	Desirability*
1	30.000	10.000	55.718	12.073	3.228	60.821	0.746
2	30.000	9.968	55.662	12.083	3.228	60.821	0.746
3	30.000	9.906	55.553	12.103	3.228	60.821	0.745
4	29.893	10.000	55.588	12.146	3.228	60.821	0.744
5	30.000	9.671	55.143	12.183	3.228	60.821	0.740

*Desirability scores (from 0 to 1) are generated by RSM and used to find the treatment that creates the most desirable response. The score nearly 1.0 is more desirable.

RSM provided recommendations for the optimum condition based on the largest D value of 0.746 obtained. The concentration of xylitol was set at 30 g while *Piper retrofractum* was at 10 g as presented in Table 4. The response provided in Table 5 showed that the optimum combination of *Piper retrofractum* Vahl extract and xylitol exhibits varied responses across four main parameters, including antioxidant activity (55.717%), solubility (12.073 minutes), pH (3.228), and colour (60.821). The predicted value for antioxidant activity was 55.717% but with a relatively high standard deviation of 25.301 and a very wide prediction interval ranging from -6.917 to 118.352. This showed a considerable level of uncertainty in the parameter even though the 42.410 was in the predicted range. The solubility time also showed a predicted value of 12.073 which was relatively stable with a standard deviation of 2.569 and a narrower prediction interval range of 4.807 to 19.338. The values suggested that the model was quite reliable in projecting solubility. The pH value showed excellent predictive performance with a value of 3.228, a low standard deviation of 0.159, and a narrow prediction interval of 2.878 to 3.578 which reflected high stability and accuracy.

Table 5: Optimal Response of Combination of *Piper retrofractum Vahl* Extract and Xylitol

	Antioxidant	Solubility	pH	Colour
Predicted Mean	55.717	12.073	3.228	60.821
Predicted Median	55.717	12.073	3.228	60.821
Standard Deviation	25.301	2.569	0.159	32.557
N	1	1	1	1
SE Predicted	28.992	3.261	0.164	33.559
95% Prediction Interval Low*	-6.917	4.807	2.878	-10.707
Data Mean	42.410	14.020	3.150	58.500
95% Prediction Interval High*	118.352	19.338	3.578	132.349

*Prediction interval (PI) low and high values indicate the range within which a single future observation is likely to fall, with a specified confidence level such as 95%.

The results for colour showed a predicted value of 60.821 which was close to the actual data of 58.500. However, the very wide prediction interval of -10.707 to 132.349 and high standard deviation of 32.557 reflected the status of colour as the most challenging parameter to predict consistently by the model. The trend showed that the predictive model was generally dependable mainly for pH and solubility while more improvements were required in the formulation to achieve more precise prediction results for antioxidant activity and colour.

The relationship between the amount of the extract and xylitol to the desirability value in the hard candy process is visualised in a contour plot of Figure 2A and a surface plot of Figure 2B. The transition of the colour gradient green to yellow showed the movement from lower to higher desirability values. The increase in the content of xylitol to 30 g raised desirability values. This showed that a higher concentration of xylitol positively influenced the desirability of the final candy product. The proportion of the extract exhibited a less pronounced but visible effect.

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A higher content of Javanese long pepper extract of 10 g combined with a high level of xylitol produced the highest desirability values as observed in the yellow region at the upper right corner. The contour plot generally shows an optimal formulation with maximised xylitol content and relatively high amount of the extract to have a product with the most favourable characteristics according to RSM analysis. The insights can guide formulation adjustments to balance sweetness, extract concentration, and general product appeal.
Xylitol is a sugar alcohol commonly used as a substitute in several food products especially sugar-free candies, chewing gums, and oral care products. [39,40] It possesses a sweetness level comparable to sucrose but with fewer calories which leads to the status as a popular ingredient for low-calorie and diabetes-friendly formulations. Chemically, xylitol is a polyol with a five-carbon backbone which causes the distinctive properties such as a low glycaemic index and tooth-friendly characteristics. It is also not readily fermented by oral bacteria which aids in the prevention of dental caries. [22,25]

The roles of xylitol in the context of hard candy formulation are important. These are associated with the function as a sweetener in line with consumer-expected flavour profiles while reducing the risk of tooth decay. Xylitol also influences the physical properties of the candy such as solubility and texture. Moreover, the chemical composition contributes to the production of a finer crystalline structure and the prevention of excessive crystallisation to ensure a desirable texture for hard candy. Xylitol interacts effectively with the extracts derived from plant resources such as *Piper retrofractum* Vahl to preserve functional benefits such as antioxidant properties while maintaining sweetness level. The application as a sucrose replacement is in line with the goal of producing sugar-free hard candy that appeals to health-conscious consumers and those concerned with the dental system. This shows the unique sweetness, health benefits, and role of sweetener in texture modification.

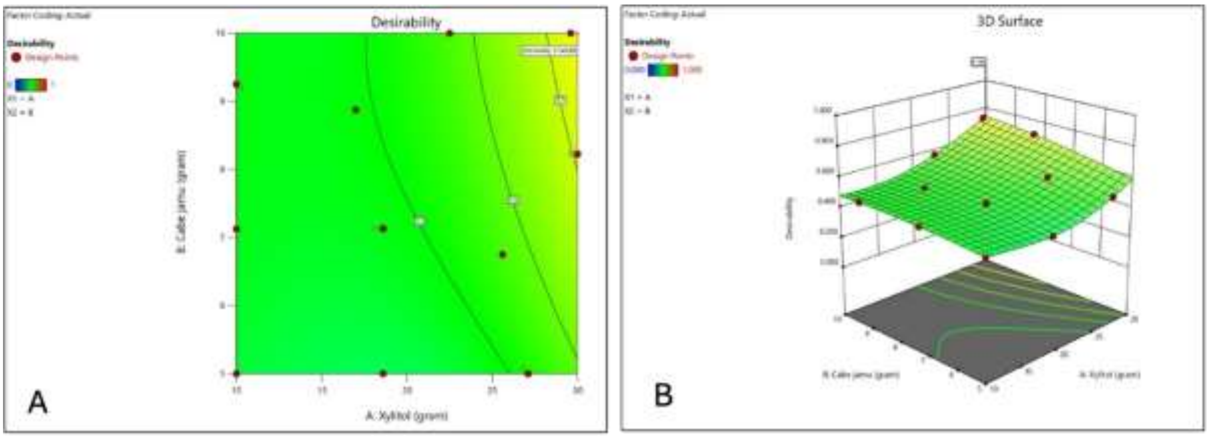

Figure 2: Contour plot (A) and surface plot (B) showing the correlation between factors and desirability value

Conclusion

In conclusion, this research showed that the optimal conditions for preparing candy were achieved by adding 10 g of *Piper retrofractum* extract and 30 g of xylitol to produce a D value of 0.746. Moreover, the responses obtained at the optimal condition included 55.718% antioxidant activity, 12.073 min of solubility time, pH 3.228, and RGB colour of 60.821.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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