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Original Article

Nutritional Content Analysis Of Yellow Pumpkin Brownies (*Cucurbita Moschata L.*) With Stevia Sweetener (*Stevia Rebaudiana*) As A Sugar Cane Substitution

Analisis Nilai Gizi Pada Brownies Labu Kuning (Cucurbita Moschata L) Dengan Pemanis Stevia (Stevia Rebaudiana) Sebagai Pengganti Gula Tebu

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Abstract: *An unhealthy diet that includes sugary snacks may promote a rise in glucose levels and enhance the risk of Type 2 Diabetes Mellitus (T2DM). Brownies are high in sugar and should be replaced with healthier ingredients to reduce their sugar content and glycemic index with stevia as natural sweeteners and yellow pumpkin which are low in calories and carbohydrates. To determine the nutritional content of brownies products with the substitution of cane sugar with stevia and yellow pumpkin. This study utilized a quasi-experimental design using completely randomized design (CRD). We experimented to evaluate the effects of four different treatments, comparing the use of cane sugar and stevia in different ratios: 100% cane sugar (P0), 50% cane sugar and 50% stevia (P1), 25% cane sugar and 75% stevia (P2), and 100% stevia (P3). Moisture, ash, protein, fat, carbohydrate and energy variables were tested using by different method, gravimetric, kjehdahl, soxhlet, by difference, and atwater which was repeated 3 times. We conducted data analysis using a one-way ANOVA and Duncan test to determine the treatment pairs with the highest significance level. The findings demonstrated that replacing cane sugar with stevia significantly affect the nutritional content of yellow pumpkin brownies products ($p<0.05$, for all comparisons). The treatments with the highest average of nutritional contents were as follows: P3 had the highest moisture content (48.64±0.04%), ash content (1.06±0.06%), protein (6.93±0.11%), and fat (14.49±0.34%), whereas P0 had the highest carbohydrate content (39.45±0.23%) and total energy (281.19±0.76 kcal/100g). The nutritional content of yellow pumpkin brownies has significantly modified by substituting sugar cane with stevia. However, additional investigation is required to determine other nutritional contents and assess its potential as functional food.*

Key word: Nutritional content, brownies, stevia, yellow pumpkin

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic condition characterized by hyperglycemia due to abnormalities in insulin secretion, insulin action, or both (1). Type 2 Diabetes Mellitus (T2DM) is the most commonly found diabetes and is marked by increased blood glucose levels. Unhealthy dietary factors accelerate the rise in blood glucose levels in T2DM (2). In Indonesia, the prevalence of DM has varied from 1.5% in 2013 to 2.0% in 2018, and then decreased to 1.7% according to the Indonesian Health Survey (SKI) 2023. DMT2 has the highest prevalence, reaching 50.2% in Indonesia (3,4).

One of the risk factors that can increase T2DM is diet. A high-calorie diet sourced from simple carbohydrates but low in consumption can increase the risk of developing type

2 diabetes (DMT2) (5). The risk of diabetes will increase if the body consumes a large amount of carbohydrates because more glucose enters the body (6). Energy consumption that exceeds needs will also cause a lot of glucose to enter the body due to high blood glucose levels influenced by high energy consumption (7).

The development of functional foods is carried out to provide additional active nutrients in products that have health benefits, such as being sources of, high in, or low in certain active substances (8). Functional foods can be used to prevent various diseases such as obesity, diabetes, hypertension, coronary heart disease, and cancer (9). Therefore, research is needed to modify food ingredients that have the potential to be functional foods in order to reduce the incidence of type 2 diabetes (DMT2).

Unhealthy eating patterns include the habit of consuming snacks that do not align with balanced nutrition guidelines (10). One of the most popular snacks is brownies (11). The sugar content in brownies can provide energy but has the potential to increase glucose levels if consumed excessively (12). Therefore, the brownie product recipe needs to be modified with safe food ingredients to avoid the occurrence of diabetes.

Modifying the product recipe, such as substituting sugar with stevia sweetener, can help reduce the risk of T2DM. Stevia is a low-calorie natural sweetener derived from the leaves of *Stevia rebaudiana*, which has been proven to have antihyperglycemic effects through its flavonoid content (13,14). The use of stevia in butter cake shows that the higher the stevia content, the lower the energy and carbohydrate levels of the product (15). The next recipe modification for the brownies is to use pumpkin as a substitute for part of the wheat flour as a source of carotene but with low calories and carbohydrates, thus having the potential to address DM issues (16). Based on research regarding on yellow pumpkin, it explains that the administration of yellow pumpkin extract is effective in reducing glucose levels. Therefore, substituting part of the wheat flour can help address DM issues through the high carotene content in yellow pumpkin (17,18).

The development of steamed brownies by substituting cane sugar with stevia sweetener and adding yellow pumpkin can be an alternative snack to reduce the risk of T2DM. In the initial stage of developing the product named *"Browpumpvi"* (Brownies Yellow Pumpkin with Stevia), it is necessary to examine the effect of substituting cane sugar with stevia sweetener and adding yellow pumpkin on the moisture content, ash content, and nutritional content (energy, protein, fat, carbohydrates) of the steamed brownies.

2. METHODS

Study Design

This is a quasi-experimental study design using a Completely Randomized Design (CRD) with the treatment of substituting cane sugar with stevia sweetener. This study uses one control group and three treatment groups with four formulations: 100% cane sugar:0% stevia (P0), 50% cane sugar: 50% stevia (P1), 25% cane sugar:75% stevia (P1), and 0% cane sugar:100% stevia (P3). These formulations were obtained from modifications of the reference formulation from the study by Pratiwi et al., with several experimental tests on brownie products using a combination of cane sugar and stevia (19).

Table 1. Experimental Design

Treatment (%) (Sugar cane: Stevia sweetener)	Replication		
	1	2	3
P0 (100 : 0)	X_{01}	X_{02}	X_{03}
P1 (50 : 50)	X_{11}	X_{12}	X_{13}
P2 (25 : 75)	X_{21}	X_{22}	X_{24}
P3 (0 : 100)	X_{31}	X_{32}	X_{33}

This research was conducted in February-March 2024 at the Culinary Laboratory at Alma Ata University Yogyakarta and proximate testing at the Chem-mix Laboratory Yogyakarta. This research has been approved by the Research Ethics Committee of Alma Ata University and has obtained an ethical clearance letter (No: KE/AA/II10111399/EC/2024).

Research Tools and Materials

The materials used in the product preparation are stevia powder, cane sugar, wheat flour, pumpkin flesh, margarine, eggs, dark chocolate, almonds, leavening agents, and vanilla. The materials used for the nutritional content test are H_2SO_4 solution, a catalyst mixture ($CuSO_4$ + $KzSO_4$), 30% NaOH, 0.1 N HCl, oxalic acid solution, and petroleum ether. The tools used for product preparation include a scale, basin, baking tray, mixer, steamer, spatula, baking paper, and packaging. The tools used for nutritional content testing include an analytical scale, oven, petri dish, desiccator, tweezers, cloth, Kjeldahl flask, Kjeldahl apparatus, biuret, measuring cup, Erlenmeyer flask, pipette, Soxhlet apparatus, and ordinary filter paper (20).

Research Procedure

The product creation procedure begins with the preparation of tools and materials, separation of the flesh, seeds, and skin of the pumpkin, the boiling process of the pumpkin flesh, mixing the ingredients, and the final steaming stage. The product preparation can be seen in Figure 1.

Nutritional content analysis of the product, which includes moisture and ash content analysis using the gravimetric method, protein content using the Kjeldahl method, and fat content using the Soxhlet method (13). Carbohydrate content is determined by difference, and energy content is determined using the Atwater method (21,22). The nutrient content tests were repeated three times for each treatment.

Statistical Analysis

Statistical analysis was conducted using Statistical Package for the Social Science (SPSS) for Windows version 25. Water, ash, fat, protein, carbohydrate, and energy content are normally distributed, so they were analyzed using One Way ANOVA followed by Duncan's post hoc test.

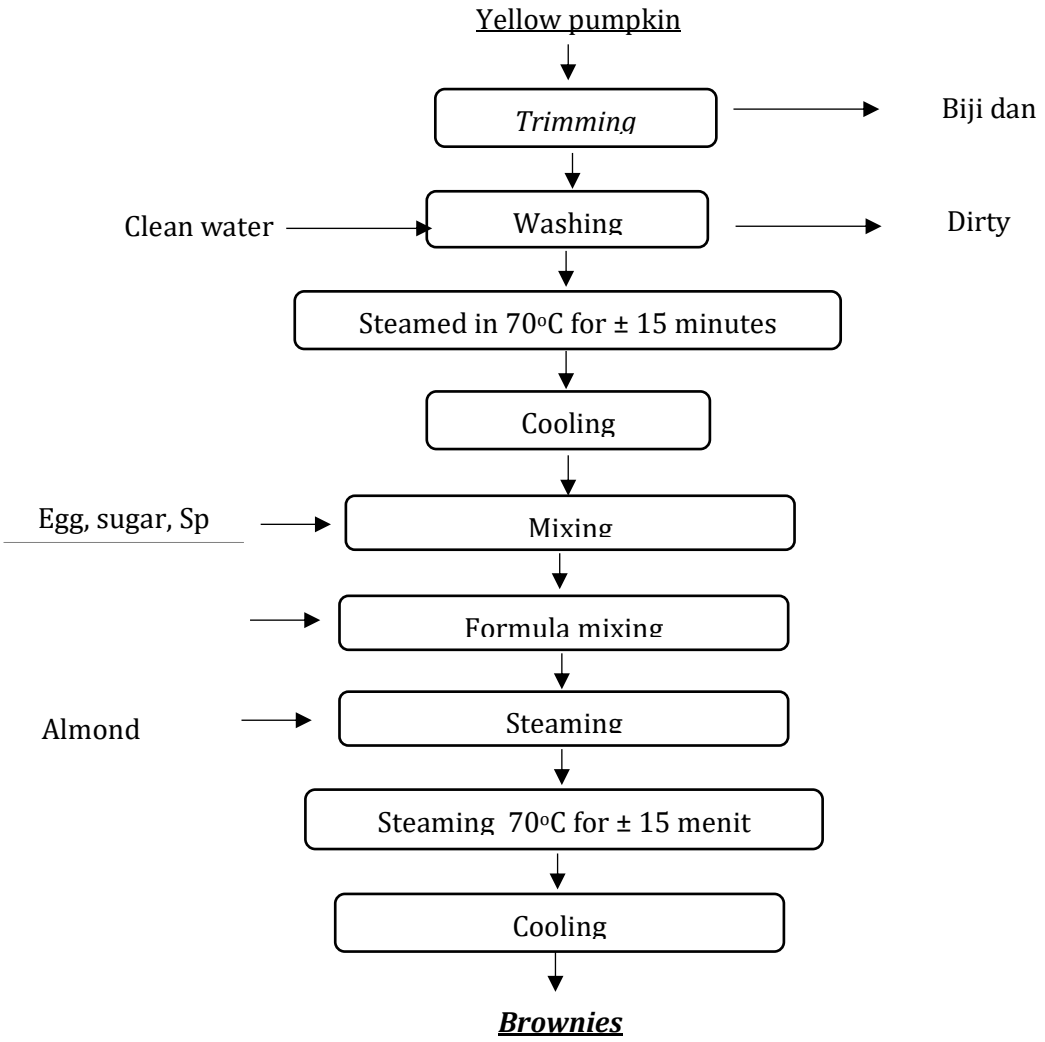

Figure 1. Product Process Development

3. RESULTS

The results of the quantitative analysis of the nutritional content in the Browpumpvi product can be seen in Table 1.

Table 2. Energy and Nutritional Content of *Browpumpvi Product* (per 100 g)

Variables	Mean ± SD	*P-value*
Water		
P0 (100:0)	43,26 ± 0,12[a]	
P1 (50:50)	44,11 ± 0,33[b]	≤ 0,001
P2 (25:75)	46,99 ± 0,41[c]	
P3 (0:100)	48,64 ± 0,04[d]	
Ash		
P0 (100:0)	0,78 ± 0,03[a]	
P1 (50:50)	0,83 ± 0,02[a]	≤ 0,001
P2 (25:75)	1,04 ± 0,02[b]	
P3 (0:100)	1,06 ± 0,06[b]	

Variables	Mean ± SD	*P-value*
Protein		
P0 (100:0)	5,04 ± 0,23[a]	
P1 (50:50)	5,63 ± 0,26[b]	≤ 0,001
P2 (25:75)	6,69 ± 0,06[c]	
P3 (0:100)	6,93 ± 0,11[c]	
Fat		
P0 (100:0)	11,47 ± 0,10[a]	
P1 (50:50)	12,01 ± 0,21[b]	≤ 0,001
P2 (25:75)	13,74 ± 0,08[c]	
P3 (0:100)	14,49 ± 0,34[d]	
Carbohydrate		
P0 (100:0)	39,45 ± 0,23[d]	
P1 (50:50)	37,06 ± 0,12[c]	≤ 0,001
P2 (25:75)	31,54 ± 0,36[b]	
P3 (0:100)	28,88 ± 0,35[a]	
Energy		
P0 (100:0)	281,19 ± 0,76[c]	
P1 (50:50)	279.02 ± 1,43[bc]	0,002
P2 (25:75)	276,65 ± 2,09[b]	
P3 (0:100)	273,68 ± 1,45[a]	

P-value α < 0.05 indicates a significant difference in the substitution of granulated sugar and stevia sweetener in the one-way ANOVA test. Numbers followed by lowercase letters in the row (a, b, c, d) that are different indicate a significant difference according to the 5% Duncan test.

The results of the statistical test show that substituting cane sugar with stevia sweetener has a significant effect on the brownie parameters. For moisture content, the value P<0.001 indicates a significant effect, with the highest moisture content in formula P3 (48.64%) and the lowest in formula P0 (43.26%). The highest content is found in formula P3, which has a sugarcane to stevia sweetener ratio of 0:100%. Therefore, the more stevia sweetener is added, the higher the moisture content will be.

Ash content also showed a significant effect (P<0.001), with the highest ash content in formula P3 (1.06%) and the lowest in formula P0 (0.78%). The highest ash content was found in formula P3, which had a sugarcane to stevia sweetener ratio of 0:100%. Therefore, it can be concluded that the more stevia sweetener is added, the higher the ash content will be.

In terms of protein content, statistical tests also showed a significant effect (P<0.001), with the highest protein content in formula P3 (6.93%) and the lowest in formula P0 (5.04%). The highest protein content was found in formula P3, which has a sugarcane to stevia sweetener ratio of 0:100%. Therefore, it can be concluded that the more stevia sweetener is added, the higher the protein content will be.

The fat content shows a significant effect (P<0.001), with the highest fat content in formula P3 (14.49%) and the lowest in formula P0 (11.47%). The highest fat content is found in formula P3, which has a sugarcane to stevia sweetener ratio of 0:100%. Therefore, it is concluded that the more stevia sweetener is added, the higher the fat content will be.

4. DISCUSSION

Nutritional Content of Browpumpvi Product

Moisture content is an important factor in determining shelf life, packaging methods, and product storage. The moisture content in food materials indicates the amount of water per unit weight in the material (23). The results of the statistical test show the influence of substituting cane sugar with stevia sweetener in each treatment. The water content in the Browpumpvi product increases with the addition of stevia leaf substitution. This condition could be caused by the sugarcane being replaced by stevia, which is hygroscopic, meaning it can attract and retain water, thereby increasing the moisture content of the product with the addition of stevia sweetener (24). Additionally, stevia contains stevioside, which has hydroxyl groups and is polar, making it easy to bond with water (25). Therefore, the addition of stevia leaves as a substitute for cane sugar can increase the water content of Browpumpvi products.

Ash content is the total amount of inorganic or mineral components present in food. (26). The results of the statistical tests show the influence of substituting cane sugar with stevia sweetener in each treatment. The ash content in Browpumpvi products increased with the addition of stevia leaf powder because the ash content in stevia is 11 g, while the ash content in sugar is 0.6 g per 100 g of each ingredient. Therefore, the addition of stevia will increase the ash content in the resulting product. This is also related to the increase in ash content in the product due to the mineral content present in stevia (27). Another study on the evaluation of dried stevia leaves explains that there are calcium, sodium, potassium, magnesium, iron, zinc, copper, and manganese in dried stevia leaves, which increases the ash content (28).

The protein content in Browpumpvi products increases with the addition of stevia leaf powder, as the protein content in stevia is higher compared to cane sugar (29). The protein content in stevia is 10 g, while the protein content in sugar is 0 g, so the addition of stevia will increase the protein content in the resulting product. The protein content in the product is influenced by the amino acid content found in stevia. There are 15 amino acids that have been identified in stevia, namely glutamate, aspartate, lysine, serine, isoleucine, alanine, proline, tyrosine, arginine, histamine, phenylalanine, leucine, valine, tryptophan, and glycine. (30). Therefore, the substitution of stevia leaf powder in the product can increase the protein content, which can be linked to the amino acid content in stevia. In this Browpumpvi product, to meet the needs based on the DM standard for one serving (25 g), the fulfillment is around ± 28%. When compared to the DM requirement, the amount of product that can be consumed to meet the protein needs is three servings.

For the fat content in the Browpumpvi product, it increases with the addition of stevia leaf powder substitution. This is because the fat content in stevia is 6 g while the fat content in sugar is 0 g, so the addition of stevia will increase the protein content in the resulting product. The increase in fat content is influenced by the high amount of fat used. This is related to the fat-soluble compounds in stevia leaves such as essential oils, chlorophyll, and carotenoids (31). Stevia also contains fatty acids such as palmitate, linolenate, oleo palmitate, stearate, and oleate. (32). Therefore, stevia contributes to the increase in the fat content of the product. The consumption of fat intake, especially by reducing saturated fats and increasing the intake of unsaturated fats, can help in the prevention and management of T2DM. Fat intake is related to glucose levels. In this Browpumpvi product, to meet the DM standards, one serving (25 g) fulfills

approximately 57% of the requirement. When compared to the DM requirement, the amount of product that can be consumed to meet the fat requirement is a maximum of two servings.

Meanwhile, the carbohydrate content in the Browpumpvi product decreases as the substitution of stevia leaves increases. This is related to the fact that stevia sweetener plays a significant role in the low carbohydrate content; the carbohydrate content in stevia is 52 g, while the carbohydrate content in sugar is 94 g per 100 g of each ingredient. Therefore, the addition of stevia will reduce the carbohydrate content in the resulting product. Stevia sweeteners do not contain sucrose, glucose, and fructose, which are carbohydrate components in cane sugar; therefore, the carbohydrate content decreases in proportion to the amount of stevia used (33,34). Stevia is known as a natural sweetener because it has a low glycemic index (GI). GI is an indicator to measure how quickly carbohydrates in food are broken down into glucose. Stevioside in stevia is a non-carbohydrate glycoside compound that is not broken down into glucose in the body (35,36). Therefore, the substitution of stevia sweetener does not contribute excess carbohydrates. In this study, the carbohydrate content was calculated by difference, influenced by other nutritional components. If the nutritional components (water, ash, fat, protein) are lower, then the carbohydrate content is higher, and vice versa (37).

Energy Content of Browpumpvi Product

The energy content in the Browpumpvi product decreases with the increasing substitution of stevia, as the energy content of stevia is 270 g while the energy content of sugar is 394 g/100 g of each ingredient. Therefore, the addition of stevia will reduce the energy content of the resulting product. The minimal caloric impact of stevia is caused by its metabolic pathway. Because steviol glycosides are not absorbed in the upper digestive tract and are only broken down in the large intestine, the glucose units released during this process are fermented by gut bacteria, resulting in minimal energy production (38). In the research results of the Browpumpvi product, it is in line with studies on products using stevia as a natural sweetener, such as the example of research on low-calorie cake making, which shows that with each treatment of adding stevia, the energy content decreases (39).

Potential of the Product as Functional Food

Functional foods are food ingredients, whether derived from animal or plant products, whole or fresh, that contain bioactive compounds to enhance a person's health, in addition to their nutritional content and taste. (40). Browpumpvi products have the potential to be functional foods. This can be seen from research results that the use of stevia as a substitute for cane sugar in Brownies can significantly reduce calorie and carbohydrate content without diminishing the sweetness of the product. The Browpumpvi product can be used as one of the snacks to prevent type 2 diabetes.

Functional foods can be used as food to prevent various diseases such as obesity, diabetes, hypertension, coronary heart disease, and cancer (9). The Browpumpvi product has the potential to be a functional food. This can be seen from the research results that the use of stevia as a substitute for cane sugar in brownies can significantly reduce the calorie and carbohydrate content without diminishing the sweetness of the product. Thus, it can be said that this product has the potential to be a functional food, but a more detailed fiber analysis is needed to specifically evaluate the impact of the

combination of pumpkin and stevia on this functional food product in preventing the occurrence of T2DM.

5. CONCLUSION

There is a significant effect of substituting cane sugar with stevia sweetener on the levels of moisture, ash, protein, fat, carbohydrates, and energy. The higher the proportion of stevia sweetener substitution, the higher the moisture, ash, protein, and fat content of the Browpumpvi product, while the carbohydrate content and energy content of the Browpumpvi product decrease as the stevia sweetener substitution increases. For the community, it is hoped that the Browpumpvi product will serve as information and a reference in making low-calorie and low-carbohydrate snacks by utilizing functional foods from pumpkin and stevia leaves. However, further research is still needed regarding other nutritional contents to assess the potential of the product as a functional food.

CONFLICT OF INTEREST

The authors declare that there were no conflicts of interest in this study.

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