

Glycemic Index and Glycemic Load of Gluten-free Brownies Made from Combination of Mocaf and Black Glutinous Rice Flour

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Abstract: Brownies are generally seen as foods that are high in sugar, then so many people are reluctant to consume them. Mocaf is gluten-free flour known to have a low to moderate glycemic index. Meanwhile, black glutinous rice is a carbohydrate source rich in amylopectin so that it can contribute to the formation of a good texture in brownies. The aim of this study is to analyze the glycemic index (GI) and the glycemic load (GL) of brownies made from a mixture of mocaf flour and black glutinous rice flour with a ratio of 1:1, 1:2, and 2:1. The research method used was a completely randomized one factor design. The chemical component test data were analyzed by One Way ANOVA test continued by Tukey test. The glycemic index of the three treatments was included in the low GI category, which was 37.66 in the ratio of flour 1:1, 41.12 in the ratio 1:2, and 31.28 in the brownies with a ratio of flour 2:1. The glycemic load obtained was also in the low category, i.e. 9.2, 9.3, and 8.2.

1 INTRODUCTION

Brownie is one of the cakes originally coming from America that has been widely produced in Indonesia. It is a featured product in several cities, not to mention one of the people's preference. However, despite its popularity, people usually hesitate to consume the cake due to its high glucose level. Besides, it also uses flour as the main ingredient. Papandreou (2017) argues that gluten in flour is one of the nutritional components in food avoided by some people, especially Celiac Disease (CD) and Non-celiac Gluten Sensitivity (NCGS) patients. Meanwhile, healthier brownies have been produced using substituted ingredients. Nevertheless, diabetic patients and other people intending to reduce their carbohydrate or sugar level rarely find brownies that may not increase their blood glucose level or cause obesity.

Mocaf (modified cassava flour) is one of the processed product of cassava that may substitute flour. In addition to that, it is the main ingredient in gluten-free food products. Kalukiningrum (2012) examined several cookie and cake products made from mocaf. The research figured out that the acceptance level of fruit cake 70%, cheese cake 83%, and chocolate cake combined with bread rolls 77%

was accepted by panelists. Firdaus (2013) investigated glycemic index of analog rice made from mocaf combined with corn starch, purple sweet potato flour, and carrot resulted analog rice with GI ranging from 59 to 64. The range was categorized into moderate IG. Furthermore, Suryaningrum (2016) produced flakes made from yellow pumpkin mocaf (40:50) with GI 54.36 and glycemic load 8.55.

Previous research indicates that brownies made from mocaf and black glutinous rice flour with a ratio 1:2 were a censor and preferred product with total sugar level 1.32% (Widanti and Mustofa, 2015). Black glutinous rice is one of the grains rich in antioxidant due to its anthocyanin color pigment. Anthocyanin has functional properties that serve as antioxidant for body, protect stomach from damage, inhibit tumor cells, increase eyesight ability, served as anti-inflammatory compound that protect brain from potential damage, and prevent obesity and diabetes. Apriani (2015) concluded that green bean porridge added with black glutinous rice contained lower GI than green bean porridge without black glutinous rice. Food with high fiber level usually contains lower IG; while higher amylose proportion in food reduces the food GI index (Arif *et al.*, 2013).

Brownie made from the combination of mocaf and black glutinous rice may result in gluten-free brownie that is healthier to consume. Therefore,

glycemic index of brownies made from mocaf and black glutinous rice should be identified to know if the brownies can be safely consumed by all people including diabetic patients.

2 MATERIALS AND METHODS

2.1 Research Design

The research design was referred to Handayani and Ayustaningwarno (2014) method using a one-factor completely randomized design in the form of three ratios of mocaf to black glutinous rice i.e. 1:1, 1:2, and 2:1. We analysed total glucose and starch level, examined the glycemic index, and measured the glycemic load.

2.2 Materials

The research materials were mocaf, black glutinous rice flour, and other brownie ingredients that were dark compound chocolate, butter, sugar, egg, chocolate powder, and baking powder. Furthermore, chemical ingredients used in analysis process were aquades, anhydrite glucose, nelson, arsenomolybdate, Cu₂O, and others.

2.3 Instruments

The research instruments included a mixer, microwave, digital scale, 10cm×15cm×8cm brownie pan, laboratory analytical instruments, blood glucose measurement kit One Touch UltraTM, and others.

2.4 Research Stages

2.4.1 Formula and Procedure

The research was initiated by making a basic baked brownies with ratios of mocaf to black glutinous rice flour 1:1, 1:2, and 2:1; while the making method was referred to that in the previous research (Widanti and Mustofa, 2015).

2.4.2 Chemical Analyses

The second stage involved chemical analyses on Total sugar content (Gustiar, 2009), Protein content applying Kjehldahl method (AOAC, 1995), Lipid content (AOAC, 1995), and Fibre content (AOAC, 1995).

2.4.3 Screening the Research Volunteer Candidates

The third stage was screening the research volunteer candidates consisting of male/female students of Faculty of Food Technology and Industry UNISRI meeting these following criteria: 18-25 years old, having normal pre-prandial blood glucose (PBG) (60-120mg/dl), having normal body mass index (BMI) (18.5-24.9kg/m²), healthy, having no DM history, having no indigestion history, not undergoing any medical treatment, not taking any psychoactive drugs, not drinking any alcoholic beverages, and willing to be a volunteer.

2.4.4 Glycaemic Index Test and Glycaemic Load Measurement

Glycaemic Index Test (Noviasari et al., 2016). In a glycaemic index test, we required at least six volunteers selected based on the criteria. In this research, we requested ten volunteers, avoiding any drop out. One day before given the treatment, they had to fast for ten hours (drinking plain water was allowed) started from 10 p.m. until 8 a.m. In the next morning, we collected ±5µl of their blood by touching the blood drop on the finger prick and measured the blood glucose using a glucometer One Touch UltraTM (finger prick capillary blood sample method). Volunteers ate the tested food (25g of pure glucose) for three subsequent days. After that, they ate the tested brownies with ratios of mocaf to black glutinous rice flour 1:1, 1:2, and 2:1 equal to 25g available carbohydrate. Sample of volunteers' blood was collected in every 30 minutes (30th, 60th, 90th, and 120th minutes). Each treatment was given in every three days to avoid any bias of each tested food. The balance of the amount of carbohydrate was obtained from brownie proximate analysis. The number of brownies consumed was calculated using this following formula:

$$\text{weigh of brownies} = \frac{25\text{g of carbohydrate} \times 100\text{g}}{\text{Carbohydrate level of brownies}} \quad (1)$$

IG was measured using Incremental Area under the Blood Glucose Response Curve (IAUC) method by dividing the area under the glycaemic response curve of sample by the area under the standard glycaemic response of glucose multiplied by 100%.

Glycaemic Load (GL) Measurement. Glycemic load was measured by multiplying GI by available carbohydrate level of brownies made from mocaf and black glutinous rice flour obtained from the test on total starch and sugar.

$$GL = \frac{GI \times \text{the amount of available carbohydrate for each portion}}{100} \quad (2)$$

2.4.5 Data Analysis

IG and BG data collected were analyzed using One Way ANOVA test with the 95% confidence interval. If a significant difference was found, we proceeded with Tukey test.

3 RESULT AND DISCUSSION

3.1 Subject Characteristics

The research used seven subjects consisting of three males and four females aged 18-20 years old with a normal nutrition status (BMI 18.4-23.8kg/m²) and normal pre-prandial blood glucose level (<110mg/dL). All subjects had signed the informed consent. Subject characteristics are listed in Table 1. Section, subsection and sub subsection first paragraph should not have the first line indent, other paragraphs should have a first line indent of 0,5-centimeter.

Table 1: Characteristics of Research Subjects.

Subject	Age (Year)	BW (kg)	BH (m)	BMI (kg/m ²)	PBG (mg/dL)
1	19	58	1.60	22.65	99
2	20	53	1.64	19.70	82
3	18	60	1.63	22.58	94
4	19	49	1.45	23.30	101
5	18	53	1.49	23.87	89
6	20	45	1.56	18.49	104
7	20	52	1.57	21.09	76
Mean	19.14±0.899	52.86±5.08	1.56±0.07	21.67±1.98	92.14±10.35

According to Table 1, the mean of subjects' age and IMT was 19.14 and 21.67 respectively. The subjects' IMT was categorized into normal and in accordance with our subject inclusion criteria. Furthermore, PBG was also categorized into normal (<110mg/dL) despite its moderate variance level (Standard Deviation = 10.35). The whole characteristics of sample did not indicate any deviation from the inclusion criteria determined.

3.2 Determination of Sample's Weight

Gluten-free brownies made from mocaf-black glutinous rice flour used as the tested food was given three treatments that were different ratios of mocaf to black glutinous rice flour 1:1, 1:2, and 2:1. The amount of sample given to subjects was determined based on sugar and starch level of each sample. It was equal to 50g of available carbohydrate. Moreover, available carbohydrate and the amount of sample were determined using the following formulae (Handayani and Ayustaningwarno, 2014).

$$\text{Available carbohydrate} = \text{total glucose level} + (1.1 \times \text{starch content})$$

$$\text{Amount of sample} = \frac{25 \text{ g}}{\text{Available carbohydrate}} \times 100$$

Standard food material used was 25 of pure glucose. The result of the calculation of the number of brownies made from mocaf-black glutinous rice flour given to subjects is presented in Table 2.

Table 2: The Amount of Brownies as the Tested Food Made from Mocaf-Black Glutinous Rice Flour Equal to 25 g of Available Carbohydrate.

Ratio of The Flours	Starch Content (%)	Total Sugar Content (%)	Available Carbohydrate (%)	Mass of Brownies Sample (g/subject)
1:1	4.79	16.47	21.74	229.99
1:2	4.15	14.05	18.61	268.66
2:1	5.52	12.71	18.78	266.18

3.3 Result of Chemical Analysis

Chemical analysis on brownies made from mocaf-black glutinous rice flour involved analyses on total sugar, starch, protein, lipid, and fiber levels. The results of analyses on total sugar and starch levels are listed in Table 2; while the results of analyses on total protein, lipid, and fiber contents are presented in Table 3.

Table 3: Protein, Lipid, and Fibre Content (%).

Ratio of The Flours	Protein Content (%)	Lipid Content (%)	Food Fiber Content (%)
1:1	6.50±0.0005	34.34±0.0018	10.46±0.0010
1:2	6.44±0.0004	33.13±0.0009	13.23±0.0017
2:1	5.17±0.0005	32.53±0.0022	14.30±0.0025

3.4 Glycemic Response, Glycemic Index, and Glycemic Load

Glycemic index indicated the blood glucose response of subjects consuming food containing carbohydrate compared to that of subjects consuming standard food that was in the form of glucose or bread (Venn and Green, 2007). High GI rapidly increased blood glucose; while low GI slowly increased blood glucose. The high GI was affected by various factors, among them were the comparison of amylose and amylopectin, monosaccharide sugar level, starch digestibility, fiber level, protein and lipid levels, processing method, and anti-nutrition compound presence (Arif *et al.*, 2013).

Blood glucose response to standard glucose food and brownies made from mocaf-black glutinous rice flour with the ratios of mocaf to black glutinous rice flour 1:1, 1:2, and 2:1 is presented in Figure 1.

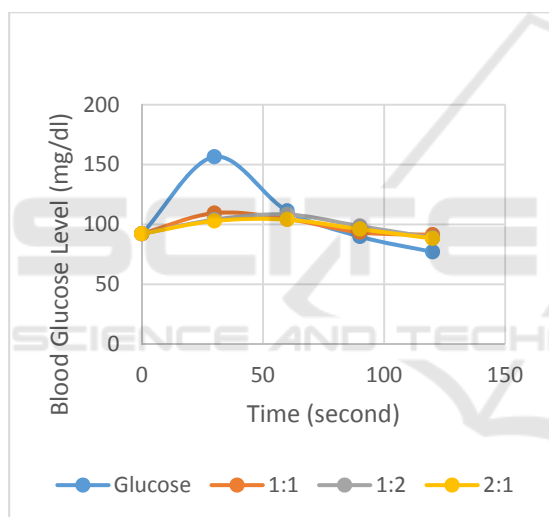


Figure 1: Blood glucose Response to Standard Food (Glucose) and Tested Food (Brownies Made from Mocaf-Black Glutinous Rice Flour).

Physiological condition of sugar blood level of someone after food consumption was called glycemic response. Meanwhile, food with low GI and that with high GI were different on the basis of their glucose digestion and adsorption rates and glucose level fluctuation in blood. Food with low GI had a slow digestion process slowing the rate of gastric emptying and causing food suspension (chime) to be slowly proceeded to the small intestine. It caused a relatively small fluctuation of blood glucose level indicated by sloping glycemic response curve. Contrastively, food with high GI indicated a rapid rate of carbohydrate digestion, glucose adsorption, and gastric emptying,

so that the fluctuation of blood glucose level was also relatively high. It was because most glucose adsorption occurred on the upper part of small intestine only. Moreover, glycemic response curve of food with high GI indicated fluctuation in the forms of more prominent peak and sharper decline curves (Arif *et al.*, 2013).

Glycemic index of gluten-free brownies made from mocaf-black glutinous rice flour was measured using Incremental Area under the Blood Glucose Response Curve (IAUC) method. We compared the area under the blood glucose response curve of standard food with the area under the blood glucose response curve of the tested food.

Formulae to measure the area under the curve by Suryaningrum (2016) was as follows:

$$L = \Delta 30t/2 + \Delta 30t + (\Delta 60 - \Delta 30)t/2 + \Delta 60 + (\Delta 90 - \Delta 60)t/2 + \Delta 90 + (\Delta 120 - \Delta 90)t/2$$

Description:

- L = area under the curve
- t = time interval of blood taking (30 minutes)
- Δ30 = difference of blood glucose level 30 minutes among after consume the sample and after fasting
- Δ60 = difference of blood glucose level 60 minutes among after consume the sample and after fasting
- Δ90 = difference of blood glucose level 90 minutes among after consume the sample and after fasting
- Δ120 = difference of blood glucose level 120 minutes among after consume the sample and after fasting

Result of glycemic index measurement (Table 4) indicated that all treatments in the form of different ratios of mocaf to black glutinous rice flour generated brownies with low-IG.

Table 4: Glycemic Index of Gluten-free Made from Mocaf-Black Glutinous Rice (BGR) Flour.

Tested Food	Glycemic Index	Category
Brownies mocaf-BGR flour 1:1	37.66	Low
Brownies mocaf-BGR flour 1:2	41.12	Low
Brownies mocaf-BGR flour 2:1	31.28	Low

Low GI (< 55), moderate GI (55-70), high GI (> 70) (Venn and Green, 2007).

The more the mocaf used, the less the brownie IG. Mocaf was cassava flour modified through a fermentation process. One of the starch modification products in cassava flour was the formation of resistant starch that would elevate the functional value of the flour (Onyango et al., 2006). Resistant starch was a starch fraction or starch degradation product that was not absorbed by a healthy individual's small intestine because the starch was formed after perfectly passing an enzyme degradation process. The higher the resistant starch in food, the lessened the starch digestibility. It indicated the lessened amount of starch that would be hydrolyzed into glucose and absorbed in digestion, reducing its effects on the increase of blood glucose.

In addition to the resistant starch, another factor influencing brownie GI was amylose and amylopectin levels in mocaf or black glutinous rice flour. Higher amylose level lowered the digestion rate, as amylose was a glucose polymer with unbranched structures (more crystalized structures with more extensive hydrogen bond). Furthermore, it had a stronger hydrogen bond than amylopectin, making digestion enzymes difficult to hydrolyze it. Such unbranched structure made amylose more tightly bound, making it difficult to be gelatinized and digested. Besides, amylose easily joined and crystalized, so it easily experienced retrogradation, making it difficult to be digested (Arif, 2013).

Amylose and amylopectin levels in mocaf were 34.75% and 39.55% respectively. Amylose level in black glutinous rice flour was lower that was 1-2%; while the amylopectin level was 98-99%. Therefore, the more the black glutinous rice flour used in brownies, the higher the GI.

Moreover, amylopectin was easier to be gelatinized and digested by human body, because a simple branched glucose polymer and having larger and more open molecules. Research indicated that food with more amylose proportion would have lower GI (Behall et al., 1998).

In addition to resistant starch and amylose-amylopectin levels, GI was also influenced by fiber level (Arif, 2013). The more the mocaf, the higher the fiber level of brownies made from mocaf-black glutinous rice flour. Fiber levels of mocaf and black glutinous rice flour were 1.9-3.4% and 6.09% (Azis et al., 2015) respectively. Both ingredients gave a contribution to the fiber level of brownies made from mocaf-black glutinous rice flour. However, higher fiber level of black glutinous rice flour than that of mocaf gave the main fiber to brownies.

Arif (2013) believed that high fiber level in food contributed to a low IG. The whole fiber food served

as a physical inhibitor in food digestion and enzyme activities, slowing the starch digestion process and lowering the blood glucose level.

Arif (2013) also clarified that lipid and protein levels were two factors affecting GI. High-fat in food inhibited the rate of gastric emptying, slowing the rate of food digestion in the small intestine. Meanwhile, high protein level stimulated insulin secretion, controlling the glucose level. Therefore, food with high fat and protein levels tended to have lower GI than similar food with low fat and protein levels.

Glycemic load was the value of blood glucose response of someone after the consumption of one food portion with a certain carbohydrate level. It was measured by multiplying food GI by the amount of carbohydrate in the food and dividing it by 100. GL might provide a more thorough description of food impacts on blood glucose level.

Table 5: Glycemic Load of Gluten-free Brownies Made from Mocaf-Black Glutinous Rice (BGR) Flour.

Tested Food	Glycemic Load	Category
Browniesmocaf-BGR flour 1:1	9.21	Low
Brownies mocaf-BGR flour 1:2	9.30	Low
Brownies mocaf-BGR flour 2:1	8.25	Low

Low GL (< 10), moderate GL (11-19), high GL (> 20) (Venn and Green, 2007).

Venn and Green (2007) concluded that higher GI in food might result in low GL if the food was consumed in a small amount; while low GI might result in high GL when consumed in an excessive amount.

Glycemic load might give a complete description of the impact of carbohydrate food portion consumed. In this research, the tested food given to respondents contained available carbohydrate equal to 25 gr. Low GL found in the three brownie products would give them low blood sugar response.

4 CONCLUSIONS

Gluten-free brownies made from mocaf-black glutinous rice flour had low glycemic index (GI) and glycemic load (GL). GI of brownies with the ratios of mocaf to black glutinous rice flour 1:1, 1:2, and 2:1 were 37.66, 41.12, and 31.28, respectively; while GL of that was 9.21, 9.30, and 8.25 respectively.

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