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Glycemic index of *Coleus tuberosus* crackers rich in resistant starch type III

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Abstract

This study aimed to analyze the glycemic index value of processed products of *Coleus tuberosus*. The study included ten healthy subjects with seven males, three females (30 ± 3.80 years on average age and 22.49 ± 1.82 on average BMI). Participants tested three different meals (D-glucose anhydrous, wheat crackers, and *Coleus tuberosus* crackers) with equal carbohydrate load (50 g). Blood glucose concentrations were measured at 0 min as well as at 30, 60, 90 and 120 min after the start of the meal for glucose levels determination. The result showed that the glycemic index of *Coleus tuberosus* crackers rich in resistant starch tipe III categorized in the low category. The glycemic index value of *Coleus tuberosus* crackers at 40.88 ± 6.42, whereas wheat crackers has high glycemic index (76.08 ± 5.36). Based on this results, *Coleus tuberosus* crackers is good for control of blood glucose profile.

Introduction

Progress in the field of economic social experienced by Indonesia caused a shift in the pattern of diseases, from infectious diseases to degenerative diseases, for example diabetes mellitus. Diabetes mellitus is a condition of elevated blood sugar levels in chronic with various metabolic disorders due to hormonal disturbances that cause a variety of chronic complications in various organs of the target. International Diabetes Federation states that in 2025 patients with diabetes mellitus is expected to reach 333 million (6.3%) people (Tabari and Larijani, 2005). The countries such as India, China, USA, Japan, Indonesia, Pakistan, Bangladesh, Italy, Russia and Brazil are top 10 countries with the highest number of people with diabetes. In the Diabetes Care (Wild, 2004) predict that Indonesia in 2000 the number 4th most diabetes (8.4 million people) and in 2030 will remain the number 4th in the world, but with 21.3 million people with diabetes. This forecast will be true if there is no effort from us to prevent or at least eliminate the factors causing an explosion that amount. Diabetes mellitus is a disease can't be cured and is closely linked to the lifestyle of modern society. However, people with diabetes can still live comfortably when can manage the diet and choose the right type of food. The need for the right kind of food, especially of the type of carbohydrate food source is the main attraction for researchers to develop research related to the glycemic index (GI).

Starch is the most important carbohydrate and considered as the major dietary source and found in the form of polysaccharide in plants, such as seeds, pulses and tubers (Sajilata *et al*., 2006). Resistant starch is in nature found in all starchy food, cereal grains, and seeds (Charalampopoulos *et al*., 2002). When Starch granules are heated in excess water that becomes disrupted and whole process is known as gelatinization that prevents the starch molecules fully accessible to digestive enzymes. After heating then cooling of starch is done then relatively slow re-association of starch molecules is occur this process is commonly termed retrogradation of starch (Colonna *et al*., 1992) during this process starch molecules re-associate themselves and can form tightly packed structures which have strong hydrogen bonding. This structure is thermally very stable and can't easily be rehydrated.

Resistant starch is the fraction of starch that is not digested by the digestive enzymes in the small intestine of healthy humans. Resistant starch digestibility of nutrients in the diet has implications for causing a low rate of hydrolysis of starch in the digestive tract. Naturally, resistant starch digestibility contained in a food either raw or processed. In the processed foods, resistant starch digestibility can be formed by the combination of heat, humidity and sometimes pressure. One way to increase levels of resistant starch digestibility in food is to heat the starch to gelatinized then cooling it rapidly. Starches are grouped into four types, namely: (1) resistant

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starch type I is the group of starch that is trapped in the cell walls of plants, for example starch present in cereals and legumes, (2) resistant starch type II is the starch granules raw like starch potatoes, corn and so on, (3) resistant starch type III in the form of starch retrogradation or starch in the form of crystals, contained in cornflakes, potatoes are cooked refrigerated and others, (4) resistant starch type IV is a starch modified chemical (Sajilata *et al.*, 2006).

The concept of glycemic index (GI) is metabolic approach to choose good food, especially carbohydrate food. This concept is useful for fostering health, prevent obesity, choosing food to exercise, and to reduce the risk of disease metabolism. IG concept emphasizes the importance of knowing the food (especially kind of carbohydrates) based on the velocity raise blood glucose levels quickly (Foresters *et al.*, 2005). The glycemic index (GI) is the level of food according to their effect on blood sugar. Foods raise blood sugar levels quickly have a high GI. Conversely, foods raise blood sugar levels slowly have a low GI. The glycemic index of food is influenced by the content of amylose, protein, fat, fiber and starch digestibility. Digestibility of starch is starch ability to be digested and absorbed in the body. Slowly absorbed carbohydrates produce lower blood glucose levels and potentially controlling blood glucose levels (Agustin *et al.*, 2015).

Coleus tuberosus is one of the agricultural products in Indonesia as a source of carbohydrate. As a source of carbohydrates, the *Coleus tuberosus* consumption may have an impact on the increase in blood glucose levels. Efforts are made to control blood glucose levels after eating *Coleus tuberosus* is the process into a rich *Coleus tuberosus* starch resistant starch type III by means of heating and cooling. Many research proves that the content of resistant starch in foods are able to control glucose profiles, and lipids in experimental animals or humans who suffer from diabetes mellitus (Park *et al*., 2004; Nugraheni *et al*., 2014; Bodinham *et al*., 2014). Resistant starch can be generated from processing (heating and cooling starchy materials repetitive (Kingman and Englyst, 1994). The process of repeated heating and cooling can increase the levels of resistant starch in the starchy material (Dundar and Gocmen, 2013). The stages of processing can increase the levels of resistant starch of *Coleus tuberosus* (Nugraheni *et al.*, 2015a)

This study aimed to obtain information on glycemic index of *Coleus tuberosus* crackers. Given this research is expected to provide information about the ability of resistant starch type III *Coleus tuberosus* starch to the management of diabetes mellitus as well as to encourage cultivation of *Coleus tuberosus* in support of food security based on inferior food sources and not assume that *Coleus tuberosus* as a source of carbohydrates alone, but more than it has potential as a functional food that is useful in improving public health.

Materials and Methods

Raw material production of *Coleus tuberosus* crackers and wheat crackers are obtained from the market in Yogyakarta, D-glucose anhydrous (Sigma). The tools used in practical measurement of glycemic index is easy touch GCU Meter, Made in Taiwan. While the materials used for blood sampling include analysis of glucose strips, lancet, and alcohol swab. Foodstuffs used include food testing, were *Coleus tuberosus* crakers rich in resistant starch type III and wheat crackers. Foodstuffs standards used, namely D-glucose anhydrous (Sigma). While the measurement data processing glycemic index is done by using Microsoft Excel for Windows 2007.

Subjects

Selection of research subjects undertaken purposively for reasons of convenience in the study, i.e 10 people who meet the inclusion and exclusion criteria, willing to follow the study (listed in the informed consent). Inclusion criteria for subjects aged 22-40 years i.e, have a normal body mass index between 18.5-25 kg / m and healthy. The exclusion criteria that had no history of diabetes, are not experiencing indigestion, not men live treatment, do not use drugs forbidden, and do not drink alcoholic beverages. The use of ten subjects provides useful results (Brouns *et al.*, 2005).

Each subject was asked to undergo testing by eating three types of treatment in the span of three days, from glucose anhydrous D solution (as a control), *Coleus tuberosus* crackers rich in resistant starch type III and wheat flour crackers. To know the Glycemic Index of each treatment product, after eating the product, in a span of two hours, to subject blood samples were taken at minute 0, 30, 60, 90 and 120.

*Preparation of experimental food (*Coleus tuberosus *crackers rich in resistant starch type III and wheat flour crackers).*

The process of making *Coleus tuberosus* flour rich in resistant starch type III was by steaming process treatment (temperature of 100^{o}C for 15 minutes) and continued the process of refrigeration (temperature of 4^{o}C for 24 hours). The next process stage were drying (temperature of 60^{o}C for 12 hours),

grinding and screening (Tyler 80 mesh) so obtained *Coleus tuberosus* flour rich in resistant starch type III. And then analyzed the levels of resistant starch and amylose to know the influence of processing on the formation of resistant starch.

Composition of *Coleus tuberosus* crackers rich in resistant starch type III were composite flour, margarine, baking soda. Composite flour is flour mixture *Coleus tuberosus* flour rich in resistant starch type III with wheat flour in the ratio 1:4 (Table 1). Formulation of *Coleus tuberosus* crackers rich in resistant starch type III 1:4 chosen based on sensory evaluation conducted by the panelists were untrained as many as 80 people (Nugraheni *et al.*, 2015b)

The processing step are: Mix the flour, yeast, salt, baking soda and cream of tartar. On the other place mixed hot water, molasses and shortening. Mix well, then add to the flour mixture. Mix until blended for 4 minutes so the dough is elastic and smooth. Place the dough in basin and cover all sides with plastic and refrigerate for 18 hours. Roll dough until thin. Cut and shape the hole. Baking 180°C for 20 minutes. Remove from oven and spread with butter, and chill in room temperature. Processing methods of wheat flour crackers such as *Coleus tuberosus* cracker, the difference between them is a composition of 100% wheat flour.

Glycemic index determination

The product is given to volunteers who have undergone full fasting (except water) for overnight (about 22.00 until 08.00 the next day). Treatment is aimed at letting fasting blood sugar levels back to normal so that when analyzing no influence from other carbohydrates. The subjects were 10 healthy individuals. It can be seen that use of ten subjects provides useful results (Brouns *et al*., 2005). Before consuming the food test, the respondents have blood drawn through the fingertips and measured glucose levels. The results are expressed as a fasting blood glucose level (glucose minute 0). After consumption of the products, blood sample taken from the fingertip back every 30 minutes to measure the levels of glucose (glucose measurement minute 30, 60, 90, and 120). As standard, respondents were given 50 grams of pure glucose.

Blood glucose response curve was constructed from the average blood glucose concentration obtained pre- and post- eating experimental food as a function of time. The incremental area under the curve (IAUC) was calculated for each tested food (glucose, *Coleus tuberosus* crackers or wheat flour crackers) in each subject, as the sum of the surface triangles and trapezoids between the blood glucose curve and the horizontal baseline running in parallel to the time axis from the beginning of the curve to the point at 120 min. The IAUC for 50 g of pure glucose was obtained in a similar way (Camille *et al*., 2014). The GI for *Coleus tuberosus* was finally calculated as the mean of the average of the GI in ten subjects in the group. Each crackers servings will be determined his IG contains 50 g of carbohydrates. The area under the curve of blood glucose response after eating *Coleus tuberosus* crackers rich in resistant starch type III. The results are then categorized low <55; medium 55-70 and high > 70.

Table 1.Formulation of wheat crackers and *Coleus tuberosus* crackers rich in resistant starch type III

Ingredient	Wheat crackers (Control)	*Coleus tuberosus* crackers (1:4)
Wheat flour (g)	187.5	150
Coleus tuberosus flour rich in resistant starch (g)	-	46.875
Yeast (g)	9.56	9.56
Salt (g)	1.25	1.25
Baking soda (g)	1.15	1.15
Cream of tartar (g)	1.19	1.19
Hot water (ml)	166.67	166.67
Molase	¼ sdt	¼ sdt
Shortening (g)	26 g	26 g
Butter, melted (ml)	30 ml	30 ml

Processing and data analysis

Data results then processed using Microsoft Excel 2007 and analyzed descriptively. Blood glucose response data is processed to obtain glycemic index value. Influence of processing on the glycemic index values were analyzed using analysis of variance (one way ANOVA) with software SPSS 16.0 for Windows.

Results and Discussion

Descriptive characteristics

The selected respondents consisted of 10 people consisting of 7 men and 3 women with normal health status. Normal conditions is meant here is that they have good nutritional status assessed from BMI in the normal range fit the inclusion criteria. The identity of the sample such as age, weight, and height were collected to determine compliance with the study inclusion and exclusion criteria. Criteria for people with diabetes mellitus not be obtained with the fulfillment of absence family history of the disease. The absence of a family member with diabetes, especially in the closest relatives of the generation above us. Age of respondents are in the range of 20-

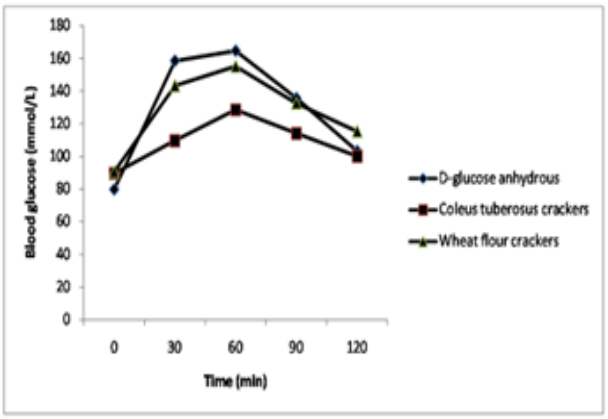

Figure 1. Blood glucose level (mmol/L) for 120 min after consumption of D-glucose anhydrous, *Coleus tuberosus* crackers and wheat flour crackers tested in normal subjects (n = 10).

40 years, with an average body weight 58 kg. On average fasting blood glucose levels 90.125 mg / dL.

Blood glucose response

Results of the blood glucose response of each respondent to administration of the test food ingredients can be seen in Figure 1. Based on Figure 1, the consumption of pure glucose, *Coleus tuberosus* crackers and wheat crackers raise blood glucose with different levels. Either pure glucose (D-glucose anhydrous), *Coleus tuberosus* crackers and wheat crackers have the same peak time is in the 60 minute, but with different quantity. Base on Figure 1 shows that the blood glucose level of *Coleus tuberosus* crackers lower than D-glucose anhydrous and wheat flour crackers.

The difference in raise of blood glucose in *Coleus tuberosus* crackers and wheat crackers are allegedly more affected by difference in amylose content and resistant starch. The higher amylose content, then the digestion becomes slower. Resistant starch is starch that can't be hydrolized by digestive enzymes and has an impact on the thickness of the bowel contents led to a decrease in the activity of α-amylase so that slow down the absorption of glucose. Resistant starch content on *Coleus tuberosus* crackers is 15.37%, while in wheat crackers is 7.35%. Amylose content on *Coleus tuberosus* cracker contains 10.67% amylose, while the wheat cracker contains 6.43% amylose (Nugraheni *et al*., 2015b).

Glycemic index

Glycemic response is a physiological condition of blood glucose levels during a certain period after a person consumes food. A carbohydrate derived from different plants, have different glycemic responses (Frei *et al.*, 2003). Differences in glycemic response may also occur in carbohydrates derived from the same plant, but different varieties. Like the previous, food raises blood glucose level quickly have a high GI, otherwise food raise glucose levels Blood slowly have a low GI (Ragnhild *et al*., 2004; Atkinson *et al.*, 2008). IG value is calculated based on the ratio between the area of the curve rise in blood glucose after eating the food that was tested by the rise in blood glucose after eating a standardized reference food, such as glucose or white bread (Brouns *et al*., 2005). Glycemic response curve shown by the fluctuations of the absorption of glucose in the blood.

Low and high GI foods can be distinguished by the speed of digestion and absorption of glucose and fluctuation levels in the blood. The glycemic index of pure glucose is set 100 to pure glucose as the reference food to other food glycemic index determination (Brouns *et al.,* 2005). Food categories according to the glycemic index range with pure glucose as the reference food, namely: low GI (<55), IG medium / intermediate (55-70), and high GI (> 70).

This study uses two types of crackers i.e. *Coleus tuberosus* crackers and wheat crackers. Based on sensory evaluation carried out by 80 people untrained pointed out that from the flavors, aromas, colors and textures are included in the category of preferred. Sensory profile *Coleus tuberosus* and wheat crackers are, taste is savory, color on orange chocolate, crispy texture, the aroma of savory crackers (Nugraheni *et al.*, 2015b).

The measurement results show that the glycemic index of *Coleus tuberosus* crackers rich in resistant starch type III has a low glycemic index (40.88 ± 6.42), whereas wheat crackers has high glycemic index (76.08 ± 5.36). Results of analysis of variance of the data glycemic index value indicates that the substitution of *Coleus tuberosus* flour rich in resistant starch type III showed differences in glycemic response (P <0.05) (Table 2). *Coleus tuberosus* crakers has low glycemic index. This means that this crackers experiencing a slow digestive process, so that the rate was a slow stomach emptying. This led to the suspension of food (chyme) slower reach the small intestine, so that absorption of glucose in the small intestine becomes slow. Finally, fluctuations in blood glucose levels was relatively small.

Wheat flour crackers has a high glycemic index (76.08 ± 5.36). This means that the rate of stomach emptying, carbohydrate digestion and absorption of glucose that goes faster, so that fluctuations in blood glucose levels are also relatively high. This is because most of the glucose absorption occurs only in the upper small intestine. This results consistent with other studies that saltine cracker or soda from wheat flour included in the high glycemic index

Table 2. Glycemic Index of D- glucose anhydrous, *Coleus tuberosus* crackers and wheat flour crackers in healthy people

Foodstuff	Glycemic Index
D-glucose anhydrous	100[a]
Wheat flour crackers	76.08 ± 5.36[b]
Coleus tuberosus crackers	40.88 ± 6.42[c]

Means ±SD within columns followed by the different letter are significant difference at $p<0.05$

(Glassman, 2002). Canadian Diabetes Association and the Vancouver Coastal Health also published that soda crackers categorized products that have a high glycemic index (> 70).

This difference is influenced by several factors, including the method of processing (level of gelatinization of starch and measuring late particles), the ratio of amylose to amylopectin, acidity and power osmotic, fiber content, fat content and protein, as well as the levels of anti-nutritional food (Boers *et al*., 2015). Resistant starch type III is form retrograded amylose and starch. Because amylose molecules have linear structures, they have a great tendency to form double helices, particularly near refrigeration temperatures (4–5°C) and with adequate moisture content. Retrograded amylose has high gelatinization temperatures, up to 170°C, and cannot be dissociated by cooking. The gelatinization temperature of retrograded amylose, however, decreases with shortening of the amylose chain length. After starchy foods are stored, particularly in a refrigerator, amylose molecules and long branch chains of amylopectin form double helices and lose their water-binding capacity. The double helices of starch molecules do not fit into the enzymatic binding site of amylase, thus they cannot be hydrolyzed by this enzyme.

It is believed that glycemix index on *Coleus tuberosus* and wheat crackers to be related to content of resistant starch type III. The level of resistant starch type III in *Coleus tuberosus* crackers is 15.37%, while in wheat crackers is 7.35%. The resistant starch levels in line with the levels of amylose on both types of crackers, where *Coleus tuberosus* cracker contains 10.67% amylose, while the wheat cracker contains 6.43% amylose (Nugraheni *et al*., 2015b). Amylose levels is one of the factors that affect the retrogradasi starch, because the most resistant starch type III is formed from retrogradation of amylose (Leszczyñski, 2004).

Coleus tuberosus crackers contain amylose higher than wheat crackers, so the content of resistant starch on *Coleus tuberosus* carcker is also higher than that of wheat crackers. This gave the impact that *Coleus tuberosus* crackers is more resistant to hydrolized by the digestive enzyme than wheat crackers, so it has a low digestibility. Digestibility of starch is the ease of a kind starch to hydrolyzed by the enzyme that breaks down starch into units simpler (Mercier and Colonna 1988). Low starch digestibility means that only a little amount of starch that can be hydrolyzed by digestive enzymes in a certain time. Thus, blood glucose levels did not increase drastically shortly after the food is digested and metabolized by the body.

This research used one cycle process of heating-cooling process. The increase in the number of resistant starch with 1 heating-cooling cycle of 27% than the native *Coleus tuberosus* (Mutiara, 2015a). *Coleus tuberosus* flour rich in resistant starch type III made by steaming 100°C for 15 minutes then cooled at a temperature of 5°C for 24 hours. On the formation of resistant starch digestibility of type III, perfectly hydrated starch granules. Amylose out of the granules into the polymer solution in the form of a random coil. As a result of cooling, the polymer chains begin to associate set up a double helix through hydrogen bonding (Haralampu, 2000). Resulting starch undergo gelatinization and continued with cooling of the starch which has undergone gelatinization, the starch structure change that leads to the formation of new crystals are insoluble form of starch retrogradated, thus causing changes in the value of IG. The formation of resistant starch digestibility is increased when the material is stored at cold temperatures, but their influence on glycemic index value is determined by the nature of the carbohydrates in the material (Carreira *et al.*, 2004).

Figure 1 shows that *Coleus tuberosus* crackers rich in resistant starch type III be able to control the rise in blood glucose levels after consumption compared with wheat crackers. Physiological effects of resistant starch to blood glucose levels can be explained through two mechanisms, namely the inhibition of α-amylase enzyme activity in the intestine and increased production of short-chain fatty acid, propionic acid, especially by anaerobic bacteria in the colon. Resistant starch is starch that can't be hydrolized by digestive enzymes. This affects the thickness of the bowel contents led to a decrease in the activity of α-amylase so that slow down the absorption of glucose.

In addition, the type of short chain fatty acids are produced from the fermentation of resistant starch in the colon can also inhibit the work of HMG CoA (3 Hydroxy 3 methyl glutaril Coenzyme A) reductase thus synthesis cholesterol decreases. The concentration of propionate on rat digest is 18.35±1.10 mL mol (Nugraheni *et al*., 2015a).

Propionate also can inhibit gluconeogenesis via conversion of HMG CoA methylmalonyl CoA and succinyl into the CoA as well as reduce plasma levels of free fatty acids. Plasma levels of free fatty acids can lower high glucose utilization and cause the onset of insulin resistance in the adipose tissue. The work of the propionate causes increased insulin secretion and insulin sensitivity in adipose tissue (Cheng *et al*., 2000; Robertson *et al*., 2005).

Resistant starch delay increases in blood glucose when would have to take a meal by slowing the rate and amount of carbohydrate digestion and making it ideal for people with diabetes. This more controlled glycemic response also helps to suppress hunger and maintain energy levels up all over the day. Foods that contain resistant starch can decrease the rate of digestion, so it can control the release of glucose. Digestion occurs over a 5- to 7-h period reduces postprandial glycemia and insulinemia and has the potential for increasing the period of satiety (Reader *et al*., 1997). Consumption of foods that contain resistant starch can control the glucose profile in diabetic experimental animal (Nugraheni *et al*., 2015a)

Conclusion

Based on this research shows that *Coleus tuberosus* crackers have a low glycemic index. Factors that possible influence is the presence of resistant starch content of the constituent materials. So, *Coleus tuberosus* crackers potential to control blood glucose level.

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