Dear Dr. Nugraheni,

Your manuscript entitled 'Coleus tuberosus Crackers Rich Resistant Starch Type III: Glycemic index evaluation' has been successfully submitted online and will be given full consideration for publication in International Food Research Journal.

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06-Aug-2016

Dear Dr. Nugraheni

Manuscript IFRJ16230, entitled 'Effect consumption of Coleus tuberosus crackers rich in resistant starch type III on the glycemic index', which you submitted to International Food Research Journal, has been reviewed. The comments of the reviewers appear below.

The reviewers believe that the manuscript requires major revision before it could be published. Therefore, I invite you to respond to the comments below and revise your manuscript.

Thank you for submitting your manuscript to International Food Research Journal; I look forward to receiving your revision. Instructions on how to revise your manuscript are included at the end of this letter.

Sincerely,
Prof. Son Radu
Editor, International Food Research Journal

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Reviewers' Comments to Author:

Reviewer: 1

Comments to the Author
Dear International Food Research Journal Editor,

Manuscript number: IFRJ 16230 entitled "Effect consumption of Coleus tuberosus crackers rich in resistant starch type III on the glycemic index".

This manuscript presents the information of effect of addition Coleus tuberosus flour on the glycemic index of crackers. Overall, the results show the positive response of Coleus tuberosus on the GI. However, more information should be added to make the manuscript clearer. Several discussion were unnecessary. Mechanism lowering GI in this issue should be more explained. Other comments are:

Topic
- should be revised, for example, Glycemic index of Coleus tuberosus crackers rich in resistant starch type III.

Abstract
- L. 15, please revise to make clearer for blood sampling.
- L. 18, should add the comparison to control (normal cracker).

L. 33
- Add reference.

L. 57
- Delete "a new"
L 87
- Add reference.

Materials and methods
- The raw material and equipment specification should be added.
- The formula of cracker must declare.

L 114
- What is the resistant starch content in Coleus tuberosus?
- How did you prepare the starch?
- Why the ratio 1:4 was use?
- How did you declare rich in resistant starch?

L 170-176
- Should delete. There are no meaning and redundant.

L 225
- Add the reference.

L 238-241
- These sentence should be checked and revised. Did not clear. Why did you said that gelatinized starch is partially more resistant to enzyme hydrolysis?

L 242-251
- Should delete. It is common and should be placed in the Introduction.

L 291
- Revise "glycemic" and "resistant"

Authors should declare some taste or sensory properties of crackers.

Table 1 can be deleted.
Table 2 and Fig.1 were the same. Should select one.

Reviewer: 2

Comments to the Author
The work is a good attempt to popularise a neglected crop. However there are many points which need clarifications
1. Regarding the production of RSIII from the starch, you are not carrying out repeated heat-freeze operations on the starch. So how do you say that RS III has been achieved?
2. Is the number of Respondens (10) enough to get reliable result?
3. You have not given the amylose content of the two starches though mentioned that amylose content may be a factor
4. Table 2 needs clarification
5. Language needs lot of editing

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The work is a good attempt to popularise a neglected crop. However there are many points which need

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25-Aug-2016

Dear Dr. Nugraheni,

Your revised manuscript entitled ‘Glycemic index of Coleus tuberosus crackers rich in resistant starch type III’ has been successfully submitted online and will be given full consideration for publication in International Food Research Journal.

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30-Oct-2016

Dear Dr. Nugraheni:

Recently, you received a decision on Manuscript ID IFRJ16230, entitled "Effect consumption of Coleus tuberosus crackers rich in resistant starch type III on the glycemic index." The manuscript and decision letter are located in your Author Center at https://mc.manuscriptcentral.com/upm-ifrj.

This e-mail is simply a reminder that your revision is due in one (1) week from 05-Nov-2016. If it is not possible for you to submit your revision within two weeks, your paper will be withdrawn and we may consider your paper as a new submission.

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Sincerely,

Son Radu
International Food Research Journal Editorial Office
ifrj@upm.edu.my

- mutiara_nugraheni <mutiara_nugraheni@uny.ac.id> 1 November 2016 00.05
Kepada: "ifrj@upm.edu.my" <ifrj@upm.edu.my>

Dear Prof. Son Radu

I sent my revised article on Sunday, 30 October 2016. Thanks you for the reminder and your attention.

Sincerely yours,

Mutiara Nugraheni
[Kutipan teks disembunyikan]
International Food Research Journal - Decision on Manuscript
IFRJ16230.R1

25-Nov-2016

Dear Dr. Nugraheni

Thank you for sending the revised version of this paper ("Glycemic index of Coleus tuberosus crackers rich in resistant starch type III"). I have now had the opportunity to examine your revised manuscript and I am pleased to accept it for publication in International Food Research Journal. Kindly refer to the attachment for the acceptance letter.

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Thank you again for your contribution to the Journal.

Sincerely,
Prof. Son Radu
Editor, International Food Research Journal

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I would like to ask whether the copyright agreement that has been signed from me, was received in IFRJ editor?
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Mutia Nugraheni

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Professor Dr. Son Radu
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Kepada: Son Radu <daniel_lian@hotmail.com>

Dear Prof Son Radu

Here this the correction of my article. thank you

Sincerely yours,

Mutiara Nugraheni
The potential of gluten free enriched resistant starch type 3 from Canna edulis flour for the management profile of glucose, lipida and short chain fatty acid in healthy rats
The potential of gluten free enriched resistant starch type 3 from *Canna edulis* flour for the
management profile of glucose, lipida and short chain fatty acid in healthy rats

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University, Karangmalang, Depok, Sleman, Yogyakarta 55281, Indonesia.

*Corresponding author: mutiara_nugraheni@uny.ac.id*

**Abstract**

This research aims at modifying the flour of *Canna edulis* with autoclaving-cooling 3 cycles
to improve the levels of resistant starch and identifying the consumption of gluten-free
enriched resistant starch type 3 (RS3) from *Canna edulis* flour against the profiles of glucose,
lipida and short chain fatty acids in healthy rats. The research began with the production of
*Canna edulis* flour with modifications of 3 cycle autoclaving-cooling. The levels of resistant
starch *Canna edulis* flour were analyzed and then proceed with the formulation of the 3 types
of gluten-free enriched RS3 from *Canna edulis* flour. Two components always present are the
native *Canna edulis* flour and *Canna edulis* flour rich in RS3. Analysis of the bio-assay was
performed using the 24 rats wistar divided into 4 groups: the standard feed, high protein
gluten free flour, high fiber gluten-free flour, and all purposes gluten-free flour. Glucose was
determined with GOD-PAP method. Total cholesterol, triglycerides, HDL, and LDL were
determined enzymatically with the CHOD-PAP method. Short Chain Fatty Acid analysis was
done with gas chromatography (GC). The results show that the modification process of 3
cycle autoclaving-cooling can increase the level of resistant starch at 39.5%. Diet feeding of
the three types of gluten free enriched RS3 from *Canna edulis* flour i.e. high protein gluten-
free flour, high fiber gluten-free flour and all purposes gluten free flour, can control the profile of glucose, lipida (total cholesterol, triglycerides, HDL, and LDL) compared to controls. The main short chain fatty acids in the three types of gluten free enriched RS3 from *Canna edulis* were acetic acid, propionic acid and butyric acid. This indicates that consumption of three types of gluten-free flour enriched RS3 from *Canna edulis* flour provides positive benefits for profiles of glucose, lipida and short chain fatty acids.

Keywords: *Canna edulis* flour, resistant starch type 3, gluten free flour, glucose, lipida, and SCFA

**Introduction**

Indonesia has great potentials of natural cereals, legumes, or tubers as a source of fat, protein and carbohydrates. However, Indonesia has become a wheat grain importer in the last ten years with imports reaching 10 million tons per year. The increase of the imports of wheat is due to the rapid development of food-based processing and the type of flour that contains gluten.

Some effort needs to be done to produce flour that can be used for a variety of food products as a substitute for wheat. It should be developed based on potential-based raw materials that exist in Indonesia (tubers, grain and legume). The resulting flour must have the advantage and the ability to provide similar sensory properties of products made from wheat. One of the efforts is to make gluten free flour based on local potentials, yet containing starch, fat and protein to support the formation of sensory properties similar to processed wheat-based products. Gluten-free flour is known as gluten free flour (GFF).
Gluten-free flour is flour deployment made without gluten content. Gluten is a protein found in wheat, rye, barley and triticale, which when added to water or liquid materials, can make the dough elastic. Gluten-free flour can be made from a source of carbohydrates, proteins with a particular composition so that certain characteristics similar to flour that contains gluten can be obtained. Raw materials used in the production of gluten-free flour is the local raw materials that are the source of fat, protein and carbohydrates. One of the raw materials used as sources of carbohydrates is *Canna edulis*.

*Canna edulis* is a herbaceous plant- which belongs to the group of tubers. *Canna edulis* tuber is used as a source of food and raw material for industry. In Indonesia, two kinds of *Canna edulis* cultivars can be found, namely red and white *Canna edulis*. *Canna edulis* is a plant that is efficient in the use of nitrogen, drought-tolerant and high-productivity (Herman *et al.*, 1996). *Canna edulis* store food in the form of starch reserves in the roots which can be consumed. Before consumed, *Canna edulis* should be boiled for a few hours. So, it is rarely consumed in the community.

Tubers as a source of carbohydrates can be physically modified to increase the value of functionality i.e. resistent starch type 3 (RS3) content with autoclaving-cooling. Resistant starch can be generated and enhanced from starch processing (heating and cooling repeated) (Kingman and Englyst, 1994, Dundar and Gocmen, 2013). Nugraheni (2015) proved that the processing stages can increase the levels of resistant starch in *Coleus tuberosus*. Nugraheni *et al.* (2012) proved that the consumption of food sources of carbohydrates, i.e. boiled *Coleus tuberosus* and *Coleus tuberosus* flake can create a glucose profile and influence the formation of lipida short-chain fatty acids (SCFA) on colonic rats.

Several studies proved that the content of resistant starch in foodstuffs is able to control the profile of glucose, and lipida animal or human suffering from diabetes mellitus (Kay, 2006; Yamada *et al.*, 2005; Nugraheni *et al.*, 2014). So, it is expected that gluten-free
enriched RS3 from *Canna edulis* flour can create profiles of glucose, lipida and short-chain fatty acid of experimental animals.

Gluten-free flour is a blend of carbohydrates, proteins, fats derived from legumes, cereals and tubers. Based on the raw materials, it can be observed gluten-free flour contains resistant starch and dietary fiber. Dietary fiber is similar to resistant starch in relation to the control of the profile of glucose and lipida. The fibers cause the formation of a complex carbohydrates, so that power decreases carbohydrate cerna. The state of being able to control the rise in blood glucose and blood glucose remains controlled. Water soluble fiber can bind fat in the intestine, thus lowering the cholesterol level in the blood. In addition, the digestive tract of fiber can bind bile salts (the end product of cholesterol) later issued along with the stool. Thus, dietary fibers are capable of reducing cholesterol levels in the blood plasma. The presence of a combination of resistant starch type 3 and fiber found in gluten free flour is expected to control glucose and lipida profile in the body.

This research aims at modifying the *Canna edulis* flour with 3 cycle autoclaving-cooling to improve the levels of resistant starch and identify the effect of the consumption of gluten-free enriched RS 3 from *Canna edulis* flour on the profiles of glucose, lipida and short chain fatty acid in healthy rats.

**Materials and Methods**

*Production of Canna edulis flour*

*Canna edulis* tuber aged 6-10 months was obtained from farmers in Clereng, Kulon Progo, special region of Yogyakarta. The *Canna edulis* tuber was cleaned, then sliced thinly and dried using a cabinet dryer in a temperature of 45°C for 24 hours. The *Canna edulis* already dried was then crushed and sifted using a sieve with a mesh size of 80.
Physical modification of *Canna edulis* flour and *Coleus tuberosus* flour with 3 cycle autoclaving-cooling

Physical modification of *Canna edulis* and *Coleus tuberosus* flour was intended to obtain flour with higher levels of resistant starch. The flour was suspended in water 20% (b/v) and then heated at a temperature of 70°C while stirred until homogeneous. Then, autoclaving process was done for 15 minutes at a temperature of 121°C. After the sample was cooled for 1 hour at a room temperature, it was stored for 24 hours at a temperature of 4°C to trigger the process of retrogradation. Heating with an autoclave cooling at 4°C was repeated twice (3 cycles of autoclaving-cooling). After that, the starch was dried in an oven temperature of 50°C, ground and sifted a sieve with a mesh of 80.

Gluten-free enriched resistant starch type 3 from *Canna edulis* flour formulation

There were three types of gluten-free flours produced: high protein gluten free flour, high fiber gluten free flour, and all purposes gluten-free. Gluten-free flour formulation was made based from the combination of the source of protein, fats, and carbohydrates with raw materials that exist in Indonesia, such as grains, legumes or tubers (Table 1). It is expected that this gluten-free flour can provide sensory properties that are similar to wheat flour. There were two raw materials that must be present in the formulation of the three types of gluten-free: *Canna edulis* flour and *Canna edulis* rich in RS3. The purpose of this formulation is getting gluten-free flour that has sensory characteristics similar to wheat flour.

Total starch, amylose, amylopectin, dietary fiber and resistant starch content
Total starch content (AOAC 1984), amylopectin (AOAC 1995), dietary fiber content were referred to AOAC (1995), and resistant starch (Englyst et al., 1992).

**Animal and diet**

Animal diet refers to the standard feed composition of AIN 1993 (Reeves, 1997). One kilogram of feed is made of the standard composition of corn starch (560.70 g), casein (>85% protein, 200 g), saccharose (100 g), Carboxy methyl cellulose (50 g), corn oil (40 g), mineral mix (AIN-93 MX, 35 g), vitamin mix (93AIN VX, 10 g), choline bitartrate (2.5 g), and L-cysteine (1.8 g). Another fed were high protein gluten-free flour, all purpose gluten free flour, and high fiber gluten free flour which must contain two raw materials: *Canna edulis* flour and *Canna edulis* flour rich in RS3.

Twenty-four male white rats (wistar) weighing 130-180 g were supplied by the Animal Care laboratory, Gadjah Mada University, Yogyakarta, Indonesia. The rats were kept under control at a temperature of 20-24°C and 12-h light/dark cycle. They were fed with standard laboratory feed and water ad libitum.

The gluten-free flour mix and standard feed AIN 93 were used to feed the rats for 21 days. The rats were divided into four groups, consisting of six animals respectively. The rats were treated for 21 days as follows: Group 1 received the standard diet, Group 2 received high fiber gluten free enriched RS3 from *Canna edulis* flour, Group 3 received high protein gluten free enriched RS3 from *Canna edulis* flour, and group 4 received all purposes gluten free enriched RS3 from *Canna edulis* flour. Drinking water was provided ad libitum. The rats were fed every morning. Weighing was performed every 2 day. The blood was collected from retro-orbitally Anthus in the eye with the ether anesthesia using capillary tubes (Hoff,
The total cholesterol was determined enzymatically with the CHOD-PAP method (Richmond, 1973). LDL-cholesterol was determined enzymatically with the CHOD-PAP method (Wieland and Siedal, 1983). HDL cholesterol was determined enzymatically with the CHOD-PAP method. Triglycerides were determined using GPO-PAP method (McGowan et al., 1983). SCFA was determined with chromatography gas.

Statistical analysis

The results were presented as the average and standard deviation of six experiments. One-way ANOVA was used to analyze differences in means between the samples followed by Least Significant Difference multiple comparison test to compare the mean values at p < 0.05. The value of p indicates 0.05 which was considered a significant difference p < 0.05. SPSS version 16.0 (SPSS Inc., South Wacker Drive, Chicago, United State of America) was used.

Results and Discussion

Production of Canna edulis flour, physical modification process of canna edulis flour and resistant starch content

Canna edulis flour was made through several stages; Canna edulis tuber was cleaned, then sliced thinly and dried using a dryer in a cabinet at the temperature of 45°C for 24 hours. Canna edulis already dried was then crushed and sifted using a sieve with a mesh size of 80. The yield obtained was 32%. Physical modification process was done by autoclaving-cooling process. Using the principle of autoclaving-cooling process, first, Canna edulis flour was suspended in water with 20% comparison (b/b). The Canna edulis suspension was then
heated using an autoclave, which resulted in gelatinized starch and the fraction of amylose out of starch granules. Next, the flour paste was cooled down, leading to the fraction of amylose experiencing retrogradation. The process of heating at a high temperature in an autoclave caused the suspensions of *Canna edulis* flour to experience gelatinization. The process of heating the suspension of starch at a temperature above the gelatinization temperatures can lead to the occurrence of the dissociation hydrogen bonding of the double helix structure of amylopectin, melting (melting) part of crystallites, and the release of amylose from granule (amylose leaching) (Sajilata et al. 2006; Zabar et al. 2008; Zaragoza Fuentes et al. 2010). The fraction of amylose bonded with the fraction of amylose through hydrogen bonds to form the structure of the double helix. The structure of the double helix was bound to other crystallites which was recrystallization fraction of amylose, known as the formation process of the RS3 (Mutungi et al. 2009).

Table 2 provides information that 3 cycle autoclaving-cooling can increase the levels of RS3 of *Canna edulis* flour about 39.5% and *Coleus tuberosus* flour about 115.56%. Autoclaving-cooling repeatedly caused the formation of more retrogradated or crystalized amylose fraction, so that it can increase the levels of RS3 (Sagulian-Aparicio et al. 2005). The heating process continued with the cooling causing the polymer chain amylose to dissolve because gelatinized will have re-asosiation back form the structure of the double helix stabilized by hydrogen bonds, which result in starch being difficult to be digested by the amylase enzyme, so the impact on the formation of resistant starch. The most resistant starch formed is from amylose, although amylopectin can also be retrogradated but it will take a long time (Huang and Rooney, 2001). The levels of resistant starch is directly proportional to the levels of amylose. This study shows that the levels of resistant starch *Canna edulis* by 3 cycle autoclaving-cooling are higher than the levels of resistant starch of native *Canna edulis*. 

http://www.publish.csiro.au/journals/hras
This is in line with the content of the amylosa, where the levels of *Canna edulis* amylose by 3 cycle autoclaving-cooling are higher than levels of native *Canna edulis* amylose.

Resistance starch, starch, amylose, amilopectin and dietary fiber content of Gluten-free enriched RS3 of *Canna edulis* flour

Three types of gluten-free flours were all purpose, high fiber and high in protein. These three types of gluten-free flour are analyzed in terms of the content of resistant starch, starch, amylose, amilopectin and dietary fiber (Table 3).

Resistant starch content

Table 3 shows that the levels of RS3 on the three flour types differ markedly, with the greatest sequence from high protein, high fiber to all purpose. The different levels of RS3 on the three type of gluten-free flour are due to the difference of materials constituting. Although these three types of gluten-free flour mix have two raw materials that must be present, namely, native *Canna edulis* flour, *Canna edulis* flour rich in RS3 and *Coleus tuberosus* rich in RS3, other materials that make up the gluten free flour also contain resistant starch.

This was confirmed by Sajilata et al (2006) and Leszczyńskiski (2004), explaining that the different types of food will have an impact on the different levels of resistant starch. The levels of resistant starch on these three kinds of gluten-free flour are expected to be further increased when used as raw materials for processing various gluten-free based products enriched RS3 from *Canna edulis* flour. The existence of the processing of a product that uses multiple stages of heating and cooling will cause the occurrence of gelatinization process and retrogradation of amylose. This has an impact on the increasing levels of RS3.
Amylose and amylopectin content

The ratio of amylose and amylopectin influence the resistant starch content (Sajilata et al., 2006). Canna edulis flour has a higher amylose content, and therefore has higher levels of resistant starch after modification. Each type of starch has different a ratio of amylopectin and amylose. The different characteristics of each type of starch are affected by the botanical source, the shape and size of the starch granules, amylose and amylopectin ratio, the content of the non-starch components, as well as crystalline and amorphous structures (Mali et al., 2005). High content of resistant starch on Canna edulis flour is related to the crystalline structure of type-B in the original native starch, such as raw potatoes, bananas and high-amyllose corn starch (Hung and Morita, 2005). Crystalline region is well-formed by amylose molecules, whereas amorphous region is well-formed by amylopectin.

Table 3 shows that the level of amylosa and amylopectin is not directly proportional to the levels of resistant starch, it is supposedly because the starch undergoes modification autoclaving-cooling only two types namely Canna edulis flour and Coleus tuberosus flour, whereas the other ingredients were native flour. Resistant starch type 3 occurred with heating-cooling. Meanwhile, the natural starch has not experienced the process of gelatinization and included in resistant starch type 2.

Further processing into various gluten-free-based products is expected to increase the levels of resistant starch and facilitate the occurrence of gelatinization and retrogradation of amylosa factions. So, it is possible that the high levels of amylosa on a material are comparable to the high levels of resistant starch in the product. Some researchers have also proved that the levels of amylose is correlated with the formation of resistant starch (Hallstrom et al., 2011; Sestili et al., 2010).
Physiologically, dietary fiber is defined as the component of plants that is not enzymatically degraded into sub-units, which can be absorbed by the stomach and small intestine. The modification produces resistant starch which has physiological effects that are beneficial to health (Sajilata et al., 2006). Nugent (2005) stated that resistant starch has characteristics similar to dietary fiber. It resists hydrolysis and digestive enzymes. The starch cannot be digested in the small intestine but can be fermented in the colon. Therefore, resistant starch is also classified as dietary fiber. Total dietary fiber is the sum of soluble dietary fibre (SDF) and insoluble dietary fibre (IDF). The SDF can be dissolved in water, including pectin and gums. The IDF is composed by cellulose, lignin and hemicellulose.

This study shows that the total dietary fiber of high fiber gluten free flour was higher than the high protein and all purpose gluten free flour (Table 3). The SDF and the IDF also contributed to the increase of the total dietary fiber. Haralampu et al. (2000) stated that resistant starch will be detected as dietary fiber, insoluble but its physiological function acts as soluble fiber. The main property that distinguishes the SDF from the IDF is its ability to absorb water. The SDF can absorb water and form a gel. Therefore, it will inhibit stomach. On the other hand, the IDF is not soluble in water and does not form a gel. Thus, the fiber may pass through the digestive tract intact.

Table 3 shows the content of dietary fiber on the three types of gluten-free flour. The difference is due to the composition of different gluten-free flour. High fiber gluten free flour contains the highest total dietary fiber (17.112 ± 0.134 %) than all purpose and high protein. This is supported by the high levels of insoluble dietary fiber and soluble dietary fiber. High fiber gluten free flour has high insoluble dietary fiber due to the presence of constituents in the form of beans, where insoluble dietary fiber is found in seralia, nuts and vegetables.
Treatment with animals

Twenty-four rats were divided into four groups. The first group was given the standard feed; the second group was given high fiber gluten free enriched RS3 from *Canna edulis* flour; the third group was given high protein gluten free enriched RS3 from *Canna edulis* flour, and the fourth group was given all purpose gluten free enriched RS3 from *Canna edulis* flour.

Profile of glucose

The animals (wistar) were given standard feed and three types of gluten free enriched RS3 from *Canna edulis* flour for 21 days. The profile of glucose was evaluated at the beginning and at the end of the treatment. The glucose profiles of the wistar rat with four types of diets were presented in Table 4.

Table 4 shows that the diet treatment for 21 days with the standard feed and three types of gluten-free enriched RS3 from *Canna edulis* flour, namely all purpose, high fiber and high protein can keep the blood glucose profile of wistar rats under normal conditions (<135 mg/dL). Although there was an increase compared to before the treatment, the increase was still in the range of normal levels.

Some types of flour used to make all purpose gluten free were able to control blood glucose levels, namely corn flour, *Coleus tuberosus* rich in RS3, native *Canna edulis* flour, and *Canna edulis* rich in RS3. *Coleus tuberosus* flour rich in RS3 and *Canna edulis* flour rich in RS3 flour contain RS and fiber related to blood sugar control. Corn contains fiber and has low glycemic index. Dietary fiber corn was 10.46% (Naves et al., 2011). Soluble fiber food components are associated with the decrease of cholesterol and blood sugar control.
High fiber Gluten free has some types of flour capable of controlling blood glucose profile i.e. sorghum flour, flour millet, corn starch, native *Canna edulis* flour, *Canna edulis* flour rich in RS3, *Coleus tuberosus* rich in RS3. According to a study of Prabowo (2010), the fiber level of yellow millet flour is 2.01%. This is also supported by the results of a study by Souilah dkk (2012), which proved that the coarse fiber flour millet Pennisetum glaucum is 6.64%. Meanwhile, according to Suarni (2001), rough fiber, on the type of hard wheat flour is only 1.92%. Shorgum has water insoluble fiber and dietary fiber useful for controlling blood glucose levels (Suarni, et al., 1995), coarse fibers and fiber levels in food in sorghum respectively amounted to 6.5%-7.9% and 1.1%-1.23% (Haryani et al., 2000).

High protein gluten free flour also has constituents which are capable to control profiles of glucose i.e., soy flour, native *Canna edulis* flour, *Canna edulis* flour rich in RS3, *Coleus tuberosus* flour rich in RS3, and rice flour. The protein content of soybean flour was 46.39%. Protein levels can control blood glucose. A high-protein diet may improve blood glucose control in persons with type 2 diabetes. (Gannon et al., 2003). Diet free amino acids and a mixture of proteins can increase insulin secretion reported in type 2 DM patients, so that it can control blood glucose (Van Loon et al., 2003). According to Zuheid et al., (2000), soy protein helps to decrease blood sugar through induced release of insulin by the amino acids. The presence of anti-trypsin on soybeans also gave two positive effects. The first effect is that insulin secretion stimulates aid due to the content of the amino acid methionine; the second influence i.e. induce a working pancreas to produce more trypsin which can induce the secretion of enzymes and pancreatic hormones include insulin. The soluble fiber content is high enough on the seaweed species e. cottoni which shows the tendency effect synergistic with soy protein in lowering blood sugar. Supplementing the diet with soybean shows a beneficial effect on the improvement of blood glucose control, lipid metabolism and antioxidant enzyme activities in type 2 DM patients. (Chang et al., 2008).
Mechanisms that could explain the influence of fiber consumption toward the decrease in blood glucose level is through the formation of the gel resulting in delays gastric emptying, and ultimately lower the speed of absorption of glucose and plasma insulin levels (Chandalia et al., 2000).

Resistant starches affect postprandial glucose levels through three common mechanisms: inhibiting α-amylase from digesting starch into glucose, increasing the viscosity of chyme in the small intestine which slows the rate of glucose uptake, and binding glucose which prevents its diffusion into the mucosal cells (Ou et al., 2001); regulated through promoting glycogen synthesis and inhibiting gluconeogenesis (Zhou et al., 2015) and improved glucose tolerance, insulin sensitivity, and satiety have resulted from the consumption of RS in healthy humans (Murphy et al., 2008).

Profile of total cholesterol, triglyceride, LDL and HDL

Feed treatment using three types of gluten-free flour, namely all purpose, high fiber and high protein in healthy rats showed that the levels of total cholesterol showed a rising trend more than the first day of the treatment (Table 4). However, the levels of total cholesterol after 21 days of treatment still showed normal levels (< 130 mg/dl). According to Jae (2008), the levels of LDL less than 100 mg/dl is optimal LDL levels; 100-129 mg/dl is the LDL levels approaching the optimal; 130 to 159 mg/dl is the highest normal limit; 160-189 mg/dl is high LDL levels including categories; more than 190 mg/dl is considered very high. When compared to the standard feed, the total cholesterol levels in the three groups of mice with gluten-free flour diet were lower. This suggests that gluten-free flour can control and improve the profile of total cholesterol.
The ability of controlling the levels of total cholesterol in these three types of gluten-free flour is caused by several factors, namely the presence of resistant starch type 3, fiber, and protein (Ranhotra et al., 2012; Pande et al., 2012; Kristensen, et al., 2012). Rats fed with the diet of three types of gluten-free flour were found to have increased triglyceride compared to the first day of the treatment (Table 4). However, the increase in the levels of triglycerides is still in the normal range. If compared to the standard feed, gluten-free flour has triglyceride levels which tend to be lower. The profile of triglycerides indicates that gluten-free flour diet can improve the profile of the triglycerides in rats.

The diet of the rats with three types of gluten-free flour has a normal HDL levels (Table 4). According to Jae (2008), the levels of plasma HDL less than 40 mg/dl are considered low, whereas above 60 mg/dl is considered high. Although there is a tendency to decrease compared to the first day, if compared to the standard feed diet, HDL levels in the rats given flour gluten-free diet are better. This proves that flour gluten-free diet on rats improve HDL levels.

Animals fed given all purpose gluten free flour, high fiber flour which is gluten-free or high protein flour gluten-free for 21 days show a trend of lower LDL levels than standard feed (Table 4). LDL levels in rats fed, namely gluten-free flour has been lower 40 mg/dl. This suggests that gluten-free flour could improve LDL levels in rats.

The profile of SCFA in Table 5 indicates that the fermentation of the diet of rats with the standard diet, all purposes gluten free enriched RS3 from Canna edulis flour, high fiber gluten free enriched RS3 from Canna edulis flour or high protein enriched RS3 from Canna edulis flour produces three types of short-chain fatty acids namely acetic acid, propionic acid and butyric acid. Short-chain fatty acids (SCFA) are formed when polysaccharide is fermented by anaerobic bacteria found in the colon. There are many forms of polysaccharides
in the colon, one of which is resistant starch. The major SCFA produced in the human intestine is butyric, propionic, and acetate.

Short-chain fatty acids have health benefits for the body and metabolism of glucose and fat. Acetic acid is metabolized in the liver, muscle, brain tissue. Propionic acid metabolized in the liver as well as being able to lower their cholesterol synthesis. Butyric acid shows the ability to inhibit the growth of colorectal cancer. Some studies mention that butyric can inhibit the growth of colorectal cancer cells by way of inhibiting the proliferation of cells, as well as improve the ability of differentiation and apoptosis of cells (Besten et al., 1995).

Decrease in the profile of total cholesterol, triglycerides and LDL is through several mechanisms. The first mechanism is related to the role of dietary fiber and resistant starch. Some types of soluble dietary fiber, insoluble dietary fiber and resistant starch can affect fat absorption by binding of fatty acids, cholesterol and bile salts in the digestive tract. Fatty acids and cholesterol bound with fibre cannot pass much needed micelle for absorption of fat so that they can pass through the unstirred water layer entry to enterosit. As a result, the fat bound to the fibers cannot be absorbed and will continue into the colon to be excreted through the stool or degraded by intestinal bacteria (Lattimer et al., 2010). The hypocholesterolaemic effect of dietary fibre has been attributed to its ability to inhibit intestinal absorption of bile acids and neutral steroids, resulting in greater faecal bile acid and total steroid excretion. Increased excretion of bile salts and cholesterol through bile salts, thus stool that is experiencing the cycle enterohepatik is also reduced. The reduced bile salts entering the liver and decreased absorption of cholesterol will lower cholesterol levels liver cells. This will increase the uptake of cholesterol from the blood that will be used for the synthesis of new bile salts, consequently lowers blood cholesterol levels and LDL serum. (Romero et al., 2002; Romeyra et al., 2005)
The second mechanism related to short-chain fatty acids is produced from the fermentation of resistant starch and dietary fiber. SCFA is the result of fermentation of dietary fiber in the colon such as acetate, propionate and butyrate (Fotschki et al., 2014). Propionic acid is produced from the fermentation of fiber on colon will inhibit the synthesis of cholesterol (Han et al., 2003; Chen et al., 2003). Propionic acid after entering the bloodstream and liver can inhibit the action of the enzyme HMG-CoA reductase and reduce cholesterol synthesis (Lupton and Turner, 2000; Saravanan and Ignacimuthu, 2015). The decrease in plasma cholesterol levels may be resulted because the induction of cholesterol synthetic activity with the higher bile acid excretion is canceled by fermentation products. In turn, the enhancement of bile acid excretion worked effectively to lower plasma cholesterol concentration (Hara et al., 1999).

Conclusion

Treatment with 3 cycle autoclaving cooling can increase the content of resistant starch in the *Canna edulis* flour at 39.5%. The formulation of three types of gluten-free enriched RS3 from *Canna edulis* flour provides the content of different chemical compositions, which depend on ingredients constituting. High fiber gluten free enriched RS3 from *Canna edulis* flour, high protein gluten free enriched RS3 from *Canna edulis* flour or all purposes gluten-free enriched RS3 from *Canna edulis* flour give a positive influence and able to control the profile of glucose, lipida and short chain fatty acids. Gluten free flour enriched RS3 from *Canna edulis* flour can potentially be used as functional food for people who are allergic to gluten or people who require the control of glucose and lipida profile.

Acknowledment
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References


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Table 1. The composition of the three types of gluten-free flour

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>All Purposes</th>
<th>High protein</th>
<th>High Fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td>composition</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Rice flour</td>
<td>51</td>
<td>Soy flour</td>
<td>32</td>
</tr>
<tr>
<td>Tapioca</td>
<td>10</td>
<td>Maize starch</td>
<td>13</td>
</tr>
<tr>
<td><em>Canna edulis</em> flour</td>
<td>11</td>
<td>Native <em>Canna edulis</em> flour</td>
<td>13</td>
</tr>
<tr>
<td><em>Coleus tuberosus</em> flour</td>
<td>1</td>
<td>Tapioca</td>
<td>9</td>
</tr>
<tr>
<td>Maize starch</td>
<td>14</td>
<td><em>Canna edulis</em> flour rich in RS3</td>
<td>10</td>
</tr>
<tr>
<td>Native <em>Canna edulis</em> flour</td>
<td>14</td>
<td><em>Coleus tuberosus</em> flour rich in RS3</td>
<td>1</td>
</tr>
<tr>
<td>Brown rice flour</td>
<td>23</td>
<td>Maize starch</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 2. Resistant starch and amylose content of native Canna edulis flours and 3 cycle of autoclaving-cooling *Canna edulis* flours

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Native <em>Canna edulis</em> flours</th>
<th>3 cycle autoclaving-cooling process</th>
<th>Percentage of increasing resistant starch content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant starch content</td>
<td>0.820 ± 0.005</td>
<td>1.144 ± 0.004</td>
<td>39.5%</td>
</tr>
</tbody>
</table>

Values are expressed as mean
Table 3. Resistant starch, starch, amylose, amylopectin and dietary fiber content

<table>
<thead>
<tr>
<th>Kind of Gluten free flour</th>
<th>Resistant starch (%)</th>
<th>Starch (%)</th>
<th>Amylose (%)</th>
<th>Amylopectin (%)</th>
<th>Soluble dietary fiber</th>
<th>Insoluble dietary fiber</th>
<th>Dietary Fiber (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All purpose gluten free flour</td>
<td>0.655±0.008a</td>
<td>68.875±0.148b</td>
<td>18.778±0.067c</td>
<td>50.098±0.211b</td>
<td>0.783±0.099b</td>
<td>5.401±0.087b</td>
<td>6.129±0.136b</td>
</tr>
<tr>
<td>High fiber gluten free flour</td>
<td>1.354±0.09b</td>
<td>69.464±0.148c</td>
<td>10.150±0.037a</td>
<td>59.315±0.114c</td>
<td>2.317±0.054c</td>
<td>14.796±0.082c</td>
<td>17.112±0.134c</td>
</tr>
<tr>
<td>High protein gluten free flour</td>
<td>1.399±0.005c</td>
<td>62.343±0.084c</td>
<td>14.586±0.038a</td>
<td>47.758±0.0514a</td>
<td>0.717±0.035a</td>
<td>4.953±0.011a</td>
<td>5.670±0.045a</td>
</tr>
</tbody>
</table>
Values are expressed as mean. Values followed by the same letters in the same column are not significantly different at 95% confidence level.

Table 4. Profile of glucose and lipida in rats with diet feed standards and gluten free flour (GFF)

<table>
<thead>
<tr>
<th>Kind of feed</th>
<th>Glucose</th>
<th>Cholesterol total</th>
<th>Triglycerida</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 28</td>
<td>Day 1</td>
<td>Day 28</td>
<td>Day 1</td>
</tr>
<tr>
<td>Feed Standard</td>
<td>63.17 ± 2.041 ab</td>
<td>98.33 ± 1.86e</td>
<td>89.17 ± 2.23a</td>
<td>116.00 ± 3.16d</td>
<td>77.83 ± 2.04b</td>
</tr>
<tr>
<td>All purpose</td>
<td>61.50 ± 1.22a</td>
<td>70.00 ± 2.19c</td>
<td>85.83 ± 1.60a</td>
<td>100.00 ± 4.29b</td>
<td>66.17 ± 3.31a</td>
</tr>
<tr>
<td>GFF</td>
<td>64.50 ± 2.95b</td>
<td>74.67 ± 2.16d</td>
<td>88.67 ± 2.33a</td>
<td>109.17 ± 4.36c</td>
<td>68.67 ± 4.27a</td>
</tr>
<tr>
<td>High Fiber</td>
<td>64.83 ± 2.93b</td>
<td>65.83 ± 2.79b</td>
<td>86.83 ± 4.22a</td>
<td>87.500 ± 4.23a</td>
<td>74.67 ± 5.75b</td>
</tr>
<tr>
<td>GFF Protein</td>
<td>64.83 ± 2.93b</td>
<td>65.83 ± 2.79b</td>
<td>86.83 ± 4.22a</td>
<td>87.500 ± 4.23a</td>
<td>74.67 ± 5.75b</td>
</tr>
</tbody>
</table>
Values are expressed as mean. Values followed by the same letters in the same row and column on one parameter are not significantly different at 95% confidence level.

Table 5. Profil of short chain fatty acid rats with diet feed standard and gluten free flour (GFF)

<table>
<thead>
<tr>
<th>Type of diet</th>
<th>Acetic acid</th>
<th>Propionic acid</th>
<th>Butyric acid</th>
<th>Total SCFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>41,278 ± 2,612ab</td>
<td>37,538 ± 1,714d</td>
<td>10,139 ± 0,437d</td>
<td>88,956 ± 4,680c</td>
</tr>
<tr>
<td>All purpose GFF</td>
<td>27,853 ± 0,548a</td>
<td>13,999 ± 0,428a</td>
<td>4,897 ± 0,438a</td>
<td>46,749 ± 0,510a</td>
</tr>
<tr>
<td>High fiber GFF</td>
<td>42,740 ± 9,214b</td>
<td>30,784 ± 2,179c</td>
<td>8,006 ± 0,289c</td>
<td>81,529 ± 10,637bc</td>
</tr>
</tbody>
</table>

http://www.publish.csiro.au/journals/hras
<table>
<thead>
<tr>
<th></th>
<th>High protein</th>
<th>36,386 ± 9,855ab</th>
<th>25,625 ± 2,722b</th>
<th>6,736 ± 0,264b</th>
<th>68,747 ± 12,315b</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFF</td>
<td>9,855ab</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean. Values followed by the same letters in the same column are not significantly different at 95% confidence level.
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 15, 30, 45, 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 type 3 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.

Keywords

Glycemic index
Crackers
*Coleus tuberosus*
Resistant starch

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 (Charalamopoulos *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 1 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 2 is the starch granules raw like 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 4 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 is 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 crackers 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°C for 15 minutes) and continued the process of refrigeration (temperature of 4°C for 24 hours). The next process stage were drying (temperature of 60°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 fingertips 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.

**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-
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* Crackers 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

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).
Coleus tuberosus crackers is 15.37%., 2015). Resistant starch type III C, and C for 15 minutes then cooled Coleus tuberosus carcker is also higher than that wheat crackers, so the content of resistant starch levels is one of the factors that affect the retrogradasi formed from retrogradation of amylose (Leszczyñski, 2004). 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.

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

<table>
<thead>
<tr>
<th>Foodstuff</th>
<th>Glycemic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-glucose anhydrous</td>
<td>100</td>
</tr>
<tr>
<td>Wheat flour crackers</td>
<td>76.08± 5.39</td>
</tr>
<tr>
<td>Coleus tuberosus crackers</td>
<td>40.88± 6.42</td>
</tr>
</tbody>
</table>

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).

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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 propionic acid 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 propionic acid on rat digest is 18.35±1.10 mL mol (Nugraheni et al.,
Propionic acid 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.

**Acknowledgment**

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**References**


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