BUKTI PROSES REVIEW

CURRENT RESEARCH in Nutrition and Food Science

Antioxidant Activity And Resistant Starch Content Of *C. tuberosus* on Different Cooking Method And Its Potential On Glucose Management In Diabetic Mice

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ABSTRACT

This research aims to know the antioxidant activity and the levels of resistant starch of C. tuberosus on different processing methods. Processing methods used were boiling and baking. Bioactive compounds being evaluated is the number of total phenolic and flavonoid. Evaluation of antioxidant activity is performed with the DPPH method. The evaluation of the levels of resistant starch was done in enzymatic method. The results showed that levels of total phenolic and flavonoid demonstrate a tendency to decline with the processing. Antioxidant activity of boiled C. tuberosus and C. tuberosus flake were increased by the existence of the processing process. The processing increases the levels of resistant. The levels of resistant starch in raw C. tuberosus 10.24 ± 0.37%; boiled C. tuberosus 15.42 ± 0.96%; C. tuberosus flake 44.09 ± 0.07%. The decrease in serum glucose in boiled C. tuberosus was 47.41%. whereas C. tuberosus flake was 54.94%.

Keyword: antioxidant activity, resistant starch, C. tuberosus, cooking method, diabetic mice

Introduction

Increasing the growing public awareness of the importance of healthy living, the claim against consumer foodstuffs also increasingly shifted. Food that is now starting to great demand not only that consumers have a good nutritional composition as well as the appearance and interesting flavors, but also must have certain physiological functions for the body. Such a function is known as the tertiary function. Foods that have a function known as tertiary is known as functional foods. Functional foods are

foods that can maintain health and prevent disease. This is because it has an active component in the biology that has benefits for health.

Research shows that there is a link between components in the food consumed with health [1]. Functional components in plants, for example, phytochemical has a biological activity to prevent disease [2]. Phytochemical compounds contained in legume, cereal, fruit, vegetables have antioxidant activity are phenolic and flavonoid [3]. Antioxidants are groups of compounds which neutralize free radicals and reactive oxygen species in the cell so as to prevent the occurrence of oxidative stress in human cells.

Resistant starch (RS) much developed and consumed because of the value of its current status. Hydrolysis resistant starch by digestive enzymes needs longer periods of time so that the production of glucose becomes slower. Indirectly, the RS has a value of functional for patients with diabetes. RS has three systems related to the functional value of the metabolism and effects in the body, i.e. as an ingredient for the fortification of fiber, lower calories, and fat oxidation. RS there are naturally in food products and can be used in a modified form as well as added in food [4].

C. tuberosus (C. tuberosus) is one of the potential food ingredients in Indonesia as a source of carbohydrates that come from minor tubers. Some research suggests that C. tuberosus has the potential to be developed as functional foods based on the content of compound bioactive and RS that can be obtained by modification of the processing. C. tuberosus contain flavonoid, ascorbic acid, which can increase the antioxidant enzyme activity and lowering products peroxide in rats fed a high-fat diet. C. tuberosus extract contains bioactive compounds that has antioxidant activity [5]. C. tuberosus processing into will bring the physical and chemical changes will have an impact on its potential as a functional food. The purpose of this research is to

evaluate the activity of antioxidants and resistant starch of C. tuberosus on different cooking methods and to know effect the consumption of C. tuberosus on glucose profile in diabetic mice

2 Materials and Methods

This research did experimentally in the laboratory, Department of Culinary Art Education, Yogyakarta State University, Center for Food & Nutrition Studies Gadjah Mada University. C. tuberosus flake making process is done by adding other ingredients namely soy flour. Study of antioxidant activity and levels of resistant starch were done on raw C. tuberosus, boiled C. tuberosus, and C. tuberosus flake.

2.1 Sample preparation

Sample preparation of antioxidant activity. Preparation of the raw C. tuberous: C. tuberosus separated the peel and flesh by peeler. The thickness of the stripping of the peel (1-1.5 mm) so that the retrieved flesh and skin the potatoes raw black. Boiled C. tuberosus: C. tuberosus boiled for 30 minutes, then skin and flesh were separated. The peel and flesh of tubers were dried using the cabinet drier at temperature 40oC for 24 hours. Then ground and shift with the sieve mesh Tyler 80 size. C. tuberosus flake made by doing the three formulations, the most preferred formulation based on hedonic test of 25 semi trained panelists used for samples. C. tuberosus flake was made from C. tuberosus flour, tapioca flour, soy flour, sorbitol, margarine, salt, cocoa powder, and water. Whereas the control of C. tuberosus flake made from C. tuberosus flour, tapioca flour, sorbitol, margarine, salt, cocoa powder, and water (without soy flour).

Sample preparation for resistant starch analysis: raw C. tuberosus sample, prepared from the all of the part of C. tuberosus, sliced and then dried it with a cabinet drier at 40oC for 24 hours. Dried C. tuberosus is used as a sample analysis of resistant

starch. Boiled C. tuberosus, prepared by boiling all of part of C. tuberosus for 30 minutes, and the used as a sample analysis of resistant starch.

2.2 Extraction process

The peel and flesh flour of raw and boiled C. tuberous, C. tuberosus flake macerated with methanol during 7 days (1:5), then filtered using Whatman No.1, evaporating the solvent with gas N2. The Extract stored in the freezer temperature -22oC.

2.3 Determination of total phenolic compounds

The methanol extract of C. tuberous were determined using spectrophotometric method [6]. As much as 0.2 mL different extract with a concentration of 100 mg/L, Folin-Ciocalteu reagent 10% as much as 2.5 mL, and 2 mL 7.5% Na2CO3 are mixed and allowed for 15 minutes at a temperature of 45oC. The absorbance of the solution was measured using the spectrofotometer at a wavelength of 765 nm. The total phenolic compounds of expressed as mg GAE/g extract. The total phenolic compounds expressed as mg GAE/g extract. The measurement was triplicate.

2.4 Determination of flavonoid contents

Determination of flavonoid contents using spectrophotometric method [7]. Determination of flavonoid contents was carried out by spectrophotometry using reagent aluminium chloride. As many as 1 mL aqueous solution extracts with a concentration of 1000 mg/L, at add with 1 mL 2% AlCl dissolved with ethanol 50% homogenized, and then use the vortex during the 20-minute incubation, mix the solution for 24 hours. Measure absorbance at 415 nm. Flavonoid contents was expressed in mg of quercetin/g extract and calculation with triplicate measurement.

2.5 Evaluation of antioxidant activity based on DPPH method

DPPH method using synthetic radical 1.1,-diphenyl picryl hydrazyl (DPPH) [8]. As many as two ml of DPPH (0.1 mM in methanol solution), plus 40 µg/ml methanol

extract of the peel and flesh of raw or boiled C. tuberous or C. tuberosus flake. Change the color after 30 minutes read at 517 nm. Percentage radical scavenging activity determined:

(A0-A1)/A0 x 100%

In this case, Ao was absorbance control and A1 was absorbance methanol extract of the peel and flesh of raw, boiled C. tuberous or C. tuberosus flake.

2.6 Evaluation of resistant starch

Resistant starch was determined by enzymatic reactions [9]. Raw C. tuberosus, boiled C. tuberosus or C. tuberosus flake (100 mg) was incubated with a solution containing pepsin as muc as 20 mg at temperature 40oC for 60 min. A tris-maleate solution containing pancreatic α-amylase as much as 40 mg then added and the mixture incubated at temperature 37oC for 16 hr to hydrolyse the digestible starch. The hydrolysate was centrifuged and the residue was solubilised with KOH 4M and incubated with 80 µL amyloglucosidase at temperature 60oC for 45 min to hydrolyse RS. A glucose oxidase-peroxidase kit used to measured the glucose content. The RS content, comprising fractions of types I and II, was calculated as mg of glucose x 0.9.

2.7. In vivo assay

In vivo evaluation was done by setting up an experimental animal conducted in Laboratory Animal Maintenance (UPHP) Gadjah Mada University. The number of animals as much as 18 wistar type males weighing 110-150 grams and maintened in closed condition the enclosure that includes the light is not controlled, air vents in a cage enough, the air temperature on the temperature the room. Standard feed is

given for three days by using standard AIN 1993. Intraperitoneal injection of alloxan is done through with a dose of 125 mg/kg body weigh of mice to make the mice became diabetic. Mice given standard feed. After the third day, mice suffering diabetes mellitus. Next up is done weighing weight and groups divided. The mice were divided into three groups, namely diet 6 mice to a standard diet AIN 1993, 6 mice to boiled C. tuberosus and 6 mice to C. tuberosus flake. Given in drinking water ad libitum. Cages are cleaned on a daily basis from dirt or stool that is inherent, residual feed weighed every day. Feed mice given each morning. Blood glucose analysis done with the method GOD Glucose PAP: enzymatic reactions photometric test.

2.8. Statistical analysis

The analysis was conducted on three replications (untuk the level of total phenolic compounds, flavonoid contents and resistant starch content) and six replictions (untuk in vivo assay) by observing their mean ± SD. The testing was performed by ANOVA, if it appeared to have a real difference, the test is followed by LSD.

3 Result and discussion

3.1 The level of total phenolic compounds dan flavonoid contents

Analysis of the levels of total phenolic compounds and flavonoid contents in the methanol extract of the peel and flesh of raw, boiled of C. tuberosus and C. tuberosus flake showed a tendency to decrease (Table 1).

Tabel 1. Level of total phenolic compounds and flavonoid content in methanol extract of The flesh and peel of raw, boiled C. tuberosus and C. tuberosus flake

Treatment	Part of sample	Level of total phenolic compounds (mg GAE/g extract)	Level of flavonoid content (mg quercetin/g of extract)

Raw *C. tuberosus*	Peel	7.73±0.08[f]	8.55±0.07[f]
	Flesh	7.24±0.10[e]	2.31±0.13[e]
Boiled *C. tuberosus*	Peel	2.17±0.01[a]	0.07±0.00[a]
	Flesh	6.51±0.02[d]	1.22±0.01[c]
C. tuberosus Flake (Control)		3.83±0.02[b]	2.17±0.01[d]
C. tuberosus Flake		4.62±0.03[c]	0.85±0.01[b]

Different letters within the same column indicate significant differences at $P<0.05$.

The research indicates that cooking process can cause significant changes in the total phenolic compounds and flavonoid contents. Treatment with high temperature can cause damaging of total phenolic compounds and flavonoid contents linked to they are highly unstable compounds [10]. Table 1 shows that the levels of total phenolic compounds decreased at the time of the processing is done. This is possible because, during the boiling water using the media as a heat conductor, some compounds are polyphenol (condensed tannins) on tuber hydrolyzed and then diffused into the boiling water. Boiling process reduce the number of total phenolic compounds on either the flesh or the peel of the making of C. tuberosus flake led to a decrease in the number of phenolic compounds. Another research proved that the boiling process impact levels decrease phenolic compounds and flavonoid on several types of vegetables [11,12].

The levels of flavonoid contents in C. tuberosus on boiling and baking process shows a tendency to decline (Table 1). This was likely caused by degradation or decomposition of flavonoid contents during the thermal processing. The possibility of flavonoid on C. tuberosus is anthocyanin. This compound is soluble in water, so by the boiling process caused anthocyanin leaching in the boiling water. It demonstrated the existence of dark color (purple, blue/black) in the water boiling of C. tuberosus. [13]. Anthocyanins was not stable during processing that used heat

treatment [14]. Increasing temperature and activity of enzymatic reactions may result in a destruction of phenolic compounds [15].

3.2 Evaluation of antioxidant activity

The antioxidant activity was evaluated with the DPPH method indicate that processing has a tendency to increase the ability of antioxidant activity in samples (Table 2). Method of processing by way of boiling can increase antioxidant activity either on the peel or flesh of C. tuberosus.

Table 2. Antioxidant activity (DPPH) on raw or boiled C. tuberosus and C. tuberosus flake

Treatment	Part of sample	Antioxidant activity (DPPH) (%)
Raw *C. tuberosus*	Peel	62.82±0.32^{c}
	Flesh	26.34±0.09^{a}
Boiled *C. tuberosus*	Peel	92.70±0.47^{e}
	Flesh	56.29±0.37^{b}
C. tuberosus flake (control)		91.11±0.51^{d}
C. tuberosus flake		92.57±0.47^{e}

Different letters within the column indicate significant differences at $P<0.05$.

Table 2 shows that the processing can increase antioxidant activity. Several things made possible the presence of phenolic compounds such as conversion to a form that has a higher antioxidant activity suppose the formation of aglycon that has the ability of higher antioxidant activity. Increased antioxidant activity caused by the transformation of compound phytochemicals becoming more active compounds e.g. polymerization of polyphenolic.

Maillard reaction i.e. reactions involving amino Carbonyl groups and so arose a new compound that Brown i.e. Maillard reaction products (MRPs) that have a greater

antioxidant property [16]. Maillard reaction can produce a variety of products, intermediate products and brown product (melanoidin), which has contributed to the color, flavor and aroma as well as having potential as antioxidants in processed food [17]. Some research suggests that treatment with boiling and baking can increase antioxidant activity in a food despite the declining levels of phenolic and flavonoid [18].

3.3 The level of Resistant Starch

Resistant starch was defined as starch can escape digestion in the intestine and as a source of fermentation substrate for colonic microflora. An aerobic fermentation generates short chain fatty acids which can be used as additional energy for animals [19].

The results of the analysis of the RS on the raw C. tuberosus, boiled C. tuberosus, and C. tuberosus flake were present on Table 3.

Table 3. Level of resistant starch on raw, boiled C. tuberosus and C. tuberosus flake

Materials	Level of resistant starch (%)
Raw *C. tuberosus*	10.2352 ± 0.3680^{a}
Boiled *C. tuberosus*	15.4218 ± 0.9570^{b}
C. tuberosus flake	44.0853 ± 0.0724^{c}

Different letters within the same column indicate significant differences at $P<0.05$.

Table 3 shows that processing can increase the levels of RS. The source of carbohydrate if experience thermal process can result in resistant starch. The more stages of processing increasingly large resistant starch that was formed. Processing by way of steaming, boiling, and roasting can raise resistant starch. The potatoes are boiled then cooled impact on increasing the levels of resistant starch (RS3) [19]. Processing method: steaming, boiling and roasting can raise resistant starch

3.5. Glucose profile

The mice suffering from diabetes after feeding Boiled and flake C. tuberosus shows a decrease in serum glucose levels (Figure 1). The decrease in serum glucose in boiled C. tuberosus was 47.41%. whereas C. tuberosus flake was 54.94%. Standard feed does not occur on a decrease in serum glucose. These data indicate that the feed that contain resistant starch was higher (Table 3), have the greater ability of lowering glucose profile.

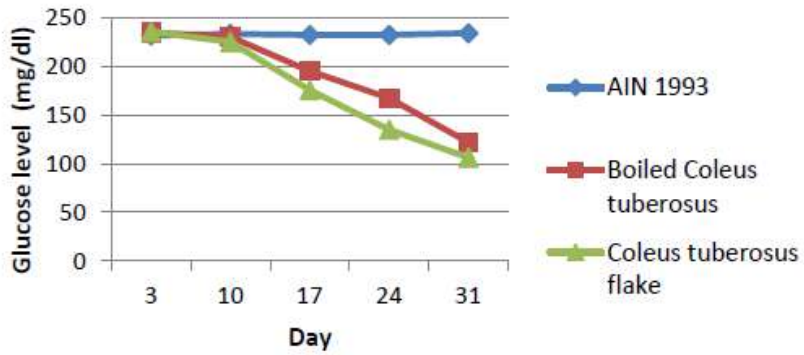

Fig. 1. Profile glucose of diabetic mice for 28 days treatment

Foods containing resistant starch will be didigesti slow, this gives implications for controlling the release of glucose [20]. This is in line with studies showing that consumption of foods containing resistant starch type 3 to control the release of glucose because it has a low glycemic index (Nugraheni et al., 2018) [21]. Commercial RS3 blood glucose was significantly lower than that of other simple carbohydrates [21] [22]. RS3 lowered postprandial blood glucose and to play a part in keeping control of metabolic in type II diabetic patients. Research also shows that foods containing resistant starch type 3 may improve glucose profiles in mice injected alloksan (Nugraheni, 2017) [23]. Consumption of resistant starch can rectify the beta cells of the pancreas, insulin sensitivity, increase levels of insulin the

pancreas, the total level of GLP-1, SCFA concentration so as to improve control of blood glucose levels (Shen et al., 2011) [24].

Current research finding that there is an increase in ROS or concentration of oxidative stress and lipida on several animal models. Alloxan led to free radicals that caused cells damage cells. During the redox process, ROS are formed and the beta cell damage caused on the island of langerhans [22] [25].

The research that has been done suggests that flavonoids and phenols has the capability of capturing free radicals, which can protect the oxidative stress that cause cell damage (such as the concentration of lipida membrane and membrane degradation). Flavonoid glycosides can stimulate insulin secretion in beta cells of the pancreas. In animals that suffer from diabetes, and given feed containing flavonoids, then it is possible that the compound acts as an insulin secretion in the pancreas penstimulasi or increase the uptake of glucose [23][26].

The ability of flavonoids as anti diabetic is able to improve glucose profile is related to the effect his ability to stimulate insulin secretion, reduces the apoptosis and proliferation of pancreatic beta cells spur, lowering insulin resistance, inflammatory and oxidative stress on the muscles and mempromot translocasi GLUT4 via PI3K/AKT and AMPK pathways (Viyanagam and Xu, 2015) [27]. Consumption of phenols may inhibit the α-amylase and α-glucosidase, able to inhibit the absorption of glucose in the intestine by sodium-dependent glucose transporter 1 (SGLT1), can stimulate the secretion of insulin and reduce hepatic glucose output (Kim et al., 2016) [28].

4 Conclusions

The process of boiling of C. tuberosus and making C. tuberosus flake have an impact on decreasing the level of phenol compounds and flavonoid. Antioxidant

activity of the flesh and peel of boiled C. tuberosus and C. tuberosus flake increased compared to raw C. tuberosus. Processing can increase the levels of resistant starch. The resistant starch content on raw C. tuberosus 10.24 ± 0.37%; boiled C. tuberosus 15.42 ± 0.96%; C. tuberosus flake 44.09 ± 0.07%. The decrease in serum glucose in boiled C. tuberosus was 47.41%. whereas C. tuberosus flake was 54.94%.

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Methodology	Clear enough. However, some corrections are needed to improve the understanding of the reader and the consistency of the information. For instance: 1- **In the first paragraph of materials and methods** (line 63, the full name of the laboratory should be written). 2- **In the sample preparation section:** rephrase the sentences and use the past participle tense in referring to the methods. 3- Also, **in the determination of total phenolic compounds**, the abbreviation of GAE was not mentioned in full (Gallic acid equivalent), whereas, **in the determination of flavonoid contents**, the chemical formula of aluminum chloride was written wrong (AlCl, it should be $AlCl_3$). 4- **In the evaluation of resistant starch content**, the authors did not mention the information of the glucose oxidase-peroxidase kit (catalog or lot number, company name, country). 5- **In the in vivo assay section**, in vivo should be written in italic (*in vivo*) and no information about the animal ethical approval was mentioned. More information about the ethical approval should be mentioned in this section. 6- **Statistical analysis,** no information about the name of the statistical analysis software used, what type of ANOVA test was used and did you perform a t-test when compared between the peel and flesh part of sample in the same treatment as it statistically more accurate?. Moreover, no information about P-values significance was mentioned.
Results and Discussion	Just add limitations of the study and future work
References (Appropriateness)	Rewrite the references according to the journal style especially in writing the authors names. However, the used references are good and related to topic.

Rating (1 to 5) 1: Excellent, 5: Poor

Originality	2
Depth of research	2
Technical quality	2

Recommendation:

☑ Accept conditionally, subject to minor revision, according to my accompanying comments

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Review Reports

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Dear
Managing Editor

Here this, I submit revisions to the article. Apologize for delay of the revision of this article. I hope this revision in accordance with the comment of reviewers. Thank you

Sincerly yours,

Mutiara Nugraheni

[Kutipan teks disembunyikan]

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Current Research in
Nutrition and Food Science

Author's Response to Reviewer's Comments

Reviewer number:

Paper title: Antioxidant Activity and Resistant Starch Content Of Coleus tuberosus on Different Cooking Method And Its Potential On Glucose Management In Diabetic Mice

Title	Reviewer's Comments	Author's Response
Abstract	The abstract is well comprehensively structured and clear	
Keywords		
Introduction	This portion of the paper is appropriate, clear and has an adequate length.	
Methodology	The methodology of this research paper has been well presented. It is concise and clear. The authors did not state any ethical approval for the animal laboratory experimental procedure. Were the animal treated according to laboratory experimental guidelines?	Has been added in line... 147. Treatment of animals experimental in the laboratory animal maintenance, Center for food and nutrition, Gadjah Mada University. All procedures performed involving animal were approved by the Gadjah Mada University Animal Ethics Committee.
Results	The conclusions of this study are supported by the data carried out and analyzed by the authors. Tables and	Has been added in line..169.. Significant differences between groups were

	Figures are clearly presented. However, different letters indicating the statistical significance should have been given their methods of analysis or degree of significance.	determined at $p<0.05$.
Discussion		
Conclusion		
References (Appropriateness)	The references are appropriate and sound	

Current Research in Nutrition and Food Science

Author's Response to Reviewer's Comments

Reviewer number:

Paper title: Antioxidant Activity and Resistant Starch Content Of Coleus tuberosus on Different Cooking Method And Its Potential On Glucose Management In Diabetic Mice

Title	Reviewer's Comments	Author's Response
Abstract	Language editing and writing a conclusion at the end of the abstract	Has been added in Line 26: The results of this study indicate that processing (boiling and baking) can increase the antioxidant activity and the levels of resistant starch.
Keywords		
Introduction	Language editing as many sentences need a rephrasing and grammar corrections. Moreover, other recent references can be added.	Language has been edited
Methodology	Clear enough. However, some corrections are needed to improve the understanding of the reader and the consistency of the information. For instance: 1- **In the first paragraph of**	1. Has been added .... the

	materials and methods (line 63, the full name of the laboratory should be written).	full name of the laboratory should be written in line 77: Treatment of animals experimental in the laboratory animal maintenance, Center for food and nutrition, Gadjah Mada University.
	2- **In the sample preparation section:** rephrase the sentences and use the past participle tense in referring to the methods.	2. rephrase the sentences and use the past participle tense in referring to the methods is revised
	3- Also, **in the determination of total phenolic compounds**, the abbreviation of GAE was not mentioned in full (Gallic acid equivalent), whereas, **in the determination of flavonoid contents**, the chemical formula of aluminum chloride was written wrong (AlCl, it should be $AlCl_3$).	3. The abbreviation of GAE was not mentioned in full (Gallic acid equivalent), was revised in line 111. **in the determination of flavonoid contents**, the chemical formula of aluminum chloride was written wrong (AlCl, it should be $AlCl_3$)....has been revised.....in line 118
	4- **In the evaluation of resistant starch content**, the authors did not	4. the glucose oxidase-peroxidase kit has been added in line 141... glucose

	mention the information of the glucose oxidase-peroxidase kit (catalog or lot number, company name, country).	(go) assay kit product number gago-20, Sigma.
	5- **In the in vivo assay section**, in vivo should be written in italic (*in vivo*) and no information about the animal ethical approval was mentioned. More information about the ethical approval should be mentioned in this section.	5. More information about the ethical approval should be mentioned in this section.,..... has been added in line 148.... Treatment of animals experimental in the laboratory animal maintenance, Center for food and nutrition, Gadjah Mada University. All procedures performed involving animal were approved by the Gadjah Mada University Animal Ethics Committee.
	6- **Statistical analysis,** no information about the name of the statistical analysis software used, what type of ANOVA test was used and did you perform a t-test when compared between the peel and flesh part of sample in the same	6. Software has been added... The software SPSS for Windows v15.0 (SPSS Inc., Chicago, Illinois) was used for analysis of variance and mean separation. LSD test was used to compare treatments when ANOVA was significant ($p \leq 0.05$). To

	treatment as it statistically more accurate?. Moreover, no information about P-values significance was mentioned.	compare the results on the peel and the flesh using t-test. Already added to the statistical analysis...line 167
Results	Just add limitations of the study and future work	Add limitation of the study and future work, has been added..... in line 269.
Discussion		
Conclusion		
References (Appropriateness)	Rewrite the references according to the journal style especially in writing the authors names. However, the used references are good and related to topic.	The writing references have been revised

Antioxidant Activity and Resistant Starch Content Of *C. tuberosus* on Different Cooking Method And Its Potential On Glucose Management In Diabetic Mice

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ABSTRACT

This research aims to know the antioxidant activity and the levels of resistant starch of C. tuberosus on different processing methods. Processing methods used were boiling and baking. Bioactive compounds being evaluated is the number of total phenolic and flavonoid. Evaluation of antioxidant activity is performed with the DPPH method. The evaluation of the levels of resistant starch was done in an enzymatic method. The results showed that levels of total phenolic and flavonoid demonstrate a tendency to decline with the processing. The existence of the processing process increased the antioxidant activity of boiled C. tuberosus and C. tuberosus flake. The processing increases the levels of resistant. The levels of resistant starch in raw C. tuberosus were10.24 ± 0.37%; boiled C. tuberosus 15.42 ± 0.96%; and C. tuberosus flake 44.09 ± 0.07%. The decrease in serum glucose in boiled C. tuberosus was 47.41% whereas C. tuberosus flake was 54.94%. The results of this study indicate that processing (boiling and baking) can increase the antioxidant activity and the levels of resistant starch.

Keyword: antioxidant activity, resistant starch, C. tuberosus, cooking method, diabetic mice

Introduction

Increasing the growing public awareness of the importance of healthy living, the claim against consumer foodstuffs also increasingly shifted. Food that is now starting to great demand not only that consumers have an excellent nutritional composition as well as the appearance and exciting flavors, but also must have specific physiological functions for the body. Such a function is known as the tertiary function. Foods that have a function known as tertiary is known as functional foods. Functional

foods are foods that can maintain health and prevent disease because it has an active component in the biology that has benefits for health.

Research shows that there is a link between components in the food consumed with health[1]. Functional components in plants, for example, phytochemical has a biological activity to prevent disease[2]. Phytochemical compounds contained in legume, cereal, fruit, vegetables have antioxidant activity phenolic and flavonoid[3]. Antioxidants are groups of compounds which neutralize free radicals and reactive oxygen species in the cell to prevent the occurrence of oxidative stress in human cells.

Resistant starch (RS) much developed and consumed because of the value of its current status. Hydrolysis resistant starch by digestive enzymes needs more extended periods and give an impact on the production of glucose becomes slower. Indirectly, the RS has a value of the function for patients with diabetes. RS has three systems related to the functional value of the metabolism and effects in the body, i.e., as an ingredient for the fortification of fiber, lower calories, and fat oxidation. RS there are naturally in food products and can be used in a modified form as well as added in food[4].

C. tuberosus is one of the possible food ingredients in Indonesia as a source of carbohydrates that come from minor tubers. Some research suggests that *C. tuberosus* has the potential to be developed as functional foods based on the content of compound bioactive and RS that can be obtained by modification of the processing. *C. tuberosus* contain flavonoid, ascorbic acid, which can increase the antioxidant enzyme activity and lowering products peroxide in rats fed a high-fat diet. *C. tuberosus* extract contains bioactive compounds that have antioxidant activity[5]. *C. tuberosus* processing into will bring the physical and chemical changes will have an

impact on its potential as a functional food. The purpose of this research is to evaluate the activity of antioxidants and resistant starch of *C. tuberosus* on different cooking methods and to know the effect the consumption of *C. tuberosus* on glucose profile in diabetic mice.

Materials and Methods

This research did experimentally in the laboratory, Department of Culinary Art Education, Yogyakarta State University, Center for Food & Nutrition Studies Gadjah Mada University. The process *C. tuberosus* flake was making process done by adding other ingredients namely soy flour. Study of antioxidant activity and levels of resistant starch done on raw *C. otuberosus*, boiled *C. ftuberosus*, and *C. tuberosus* flake. Treatment of animals experimental in the laboratory animal maintenance, Center for food and nutrition, Gadjah Mada University.

Sample preparation

Sample preparation of antioxidant activity. Preparation of the raw *C. tuberous*: *C. tuberosus* separated the peel and flesh by Peeler. The thickness of the stripped of the peel (1-1.5 mm) so that the retrieved peel and flesh of *Coleus tuberosus*. Boiled *C. tuberosus*: *C. tuberosus* boiled for 30 minutes, then peel and flesh were separated. The peel and flesh of tubers dried with the cabinet drier at temperature 40^{o}C for 24 hours. Then ground and shift with the sieve mesh Tyler 80 size. *C. tuberosus* flake made with the three formulations, the most preferred formulation based on the hedonic test of 30 semi-trained panelists used for samples. *C. tuberosus* flake made from *C. tuberosus* flour, tapioca flour, soy flour, sorbitol, margarine, salt, cocoa powder, and water. Whereas the control of C. tuberosus flake

made from *C. tuberosus* flour, tapioca flour, sorbitol, margarine, salt, cocoa powder, and water (without soy flour).

Sample preparation for resistant starch analysis: raw *C. tuberosus* sample, prepared from the all of the parts of *C. tuberosus*, sliced and then dried it with a cabinet drier at 40^{o}C for 24 hours. Dried *C. tuberosus* used as a sample analysis of resistant starch. Boiled *C. tuberosus*, prepared by boiling all of part of *C. tuberosus* for 30 minutes, peeled and the using as a sample analysis of resistant starch.

Extraction process

The peel and flesh flour of raw and boiled *C. tuberous*, *C. tuberosus* flake macerated with methanol during seven days (1:5), then filtered using Whatman No.1, evaporating the solvent with gas N_2. The Extract stored in the freezer temperature -22^{o}C.

Determination of total phenolic compounds

The methanol extract of *C. tuberous* determined using spectrophotometric method[6]. As much as 0.2 mL different extract with a concentration of 100 mg/L, Folin-Ciocalteu reagent 10% as much as 2.5 mL, and two mL 7.5% Na_2CO_3 are mixed and allowed for 15 minutes at a temperature of 45^{o}C. The absorbance of the solution was measured using the spectrophotometer at a wavelength of 765 nm. The total phenolic compounds expressed as mg Galic Acid Equivalent/g extract (mg GAE/g extract). The measurement was in triplicate.

Determination of flavonoid contents

Determination of flavonoid contents used spectrophotometric method[7]. Determination of flavonoid contents was carried out by spectrophotometry using reagent aluminium chloride. As many as one mL aqueous solution extracts with a concentration of 1000 mg/L, at add with one mL 2% $AlCl_3$ dissolved with ethanol 50% homogenized, and then use the vortex during the 20-minute incubation, mix the solution for 24 hours. Measure absorbance at 415 nm. Flavonoid contents expressed in mg of Quercetin Equivalent/g extract and calculation with triplicate measurement.

Evaluation of antioxidant activity based on DPPH method

DPPH method using synthetic radical 1.1,-diphenyl picrylhydrazyl (DPPH)[8]. As many as two ml of DPPH (0.1 mM in methanol solution), plus 40 µg/ml methanol extract of the peel and flesh of raw or boiled *C. tuberous* or *C. tuberosus* flake. Change the color after 30 minutes read at 517 nm. Percentage radical scavenging activity determined:

(A0-A1)/A0 x 100%

In this case, Ao was absorbance control, and A1 was the absorbance methanol extract of the peel and flesh of raw, boiled *C. tuberous* or *C. tuberosus* flake.

Evaluation of resistant starch content

Resistant starch determined by enzymatic reactions[9]. Raw *C. tuberosus*, boiled *C. tuberosus* or *C. tuberosus* flake (100 mg) incubated with a solution containing pepsin as much as 20 mg at temperature 40^{o}C for 60 min. A tris-maleate solution containing pancreatic α-amylase as much as 40 mg then added and the mixture incubated at temperature 37^{o}C for 16 hr to hydrolyze the digestible starch. The hydrolysate centrifuged, and the residue was solubilized with KOH 4M and incubated with 80 µL

amyloglucosidase at temperature 60^{o}C for 45 min to hydrolyze RS. A glucose oxidase-peroxidase kit used to measure the glucose content (glucose assay kit product number gago-20, Sigma). The RS content, comprising fractions of types I and II, was calculated as mg of glucose x 0.9.

In vivo assay

In vivo evaluation was done by setting up an experimental animal conducted in Treatment of animals experimental in the laboratory animal maintenance, Center for food and nutrition, Gadjah Mada University. All procedures performed involving animal were approved by the Gadjah Mada University Animal Ethics Committee. The number of animals as much as 18 Wistar type males weighing 110-150 grams and maintained in the closed condition the enclosure that includes the light did not control, air vents in a cage enough, the air temperature on the temperature the room. Standard feed has given for three days by using standard AIN 1993. Intraperitoneal injection of alloxan was done through with a dose of 125 mg/kg body weight of mice to make the mice became diabetic. Mice were given standard feed. After the third day, mice suffering from diabetes mellitus. Next up is done weighing weight and groups divided. The mice were divided into three groups, namely diet six mice to a standard diet AIN 1993, six mice to boiled *C. tuberosus* and six mice to *C. tuberosus* flake. Given in drinking water ad libitum. Cages cleaned daily from dirt or stool that is inherent, and residual feed weighed every day. Feed mice were given each morning. Blood glucose analysis was done with the method GOD Glucose PAP: enzymatic reactions photometric test.

Statistical analysis

The analysis was conducted on three replications (the level of total phenolic compounds, flavonoid contents, and resistant starch content) and six replications (in vivo assay) by observing their mean ± SD. A t-test used when compared between the peel and flesh part of sample in the same treatment. The software SPSS for Windows v15.0 (SPSS Inc., Chicago, Illinois) was used for analysis of variance and mean separation. LSD test was used to compare treatments when ANOVA was significant ($p \leq 0.05$).

Result and discussion

The level of total phenolic compounds dan flavonoid contents

Analysis of the levels of total phenolic compounds and flavonoid contents in the methanol extract of the peel and flesh of raw, boiled of *C. tuberosus* and *C. tuberosus* flake showed a tendency to decrease (Table 1).

The research indicates that cooking process can cause significant changes in the total phenolic compounds and flavonoid contents. Treatment with high temperature can cause damaging of total phenolic compounds and flavonoid contents linked to they are highly unstable compounds[10]. Table 1 shows that the levels of total phenolic compounds decreased at the time of the processing is done. The decrease is possible because, during the boiling water using the media as a heat conductor, some compounds are polyphenol (condensed tannins) on tuber hydrolyzed and then diffused into the boiling water. Boiling process reduce the number of total phenolic compounds on either the flesh or the peel of the making of *C. tuberosus* flake led to a decrease in the number of phenolic compounds. Another research proved that the boiling process impact levels decrease phenolic compounds and flavonoid on several types of vegetables[11,12].

The levels of flavonoid contents in *C. tuberosus* on boiling and baking process shows a tendency to decline (Table 1). Degradation or decomposition of flavonoid contents likely caused this during the thermal processing. The possibility of flavonoid on *C. tuberosus* is anthocyanin. This compound is soluble in water, so by the boiling process caused anthocyanin leaching in the boiling water. It demonstrated the existence of dark color (purple, blue/black) in the water boiling of *C. tuberosus*[13]. Anthocyanins were not stable during processing that used heat treatment[14]. Increasing temperature and activity of enzymatic reactions may destroy phenolic compounds[15].

Evaluation of antioxidant activity

The antioxidant activity evaluated with the DPPH method indicate that processing tends to increase the ability of antioxidant activity in samples (Table 2). Method of processing by way of boiling can increase antioxidant activity either on the peel or flesh of *C. tuberosus*.

Table 2 shows that processing can increase antioxidant activity. Several things made possible the presence of phenolic compounds such as conversion to a form that has a higher antioxidant activity suppose the formation of aglycon that has the ability of higher antioxidant activity. Increased antioxidant activity caused by the transformation of compound phytochemicals becoming more active compounds, e.g., polymerization of polyphenolic.

Maillard reaction, i.e., reactions involving amino Carbonyl groups and so arose a new compound that Brown, i.e., Maillard reaction products (MRPs) that have a higher antioxidant property[16]. Maillard reaction can produce a variety of products, intermediate products and brown product (melanoidin), which has contributed to the

color, flavor, and aroma as well as having potential as antioxidants in processed food[17]. Some research suggests that treatment with boiling and baking can increase antioxidant activity in food despite the declining levels of phenolic and flavonoid[18].

3.3 The level of Resistant Starch

Resistant starch defined as starch can escape digestion in the intestine and as a source of fermentation substrate for colonic microflora. Anaerobic fermentation generates short chain fatty acids which can be used as additional energy for animals[19].

The results of the analysis of the RS on the raw *C. tuberosus*, boiled *C. tuberosus*, and *C. tuberosus* flake was present on Table 3. Table 3 shows that processing can increase the levels of RS. The source of carbohydrate if experience thermal process can result in resistant starch. The more stages of processing increasingly high resistant starch that was formed. Processing by way of steaming, boiling, and roasting can raise resistant starch. The potatoes boiled then cooled impact on increasing the levels of resistant starch (RS3)[19]. Processing method: steaming, boiling and roasting can raise resistant starch.

The glucose profile

The mice suffering from diabetes after feeding Boiled and flake *C. tuberosus* shows a decrease in serum glucose levels (Figure 1). The decrease in serum glucose in boiled *C. tuberosus* was 47.41%. Whereas *C. tuberosus* flake was 54.94%. Standard feed does not occur on a decrease in serum glucose. These data indicate that the feed that contains resistant starch was higher (Table 3), have the greater ability to lower glucose profile.

Foods containing resistant starch will be digested slow; this gives implications for controlling the release of glucose[20]. This is in line with studies showing that consumption of foods containing resistant starch type 3 to control the release of glucose because it has a low glycemic index[21]. Commercial RS3 blood glucose was significantly lower than that of other simple carbohydrates[22]. RS3 lowered postprandial blood glucose and to play a part in keeping control of metabolism in type II diabetic patients. Research also shows that foods containing resistant starch type 3 may improve glucose profiles in mice injected alloxan[23]. Consumption of resistant starch can rectify the beta cells of the pancreas, insulin sensitivity, increase levels of insulin the pancreas, the total level of GLP-1, SCFA concentration on improving control of blood glucose levels[24].

The current research was finding that there is an increase in ROS or concentration of oxidative stress and lipids on several animal models. Alloxan led to free radicals that caused cells damage cells. During the redox process, ROS are formed and the beta cell damage caused on the island of Langerhans[25].

The research that has been done suggests that flavonoids and phenols have the capability of capturing free radicals, which can protect the oxidative stress that causes cell damage (such as the concentration of lipids membrane and membrane degradation). Flavonoid glycosides can stimulate insulin secretion in beta cells of the pancreas. In animals that suffer from diabetes, and given feed containing flavonoids, then it is possible that the compound acts as an insulin secretion in the pancreas stimulated or increase the uptake of glucose[26].

The ability of flavonoids as antidiabetic can improve glucose profile is related to the effect his ability to stimulate insulin secretion, reduces the apoptosis and proliferation of pancreatic beta cells spur, lowering insulin resistance, inflammatory and oxidative

stress on the muscles and promotes translocate of GLUT4 via PI3K/AKT and AMPK pathways[27]. Consumption of phenols may inhibit the α-amylase and α-glucosidase, able to inhibit the absorption of glucose in the intestine by sodium-dependent glucose transporter 1 (SGLT1), can stimulate the secretion of insulin and reduce hepatic glucose output[28].

This study provides information that the existence of two processing techniques, i.e. boiling and baking can increase antioxidant activity and levels of resistant starch. Nevertheless still required information related to the antioxidant activity and the levels of resistant starch for various processing techniques (steaming, roasting) or Coleus tuberosus processing to a wide range of processed products, so that the community has more options in Coleus tuberosus processing as a product ready to eat.

Conclusions

The process of boiling of *C. tuberosus* and making *C. tuberosus* flake have an impact on decreasing the level of phenol compounds and flavonoid. Antioxidant activity of the flesh and peel of boiled *C. tuberosus* and *C. tuberosus* flake increased compared to raw *C. tuberosus*. Processing can increase the levels of resistant starch. The resistant starch content on raw *C. tuberosus* 10.24 ± 0.37%; boiled C. *tuberosus* 15.42 ± 0.96%; *C. tuberosus* flake 44.09 ± 0.07%. The decrease in serum glucose in boiled *C. tuberosus* was 47.41%. Whereas *C. tuberosus* flake was 54.94%.

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Table 1. Level of total phenolic compounds and flavonoid content in the methanol extract of The flesh and peel of raw, boiled *C. tuberosus* and *C. tuberosus* flake

Treatment	Part of sample	Level of total phenolic compounds (mg GAE/g extract)	Level of flavonoid content (mg quercetin/g of extract)
Raw *C. tuberosus*	Peel	7.73±0.08^{fB}	8.55±0.07^{fB}
	Flesh	7.24±0.10^{eA}	2.31±0.13^{eA}
Boiled *C. tuberosus*	Peel	2.17±0.01^{aA}	0.07±0.00^{aA}
	Flesh	6.51±0.02^{dB}	1.22±0.01^{cB}
C. tuberosus Flake (Control)		3.83±0.02^{b}	2.17±0.01^{d}
C. tuberosus Flake		4.62±0.03^{c}	0.85±0.01^{b}

Different letters (a-f) within the column indicate significant differences in different treatment at $P < 0.05$.
Different capital letters (A-B) within the column indicate significant differences in different part of sample in the same teratment at $P < 0.05$.

Table 2. Antioxidant activity (DPPH) on raw or boiled *C. tuberosus* and *C. tuberosus* flake

Treatment	Part of sample	Antioxidant activity (DPPH) (%)
Raw *C. Tuberosus*	Peel	62.82 ± 0.32^{cB}
	Flesh	26.34 ± 0.09^{aA}
Boiled *C. Tuberosus*	Peel	92.70 ± 0.47^{eB}
	Flesh	56.29 ± 0.37^{bA}
C. tuberosus flake (control)		91.11 ± 0.51^{d}
C. tuberosus flake		92.57 ± 0.47^{e}

Different letters (a-e) within the column indicate significant differences in different treatment at $P < 0.05$.
Different capital letters (A-B) within the column indicate significant differences in different part of sample in the same treatment at $P < 0.05$.

Table 3. Level of resistant starch on raw, boiled *C. tuberosus* and *C. tuberosus* flake

Materials	Level of resistant starch (%)
Raw *C. tuberosus*	10.24 ± 0.37^{a}
Boiled *C. tuberosus*	15.42 ± 0.96^{b}
C. tuberosus flake	44.09 ± 0.07^{c}

Different letters within the column indicate significant differences at $P<0.05$.

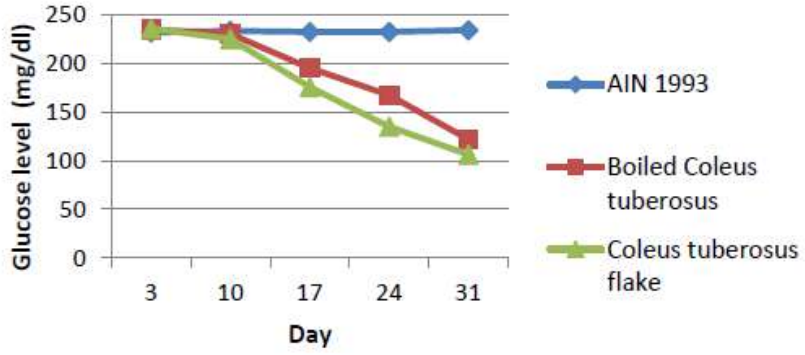

Fig. 1. Profile glucose of diabetic mice for 28 days treatment

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Antioxidant Activity and Resistant Starch Content of *C. tuberosus* on Different Cooking Method and its Potential on Glucose Management in Diabetic Mice

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Abstract

This research aims to know the antioxidant activity and the levels of resistant starch of *C. tuberosus* on different processing methods. Processing methods used were boiling and baking. Bioactive compounds being evaluated is the number of total phenolic and flavonoid. Evaluation of antioxidant activity is performed with the DPPH method. The evaluation of the levels of resistant starch was done in an enzymatic method. The results showed that levels of total phenolic and flavonoid demonstrate a tendency to decline with the processing. The existence of the processing process increased the antioxidant activity of boiled *C. tuberosus* and *C. tuberosus* flake. The processing increases the levels of resistant. The levels of resistant starch in raw *C. tuberosus* were10.24 ± 0.37%; boiled *C. tuberosus* 15.42 ± 0.96%; and *C. tuberosus* flake 44.09 ± 0.07%. The decrease in serum glucose in boiled *C. tuberosus* was 47.41% whereas *C. tuberosus* flake was 54.94%. The results of this study indicate that processing (boiling and baking) can increase the antioxidant activity and the levels of resistant starch.

Article History

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Keywords

Antioxidant Activity;
C. Tuberosus;
Cooking Method;
Diabetic Mice;
Resistant Starch.

Introduction

Increasing the growing public awareness of the importance of healthy living, the claim against consumer foodstuffs also increasingly shifted. Food that is now starting to great demand not only that consumers have an excellent nutritional composition as well as the appearance and exciting flavors, but also must have specific physiological functions for the body. Such a function is known as the tertiary function. Foods that have a function known as tertiary is known as functional foods. Functional foods are foods that can maintain health and prevent disease

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because it has an active component in the biology that has benefits for health.

Research shows that there is a link between components in the food consumed with health.[1] Functional components in plants, for example, phytochemical has a biological activity to prevent disease.[2] Phytochemical compounds contained in legume, cereal, fruit, vegetables have antioxidant activity phenolic and flavonoid.[3] Antioxidants are groups of compounds which neutralize free radicals and reactive oxygen species in the cell to prevent the occurrence of oxidative stress in human cells.

Resistant starch (RS) much developed and consumed because of the value of its current status. Hydrolysis resistant starch by digestive enzymes needs more extended periods and give an impact on the production of glucose becomes slower. Indirectly, the RS has a value of the function for patients with diabetes. RS has three systems related to the functional value of the metabolism and effects in the body, i.e., as an ingredient for the fortification of fiber, lower calories, and fat oxidation. RS there are naturally in food products and can be used in a modified form as well as added in food.[4]

C. tuberosus is one of the possible food ingredients in Indonesia as a source of carbohydrates that come from minor tubers. Some research suggests that *C. tuberosus* has the potential to be developed as functional foods based on the content of compound bioactive and RS that can be obtained by modification of the processing. *C. tuberosus* contain flavonoid, ascorbic acid, which can increase the antioxidant enzyme activity and lowering products peroxide in rats fed a high-fat diet. *C. tuberosus* extract contains bioactive compounds that have antioxidant activity.[5] *C. tuberosus* processing into will bring the physical and chemical changes will have an impact on its potential as a functional food. The purpose of this research is to evaluate the activity of antioxidants and resistant starch of *C. tuberosus* on different cooking methods and to know the effect the consumption of *C. tuberosus* on glucose profile in diabetic mice.

Materials and Methods

This research did experimentally in the laboratory, Department of Culinary Art Education, Yogyakarta State University, Center for Food & Nutrition Studies Gadjah Mada University. The process *C. tuberosus* flake was making process done by adding other ingredients namely soy flour. Study of antioxidant activity and levels of resistant starch done on raw *C. otuberosus*, boiled *C. ftuberosus*, and *C. tuberosus* flake. Treatment of animals experimental in the laboratory animal maintenance, Center for food and nutrition, Gadjah Mada University.

Sample Preparation

Sample preparation of antioxidant activity. Preparation of the raw *C. tuberous*: *C. tuberosus* separated the peel and flesh by Peeler. The thickness of the stripped of the peel (1-1.5 mm) so that the retrieved peel and flesh of Coleus tuberosus. Boiled *C. tuberosus*: *C. tuberosus* boiled for 30 minutes, then peel and flesh were separated. The peel and flesh of tubers dried with the cabinet drier at temperature 40 ^{o}C for 24 hours. Then ground and shift with the sieve mesh Tyler 80 size. *C. tuberosus* flake made with the three formulations, the most preferred formulation based on the hedonic test of 30 semi-trained panelists used for samples. *C. tuberosus* flake made from *C. tuberosus* flour, tapioca flour, soy flour, sorbitol, margarine, salt, cocoa powder, and water. Whereas the control of *C. tuberosus* flake made from *C. tuberosus* flour, tapioca flour, sorbitol, margarine, salt, cocoa powder, and water (without soy flour).

Sample preparation for resistant starch analysis: raw *C. tuberosus* sample, prepared from the all of the parts of *C. tuberosus*, sliced and then dried it with a cabinet drier at 40 ^{o}C for 24 hours. Dried *C. tuberosus* used as a sample analysis of resistant starch. Boiled *C. tuberosus*, prepared by boiling all of part of *C. tuberosus* for 30 minutes, peeled and the using as a sample analysis of resistant starch.

Extraction Process

The peel and flesh flour of raw and boiled *C. tuberous*, *C. tuberosus* flake macerated with methanol during seven days (1:5), then filtered using Whatman No.1, evaporating the solvent with gas N_2. The Extract stored in the freezer temperature -22 ^{o}C.

Determination of Total Phenolic Compounds

The methanol extract of *C. tuberous* determined using spectrophotometric method.[6] As much as 0.2 mL different extract with a concentration of 100

mg/L, Folin-Ciocalteu reagent 10% as much as 2.5 mL, and two mL 7.5% Na_2CO_3 are mixed and allowed for 15 minutes at a temperature of 45 °C. The absorbance of the solution was measured using the spectrophotometer at a wavelength of 765 nm. The total phenolic compounds expressed as mg Galic Acid Equivalent/g extract (mg GAE/g extract). The measurement was in triplicate.

Determination of Flavonoid Contents

Determination of flavonoid contents used spectrophotometric method.[7] Determination of flavonoid contents was carried out by spectrophotometry using reagent aluminium chloride. As many as one mL aqueous solution extracts with a concentration of 1000 mg/L, at add with one mL 2% $AlCl_3$ dissolved with ethanol 50% homogenized, and then use the vortex during the 20-minute incubation, mix the solution for 24 hours. Measure absorbance at 415 nm. Flavonoid contents expressed in mg of Quercetin Equivalent/g extract and calculation with triplicate measurement.

Evaluation of Antioxidant Activity Based on Dpph Method

DPPH method using synthetic radical 1.1,-diphenyl picrylhydrazyl (DPPH)8. As many as two ml of DPPH (0.1 mM in methanol solution), plus 40 µg/ml methanol extract of the peel and flesh of raw or boiled *C. tuberous* or *C. tuberosus* flake. Change the color after 30 minutes read at 517 nm. Percentage radical scavenging activity determined:

(A0-A1)/A0 x 100%

In this case, Ao was absorbance control, and A1 was the absorbance methanol extract of the peel and flesh of raw, boiled *C. tuberous* or *C. tuberosus* flake.

Evaluation of Resistant Starch Content

Resistant starch determined by enzymatic reactions9. Raw *C. tuberosus*, boiled *C. tuberosus* or *C. tuberosus* flake (100 mg) incubated with a solution containing pepsin as much as 20 mg at temperature 40oC for 60 min. A tris-maleate solution containing pancreatic α-amylase as much as 40 mg then added and the mixture incubated at temperature 37oC for 16 hr to hydrolyze the digestible starch. The hydrolysate centrifuged, and the residue was solubilized with KOH 4M and incubated with 80 µL amyloglucosidase at temperature 60oC for 45 min to hydrolyze RS. A glucose oxidase-peroxidase kit used to measure the glucose content (glucose assay kit product number gago-20, Sigma). The RS content, comprising fractions of types I and II, was calculated as mg of glucose x 0.9.

In vivo Assay

In vivo evaluation was done by setting up an experimental animal conducted in Treatment of

Table 1: Level of total phenolic compounds and flavonoid content in the methanol extract of The flesh and peel of raw, boiled *C. tuberosus* and *C. tuberosus* flake

Treatment	Part of sample	Level of total phenolic compounds (mg GAE/g extract)	Level of flavonoid content (mg quercetin /g of extract)
Raw *C. tuberosus*	Peel	7.73 ± 0.08^{fB}	8.55 ± 0.07^{fB}
	Flesh	7.24 ± 0.10^{eA}	2.31 ± 0.13^{eA}
Boiled *C. tuberosus*	Peel	2.17 ± 0.01^{aA}	0.07 ± 0.00^{aA}
	Flesh	6.51 ± 0.02^{dB}	1.22 ± 0.01^{cB}
C. tuberosus Flake (Control)		3.83 ± 0.02^{b}	2.17 ± 0.01^{d}
C. tuberosus Flake		4.62 ± 0.03^{c}	0.85 ± 0.01^{b}

Different letters (a-f) within the column indicate significant differences in different treatment at $P < 0.05$.
Different capital letters (A-B) within the column indicate significant differences in different part of sample in the same teratment at $P < 0.05$

animals experimental in the laboratory animal maintenance, Center for food and nutrition, Gadjah Mada University. All procedures performed involving animal were approved by the Gadjah Mada University Animal Ethics Committee. The number of animals as much as 18 Wistar type males weighing 110-150 grams and maintained in the closed condition the enclosure that includes the light did not control, air vents in a cage enough, the air temperature on the temperature the room. Standard feed has given for three days by using standard AIN 1993. Intraperitoneal injection of alloxan was done through with a dose of 125 mg/kg body weight of mice to make the mice became diabetic. Mice were given standard feed. After the third day, mice suffering from diabetes mellitus. Next up is done weighing weight and groups divided. The mice were divided into three groups, namely diet six mice to a standard diet AIN 1993, six mice to boiled *C. tuberosus* and six mice to *C. tuberosus* flake. Given in drinking water ad libitum. Cages cleaned daily from dirt or stool that is inherent, and residual feed weighed every day. Feed mice were given each morning. Blood glucose analysis was done with the method GOD Glucose PAP: enzymatic reactions photometric test.

Statistical Analysis

The analysis was conducted on three replications (the level of total phenolic compounds, flavonoid contents, and resistant starch content) and six replications (in vivo assay) by observing their mean ± SD. A t-test used when compared between the peel and flesh part of sample in the same treatment. The software SPSS for Windows v15.0 (SPSS Inc., Chicago, Illinois) was used for analysis of variance and mean separation. LSD test was used to compare treatments when ANOVA was significant ($p \leq 0.05$).

Result and Discussion

The Level of Total Phenolic Compounds Dan Flavonoid Contents

Analysis of the levels of total phenolic compounds and flavonoid contents in the methanol extract of the peel and flesh of raw, boiled of *C. tuberosus* and *C. tuberosus* flake showed a tendency to decrease (Table 1).

The research indicates that cooking process can cause significant changes in the total phenolic compounds and flavonoid contents. Treatment with high temperature can cause damaging of total phenolic compounds and flavonoid contents linked to they are highly unstable compounds.[10] Table 1 shows that the levels of total phenolic compounds decreased at the time of the processing is done. The decrease is possible because, during the boiling water using the media as a heat conductor, some compounds are polyphenol (condensed tannins) on tuber hydrolyzed and then diffused into the boiling water. Boiling process reduce the number of total phenolic compounds on either the flesh or the peel of the making of *C. tuberosus* flake led to a decrease

Table 2: Antioxidant activity (DPPH) on raw or boiled *C. tuberosus* and *C. tuberosus* flake

Treatment	Part of sample	Antioxidant activity (DPPH) (%)
Raw *C. Tuberosus*	Peel	62.82±0.32cB
	Flesh	26.34±0.09aA
Boiled *C. Tuberosus*	Peel	92.70±0.47eB
	Flesh	56.29±0.37bA
C. Tuberosus flake (control)		91.11±0.51d
C. Tuberosus flake		92.57±0.47e

Different letters (a-e) within the column indicate significant differences in different treatment at $P < 0.05$.

Different capital letters (A-B) within the column indicate significant differences in different part of sample in the same treatment at $P < 0.05$.

in the number of phenolic compounds. Another research proved that the boiling process impact levels decrease phenolic compounds and flavonoid on several types of vegetables.[11,12]

The levels of flavonoid contents in *C. tuberosus* on boiling and baking process shows a tendency to decline (Table 1). Degradation or decomposition of flavonoid contents likely caused this during the thermal processing. The possibility of flavonoid on *C. tuberosus* is anthocyanin. This compound is soluble in water, so by the boiling process caused anthocyanin leaching in the boiling water. It demonstrated the existence of dark color (purple, blue/black) in the water boiling of *C. tuberosus*.[13] Anthocyanins were not stable during processing that used heat treatment14. Increasing temperature and activity of enzymatic reactions may destroy phenolic compounds.[15]

Evaluation of Antioxidant Activity

The antioxidant activity evaluated with the DPPH method indicate that processing tends to increase the ability of antioxidant activity in samples (Table 2). Method of processing by way of boiling can increase antioxidant activity either on the peel or flesh of *C. tuberosus*.

Table 2 shows that processing can increase antioxidant activity. Several things made possible the presence of phenolic compounds such as conversion to a form that has a higher antioxidant activity suppose the formation of aglycon that has the ability of higher antioxidant activity. Increased antioxidant activity caused by the transformation of compound phytochemicals becoming more active compounds, e.g., polymerization of polyphenolic.

Maillard reaction, i.e., reactions involving amino Carbonyl groups and so arose a new compound that Brown, i.e., Maillard reaction products(MRPs) that have a higher antioxidant property.[16] Maillard reaction can produce a variety of products, intermediate products and brown product (melanoidin), which has contributed to the color, flavor, and aroma as well as having potential as antioxidants in processed food.[17] Some research suggests that treatment with boiling and baking can increase antioxidant activity in food despite the declining levels of phenolic and flavonoid.[18]

The level of Resistant Starch

Resistant starch defined as starch can escape digestion in the intestine and as a source of fermentation substrate for colonic microflora. Anaerobic fermentation generates short chain fatty acids which can be used as additional energy for animals.[19]

The results of the analysis of the RS on the raw *C. tuberosus*, boiled *C. tuberosus*, and *C. tuberosus* flake was present on Table 3. Table 3 shows that processing can increase the levels of RS. The source

Table 3: Level of resistant starch on raw, boiled *C. tuberosus* and *C. tuberosus* flake

Materials	Level of resistant starch (%)
Raw *C. tuberosus*	10.24 ± 0.37a
Boiled *C. tuberosus*	15.42 ± 0.96b
C. tuberosus flake	44.09 ± 0.07c

Different letters within the column indicate significant differences at P<0.05.

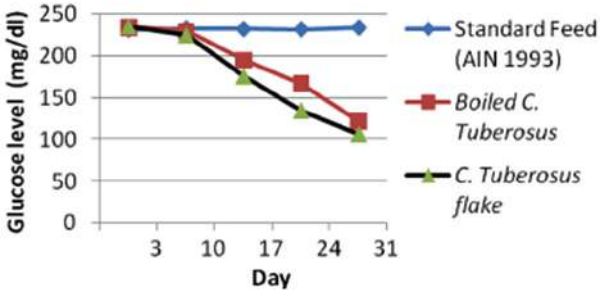

Fig. 1: Profile glucose of diabetic mice for 28 days treatment

of carbohydrate if experience thermal process can result in resistant starch. The more stages of processing increasingly high resistant starch that was formed. Processing by way of steaming, boiling, and roasting can raise resistant starch. The potatoes boiled then cooled impact on increasing the levels of resistant starch (RS3).[19] Processing method: steaming, boiling and roasting can raise resistant starch.

The Glucose Profile

The mice suffering from diabetes after feeding Boiled and flake *C. tuberosus* shows a decrease in serum glucose levels (Figure 1). The decrease in serum glucose in boiled *C. tuberosus* was 47.41%. Whereas *C. tuberosus* flake was 54.94%. Standard feed does not occur on a decrease in serum glucose. These data indicate that the feed that contains resistant starch was higher (Table 3), have the greater ability to lower glucose profile.

Foods containing resistant starch will be digested slow; this gives implications for controlling the release of glucose.[20] This is in line with studies showing that consumption of foods containing resistant starch type 3 to control the release of glucose because it has a low glycemic index.[21] Commercial RS3 blood glucose was significantly lower than that of other simple carbohydrates.[22] RS3 lowered postprandial blood glucose and to play a part in keeping control of metabolism in type II diabetic patients. Research also shows that foods containing resistant starch type 3 may improve glucose profiles in mice injected alloxan.[23] Consumption of resistant starch can rectify the beta cells of the pancreas, insulin sensitivity, increase levels of insulin the pancreas, the total level of GLP-1, SCFA concentration on improving control of blood glucose levels.[24]

The current research was finding that there is an increase in Reactive Oxygen Species (ROS) or concentration of oxidative stress and lipids on several animal models. Alloxan led to free radicals that caused cells damage cells. During the redox process, ROS are formed and the beta cell damage caused on the island of Langerhans.[25]

The research that has been done suggests that flavonoids and phenols have the capability of capturing free radicals, which can protect the oxidative stress that causes cell damage (such as the concentration of lipids membrane and membrane degradation). Flavonoid glycosides can stimulate insulin secretion in beta cells of the pancreas. In animals that suffer from diabetes, and given feed containing flavonoids, then it is possible that the compound acts as an insulin secretion in the pancreas stimulated or increase the uptake of glucose.[26]

The ability of flavonoids as antidiabetic can improve glucose profile is related to the effect his ability to stimulate insulin secretion, reduces the apoptosis and proliferation of pancreatic beta cells spur, lowering insulin resistance, inflammatory and oxidative stress on the muscles and promotes translocate of GLUT4 via PI3K/AKT and AMPK pathways.[27] Consumption of phenols may inhibit the α-amylase and α-glucosidase, able to inhibit the absorption of glucose in the intestine by sodium-dependent glucose transporter 1 (SGLT1), can stimulate the secretion of insulin and reduce hepatic glucose output.[28]

This study provides information that the existence of two processing techniques, i.e. boiling and baking can increase antioxidant activity and levels of resistant starch. Nevertheless still required information related to the antioxidant activity and the levels of resistant starch for various processing techniques (steaming, roasting) or Coleus tuberosus processing to a wide range of processed products, so that the community has more options in Coleus tuberosus processing as a product ready to eat.

Conclusions

The process of boiling of *C. tuberosus* and making *C. tuberosus* flake have an impact on decreasing the level of phenol compounds and flavonoid. Antioxidant activity of the flesh and peel of boiled *C. tuberosus* and *C. tuberosus* flake increased compared to raw *C. tuberosus*. Processing can increase the levels of resistant starch. The resistant starch content on raw *C. tuberosus* 10.24 ± 0.37%; boiled *C. tuberosus* 15.42 ± 0.96%; *C. tuberosus* flake 44.09 ± 0.07%. The decrease in serum glucose in boiled *C. tuberosus* was 47.41%. Whereas *C. tuberosus* flake was 54.94%.

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Conflict of Interest
The author(s) declare no conflict of interest.

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Dear editor,

These are the revised the proof article. I sent in pdf and also revised picture (because in picture, C. tuberosus wasn't write in italic. Please uses the revised picture. Thank you

Sincerely yours,

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Antioxidant Activity and Resistant Starch Content of *C. tuberosus* on Different Cooking Method and its Potential on Glucose Management in Diabetic Mice

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Abstract

This research aims to know the antioxidant activity and the levels of resistant starch of *C. tuberosus* on different processing methods. Processing methods used were boiling and baking. Bioactive compounds being evaluated is the number of total phenolic and flavonoid. Evaluation of antioxidant activity is performed with the DPPH method. The evaluation of the levels of resistant starch was done in an enzymatic method. The results showed that levels of total phenolic and flavonoid demonstrate a tendency to decline with the processing. The existence of the processing process increased the antioxidant activity of boiled *C. tuberosus* and *C. tuberosus* flake. The processing increases the levels of resistant. The levels of resistant starch in raw *C. tuberosus* were10.24 ± 0.37%; boiled *C. tuberosus* 15.42 ± 0.96%; and *C. tuberosus* flake 44.09 ± 0.07%. The decrease in serum glucose in boiled *C. tuberosus* was 47.41% whereas *C. tuberosus* flake was 54.94%. The results of this study indicate that processing (boiling and baking) can increase the antioxidant activity and the levels of resistant starch.

Article History

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Keywords

Antioxidant Activity;
C. Tuberosus;
Cooking Method;
Diabetic Mice;
Resistant Starch.

Introduction

Increasing the growing public awareness of the importance of healthy living, the claim against consumer foodstuffs also increasingly shifted. Food that is now starting to great demand not only that consumers have an excellent nutritional composition as well as the appearance and exciting flavors, but also must have specific physiological functions for the body. Such a function is known as the tertiary function. Foods that have a function known as tertiary is known as functional foods. Functional foods are foods that can maintain health and prevent disease

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because it has an active component in the biology that has benefits for health.

Research shows that there is a link between components in the food consumed with health.[1] Functional components in plants, for example, phytochemical has a biological activity to prevent disease.[2] Phytochemical compounds contained in legume, cereal, fruit, vegetables have antioxidant activity phenolic and flavonoid.[3] Antioxidants are groups of compounds which neutralize free radicals and reactive oxygen species in the cell to prevent the occurrence of oxidative stress in human cells.

Resistant starch(RS) much developed and consumed because of the value of its current status. Hydrolysis resistant starch by digestive enzymes needs more extended periods and give an impact on the production of glucose becomes slower. Indirectly, the RS has a value of the function for patients with diabetes. RS has three systems related to the functional value of the metabolism and effects in the body, i.e., as an ingredient for the fortification of fiber, lower calories, and fat oxidation. RS there are naturally in food products and can be used in a modified form as well as added in food.[4]

C. tuberosus is one of the possible food ingredients in Indonesia as a source of carbohydrates that come from minor tubers. Some research suggests that *C. tuberosus* has the potential to be developed as functional foods based on the content of compound bioactive and RS that can be obtained by modification of the processing. *C. tuberosus* contain flavonoid, ascorbic acid, which can increase the antioxidant enzyme activity and lowering products peroxide in rats fed a high-fat diet. *C. tuberosus* extract contains bioactive compounds that have antioxidant activity.[5] *C. tuberosus* processing into will bring the physical and chemical changes will have an impact on its potential as a functional food. The purpose of this research is to evaluate the activity of antioxidants and resistant starch of *C. tuberosus* on different cooking methods and to know the effect the consumption of *C. tuberosus* on glucose profile in diabetic mice.

Materials and Methods

This research did experimentally in the laboratory, Department of Culinary Art Education, Yogyakarta State University, Center for Food & Nutrition Studies Gadjah Mada University. The process *C. tuberosus* flake was making process done by adding other ingredients namely soy flour. Study of antioxidant activity and levels of resistant starch done on raw *C. otuberosus*, boiled *C. ftuberosus*, and *C. tuberosus* flake. Treatment of animals experimental in the laboratory animal maintenance, Center for food and nutrition, Gadjah Mada University.

Sample Preparation

Sample preparation of antioxidant activity. Preparation of the raw *C. tuberous*: *C. tuberosus* separated the peel and flesh by Peeler. The thickness of the stripped of the peel (1-1.5 mm) so that the retrieved peel and flesh of Coleus tuberosus. Boiled *C. tuberosus*: *C. tuberosus* boiled for 30 minutes, then peel and flesh were separated. The peel and flesh of tubers dried with the cabinet drier at temperature 40oC for 24 hours. Then ground and shift with the sieve mesh Tyler 80 size. *C. tuberosus* flake made with the three formulations, the most preferred formulation based on the hedonic test of 30 semi-trained panelists used for samples. *C. tuberosus* flake made from *C. tuberosus* flour, tapioca flour, soy flour, sorbitol, margarine, salt, cocoa powder, and water. Whereas the control of *C. tuberosus* flake made from *C. tuberosus* flour, tapioca flour, sorbitol, margarine, salt, cocoa powder, and water (without soy flour).

Sample preparation for resistant starch analysis: raw *C. tuberosus* sample, prepared from the all of the parts of *C. tuberosus*, sliced and then dried it with a cabinet drier at 40oC for 24 hours. Dried *C. tuberosus* used as a sample analysis of resistant starch. Boiled *C. tuberosus*, prepared by boiling all of part of *C. tuberosus* for 30 minutes, peeled and the using as a sample analysis of resistant starch.

Extraction Process

The peel and flesh flour of raw and boiled *C. tuberous*, *C. tuberosus* flake macerated with methanol during seven days (1:5), then filtered using Whatman No.1, evaporating the solvent with gas N_2. The Extract stored in the freezer temperature -22 °C.

Determination of Total Phenolic Compounds

The methanol extract of *C. tuberous* determined using spectrophotometric method6. As much as 0.2 mL different extract with a concentration of 100

mg/L, Folin-Ciocalteu reagent 10% as much as 2.5 mL, and two mL 7.5% Na_2CO_3 are mixed and allowed for 15 minutes at a temperature of 45 °C. The absorbance of the solution was measured using the spectrophotometer at a wavelength of 765 nm. The total phenolic compounds expressed as mg Galic Acid Equivalent/g extract (mg GAE/g extract). The measurement was in triplicate.

Determination of Flavonoid Contents

Determination of flavonoid contents used spectrophotometric method.[7] Determination of flavonoid contents was carried out by spectrophotometry using reagent aluminium chloride. As many as one mL aqueous solution extracts with a concentration of 1000 mg/L, at add with one mL 2% $AlCl_3$ dissolved with ethanol 50% homogenized, and then use the vortex during the 20-minute incubation, mix the solution for 24 hours. Measure absorbance at 415 nm. Flavonoid contents expressed in mg of Quercetin Equivalent/g extract and calculation with triplicate measurement.

Evaluation of Antioxidant Activity Based on Dpph Method

DPPH method using synthetic radical 1.1,-diphenyl picrylhydrazyl (DPPH)8. As many as two ml of DPPH (0.1 mM in methanol solution), plus 40 µg/ml methanol extract of the peel and flesh of raw or boiled *C. tuberous* or *C. tuberosus* flake. Change the color after 30 minutes read at 517 nm. Percentage radical scavenging activity determined:

(A0-A1)/A0 x 100%

In this case, Ao was absorbance control, and A1 was the absorbance methanol extract of the peel and flesh of raw, boiled *C. tuberous* or *C. tuberosus* flake.

Evaluation of Resistant Starch Content

Resistant starch determined by enzymatic reactions9. Raw *C. tuberosus*, boiled *C. tuberosus* or *C. tuberosus* flake (100 mg) incubated with a solution containing pepsin as much as 20 mg at temperature 40oC for 60 min. A tris-maleate solution containing pancreatic α-amylase as much as 40 mg then added and the mixture incubated at temperature 37oC for 16 hr to hydrolyze the digestible starch. The hydrolysate centrifuged, and the residue was solubilized with KOH 4M and incubated with 80 µL amyloglucosidase at temperature 60oC for 45 min to hydrolyze RS. A glucose oxidase-peroxidase kit used to measure the glucose content (glucose assay kit product number gago-20, Sigma). The RS content, comprising fractions of types I and II, was calculated as mg of glucose x 0.9.

In vivo Assay

In vivo evaluation was done by setting up an experimental animal conducted in Treatment of

Table 1: Level of total phenolic compounds and flavonoid content in the methanol extract of The flesh and peel of raw, boiled C. tuberosus and C. tuberosus flake

Treatment	Part of sample	Level of total phenolic compounds (mg GAE/g extract)	Level of flavonoid content (mg quercetin /g of extract)
Raw *C. tuberosus*	Peel	7.73 ± 0.08^{fB}	8.55 ± 0.07^{fB}
	Flesh	7.24 ± 0.10^{eA}	2.31 ± 0.13^{eA}
Boiled *C. tuberosus*	Peel	2.17 ± 0.01^{aA}	0.07 ± 0.00^{aA}
	Flesh	6.51 ± 0.02^{dB}	1.22 ± 0.01^{cB}
C. tuberosus Flake (Control)		3.83 ± 0.02^{b}	2.17 ± 0.01^{d}
C. tuberosus Flake		4.62 ± 0.03^{c}	0.85 ± 0.01^{b}

Different letters (a-f) within the column indicate significant differences in different treatment at $P < 0.05$.
Different capital letters (A-B) within the column indicate significant differences in different part of sample in the same teratment at $P < 0.05$

animals experimental in the laboratory animal maintenance, Center for food and nutrition, Gadjah Mada University. All procedures performed involving animal were approved by the Gadjah Mada University Animal Ethics Committee. The number of animals as much as 18 Wistar type males weighing 110-150 grams and maintained in the closed condition the enclosure that includes the light did not control, air vents in a cage enough, the air temperature on the temperature the room. Standard feed has given for three days by using standard AIN 1993. Intraperitoneal injection of alloxan was done through with a dose of 125 mg/kg body weight of mice to make the mice became diabetic. Mice were given standard feed. After the third day, mice suffering from diabetes mellitus. Next up is done weighing weight and groups divided. The mice were divided into three groups, namely diet six mice to a standard diet AIN 1993, six mice to boiled *C. tuberosus* and six mice to *C. tuberosus* flake. Given in drinking water ad libitum. Cages cleaned daily from dirt or stool that is inherent, and residual feed weighed every day. Feed mice were given each morning. Blood glucose analysis was done with the method GOD Glucose PAP: enzymatic reactions photometric test.

Statistical Analysis

The analysis was conducted on three replications (the level of total phenolic compounds, flavonoid contents, and resistant starch content) and six replications (in vivo assay) by observing their mean ± SD. A t-test used when compared between the peel and flesh part of sample in the same treatment. The software SPSS for Windows v15.0 (SPSS Inc., Chicago, Illinois) was used for analysis of variance and mean separation. LSD test was used to compare treatments when ANOVA was significant ($p \leq 0.05$).

Result and Discussion

The Level of Total Phenolic Compounds Dan Flavonoid Contents

Analysis of the levels of total phenolic compounds and flavonoid contents in the methanol extract of the peel and flesh of raw, boiled of *C. tuberosus* and *C. tuberosus* flake showed a tendency to decrease (Table 1).

The research indicates that cooking process can cause significant changes in the total phenolic compounds and flavonoid contents. Treatment with high temperature can cause damaging of total phenolic compounds and flavonoid contents linked to they are highly unstable compounds.[10] Table 1 shows that the levels of total phenolic compounds decreased at the time of the processing is done. The decrease is possible because, during the boiling water using the media as a heat conductor, some compounds are polyphenol (condensed tannins) on tuber hydrolyzed and then diffused into the boiling water. Boiling process reduce the number of total phenolic compounds on either the flesh or the peel of the making of *C. tuberosus* flake led to a decrease

Table 2: Antioxidant activity (DPPH) on raw or boiled C. tuberosus and C. tuberosus flake

Treatment	Part of sample	Antioxidant activity (DPPH) (%)
Raw C. Tuberosus	Peel	62.82±0.32cB
	Flesh	26.34±0.09aA
Boiled C. Tuberosus	Peel	92.70±0.47eB
	Flesh	56.29±0.37bA
C. tuberosus flake (control)		91.11±0.51d
C. tuberosus flake		92.57±0.47e

Different letters (a-e) within the column indicate significant differences in different treatment at $P < 0.05$.

Different capital letters (A-B) within the column indicate significant differences in different part of sample in the same treatment at $P < 0.05$.

in the number of phenolic compounds. Another research proved that the boiling process impact levels decrease phenolic compounds and flavonoid on several types of vegetables.[11,12]

The levels of flavonoid contents in *C. tuberosus* on boiling and baking process shows a tendency to decline (Table 1). Degradation or decomposition of flavonoid contents likely caused this during the thermal processing. The possibility of flavonoid on *C. tuberosus* is anthocyanin. This compound is soluble in water, so by the boiling process caused anthocyanin leaching in the boiling water. It demonstrated the existence of dark color (purple, blue/black) in the water boiling of *C. tuberosus*13. Anthocyanins were not stable during processing that used heat treatment14. Increasing temperature and activity of enzymatic reactions may destroy phenolic compounds.[15]

Evaluation of Antioxidant Activity

The antioxidant activity evaluated with the DPPH method indicate that processing tends to increase the ability of antioxidant activity in samples (Table 2). Method of processing by way of boiling can increase antioxidant activity either on the peel or flesh of *C. tuberosus*.

Table 2 shows that processing can increase antioxidant activity. Several things made possible the presence of phenolic compounds such as conversion to a form that has a higher antioxidant activity suppose the formation of aglycon that has the ability of higher antioxidant activity. Increased antioxidant activity caused by the transformation of compound phytochemicals becoming more active compounds, e.g., polymerization of polyphenolic.

Maillard reaction, i.e., reactions involving amino Carbonyl groups and so arose a new compound that Brown, i.e., Maillard reaction products(MRPs) that have a higher antioxidant property.[16] Maillard reaction can produce a variety of products, intermediate products and brown product (melanoidin), which has contributed to the color, flavor, and aroma as well as having potential as antioxidants in processed food.[17] Some research suggests that treatment with boiling and baking can increase antioxidant activity in food despite the declining levels of phenolic and flavonoid.[18]

The level of Resistant Starch

Resistant starch defined as starch can escape digestion in the intestine and as a source of fermentation substrate for colonic microflora. Anaerobic fermentation generates short chain fatty acids which can be used as additional energy for animals.[19]

The results of the analysis of the RS on the raw *C. tuberosus*, boiled *C. tuberosus*, and *C. tuberosus* flake was present on Table 3. Table 3 shows that processing can increase the levels of RS. The source of carbohydrate if experience thermal process can result in resistant starch. The more stages of processing increasingly high resistant starch that was formed. Processing by way of steaming, boiling, and roasting can raise resistant starch. The

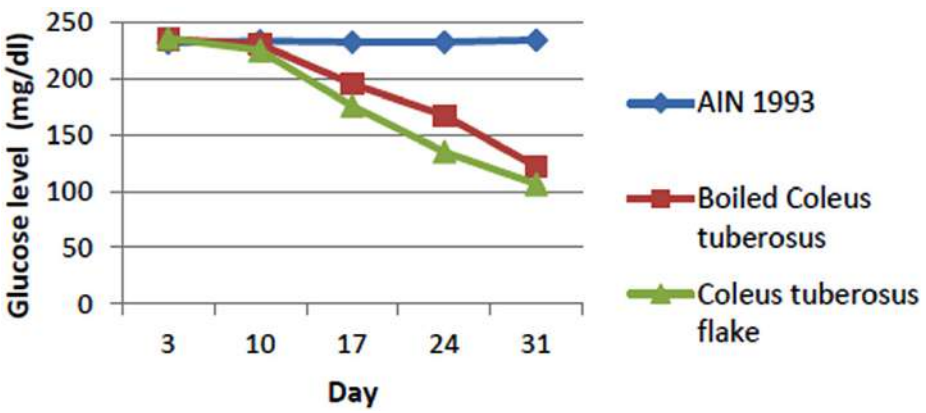

Fig. 1: Profile glucose of diabetic mice for 28 days treatment

potatoes boiled then cooled impact on increasing the levels of resistant starch (RS3).[19] Processing method: steaming, boiling and roasting can raise resistant starch.

The Glucose Profile

The mice suffering from diabetes after feeding Boiled and flake *C. tuberosus* shows a decrease in serum glucose levels (Figure 1). The decrease in serum glucose in boiled *C. tuberosus* was 47.41%. Whereas *C. tuberosus* flake was 54.94%. Standard feed does not occur on a decrease in serum glucose. These data indicate that the feed that contains resistant starch was higher (Table 3), have the greater ability to lower glucose profile.

Foods containing resistant starch will be digested slow; this gives implications for controlling the release of glucose.[20] This is in line with studies showing that consumption of foods containing resistant starch type 3 to control the release of glucose because it has a low glycemic index.[21] Commercial RS3 blood glucose was significantly lower than that of other simple carbohydrates.[22] RS3 lowered postprandial blood glucose and to play a part in keeping control of metabolism in type II diabetic patients. Research also shows that foods containing resistant starch type 3 may improve glucose profiles in mice injected alloxan.[23] Consumption of resistant starch can rectify the beta cells of the pancreas, insulin sensitivity, increase levels of insulin the pancreas, the total level of GLP-1, SCFA concentration on improving control of blood glucose levels.[24]

The current research was finding that there is an increase in ROS or concentration of oxidative stress and lipids on several animal models. Alloxan led to free radicals that caused cells damage cells. During the redox process, ROS are formed and the beta cell damage caused on the island of Langerhans.[25]

The research that has been done suggests that flavonoids and phenols have the capability of capturing free radicals, which can protect the oxidative stress that causes cell damage (such as the concentration of lipids membrane and membrane degradation). Flavonoid glycosides can stimulate insulin secretion in beta cells of the pancreas. In animals that suffer from diabetes, and given feed containing flavonoids, then it is possible that the compound acts as an insulin secretion in the pancreas stimulated or increase the uptake of glucose.[26]

The ability of flavonoids as antidiabetic can improve glucose profile is related to the effect his ability to stimulate insulin secretion, reduces the apoptosis and proliferation of pancreatic beta cells spur, lowering insulin resistance, inflammatory and oxidative stress on the muscles and promotes translocate of GLUT4 via PI3K/AKT and AMPK pathways.[27] Consumption of phenols may inhibit the α-amylase and α-glucosidase, able to inhibit the absorption of glucose in the intestine by sodium-dependent glucose transporter 1 (SGLT1), can stimulate the secretion of insulin and reduce hepatic glucose output.[28]

This study provides information that the existence of two processing techniques, i.e. boiling and baking can increase antioxidant activity and levels of resistant starch. Nevertheless still required information related to the antioxidant activity and the levels of resistant starch for various processing techniques (steaming, roasting) or Coleus tuberosus processing to a wide range of processed products, so that the community has more options in Coleus tuberosus processing as a product ready to eat.

Conclusions

The process of boiling of *C. tuberosus* and making *C. tuberosus* flake have an impact on decreasing the level of phenol compounds and flavonoid. Antioxidant activity of the flesh and peel of boiled *C. tuberosus* and *C. tuberosus* flake increased compared to raw *C. tuberosus*. Processing can increase the levels of resistant starch. The resistant starch content

Table 3: Level of resistant starch on raw, boiled *C. tuberosus* and *C. tuberosus* flake

Materials	Level of resistant starch (%)
Raw *C. tuberosus*	10.24 ± 0.37a
Boiled *C. tuberosus*	15.42 ± 0.96b
C. tuberosus flake	44.09 ± 0.07c

Different letters within the column indicate significant differences at P<0.05.

on raw *C. tuberosus* 10.24 ± 0.37%; boiled *C. tuberosus* 15.42 ± 0.96%; *C. tuberosus* flake 44.09 ± 0.07%. The decrease in serum glucose in boiled *C. tuberosus* was 47.41%. Whereas *C. tuberosus* flake was 54.94%.

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Conflict of Interest
The author(s) declare no conflict of interest.

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Antioxidant Activity and Resistant Starch Content of *C. tuberosus* on Different Cooking Method and its Potential on Glucose Management in Diabetic Mice

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Abstract

This research aims to know the antioxidant activity and the levels of resistant starch of *C. tuberosus* on different processing methods. Processing methods used were boiling and baking. Bioactive compounds being evaluated is the number of total phenolic and flavonoid. Evaluation of antioxidant activity is performed with the DPPH method. The evaluation of the levels of resistant starch was done in an enzymatic method. The results showed that levels of total phenolic and flavonoid demonstrate a tendency to decline with the processing. The existence of the processing process increased the antioxidant activity of boiled *C. tuberosus* and *C. tuberosus* flake. The processing increases the levels of resistant. The levels of resistant starch in raw *C. tuberosus* were10.24 ± 0.37%; boiled *C. tuberosus* 15.42 ± 0.96%; and *C. tuberosus* flake 44.09 ± 0.07%. The decrease in serum glucose in boiled *C. tuberosus* was 47.41% whereas *C. tuberosus* flake was 54.94%. The results of this study indicate that processing (boiling and baking) can increase the antioxidant activity and the levels of resistant starch.

Article History

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Keywords

Antioxidant Activity;
C. Tuberosus;
Cooking Method;
Diabetic Mice;
Resistant Starch.

Introduction

Increasing the growing public awareness of the importance of healthy living, the claim against consumer foodstuffs also increasingly shifted. Food that is now starting to great demand not only that consumers have an excellent nutritional composition as well as the appearance and exciting flavors, but also must have specific physiological functions for the body. Such a function is known as the tertiary function. Foods that have a function known as tertiary is known as functional foods. Functional foods are foods that can maintain health and prevent disease

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because it has an active component in the biology that has benefits for health.

Research shows that there is a link between components in the food consumed with health.[1] Functional components in plants, for example, phytochemical has a biological activity to prevent disease.[2] Phytochemical compounds contained in legume, cereal, fruit, vegetables have antioxidant activity phenolic and flavonoid.[3] Antioxidants are groups of compounds which neutralize free radicals and reactive oxygen species in the cell to prevent the occurrence of oxidative stress in human cells.

Resistant starch(RS) much developed and consumed because of the value of its current status. Hydrolysis resistant starch by digestive enzymes needs more extended periods and give an impact on the production of glucose becomes slower. Indirectly, the RS has a value of the function for patients with diabetes. RS has three systems related to the functional value of the metabolism and effects in the body, i.e., as an ingredient for the fortification of fiber, lower calories, and fat oxidation. RS there are naturally in food products and can be used in a modified form as well as added in food.[4]

C. tuberosus is one of the possible food ingredients in Indonesia as a source of carbohydrates that come from minor tubers. Some research suggests that *C. tuberosus* has the potential to be developed as functional foods based on the content of compound bioactive and RS that can be obtained by modification of the processing. *C. tuberosus* contain flavonoid, ascorbic acid, which can increase the antioxidant enzyme activity and lowering products peroxide in rats fed a high-fat diet. *C. tuberosus* extract contains bioactive compounds that have antioxidant activity.[5] *C. tuberosus* processing into will bring the physical and chemical changes will have an impact on its potential as a functional food. The purpose of this research is to evaluate the activity of antioxidants and resistant starch of *C. tuberosus* on different cooking methods and to know the effect the consumption of *C. tuberosus* on glucose profile in diabetic mice.

Materials and Methods

This research did experimentally in the laboratory, Department of Culinary Art Education, Yogyakarta State University, Center for Food & Nutrition Studies Gadjah Mada University. The process *C. tuberosus* flake was making process done by adding other ingredients namely soy flour. Study of antioxidant activity and levels of resistant starch done on raw *C. otuberosus*, boiled *C. ftuberosus*, and *C. tuberosus* flake. Treatment of animals experimental in the laboratory animal maintenance, Center for food and nutrition, Gadjah Mada University.

Sample Preparation

Sample preparation of antioxidant activity. Preparation of the raw *C. tuberous*: *C. tuberosus* separated the peel and flesh by Peeler. The thickness of the stripped of the peel (1-1.5 mm) so that the retrieved peel and flesh of Coleus tuberosus. Boiled *C. tuberosus*: *C. tuberosus* boiled for 30 minutes, then peel and flesh were separated. The peel and flesh of tubers dried with the cabinet drier at temperature 40oC for 24 hours. Then ground and shift with the sieve mesh Tyler 80 size. *C. tuberosus* flake made with the three formulations, the most preferred formulation based on the hedonic test of 30 semi-trained panelists used for samples. *C. tuberosus* flake made from *C. tuberosus* flour, tapioca flour, soy flour, sorbitol, margarine, salt, cocoa powder, and water. Whereas the control of *C. tuberosus* flake made from *C. tuberosus* flour, tapioca flour, sorbitol, margarine, salt, cocoa powder, and water (without soy flour).

Sample preparation for resistant starch analysis: raw *C. tuberosus* sample, prepared from the all of the parts of *C. tuberosus*, sliced and then dried it with a cabinet drier at 40oC for 24 hours. Dried *C. tuberosus* used as a sample analysis of resistant starch. Boiled *C. tuberosus*, prepared by boiling all of part of *C. tuberosus* for 30 minutes, peeled and the using as a sample analysis of resistant starch.

Extraction Process

The peel and flesh flour of raw and boiled *C. tuberous*, *C. tuberosus* flake macerated with methanol during seven days (1:5), then filtered using Whatman No.1, evaporating the solvent with gas N_2. The Extract stored in the freezer temperature -22 °C.

Determination of Total Phenolic Compounds

The methanol extract of *C. tuberous* determined using spectrophotometric method6. As much as 0.2 mL different extract with a concentration of 100

mg/L, Folin-Ciocalteu reagent 10% as much as 2.5 mL, and two mL 7.5% Na_2CO_3 are mixed and allowed for 15 minutes at a temperature of 45 °C. The absorbance of the solution was measured using the spectrophotometer at a wavelength of 765 nm. The total phenolic compounds expressed as mg Galic Acid Equivalent/g extract (mg GAE/g extract). The measurement was in triplicate.

Determination of Flavonoid Contents

Determination of flavonoid contents used spectrophotometric method.[7] Determination of flavonoid contents was carried out by spectrophotometry using reagent aluminium chloride. As many as one mL aqueous solution extracts with a concentration of 1000 mg/L, at add with one mL 2% $AlCl_3$ dissolved with ethanol 50% homogenized, and then use the vortex during the 20-minute incubation, mix the solution for 24 hours. Measure absorbance at 415 nm. Flavonoid contents expressed in mg of Quercetin Equivalent/g extract and calculation with triplicate measurement.

Evaluation of Antioxidant Activity Based on Dpph Method

DPPH method using synthetic radical 1.1,-diphenyl picrylhydrazyl (DPPH)8. As many as two ml of DPPH (0.1 mM in methanol solution), plus 40 µg/ml methanol extract of the peel and flesh of raw or boiled *C. tuberous* or *C. tuberosus* flake. Change the color after 30 minutes read at 517 nm. Percentage radical scavenging activity determined:

(A0-A1)/A0 x 100%

In this case, Ao was absorbance control, and A1 was the absorbance methanol extract of the peel and flesh of raw, boiled *C. tuberous* or *C. tuberosus* flake.

Evaluation of Resistant Starch Content

Resistant starch determined by enzymatic reactions9. Raw *C. tuberosus*, boiled *C. tuberosus* or *C. tuberosus* flake (100 mg) incubated with a solution containing pepsin as much as 20 mg at temperature 40oC for 60 min. A tris-maleate solution containing pancreatic α-amylase as much as 40 mg then added and the mixture incubated at temperature 37oC for 16 hr to hydrolyze the digestible starch. The hydrolysate centrifuged, and the residue was solubilized with KOH 4M and incubated with 80 µL amyloglucosidase at temperature 60oC for 45 min to hydrolyze RS. A glucose oxidase-peroxidase kit used to measure the glucose content (glucose assay kit product number gago-20, Sigma). The RS content, comprising fractions of types I and II, was calculated as mg of glucose x 0.9.

In vivo Assay

In vivo evaluation was done by setting up an experimental animal conducted in Treatment of

Table 1: Level of total phenolic compounds and flavonoid content in the methanol extract of The flesh and peel of raw, boiled C. tuberosus and C. tuberosus flake

Treatment	Part of sample	Level of total phenolic compounds (mg GAE/g extract)	Level of flavonoid content (mg quercetin /g of extract)
Raw *C. tuberosus*	Peel	7.73 ± 0.08^{fB}	8.55 ± 0.07^{fB}
	Flesh	7.24 ± 0.10^{eA}	2.31 ± 0.13^{eA}
Boiled *C. tuberosus*	Peel	2.17 ± 0.01^{aA}	0.07 ± 0.00^{aA}
	Flesh	6.51 ± 0.02^{dB}	1.22 ± 0.01^{cB}
C. tuberosus Flake (Control)		3.83 ± 0.02^{b}	2.17 ± 0.01^{d}
C. tuberosus Flake		4.62 ± 0.03^{c}	0.85 ± 0.01^{b}

Different letters (a-f) within the column indicate significant differences in different treatment at $P < 0.05$.
Different capital letters (A-B) within the column indicate significant differences in different part of sample in the same teratment at $P < 0.05$

animals experimental in the laboratory animal maintenance, Center for food and nutrition, Gadjah Mada University. All procedures performed involving animal were approved by the Gadjah Mada University Animal Ethics Committee. The number of animals as much as 18 Wistar type males weighing 110-150 grams and maintained in the closed condition the enclosure that includes the light did not control, air vents in a cage enough, the air temperature on the temperature the room. Standard feed has given for three days by using standard AIN 1993. Intraperitoneal injection of alloxan was done through with a dose of 125 mg/kg body weight of mice to make the mice became diabetic. Mice were given standard feed. After the third day, mice suffering from diabetes mellitus. Next up is done weighing weight and groups divided. The mice were divided into three groups, namely diet six mice to a standard diet AIN 1993, six mice to boiled *C. tuberosus* and six mice to *C. tuberosus* flake. Given in drinking water ad libitum. Cages cleaned daily from dirt or stool that is inherent, and residual feed weighed every day. Feed mice were given each morning. Blood glucose analysis was done with the method GOD Glucose PAP: enzymatic reactions photometric test.

Statistical Analysis

The analysis was conducted on three replications (the level of total phenolic compounds, flavonoid contents, and resistant starch content) and six replications (in vivo assay) by observing their mean ± SD. A t-test used when compared between the peel and flesh part of sample in the same treatment. The software SPSS for Windows v15.0 (SPSS Inc., Chicago, Illinois) was used for analysis of variance and mean separation. LSD test was used to compare treatments when ANOVA was significant ($p \leq 0.05$).

Result and Discussion

The Level of Total Phenolic Compounds Dan Flavonoid Contents

Analysis of the levels of total phenolic compounds and flavonoid contents in the methanol extract of the peel and flesh of raw, boiled of *C. tuberosus* and *C. tuberosus* flake showed a tendency to decrease (Table 1).

The research indicates that cooking process can cause significant changes in the total phenolic compounds and flavonoid contents. Treatment with high temperature can cause damaging of total phenolic compounds and flavonoid contents linked to they are highly unstable compounds.[10] Table 1 shows that the levels of total phenolic compounds decreased at the time of the processing is done. The decrease is possible because, during the boiling water using the media as a heat conductor, some compounds are polyphenol (condensed tannins) on tuber hydrolyzed and then diffused into the boiling water. Boiling process reduce the number of total phenolic compounds on either the flesh or the peel of the making of *C. tuberosus* flake led to a decrease

Table 2: Antioxidant activity (DPPH) on raw or boiled C. tuberosus and C. tuberosus flake

Treatment	Part of sample	Antioxidant activity (DPPH) (%)
Raw C. Tuberosus	Peel	62.82±0.32cB
	Flesh	26.34±0.09aA
Boiled C. Tuberosus	Peel	92.70±0.47eB
	Flesh	56.29±0.37bA
C. tuberosus flake (control)		91.11±0.51d
C. tuberosus flake		92.57±0.47e

Different letters (a-e) within the column indicate significant differences in different treatment at $P < 0.05$.

Different capital letters (A-B) within the column indicate significant differences in different part of sample in the same treatment at $P < 0.05$.

in the number of phenolic compounds. Another research proved that the boiling process impact levels decrease phenolic compounds and flavonoid on several types of vegetables.[11,12]

The levels of flavonoid contents in *C. tuberosus* on boiling and baking process shows a tendency to decline (Table 1). Degradation or decomposition of flavonoid contents likely caused this during the thermal processing. The possibility of flavonoid on *C. tuberosus* is anthocyanin. This compound is soluble in water, so by the boiling process caused anthocyanin leaching in the boiling water. It demonstrated the existence of dark color (purple, blue/black) in the water boiling of *C. tuberosus*13. Anthocyanins were not stable during processing that used heat treatment14. Increasing temperature and activity of enzymatic reactions may destroy phenolic compounds.[15]

Evaluation of Antioxidant Activity

The antioxidant activity evaluated with the DPPH method indicate that processing tends to increase the ability of antioxidant activity in samples (Table 2). Method of processing by way of boiling can increase antioxidant activity either on the peel or flesh of *C. tuberosus*.

Table 2 shows that processing can increase antioxidant activity. Several things made possible the presence of phenolic compounds such as conversion to a form that has a higher antioxidant activity suppose the formation of aglycon that has the ability of higher antioxidant activity. Increased antioxidant activity caused by the transformation of compound phytochemicals becoming more active compounds, e.g., polymerization of polyphenolic.

Maillard reaction, i.e., reactions involving amino Carbonyl groups and so arose a new compound that Brown, i.e., Maillard reaction products(MRPs) that have a higher antioxidant property.[16] Maillard reaction can produce a variety of products, intermediate products and brown product (melanoidin), which has contributed to the color, flavor, and aroma as well as having potential as antioxidants in processed food.[17] Some research suggests that treatment with boiling and baking can increase antioxidant activity in food despite the declining levels of phenolic and flavonoid.[18]

The level of Resistant Starch

Resistant starch defined as starch can escape digestion in the intestine and as a source of fermentation substrate for colonic microflora. Anaerobic fermentation generates short chain fatty acids which can be used as additional energy for animals.[19]

The results of the analysis of the RS on the raw *C. tuberosus*, boiled *C. tuberosus*, and *C. tuberosus* flake was present on Table 3. Table 3 shows that processing can increase the levels of RS. The source of carbohydrate if experience thermal process can result in resistant starch. The more stages of processing increasingly high resistant starch that was formed. Processing by way of steaming, boiling, and roasting can raise resistant starch. The

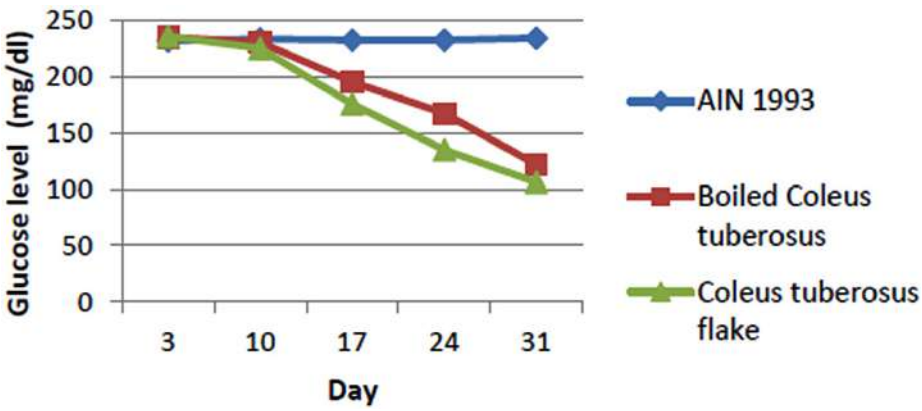

Fig. 1: Profile glucose of diabetic mice for 28 days treatment

potatoes boiled then cooled impact on increasing the levels of resistant starch (RS3).[19] Processing method: steaming, boiling and roasting can raise resistant starch.

The Glucose Profile

The mice suffering from diabetes after feeding Boiled and flake *C. tuberosus* shows a decrease in serum glucose levels (Figure 1). The decrease in serum glucose in boiled *C. tuberosus* was 47.41%. Whereas *C. tuberosus* flake was 54.94%. Standard feed does not occur on a decrease in serum glucose. These data indicate that the feed that contains resistant starch was higher (Table 3), have the greater ability to lower glucose profile.

Foods containing resistant starch will be digested slow; this gives implications for controlling the release of glucose.[20] This is in line with studies showing that consumption of foods containing resistant starch type 3 to control the release of glucose because it has a low glycemic index.[21] Commercial RS3 blood glucose was significantly lower than that of other simple carbohydrates.[22] RS3 lowered postprandial blood glucose and to play a part in keeping control of metabolism in type II diabetic patients. Research also shows that foods containing resistant starch type 3 may improve glucose profiles in mice injected alloxan.[23] Consumption of resistant starch can rectify the beta cells of the pancreas, insulin sensitivity, increase levels of insulin the pancreas, the total level of GLP-1, SCFA concentration on improving control of blood glucose levels.[24]

The current research was finding that there is an increase in ROS or concentration of oxidative stress and lipids on several animal models. Alloxan led to free radicals that caused cells damage cells. During the redox process, ROS are formed and the beta cell damage caused on the island of Langerhans.[25]

The research that has been done suggests that flavonoids and phenols have the capability of capturing free radicals, which can protect the oxidative stress that causes cell damage (such as the concentration of lipids membrane and membrane degradation). Flavonoid glycosides can stimulate insulin secretion in beta cells of the pancreas. In animals that suffer from diabetes, and given feed containing flavonoids, then it is possible that the compound acts as an insulin secretion in the pancreas stimulated or increase the uptake of glucose.[26]

The ability of flavonoids as antidiabetic can improve glucose profile is related to the effect his ability to stimulate insulin secretion, reduces the apoptosis and proliferation of pancreatic beta cells spur, lowering insulin resistance, inflammatory and oxidative stress on the muscles and promotes translocate of GLUT4 via PI3K/AKT and AMPK pathways.[27] Consumption of phenols may inhibit the α-amylase and α-glucosidase, able to inhibit the absorption of glucose in the intestine by sodium-dependent glucose transporter 1 (SGLT1), can stimulate the secretion of insulin and reduce hepatic glucose output.[28]

This study provides information that the existence of two processing techniques, i.e. boiling and baking can increase antioxidant activity and levels of resistant starch. Nevertheless still required information related to the antioxidant activity and the levels of resistant starch for various processing techniques (steaming, roasting) or Coleus tuberosus processing to a wide range of processed products, so that the community has more options in Coleus tuberosus processing as a product ready to eat.

Conclusions

The process of boiling of *C. tuberosus* and making *C. tuberosus* flake have an impact on decreasing the level of phenol compounds and flavonoid. Antioxidant activity of the flesh and peel of boiled *C. tuberosus* and *C. tuberosus* flake increased compared to raw *C. tuberosus*. Processing can increase the levels of resistant starch. The resistant starch content

Table 3: Level of resistant starch on raw, boiled *C. tuberosus* and *C. tuberosus* flake

Materials	Level of resistant starch (%)
Raw *C. tuberosus*	10.24 ± 0.37a
Boiled *C. tuberosus*	15.42 ± 0.96b
C. tuberosus flake	44.09 ± 0.07c

Different letters within the column indicate significant differences at $P<0.05$.

on raw *C. tuberosus* 10.24 ± 0.37%; boiled *C. tuberosus* 15.42 ± 0.96%; *C. tuberosus* flake 44.09 ± 0.07%. The decrease in serum glucose in boiled *C. tuberosus* was 47.41%. Whereas *C. tuberosus* flake was 54.94%.

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Conflict of Interest

The author(s) declare .no conflict of interest.

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