



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* were $10.24 \pm 0.37\%$; boiled *C. tuberosus* $15.42 \pm 0.96\%$; and *C. tuberosus* flake $44.09 \pm 0.07\%$. 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.



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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

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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. tuberosus*: *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 °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 °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. tuberosus*, *C. tuberosus* flake macerated with methanol during seven days (1:5), then filtered using Whatman No.1, evaporating the solvent with gas N₂. The Extract stored in the freezer temperature -22 °C.

Determination of Total Phenolic Compounds

The methanol extract of *C. tuberosus* 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% Na₂CO₃ 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% AlCl₃ 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. tuberosus* or *C. tuberosus* flake. Change the color after 30 minutes read at 517 nm. Percentage radical scavenging activity determined:

$$(A_0 - A_1) / A_0 \times 100\%$$

In this case, A₀ was absorbance control, and A₁ was the absorbance methanol extract of the peel and flesh of raw, boiled *C. tuberosus* 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 40°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°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°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

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 <i>C. tuberosus</i>	Peel	7.73±0.08 ^{fB}	8.55±0.07 ^{fB}
	Flesh	7.24±0.10 ^{eA}	2.31±0.13 ^{eA}
Boiled <i>C. tuberosus</i>	Peel	2.17±0.01 ^{aA}	0.07±0.00 ^{aA}
	Flesh	6.51±0.02 ^{dB}	1.22±0.01 ^{cB}
<i>C. tuberosus</i> Flake (Control)		3.83±0.02 ^b	2.17±0.01 ^d
<i>C. tuberosus</i> 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 treatment at P < 0.05

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 \pm 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

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

Treatment	Part of sample	Antioxidant activity (DPPH) (%)
Raw <i>C. Tuberosus</i>	Peel	62.82 \pm 0.32cB
	Flesh	26.34 \pm 0.09aA
Boiled <i>C. Tuberosus</i>	Peel	92.70 \pm 0.47eB
	Flesh	56.29 \pm 0.37bA
<i>C. Tuberosus</i> flake (control)		91.11 \pm 0.51d
<i>C. Tuberosus</i> flake		92.57 \pm 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 $P < 0.05$.

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 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

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

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

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

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*

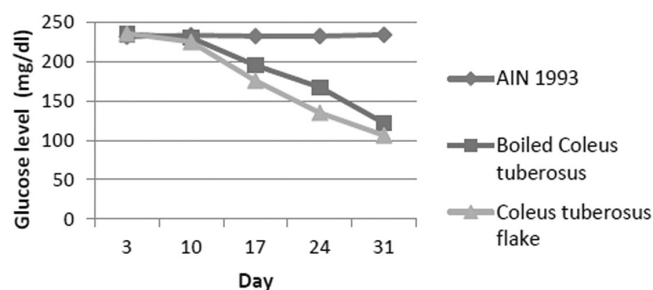


Fig. 1: Profile glucose of diabetic mice for 28 days treatment

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 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* $10.24 \pm 0.37\%$; boiled *C. tuberosus* $15.42 \pm 0.96\%$; *C. tuberosus* flake $44.09 \pm 0.07\%$. 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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