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Research Article

Effect of *Coleus tuberosus* Flour High Resistant Starch Consumption in Glucose, Lipid, Digest and Short Chain Fatty Acid Profile in Normal Rats

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Abstract: This research was conducted to study the effect of processing methods on the resistant starch content of *Coleus tuberosus* and the influence of consumption of *Coleus tuberosus* flour toward profiles of glucose, lipids (total cholesterol, triglycerides, LDL, HDL), digest and Short Chain Fatty Acids (SCFA) in normal rats. Processing method affects the levels of resistant starch in the *Coleus tuberosus* starch. The results showed the levels of resistant starch of *Coleus tuberosus* with different processing are steaming-cooling: 9.5291±0.0724%; boiling-cooling: 9.1235±0.3680% and oven-cooling: 9.0306±0.9570%; raw *Coleus tuberosus*: 7.5243±0.2054%. Effect of *Coleus tuberosus* flour consumption with steaming-cooling process controlling glucose and lipid profile in normal rats compared to the other treatment processes. Short-chain fatty acid profiles in all processes showed the greatest proportion of acetic acid, followed by acid propionate and the last is butyric acid. This study shows that *Coleus tuberosus* flour that produced by heating and followed by cooling process can increase the levels of resistant starch and physiological benefits to the management profile of glucose, lipids, digest and SCFA in normal rats.

Keywords: *Coleus tuberosus*, digest, glucose, lipids, resistant starch, SCFA

INTRODUCTION

Resistant starch is starch or starch degradation products that cannot be digested by the intestines of healthy humans (Asp and Bjorck, 1992). Resistant starch can produce short-Chain Fatty Acids which are the main end products of degradation by anaerobic bacteria in the large intestine (Cummings, 1989). One attempt to increase levels of resistant starch in starchy foods is processing (repetition of the process of heating and cooling starchy materials) (Kingman and Englyst, 1994).

Heating starch with excessive water will result starch gelatinization (Wursch, 1989). The process continued after the starch gelatinized e.g., refrigeration, freezing, baking or frying will result in retrogradation of starch that can alter the structure of starch which leads to the formation of new crystals are insoluble. Gelatinization and retrogradation which often occur in the processing of starchy materials affect the digestibility of starch in the small intestine.

Coleus tuberosus is one of the agricultural products in Indonesia as a source of carbohydrates and drugs that are expected to be a source of resistant starch through the process of heating followed by cooling. Research is needed to determine levels of resistant starch with different processing methods, as well as the effect of the consumption of *Coleus tuberosus* flour containing resistant starch on lipid, glucose and short chain fatty acids profile in normal rats. This study is expected to provide information methods of making high resistant starch type that can be used as a functional food, as well as to encourage cultivated *Coleus tuberosus* in order to support food security and useful in improving public health.

METHODOLOGY

Materials: This study used *Coleus tuberosus* that was obtained from Farmers in Clereng, Kulon Progo, Yogyakarta, Indonesia. Cholesterol kit, triglycerides kit, Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL) kit, from Diasys diagnostic system kit, Holzheim, Germany. Alloxan monohydrate, pancreatic α-amylase, amyloglucosidase enzyme from Sigma Aldrich (St. Louis, MO, USA). Glucose profiles used glucose kit (Diagnostic Bavaria Germany). Standard feed (American Institute of Nutrition, AIN 1993), all other reagents and solvents were of analytical reagent grade.

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Preparation of pre-cooked flour of *Coleus tuberosus:* Preparation of pre-cooked flour, covering the three treatments are boiling (100°C for 15 min), followed refrigeration (4°C for 24 h), steaming (100°C for 15 min), followed refrigeration (4°C for 24 h) and oven (100°C for 15 min), followed refrigeration (4°C for 24 h). *Coleus tiberosus* from each treatment reduced in size and dried with cabinet dryerat temperature 45°C for 20 h, ground and then sieved (80 mesh).

Resistant starch analysis: One hundred mg *Coleus tuberosus* flour (steaming-cooling, boiling-cooling, oven-cooling) was added 10 mL solution of pancreatic enzyme α-amylase (500 U) 0.1 mol/L tris-maleate buffer solution (calcium chloride 4 mL). It was allowed for 16 h at 37°C. After that, 40 mL of ethanol was added, allowed for 1 h and then centrifuged. The residue obtained was washed twice with 80% ethanol and dried at 60°C. The residue was dried and 1.56 mL of H_2O and 1.5 mL 4 mol/L KOH were added and allowed for 0.5 h at room temperature. Then, 12 mL H_2O was added. Obtained dispersion was taken and 1.5 of 0.65 mL 2 mol/L acetic acid (to obtain pH 4.5) and 0.1 mL amyloglucosidase enzyme (0.1 mL of 0.1 mol/L Na acetate buffer pH 4.5) were added. It was shaken for 90 min at 65°C. Glucosewasdeterminedusing glucose oxidase test. The result is a resistant starch (Champ *et al*., 1999).

Animal diet: Animal diet refers to the standard feed composition AIN 1993 (Reeves, 1997). One kilogram of feed was made of the standard composition of corn starch (560.70 g), vitamin mix (AIN 93-VX, 10 g), L-cysteine (1.8 g), Carboxy methyl cellulose (50 g), mineral mix (AIN 93-MX, 35 g), casein (>85% protein, 200 g), saccharose (100 g), corn oil (40 g) and choline bitartrate (2.5 g). Another diet were *Coleus tuberosus* flour from steaming-cooling, boiling-cooling and baking-cooling process, without the addition of other materials.

Bioassay pre-cooked of *Coleus tuberosus* flour: This stage is to determine the effect of the consumption of pre-cooked *Coleus tuberosus* on profiles of glucose, lipid and short chain fatty acids. Experimental animals used were rats type Wistar (aged 2 months with an average weight of 110 g). Diet rats during adaptation refers to the formula AIN-93 diet (Reeves *et al*., 1993). While diet in experiments using 3 kinds of diets that are *Coleus tuberosus* flour with Boiling-Cooling treatment (BC), Steaming-Cooling (SC) and Oven-Cooling (OC).

A total of 18 rats Wistar type (age 2 months, average weight 110 g) were adapted for 3 days by placing each rat in a cage individually sufficient light, ventilation and at room temperature. During adaptation, the rats were given a standard diet (AIN-93) and drink ad libitum. The rats were divided into 3 (three) groups according to the treatment, each treatment consisting of 6 rats. Dietary treatment was given 20 g/day and given drinking water ad libitum for 20 days. On day 21 performed blood sampling for analysis of lipid profile and blood glucose. Blood sampling is done by conditioning the rats in the fasting state for 12 h beforehand. LDL cholesterol by CHOP-PAP method (Wieland and Siedal, 1983), analysis of HDL cholesterol by CHOP-PAP method and the total triglycerides by GPO-PAP method (McGowan *et al*., 1983). Analysis of serum glucose using GOD-PAP method, analysis of total cholesterol using CHOD-PAP method (Richmond, 1973).

Surgery rats from each treatment group intended to take the digests in the caecum, which then continued analysis of the levels of Short-Chain Fatty Acids (SCFA). The weight of digesta was measured by using an analytical balance. The analysis of SCFA was conducted by using Chromatography Gas (GC) GC-8A Brand Simadzu, with a FID detector.

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

RESULTS AND DISCUSSION

Coleus tuberosus before eating are always undergoing a process that uses heat treatment processing. Heat treatment continued cooling for example, can cause the starch that has undergone a form of starch gelatinization retrograded. Gelatinization and retrogradation processes that occur in the processing can cause the formation of resistant starch in the starch digestibility affecting the small intestine.

Effect of cooking method on resistant starch content of *Coleus tuberosus:* Resistant starch is defined as starch escapes digestion small intestine and become the source of aerobic fermentation substrates for colonic micro flora. An aerobic fermentation produces Short Chain Fatty Acids (SCFA) which can be used as an additional energy to the animal (Kleessen *et al*., 1997). Resistant starch is formed in a process that is repeatable processing and high temperature treatment followed by a relatively low temperature. When starch is heated and undergo gelatinization, it becomes easier to digest, then continued cooling treatment it will form crystalline starch resistant to enzyme digest (retrograded). The results of the analysis of Resistant Starch in *Coleus tuberosus* with treatment Boiling-Cooling (BC), Steaming-Cooling (SC) and Oven-Cooling (OC) are in Table 1.

Table 1: Resistant starch content of *Coleus tuberosus* flour with treatment Boiling-Cooling (BC), Steaming-Cooling (SC) and Oven-Cooling (OC)

Kind of starchs	Resistant starch content (%)
Raw *Coleus tuberosus*	7.5243±0.2054[a]
Coleus tuberosus flour by Boiling-Cooling (BC)	9.1235±0.3680[c]
Coleus tuberosus flour by Oven-Cooling (OC)	9.0306±0.9570[b]
Coleus tuberosus flour by Steaming-Cooling (SC)	9.5291±0.0724[d]

Values were expressed as mean±standard deviation (n = 6); Means with different letters in same column were significantly different at level of $p<0.05$

Table 1 shows that the presence of repetitive processing (high temperature and followed by low temperature) on *Coleus tuberosus* can increase the levels of resistant starch. Raw *Coleus tuberosus* starch containing Resistant Starch (RS) 7.5243% and all of treatment combinations of heating and cooling will increase the levels of resistant starch. Boiling and cooling process can raise the level of RS from 7.5243 to 9.1235%. Steaming and cooling can increase the level of RS from 7.5243 to 9.0306% and the oven and cooling can increase the level of RS from 7.5243 to 9.5291%. This study shows that treatment with *Coleus tuberosus* steaming and continued cooling process has the highest starch content resistant stach than the other two treatments.

The results are consistent with Sajilata *et al*. (2006) research that proved that the process of boiling the potatoes, followed by cooling will increase the levels of resistant starch. Cooling process of potato (*Solanum tuberosus*) will increase levels of resistant starch type 3. Starch with the water heating process carried out in this study will lead to gelatinization (Haralampu, 2000). Heating treatment then continued with cooling process will change the structure of starch which leads to the formation of new crystals that are insoluble in the form of starch retrograded. Gelatinization and retro gradation which often occur in the processing of starchy materials affect the digestibility of starch in the small intestine (Englyst *et al*., 1992).

In the pre-cooked flour, heating process that resulted in starch gelatinization is carried in open containers may not have been perfect, so as to produce starch with low levels of resistant starch type 3. But heat treatment accompanied by cooling process (in the refrigerator for 12 h) cause changes in the structure of starch which leads to the formation of a new crystal form of insoluble starch (retrograded starch). So the combination process by high temperature and low temperature (cooling) will increase the levels of RS-type 3. Research Chiu and Stewart (2013) also explains that the formation of resistant starch type 3 associated with aggregates formed by heating and followed cooling of starch.

This study proves that the process of steaming and cooling had the highest levels of resistant starch, which is expected to have beneficial physiological effects in an effort to support the development of functional foods based on local tubers. A physiological effect of resistant starch is not only due to resistant starch itself, but also the result of resistant starch fermentation in the large intestine is Short Chain Fatty Acids (SCFA). SCFA are the major end product of the degradation of dietary fiber and resistant starch by anaerobic bacteria in the large intestine (Topping and Clifton, 2001).

According to Cummings and Bingham (1987), the product of resistant starch and dietary fiber fermentation in the form of short chain fatty acids and gases, namely CO_2, CH_4 and H_2. Acetic acid, propionic acid and butyric acid are the most widely type of SCFA, whereas isobutyrate, valerate and isovalerat very little contribution. Propionic acid has an important role in carbohydrate and lipid metabolism in the liver (Henningsson *et al*., 2001), whereas butyric acid could be expected to prevent colon cancer because of its ability to suppress the growth of abnormal cells (Aluko, 2012; Hinnebusch *et al*., 2002).

Analysis of glucose, lipid, digest and short chain fatty acids profile in normal rats: These experiments using Wistar rats were fed a diet of pre-cooked *Coleus tuberosus* flour consist of three processing (Boiling-Cooling (BC), Steaming-Cooling (SC) and Oven-Cooling (OC)). This study aimed to determine the effect of the consumption high resistant starch type 3 of *Coleus tuberosus* flour against physical and chemical properties of digests and blood of rats. Analysis of physical and chemical properties of digests include volume, weight, moisture content, pH and levels of Short Chain Fatty Acids (SCFA), whereas in blood will analyzed lipid and glucose profile.

Glucose profile: Experimental animals were selected by analyzing glucose and lipids to obtain animals with normal lipid profiles and glucose to be used in the experiment. Selected experimental animals then were divided into three diet groups based on the treatment process were: steaming-cooling, boiling-cooling and oven-cooling. Based on the analysis of glucose showed that a diet with high resistant starch of *Coleus tuberosus* flour can manage and control the glucose profiles of experimental animals in the normal range (Fig. 1).

This study shows that glucose levels in rat that fed a diet of *Coleus tuberorus* flour (steaming-cooling) is lower than diet with boiling-cooling and oven-cooling process (Fig. 2). Consumption of flour with steaming-cooling process can provide glucose lowering effect greater than the flour with the other two processes. This decrease is thought to be the amount of resistant starch in the flour with steaming and cooling process (Table 1). This result is expected to provide a positive influence for the management of diabetes and hypercholesterolemia.

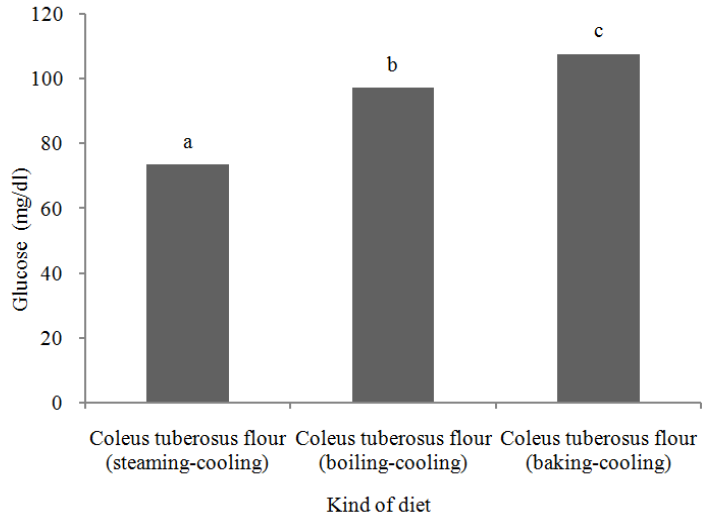

Fig. 1: Effect of *Coleus tuberosus* flour diets on glucose levels; Data are presented as mean±S.D. from six independent experiments

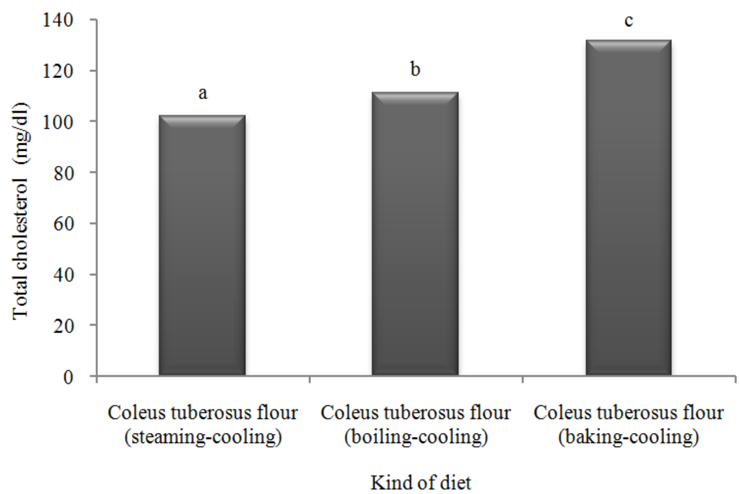

Fig. 2: Diets of *Coleus tuberosus* starch with processing variations on cholesterol levels; Data are presented as mean±S.D. from six independent experiments

The results of this study are supported by Cumming (1987) which states that resistant starch is a starch that is resistant to digestion by digestive enzymes in healthy individuals. Digestibility due to hold the generated too little glucose, thus contributing to lower postprandial response to foods containing high amylase, or resistant starch. The resistant starch has also been reported to be hypoglycemic. Resistant starch lowers the glycemic response because it is thick as well as water-soluble dietary fiber that inhibits the absorption of glucose.

Sajilata *et al*. (2006) stated that foods containing RS will be digested slowly, it implies to control the release of glucose. Resistant starch metabolism occurs 5-7 h after eating and it contrasts with the cooked starch (which is not included in the RS) can be digested quickly. Five-seven hour digestion reduces postprandial glycemia and insulemia and has the potential to increase the period of satiety. Study using 10 healthy people who were given 50 g of starch-free diet RS (0% RS) or 50 g of starch containing a high RS (54% RS) showed that a diet with high levels of RS significantly lowers postprandial blood glucose concentration, insulin and epinephrine.

Another study, Reader *et al*. (1997) using a commercial resistant starch type 3 in the diet of experimental animals showed that blood glucose profile was significantly lower than other simple carbohydrate diet (oligosaccharides and other starches). Resistant starch type 3 lowers postprandial blood glucose and plays a role in maintaining metabolic control in patients with type II diabetes.

Total cholesterol profile: This study shows that consumption of *Coleus tuberosus* flour with three processing method, steaming-cooling, boiling-cooling

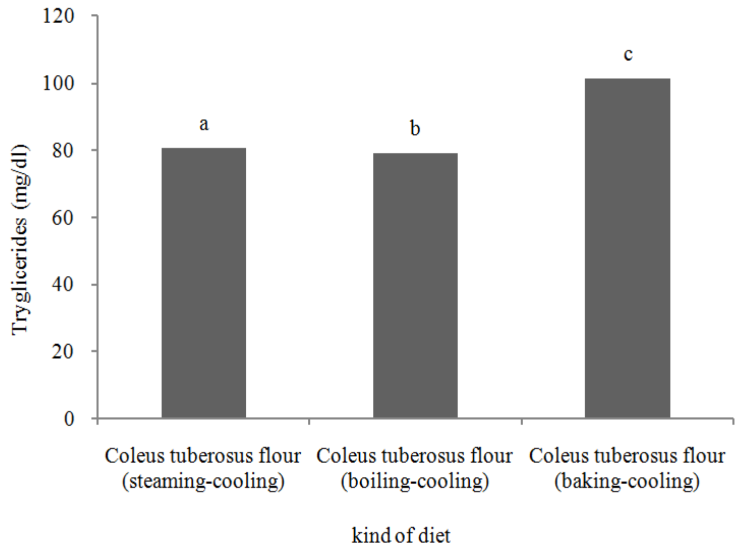

Fig. 3: *Coleus tuberosus* flour consumption with processing variations on levels of triglycerides; Data are presented as mean±S.D. from six independent experiments

and oven-cooling can control the levels of total cholesterol in normal experimental animals. Based on this research it is known that a diet of *Coleus tuberosus* flour with steaming-cooling process provide the best capability to control cholesterol levels.

Ability to control cholesterol levels in the diet of *Coleus tuberosus* flour (steaming-cooling) is expected to manage and lower the cholesterol profile in hypercholesterolemia disease. Control levels of total cholesterol are associated with higher levels of resistant starch in the feed. One mechanism of resistant starch in lowering lipid profile is resistant starch to replace the bile acid pool of Cholic acid into chenodeoxycholic acid. Chenodeoxycholic acid is an inhibitor of 3-Hydroxy-3-Methylglutaryl (HMG)-CoA reductase, a regulatory enzyme required for the biosynthesis of cholesterol. HMG CoA reductase activity resulted in lower production of cholesterol will decrease. And it causes serum cholesterol down.

This is in line with the study of Han *et al*. (2004) showed that consumption of resistant starch is adzuki beans (*Vigna angularis*), Kintoki (*Phaseolus vulgaris*, variety) and tebou (*P. vulgaris*, variety) can lower cholesterol levels. Mechanism of cholesterol lowering through the ability to sequester bile cholesterol, promoting its excretion into the feces. The cholesterol lowering effect of RS may be explained by promote caecal fermentation, with the consequent production of SCFAs, especially propionate and butyrate. Short chain fatty acids are involved in lowering cholesterol levels by counteracting the induction of hepatic cholesterol synthesis in the caused by an increase of bile acid excretion (Britesa *et al*., 2011). Ashraf *et al*. (2012) explain the resistant starch plays important role in potential health benefits similar to soluble fiber and in functional properties. Resistant starch absolutely influences the function of the digestive tract, the blood cholesterol levels and microbial flora.

Triglycerides profile: *Coleus tuberosus* flour diet with three processing able to control triglyceride profiles in experimental animals. Blood triglyceride levels should be below 100 mg/dL. However, *Coleus tuberosus* flour diet with steaming-cooling process is more effective in controlling triglyceride levels (Fig. 3). Ability to control of triglyceride levels in animals with *Coleus tuberosus* high resistant starch flour diet in line with research Ranhotra *et al*. (1996) which proved that the consumption of feed containing resistant starch can reduce triglyceride levels by 50%. This was reinforced by the study De Deckere *et al*. (1993) which states that the consumption of resistant starch may reduce triglycerides.

Low Density Lipoprotein (LDL) profile: Good blood LDL levels are below 100 mg/dL. When LDL levels over 130 mg/dL, it can lead to heart disease. The results showed that the consumption of *Coleus tuberosus* with three different processes able to control LDL levels within a safe limit (Fig. 4). Steaming-cooling process provides more effective control than the other two processes. It is thought to be related to the levels of resistant starch in the *Coleus tuberosus* flour with steaming-cooling process relatively higher (Table 1) makes one of the things that cause LDL levels can be maintained.

LDL level on experimental animals in this study can be control with diet *Coleus tuberosus* flour. This result relates to the role of resistant starch enhancing

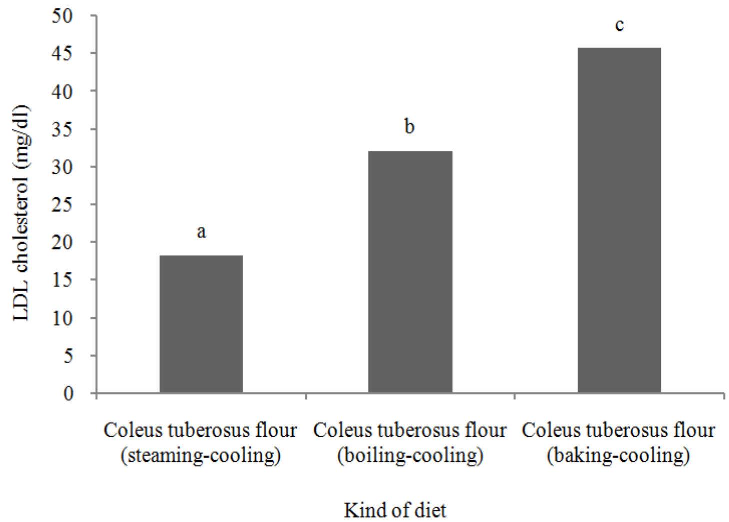

Fig. 4: *Coleus tuberosus* flour consumption with processing variations on levels of LDL; Data are presented as mean±S.D. from six independent experiments

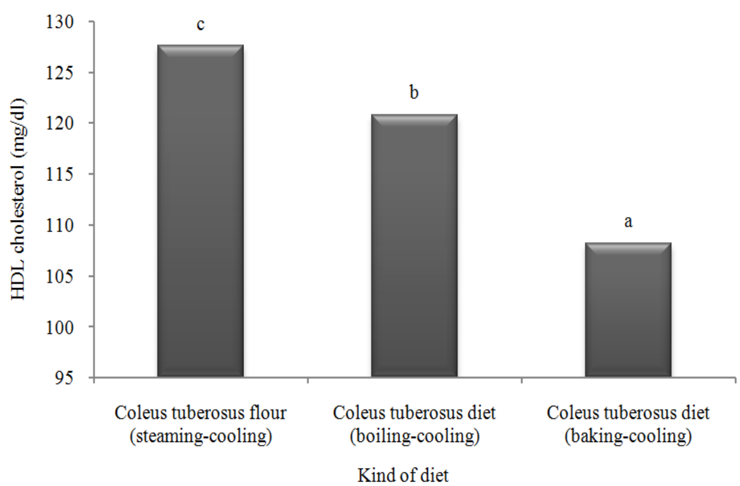

Fig. 5: *Coleus tuberosus* flour consumption with processing variations on levels of HDL; Data are presented as mean±S.D. from six independent experiments

the hepatic LDL receptor mRNA levels. This is consistent with the study of Han *et al*. (2004, 2005) that proved that the consumption of resistant starch may reduce LDL via enhancement of the hepatic LDL-receptor mRNA and cholesterol 7 alpha-hydroxylase mRNA levels and caecal bile acid excretion and a Decrease in the hepatic HMG-CoA reductase mRNA levels. Decrease on the concentration of LDL cholesterol in rats in this research, because resistant starch that contained on *Coleus tuberosus* flour has the ability to bind bile acids, increase of the viscosity of intestinal contents that can inhibit various types of macronutrient absorption, Including lipids and reduced bile acid absorption from the small intestine via the enterohepatic circulation (Fig. 5). The result of other studies conducted Finley *et al*. (2007) was known that rats fed resistant starch has hepatic LDL receptor mRNA were higher when Compared to the controls, so as to increase of the activity of the LDL receptor and SR-B1 in the liver. SR-BI (Scavenger Receptor class B, type I) is a membrane protein present on the cells, or liver or adrenal tissue. As the LDL receptor, SR-B1 functions to capture cholesterol esters from HDL in the liver. This process can encourage the transfer of cholesterol from peripheral tissues to the liver and then excreted through the feces.

High Density Lipoprotein (HDL) profile: High density lipoprotein is called good cholesterol. Blood levels of the good HDL more than 60 mg/dL, if the levels of HDL below 40 mg/dL, it can lead to heart disease. This study provides information that the ability to raise HDL cholesterol levels in diet of *Coleus tuberosus* flour with steaming-cooling process is higher than the boiling-cooling and oven-cooling. This ability is associated with high levels of resistant starch content

Table 2: Effect of dietary of pre-cooked *Coleus tuberosus* flour on digest weigh and water content

Diet	Digest weight	Water content (%)
Coleus tuberosus flour by Steaming-Cooling (SC)	2.43±0.05^{b}	85.57±2.08^{c}
Coleus tuberosus flour by Boiling-Cooling (BC)	2.52±0.13^{c}	83.31±3.71^{b}
Coleus tuberosus flour by Oven-Cooling (OC)	2.13±0.03^{a}	76.20±2.64^{a}

Values were expressed as mean±standard deviation (n = 6); Means with different letters in same column were significantly different at level of p<0.05

Table 3: The concentration of acetic acid, butyric acid and propionate in the rat digest

Kind of diet	Acetic acid (mL mol)	Propionic acid (mL mol)	Butiric acid (mL mol)
Coleus tuberosus flour by Boiling-Cooling (BC)	67.25±1.59^{a}	32.39±1.04^{b}	12.96±0.95^{b}
Coleus tuberosus flour by Oven-Cooling (OC)	73.41±0.97^{b}	30.35±1.01^{a}	6.29±0.20^{a}
Coleus tuberosus flour by Steaming-Cooling (SC)	77.76±1.05^{c}	34.86±0.98^{c}	18.35±1.10^{c}

Values were expressed as mean±standard deviation (n = 6); Means with different letters in same column were significantly different at level of p<0.05

in steaming-cooling process (Table 1). Resistant starch can increase of the expression of SR-B1 mRNA and increase concentrations of HDL cholesterol. The results of Kim and Shin (1998) study supports the research, that the increase in HDL may occur due to the content of resistant starch is able to increase the number of apolipoprotein A-1 which has a role in promoting the synthesis of HDL.

Weight and water content of digest: Weight and water content of digests Rat based on the analysis of variance showed that the dietary treatment and the type of *Coleus tuberosus* processing significantly ($p \leq 0.05$) on the volume, weight and water content of digests rats. Effect of dietary of pre-cooked *Coleus tuberosus* flour on weight and water content of digests rats are presented in Table 2.

Coleus tuberosus starch diet with steaming-cooling process has a tendency weight and water content is relatively high compared to the boiling-cooling and the oven-cooling process. This is due to the *Coleus tuberosus* starch with steaming-cooling process has relatively high levels of resistant starch (Table 1). These results provide information that the steaming-cooling process has more effective in increasing the weight and water content of digest in rats. This research proves that the process of steaming for 30 min and continued for 12 h cooling process is effective in gelatinization and retrogradation of starch resulted in increased levels of resistant starch (type-3).

According Wursch (1989), heating starch with water will result in excessive starch gelatinization, a process that includes hydration and dissolution of starch granules. But reheating and followed by cooling process of gelatinized starch can change the structure of starch which leads to the formation of a new crystal form of insoluble starch (retrograded starch). Starch storage which has gelatinized at low temperatures will accelerate of starch retrogradation. Gelatinization and retrogradation which often occurs during the processing of starchy materials can affect the digestibility of starch in the small intestine.

Digest water content is a reflection of the Water-Holding Capacity (WHC) of dietary components, especially polysacharides. Water holding capacity of polysaccharides determined by the chemical structure of polysaccharides, species and anatomy of the source material (Eastwood and Mitchell, 1976), as it also is influenced by particle size and pH. The water content of digest in the group of rats with a diet of *Coleus tuberosus* with steaming-cooling process is higher than the other two processes, probably caused by high levels of resistant starch that resistant digested by digestive enzymes. Data on water content and weight of digest in rats with diet of high resistant starch of *Coleus tuberosus* flour can give an information that *Coleus tuberosus* flour can be used as a supplement to prevent constipation and diverticulosis.

Some studies also showed that administration of a diet high in resistant starch will increase the digest weight of rats (Soral-Emietana and Wronkowska, 2004), increasing the water content of digest and increasing the amount of feces (Schultz *et al*., 1993; Damat, 2013).

Short chain fatty acid profile: Based on the analysis of variance showed that the type of treatment and the type of processing significant effect ($p \leq 0.05$) on the concentration of acetic acid, propionic, butyric and total SCFA in the digest of rats. Effect of dietary administration of *Coleus tuberosus* flour with processing variations of the concentration of acetic acid, propionic acid and butyric acid, also pH in the rat digest are presented in Table 3.

The results of this study indicate that dietary of *Coleus tuberosus* flour with steaming-cooling process gives the concentration of acetate; propionate and butyrate were relatively high compared to the other diets (Table 2). Types of short chain fatty acid fermentation products digest rats in this study is in line with research Cummings and Bingham (1987) who explained that products of fermentation of resistant starch and dietary fiber in the form of short chain fatty acids and gases, namely CO_2 , CH_4 and H_2. Acetic acid, propionic acid and butyric acid a dominant SCFA, whereas isobutyrate, valerate and isovalerat very little contribution.

Short chain fatty acids have a positive effect on the body's physiological functions. Several studies have shown propionic acid has several functions that regulate carbohydrate metabolism in the liver, as well as acetate, but has the opposite effect with propionic acid. Propionate stimulates glucose utilization or potentially to improve glucose metabolism in the liver. Propionic acids increased significantly insulin-stimulated glucose uptake in 3T3-L1 adipo-cytes and basal glucose uptake in C2C12 myotubes. These effects of both SCFAs known to be GPR41 agonists on glucose uptake are mediated via, at least in part, GPR41. GPR41 may play an important role in improving insulin sensitization for the management of type 2 diabetes and related complications (Han *et al*., 2014). Acetate is produced by the fermentation of fiber effect on insulin sensitivity that benefit from high-fiber diet than in glycemic control (Weickert and Pfeiffer, 2008). Thus acetate and propionate have different effects on glucose and fat metabolism. Acetate could be expected to decrease glucose and cholesterol while increasing the concentration of propionic acid may increase glucose production and lowering cholesterol concentration.

Meanwhile, according to Cummings and Bingham (1987) butyric acid can prevent colon cancer because of its ability to suppress the growth of abnormal cells or for its ability to inhibit carcinogenesis. According to Morita *et al*. (1999), the increase in the intake of resistant starch is fermented also enlarge the pool SCFA in the colon that can improve the intestinal microflora and prevent colon cancer. Butyric acid is critical for colonic epithelial cells and in some circumstances has demonstrated inhibition of neoplastic cell growth (Tharanathan and Mahadevamma, 2003).

CONCLUSION

The process of making pre-cooked *Coleus tuberosus* with steaming and continued with cooling for 24 h give a resistant starch levels higher than the boiling-cooling and oven-cooling process. Levels of resistant starch that is relatively higher in the steaming process-cooling to provide positive benefits to the profiles of glucose, lipids, digest and short chain fatty acids in normal rats.

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