

Hypoglycemic Effect of Modified Water Yam Flour (Dioscorea alata) on Diabetic Wistar Rats (Rattus norvegicus)

by Rosida Rosida

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Hypoglycemic Effect of Modified Water Yam Flour (*Dioscorea alata*) on Diabetic Wistar Rats (*Rattus norvegicus*)

Rosida^{1,2,*}, Harijono³, Estiasih Teti³, Sriwahyuni Endang⁴

¹Department of Food Technology, Faculty of Industrial Technology, University of Pembangunan Nasional "Veteran" Surabaya, Indonesia 60294

²Doctoral Program of Agricultural Technology, Faculty of Agriculture, Brawijaya University, Malang, Indonesia 65145

³Department of Food Science and Technology, Faculty of Agricultural Technology, Brawijaya University, Malang, Indonesia 65145

⁴Department of Medicine, Faculty of Medicine, Brawijaya University, Malang, Indonesia 65145

*Corresponding author: rosidaupnjatim@gmail.com

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Abstract The objective of this study was to evaluate the hypoglycemic effect of modified water yam flour (MWYF). To prepare MWYF, purple, yellow and white water yam were autoclaved and cooled in refrigerator (repeated up to three times) prior to drying and milling. The hypoglycemic activities were evaluated by means in vivo test on alloxan induced diabetic rats, meal tolerance test (MTT) and short-chain fatty acids (SCFAs) analysis. A nested experimental design was employed in the experiment. The research showed that water yam modification by three cycle autoclaving-cooling treatment are able to increase resistant starch and dietary fiber content, thus able to decrease blood glucose level. After four week experiment, It was found that all MWYF exhibited ability to decrease blood glucose level in hyperglycemic rats as well as inhibit glucose absorption in MTT and increase SCFAs formation. The white MWYF exhibited the most significant hypoglycemic activity.

Keywords: hypoglycemic effect, modified water yam flour, resistant starch, short-chain fatty acids

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1. Introduction

Diabetes is a chronic endocrine disease characterized by persistent hyperglycemia and associated with abnormalities of carbohydrate, protein, and lipid metabolism. Diabetes is rapidly emerging as a global health care problem that threatens to reach pandemic levels by 2030; the number of people with diabetes worldwide is projected to increase from 171 million in 2000 to 366 million by 2030 [1]. Hyperglycemia is a condition which glucose level in blood plasma over its normal limit, such as over than 120 mg/dL on fasting condition and over than 200 mg/dL on two hours after eating and caused degenerative disease, such as diabetes mellitus. Recent efforts for the complementary treatment of diabetes have focused on functional foods and their bioactive compounds [2].

Water yam (*Dioscorea alata* L) is known as ubi kelapa in Bahasa. In English, aside from purple yam, other common names include greater yam, ten-month yam, or water yam [3]. In Indonesia, water yam is usually differentiated by its flesh color, such as white, yellow and purple water yam [4]. Some studies showed that wild yam [4] and lesser yam [5] had hypoglycemic effect. The effect in reducing blood glucose level was due to some polysaccharides in yam, such as water soluble

polysaccharide, resistant starch and dietary fiber. However, there are few reports on hypoglycemic properties of water yam.

Water yam is usually boiled or steamed and consumed as staple food in village society. Being processed, Resistant Starch type III (retrograded starch) is formed [6] it has positive effect to human health. Resistant Starch (RS) is defined as the fraction of starch or starch degradation products that are not absorbed in the small intestine of healthy individuals and are resistance to amylase hydrolysis [6]. RS is categorized as a part of dietary fiber. When carbohydrates were not well digested and the absorption of sugars in the small intestine was inhibited, the digesta was transferred into the colon and then fermented by bacteria [7] producing short chain fatty acids (SCFAs). A high intake of fiber is recommended for diabetic patients. The viscous properties of fiber and gel formation may inhibit macronutrient absorption and reduce postprandial glucose response.

Physically modification of starch by heating and subsequent cooling can increase RS level. One of the processing technique that can increase RS content is autoclaving-cooling process. The changes of the structure and properties of starch under autoclaving-cooling cycle depend on botanical source. Tubers source is susceptible to the autoclaving-cooling cycling treatment compared to seeds and beans [6].

This study was carried out to examine the effect of autoclaving-cooling treatment on RS and dietary fiber content of three types of water yam and investigated the hypoglycemic effect of modified water yam flour (MWYF) on alloxan induced diabetic rats. Animal models of diabetes mellitus were used in laboratory according to the pathology of diabetic patients. Alloxan and streptozotocin are the most prominent diabetogenic chemical compounds in experimental diabetes research. Both compounds can cause insulin dependent diabetes mellitus (DM) with characteristic of DM type 1, as in human being. However, Streptozotocin can be used to induce either DM type 1 or type 2 on experimental animal [8]. This study used alloxan to induce diabetes mellitus in rats since the research target is an insulin dependent DM (DM type 1). Alloxan injection is the quick way to produce experimental diabetic condition (hyperglycemic) on experimental rats. Alloxan rapidly can reach pancreas. Alloxan can cause β cell pancreas damage so it can not produce insulin. The lack of insulin cause fast increasing of blood glucose in experimental animals [8].

2. Materials and Methods

2.1. Materials

Three types of water yam (*Dioscorea alata* L): purple, yellow, and white water yam were obtained from local farmers in Tuban, East Java, Indonesia. Reagents were analytical grade, such as 96% ethanol (pa), DiaSys (Diagnostic System) Glucose GOD FS, Na-phosphate buffer (pH 7.0), α -amylase (EEC 232-560-9), amyloglucosidase (EEC 232-877-2), pullulanase (EC 3.2.1.41) (by Sigma Chemical Co.) and alloxan monohydrate (Merck). Sixty male Wistar rats (*Rattus norvegicus*) (2-3 months old), weighing 150 ± 20 g were obtained from LPPT Gadjah Mada University, Yogyakarta.

2.2. Preparation of modified water yam flour (MWYF)

Selected purple, yellow, and white water yam tuber, weighing 100-200 g, were placed in 500 ml beaker glass and heated for 15 minutes at 121°C using an autoclave (ALP Co, Model KT-30T-3-3-10). After autoclaving, the samples were allowed to cool and stored for 24 hours in a refrigerator (4°C) which was termed as one cycles. This autoclaving-cooling cycle was repeated up to 3 times. The treated samples were peeled, sliced, and dried in a cabinet drier at 60°C for 12 hours. The dried sample were milled into fine flour (80 mesh size) which was termed as modified water yam flour (MWYF). Native water yam flour (NWYF) was made of water yam tuber which were peeled, sliced, then quickly dried in cabinet drier prior to milling and sieving (80 mesh size). Resistant starch and dietary fiber content was determined using a modified method of AOAC method 2002.02 [9].

2.3. Meal Tolerance Test

MTT was conducted using a modified method of Harijono *et al.* [4]. The aim of the test was to determine the changes of blood glucose level short after 60 min of consuming the feed. Twenty eight male Wistar rats

(*Rattus norvegicus*) were divided into seven groups (four rats per group) according to diet. MTT was conducted on overnight-fasted rats. Rats were allowed free access to their respective diets and water for 1 hour. The feed was referred to modified standard diet of AIN 93-M [10] that consisted of 67.70% corn starch, 14% casein, 10% sucrose, 4% soybean oil, 3.5% AIN mineral mix, 1.0% AIN vitamin mix, 0.25% choline bitartrate, 0.18% L-cystine, except carboxy methyl cellulose addition (fiber-free diets). NWYF and MWYF diets were made by substituting corn starch with purple, yellow and white native and modified water yam flour. Blood sample were taken from retro-orbital plexus of rats at 0, 30, 60, 90 and 120 minutes after feeding and were measured by the GOD/PAP method [11].

2.4. Hypoglycemic activity assay

Hypoglycemic activity assay was referred to method of Estiasih *et al.* [5]. This study has been approved by animal care and use committee of Brawijaya University, Indonesia. Thirty two male Wistar rats (*Rattus norvegicus*) were placed individually in stainless steel wire mesh cages. All rats were allowed 1 week to adapt to the environment before being given the treatment. The rats, except the control(-)/normal group were intraperitoneal alloxan induced of 80 mg/kg body weight so they suffered diabetic. Three days after the induction of diabetes, rats with blood glucose levels over 180 mg/dL were considered to have diabetes mellitus. The rats were divided into eight groups, each group was consisting four rats. Control group was fed AIN-93M diet [10], while others were given purple, yellow and white native (NWYF) and modified water yam flour (MYWF), as described in MTT. They were fed and given water *ad libitum* for 4 weeks. The measurement were performed on days 0, 7, 14, 21 and 28. Blood glucose were taken from a retro-orbital plexus after fasting for 16 hours and were measured by the GOD/PAP method [11]. Body weight and feed intake were monitored weekly. At the end of bioassay, the rats were anaesthetized with ether before the caecum digesta were taken, prior to analyzing for pH and Short Chain Fatty Acids (SCFAs) content by Gas Chromatography Method [12].

2.5. Statistical analysis

The *in vivo* study data were analyzed by nested experimental design with two factors by one way analysis of variance (ANOVA) and continued with DMRT (Duncan's Multiple Range Test).

3. Results

Resistant starch and dietary fiber content of native (NWYF) and modified water yam flour (MWYF) are shown in Figure 1a and Figure 1b.

Figure 1 showed that the types of water yam and autoclaving-cooling cycle affected resistant starch and dietary fiber content of flour. All the modified water yam flour had higher RS content than the native one. The native and modified purple, yellow and white water yam flour were subsequently evaluated for their hypoglycemic effects. The influence of feeding standard, native and modified water yam flour diets on the body weight, feed intake and pH of rats digesta were shown in Table 1.

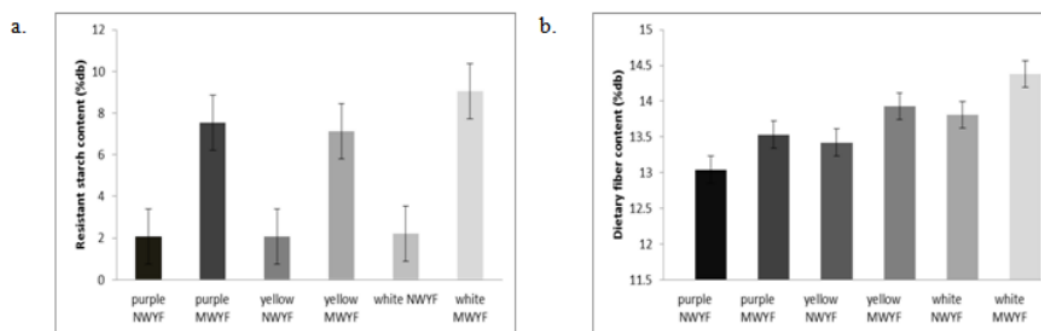


Figure 1. Resistant starch (a) and dietary fiber (b) content of purple, yellow, and white native (NWYF) and modified water yam flour (MWYF)

Table 1. Body weight, feed intake and pH of rats digesta which were fed standard, native and modified water yam flour diets

Treatment Feed	Weight gain /28 days (g)	Feed intake /day (g)	pH
Control (-)	31.22 ^a ± 7.19	9.02 ^{ab} ± 1.18	7.36 ^b ± 0.10
Control (+)	31.47 ^a ± 7.07	8.64 ^a ± 0.69	7.26 ^b ± 0.04
Purple NWYF	35.25 ^a ± 11.35	11.11 ^{ab} ± 1.62	6.34 ^a ± 0.43
Purple MWYF	42.90 ^a ± 6.36	13.78 ^{bc} ± 1.70	5.98 ^a ± 0.46
Yellow NWYF	35.10 ^a ± 8.99	10.48 ^{ab} ± 2.09	6.44 ^a ± 0.23
Yellow MWYF	39.65 ^a ± 9.21	11.79 ^b ± 2.18	5.86 ^a ± 0.73
White NWYF	38.37 ^a ± 10.05	10.49 ^{ab} ± 2.45	6.27 ^a ± 0.85
White MWYF	48.30 ^a ± 3.14	13.80 ^{bc} ± 1.81	5.85 ^a ± 0.42

*mean of 4 replication

** SD with different superscripts in a column differ significantly ($p < 0.05$)

Table 1 showed that the feed intake of the experimental groups were significantly different ($p < 0.05$), but it did not influence body weight gain of the rats significantly. The influence of feeding standard, native and modified water

yam flour diets on acetic, propionic, and butyric acid content of caecum digesta of experimental rats was shown in Figure 2, while the result of Meal Tolerance Test (MTT) was presented in Figure 3.

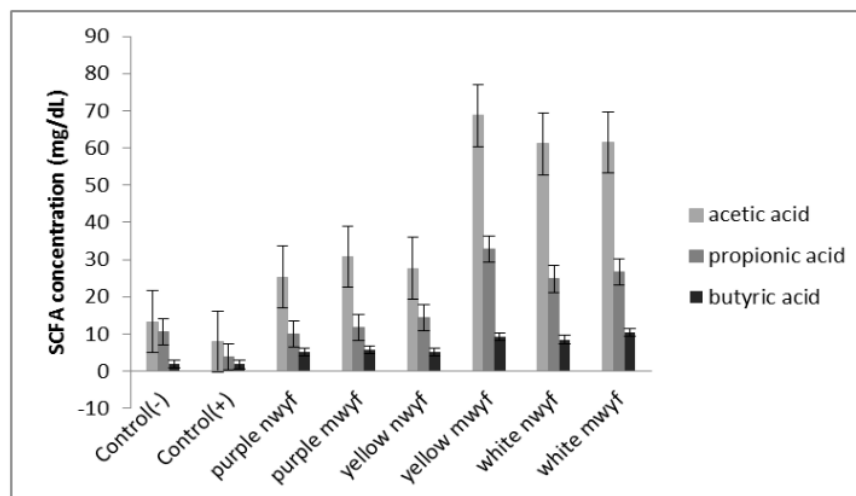


Figure 2. Acetic, propionic and butyric acid content of caecum digesta of rats which were fed standard, native and modified water yam flour diets

The results revealed that digesta of caecum of MWYF diets group had more SCFAs (Figure 2) and lower pH (Table 1) than those of NWYF and control diet groups.

The pH of the digesta decreased due to increasing in concentration of SCFAs of the digesta.

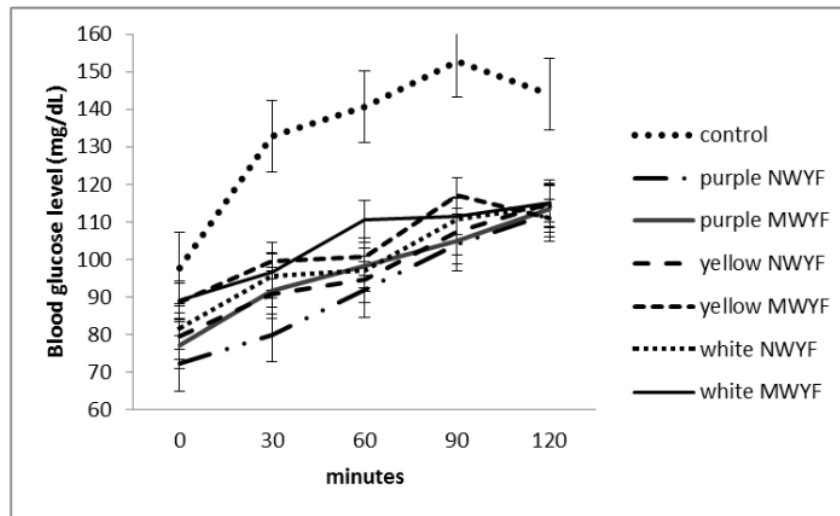


Figure 3. Changes of blood glucose level on MTT of rats fed standard, native (NWYF) and modified water yam flour (MWYF)

Meal Tolerance Test (MTT) indicated that the diets influenced blood glucose level content significantly ($p < 0.05$). Rats with standard diet revealed the highest blood glucose raising. The results demonstrated that feeding MWYF diet caused slowly blood glucose increasing especially white MWYF diet.

The weekly changes of blood glucose level of rats during 4 weeks feeding standard, native (NWYF) and modified water yam flour (MWYF) diet on diabetic rats was presented in Table 2 and Figure 4.

Table 2. Changes of blood glucose level of rats during 4 weeks feeding standard, NWYF and MWYF on diabetic rats

Treatment feed	Blood glucose levels (mg/dL)					Increasing/decreasing blood glucose level (mg/dL)
	Week 0	Week 1	Week 2	Week 3	Week 4	
Control (-)	94.30 ^a	95.50 ^a	92.38 ^a	90.80 ^a	87.43 ^a	-26.88
Control (+)	200.85 ^b	190.43 ^{ab}	181.65 ^{ab}	169.35 ^{ab}	157.90 ^a	-42.95
Purple NWYF	204.28 ^b	179.55 ^{ab}	142.05 ^{ab}	113.10 ^a	99.30 ^a	-114.98
Purple MWYF	205.83 ^c	170.48 ^{bc}	134.23 ^b	116.33 ^{ab}	99.10 ^a	-116.73
Yellow NWYF	206.78 ^b	171.78 ^b	129.28 ^a	106.35 ^a	101.78 ^a	-115.00
Yellow MWYF	204.78 ^c	161.25 ^b	123.75 ^{ab}	105.40 ^a	87.63 ^a	-117.15
White NWYF	217.13 ^c	189.15 ^{bc}	154.15 ^b	132.28 ^{ab}	111.43 ^a	-115.70
White MWYF	201.85 ^c	166.50 ^{bc}	133.00 ^b	97.85 ^{ab}	83.33 ^a	-118.53

*mean of 4 replications

** SD with different superscripts in a column differ significantly ($p < 0.05$)

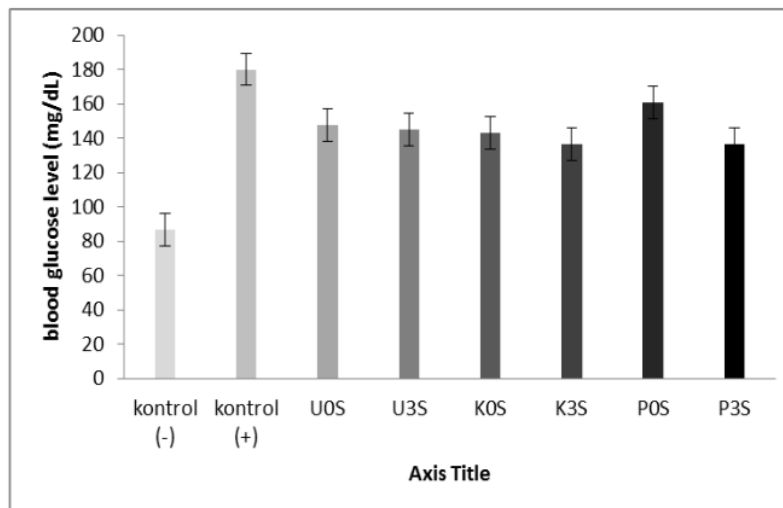


Figure 4. Change of blood glucose level of rats during four week feeding standard, native and modified water yam flour

4. Discussion

The recent data revealed that autoclaving-cooling process increase RS content of purple water yam by 3.64% (from 2.07%db to 7.55%db), yellow water yam by 3.46% (from 2.06%db to 7.14%db), and white water yam by 4.07% (from 2.22 %db to 9.04%db). Modified purple, yellow and white water yam flour had high dietary fiber content (13.53 %db, 13.93 %db, and 14.38 %db, respectively).

Previous study reported that three and five cycle treatment of arrowroot starch resulted RS content about (10.91%) and (2.15%), respectively [13]. Other researcher showed that three cycles of autoclaving-cooling of wheat starch raised RS content into (7.8%) compared to those made of one cycle treatment (6.2%) [14]. The high RS content of MWYF made it potential to be processed as high RS food or functional food.

In vivo evaluation showed the effect of experimental diet on feed intake and body weight in normal and diabetic rats. However, no significant differences were found in body weight gain for all diet groups (Table 2). Wisaniyasa *et al.* [15] reported that after alloxan injection of the rats, there was the low feed intake of experimental rats but the feed intake gradually increased during experiment. In the following day, slowly there was body weight gain of rats along with increasing in feed intake.

Figure 2 revealed that SCFAs concentration significantly increased for all water yam diet groups, either native or modified flour, compared to standard diet groups. The highest acetic and propionic acid was on the digesta of rats with yellow MWYF diet (68.70% and 32.93%, respectively) and the second was the rats with white MWYF diet (61.46% and 26.75%, respectively). The white MWYF diet exhibited the highest butyric acid content (10.42%) on the rat digesta, hence this flour is good to prevent colon cancer.

The profile and type SCFAs varied due to the type of fiber. The main SCFAs produced were acetate, propionate and butyrate [7]. The SCFAs concentration varied with the type of polysaccharides which are fermented, although generally the most SCFA is acetic acid and the least is butyric acid. Other researcher reported that the consumption of Pedada fruit flour increase body weight in diabetic rats and the order of concentration of the SCFAs was similar, such as acetic acid > propionic acid > butyric acid [16]. The previous research reported that butyric acid was produced in high amount in the fermentation of high RS diet [17]. In this research white MWYF diet had generally high butyric acid content hence it is good for colon cancer prevention.

The hypoglycemic activities were evaluated by means of meal tolerance test (MTT) and in vivo test on alloxan induced hyperglycemic rats. The objective of meal tolerance test (MTT) is to determine the best product formulation based on the lowest blood glucose raising and to study the tolerance of blood glucose increasing after feeding experimental feed. The kind of water yam flour which could prevent the increasing of blood glucose is the best treatment. Figure 3 showed the changes in glucose levels following the MTT. MTT showed that the highest glucose absorption resistance is in the group of white MWYF, followed by purple MWYF and yellow MWYF.

The results demonstrated that feeding MWYF diet caused slowly blood glucose increasing especially white MWYF diet. Thus, one way to lower blood glucose mechanism is feeding high RS and dietary fiber diet, such as MWYF. RS had low glycemic index due to its low glucose releasing so that it could lower body insulin response and help diabetic patient in normalizing his blood glucose. The low insulin response could decline the increase of blood glucose so that the need of energy is low [18]. Physical forms of fiber affect the ability to inhibit the absorption of glucose. Dietary fiber could decrease postprandial glucose because their viscous and gel forming properties that inhibits macronutrient absorption [19]. Gel structure was able to entrap glucose and other nutrient that slow down absorption.

Table 2 indicated that all water yam flour had hypoglycemic effect that was presented by decreasing blood glucose level. Sharp decrease in blood glucose level was found in white MWYF (118.53 mg/dL), followed by yellow MWYF (117.15 mg/dL) and purple MWYF (116.73 mg/dL) due to its RS and dietary fiber content. RS has functional value for diabetic patients. Consuming RS can decrease blood glucose level. RS can release energy slowly so it cannot be rapidly digested as glucose form. RS declines glycemic effect and is sensitive to insulin hormone so that it can reduce diabetic potency [20]. Dietary fiber can reduce postprandial glucose due to its properties which can form gel structure and viscous solution. Viscous solution which is caused by soluble fiber made high viscosity that hampered glucose absorption at digestive tract due to glucose entrapment in the weak gel structure [17].

5. Conclusion

Modified purple, yellow, and white water yam flour had high resistant starch content (7.04%db, 7.55%db and 9.03%db respectively). Animal study on diabetic rats revealed that consuming MWYF diets resulted higher SCFA content and lower pH of caecum digesta. The pH of the digesta decreased due to increasing in concentration of SCFA of the digesta. All MWYF diets exhibited ability to decrease blood glucose level in diabetic rats as well as inhibit glucose absorption and increase SCFAs formation. The white MWYF exhibited the most hypoglycemic activity and butyric acid content, so it had potential prospect to be developed as functional food.

Conflict of Interest

The authors declare no conflicts of interest in this article.

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