Characteristics of Retrograded Purple Sweet Potato Flour and Its Physiological Function on Healthy Mice

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Abstract— Modified purple sweet potato flour is very potential to be developed as a main diet for people with obesity and diabetes mellitus. Preparation of modified flour was done by retrogradation processed through partial gelatinization and followed by storage at 5°C for 24 hours to increase resistant starch. The aims of this research were to modified starch of purple sweet potato flour through retrogradation, and then investigate the effect of using purple sweet potato flour with a high content of resistant starch as the main diet on blood sugar level, body and faeces weight healthy mice. The experiment consisted of two treatments: the provision of ration standard on healthy mice, provision of ration with the addition of retrograded purple sweet potato flour on healthy mice. The parameters observed were the blood sugar levels, body weight, and faeces. The results showed that rationing of retrograded purple sweet potato were able to normalize blood sugar levels, maintained the body weight, and increased the feces weight of healthy mice.

Keywords— mice, purple sweet potato flour, retrograded.

I. INTRODUCTION

Local variety of purple sweet potato contains appreciable amount of phenolic anthocyanin [1] which is very potential to be used as sources of food coloring and bioactive compound that benefit to health; however it has not been exploited optimally. Purple sweet potato may be processed in to flour to prolong its shelf life and to widen its uses. The predominant content of the flour is starch. The in vitro digestibility of the starch has been reported to be high which lead to high glycemic index (GI)[2]. This high GI might not be favorable not only to people who suffer from diabetes, and obesity but also healthy people [3]. Therefore it is important to modify the starch properties primarily its resistibility for digestion as well as to preserve the bioactive compound in order to utilize it as novel food ingredients. The aim of this study were to investigate the effect of retrogradation on digestibility and physicochemical properties of the purple sweet potato flour, and to investigate its physiological function on healthy mice.

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II. MATERIALS AND METHODS

A. *Materials*

A-amylase activity of 30U/mg and glucoamylase activity of 70 U/mg were purchased from Sigma (St. Louis, MO, USA), other reagents were purchased from commercial sources. Local variety purple sweet potatoes (PSP), harvested 120 days after planting, were obtained from Seedling Farm, Agriculture Training Center Lampung**.**

B. *Preparation of retrograded purple sweet potato flour*

Freshly harvested PSP were washed under running tap water and grated using a food chopper. The grated PSP was heated in a an electric oven equipped with a single rotary drum to at 90 °C for 30 min, cooled at room temperature, then stored at 5 °C for 48 h. The cold-stored shredded PSP were dried in an oven (Memmert) at 60 °C for 8 hrs to achieve moisture content of 10%, then ground using a hammer mill (FCT Z500, Ramesia) and sieved to pass 80 mesh siever, stored in a sealed plastic bag until further analysis.

Manuscript received August 4th , 2017. (Please write the exact date on which you submitted your paper. Please do not delete this information.) This research was supported by The Ministry of Research , Technology and Higher Education of The Republic of Indonesia for funding this project.

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C. Determination of resistant starch content

The content of RS in the flour was determined according to Association of Official Analytical Chemists (AOAC) methods 2002.02 [4]. The starch in the samples were digested by actions of α-amylase and glucosidase for 16 h at 37°C. The reaction was stopped by adding ethanol 96% and then centrifuged to recover the RS. The RS was dissolved in KOH (2 M) and neutralized with acetate buffer, hydrolyzed with glucosidase, and the resulted glucose, which is a measure of RS was quantified using phenol-sulphuric acid method [5].

D. Determination of total anthocyanin

Total content of anthocyanin was quantified using the colorimetric pH-differential method described by [6] using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 mol/L) (125 mL of 0.2 mol/L KCl and 375 of 0.2 mol/L HCl) and sodium acetate buffer, pH 4.5 (0.4 mol/L) (400 mL of 1 mol/L sodium acetate, 240 mL of 1 mol/L HCl and 360 mL of water) with minor modification . One mL of extracted PSP flour was diluted in 4 mL of each buffer to achieve the absorbance readings at 520 nm or at 700 nm between 0.2 and 0.8. The diluted samples were allowed to stand for 15 min at the dark room before the absorbance was read at 520 and 700 nm using a GENESYS UV-Visible spectrophotometer, with distilled water used as the blank. Anthocyanin pigment concentration is expressed as cyaniidin-3-glucoside equivalents as follows:

$$\text{Anthocyanin pigment (cyanidin-3-glucoside equivalentsmg/L)} = \frac{A \times MW \times DF \times 1000}{\epsilon \times l} \quad (1)$$

Where:

A is the absorbance of the sample calculated as: $(A_{520} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH\ 4.5}$
MW is the molecular weight for cyanidin - 3- glucoside (449.2g/mol).
DF = is the dilution factor
ϵ is the molar absorbance of cyanidin-3-glucoside (26900L/(cm x mol)
l is cell path length (1 cm), and
1000 is the conversion factor from g to mg

E. Scanning Electron Microscope(SEM) analysis

Retrograded PSPF granule micrographs were acquired using FEI SEM type Inspect S50, EDAX AMETEK. Sample powder was placed in a double-sided carbon taped holder, then coated with Au-Pd using sputter coater (Emitech SC7620). The micrographs were obtained with an accelerating voltage of 10.00 kV.

F. Animal Experiments

Six-week old male mice were purchased from the Veterinary Laboratory Center of Lampung. All animal experiments were conducted in accordance with the guideline for animal welfare . Mice were maintained in cages (10 mice in each cage) in an environmentally controlled room at 25±2°C and 70±5% relative humidity with 12 h light/dark for one week, fed with local commercial diet (bought from a local Pet shop) for acclimation. After acclimating, the mice were randomly divided into two groups as follows: the normal control diet and the diet containing retrograded PSPF-treated. Mice in each group were given diet as shown in Table 1. The blood glucose levels were measured from tail vein blood by the Glucose meter, the body weight of the mice were taken for measurement each week for 4 weeks, whereas the faeces were collected for 24 , at day 12, 13 and 14.

Table 1. Composition of the diet

Composition	Diet	
	Control	Diet containing retrograded PSPF
Casein	0. 9	0.19
Cellulose	0.05	0.05
Corn Starch	0.49	-
RS rich PSPF	-	0.49
Corn Oil	0.18	0.18
Sucrose	0.05	0.05
Mineral Mix	0.035	0.035
Vitamin Mix	0.01	0.01

Source: Report of the American Institute of Nutrition Adhoc Committee on Standards for Nutritional Studies (1977) with minor modification

G. Startistical Analysis

Data were presented as the mean ± standard deviation.

III. RESULT AND DISCUSSION

The chemical composition of retrograded PSPF are shown in Table 2,

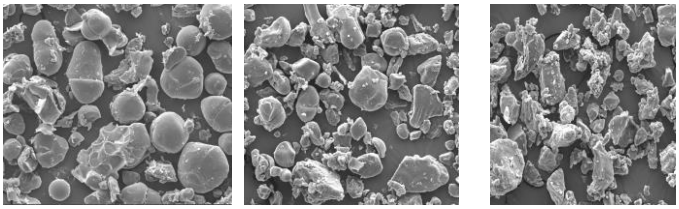
Table 2. The chemical composition of retrograded PSP Flour

Composition	Content*
Moisture (%)	7.20 ± 0.15
Ash (%)	2.20 ±0.32
Protein (%)	2.72 ±0.22
Fat (%)	0.82 ± 0.12
Carbohydrate (%)	87.06 ± 0.45
Total Starch (%)	73,34 ± 0.65
Resistant starch (%)	31.89 ± 0.23
Anthocyanin (mg/100 gram)	78.00 ± 0.40

*Values are means of 3 replications ± std

SEM results

Scanning electron micrographs of native , gelatinized and retrograded PSPF showed structural differences. Native flour contains starch granule which are round, spherical and surrounded by cell wall material (Figure 1a). While in gelatinized PSPF the granular characteristic partly changed (Fig 1b) and become bigger and less structured (Figure 1c), and retrograded PSPF became more compact. The change in granular structure may lead to the changes in starch functional properties and physiology function when starch is used for food non food and pharmaceutical purposes.



a. Original PSP F b. Gelatinized PSPF c. Retrograded PSPF

Figure 1. SEM appearance of PSPFgranule

Blood glucose levels and body weight after acclimation

The functional retrograded PSPF was examined to find its effect on the blood glucose levels of the mice. The retrograded PSPF contained resistant starch 31.8%, whereas the corn starch contained in the standard diet was not detected. During the first three weeks, non-fasting glucose levels in PSPF containing diet were slightly higher compared to those in the standard diet. However, in week 4 and 5, the blood glucose levels in PSPF group were almost 50% lower than those in standard group (Figure. This indicates that retrograded PSPF containing diet may prevent the rise in blood glucose of healthy mice , probably by the mechanism of suppressing insulin resistant development.

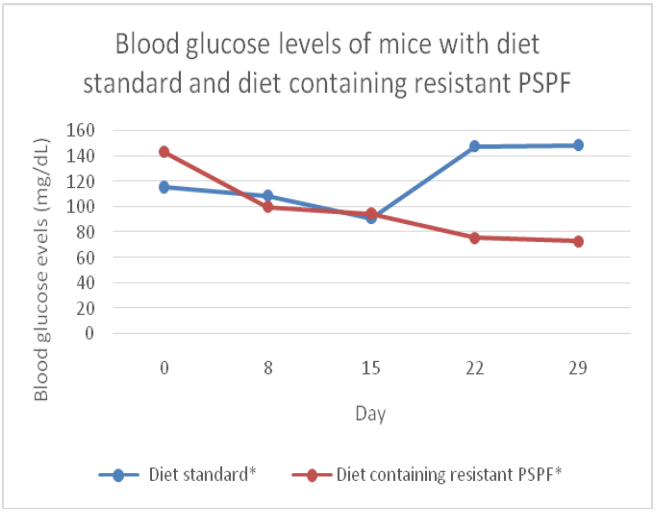


Figure 2. Blood glucose levels of mice fed with diet standard and diet containing retrograded PSPF

Body weight

During the experiment it was observed that the body weight of mice in the two groups decreased from 38.8 g (initially) to 18.3 g at day 29 in the standard group, and from 34.2g to 21.8 g in PSPF group (Figure 3); However the decrease was lower in the PSPF group. This suggest that resistant starch contained in the retrograded PSPF may help stabilize lipid absorbtion in the body.

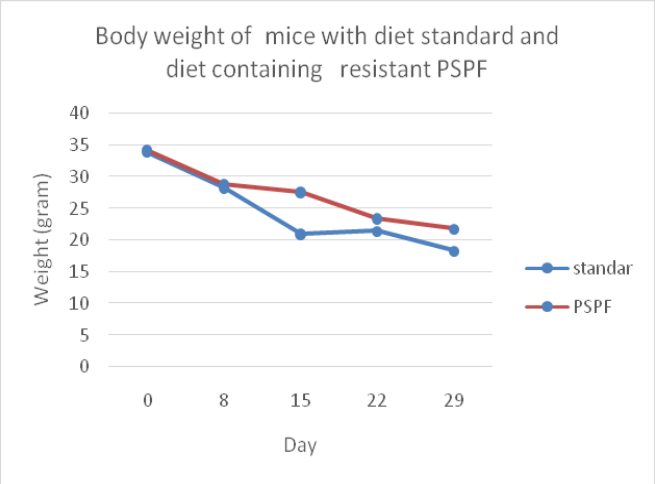


Figure 3. Body weight of mice of mice fed with diet standard and diet containing PSPF

Faeces weight and texture

The results showed that the faeces in standar group was 0,247413 g/ day , whereas those in PSPF group was 0,311393 g/day (Figure 4). This indicates that the inclusion of retrograded PSPF into the diet had increased the faeces as much as 25,86 % . In addition the faeces of the mice in two groups appeared difference. Mice faeces in standard group was soggy , whereas those in PSPF group was more homogen in shape and firmer texture. (Figure 5), this was due to higher fiber content in PSPF compared to those in standard diet.

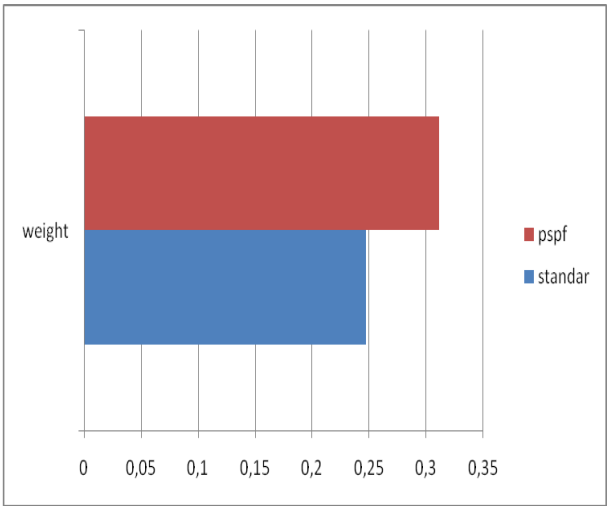


Figure 4. The weight of the mice faeces in diet standard group and diet containing retrograded PSPF.

(a) standard (b) PSPF

Figure 5. Appearance of mice faeces

IV. CONCLUSIONS

Inclusion of retrograded purple sweet potato flour into the diet has the beneficial effects in maintaining blood glucose level, body weight, blood , increase faeces weight. It can be expected that retrograded purple sweet potato flour can be use as novel food ingredients with functional properties.

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