In vivo bioassay of composite flour made of growol and cowpeas (*Vigna unguiculata*) sprout flour for determining the hypoglycemic properties

B Kanetro, D H Swasono and T D Astuti

Food Technology Department, Faculty of Agroindustry, Agrotechnology Department. Faculty of Agroindustry, Accounting Department, Faculty of Economic, University of Mercu Buana Yogyakarta, Yogyakarta, Indonesia

Jl. Raya Wates - Jogjakarta, Karanglo, Argomulyo, Kec. Sedayu, Bantul, Daerah Istimewa Yogyakarta 55752

Email: bayu_kanetro@yahoo.co.id

Abstract. Growol was traditional food that was produced by spontaneous fermentation of cassava in water for 3–5 days. In preliminary research showed that cowpeas sprout protein had hypoglycemic properties. This research was aimed to determine the hypoglycemic properties through in vivo bioassay of composite flour that was made of growol and cowpeas sprout flour by using normal and diabetic Sprague Dawley male rats. There were two of research treatments, that were rat condition and feed treatment. The first treatments (rat condition) were normal rats and diabetic rats which was induced by alloxan injection, and the second treatments (feed) were standard feed and composite flour feed which was prepared by substitution of corn starch in standard feed with the composite flour. The blood glucose of rats were analysed on 3rd, 7th, and 18th days for the treatment and before the treatment as control (0th). The result of this research indicated that the potency of hypoglycemic were shown by decreasing of blood glucose level in diabetic rats with growol treatment. On 18th days treatment, the blood glucose of diabetic rats with standard feed increased and they were still diabetic. While the blood glucose of diabetic rats with composite feed treatment reduced 20% on 18th days after the treatment, although they are still diabetic. The composite flour might decrease blood glucose for more than 18 days feed treatment. That was indicated that the composite flour that was made of growol and cowpeas sprout flour might be used as functional food for type 2 diabetic therapy

Key words: composite flour, growol, cassava, cowpeas sprout, hypoglycemic

1. Introduction

Growol was traditional food from Kulonprogo Yogyakarta Indonesia as staple food providing energy especially in dried climate condition that was high price of rice. Growol was produced by spontaneous fermentation of cassava in water for 3–5 days, and then the fermented cassava were pressed to remove water, formed, steamed, and dried [9]. The same product as growol in the other country was gari or rale that was made of cassava by fermentation using lactic acid bacteria [2]. The protein of growol was lower than rice, so the addition of legumes flour into growol was important to increase protein.
many local legumes in Indonesia, such as cowpeas. Cowpeas may be potential for increasing protein of food, such as growol. In the preliminary research was known that growol with addition of cowpeas sprout flour 30% could increased protein, that was the same as rice [6].

The potency of functional food could be increased by germination. The potency of soybean sprout protein to stimulate insulin secretion was higher than soybean protein [5]. Germination of soybean [12], and rice [15] could increase the potency for decreasing blood glucose. Soybean sprout protein also showed the role as insulin-like protein [13]. The protein of cowpeas sprout contained high of arginine (Arg) that was the same as protein soybean [5]. The specific amino acids such as Leu, Arg, Ala, Phe, Ile, and Lys may influence insulin secretion via a number of possible mechanisms, including generation of metabolic coupling factor, depolarization of the plasma membrane, or enhancement of mitochondrial function [10]. The specific amino acids especially Arg and Leu can activate mitochondrial metabolism in pancreatic β-cell via the tricarboxylic acid (TCA) cycle, resulting in the formation of ATP. The rise in ATP levels leads to closure of ATP-dependent K+ channels, which in turn depolarizes the cell membrane, thus opening of voltage-dependent Ca2+ channels and increasing intracellular Ca2+ concentration, which triggers insulin exocytosis and hence facilitating insulin secretion from pancreatic β-cell [1, 11]. Germinated soybean contained the specific amino acids more than the original seed [8]. In the preliminary research also showed that protein isolate of cowpeas sprout had hypoglycemic properties based on in vivo bioassay by using Sprague Dawley male [7]. Therefore growol with addition of cowpeas sprout might be potential as functional food due to its hypoglycemic component especially Arg, so it was potential as functional food. This research was aimed to determine hypoglycemic properties of the composite flour that was made of growol and cowpeas sprout flour through in vivo bioassay in normal and diabetic rats.

2. Material and methods

The cowpea (*Vigna unguiculata*) were obtained from Beringharjo market in Yogyakarta. Chemical agents, such as alloxan, corn starch, casein, vitamin mix, mineral mix, sucrose, choline bitartat, soy oil, glucose kit (*DiaSys Diagnostic System GmBH & Co*) were purchased from Sigma Chemical Co. The rats were obtained from Animal Experiment Development Unit, Gadjah Mada University, Yogyakarta. The sequences of experimental activities were performed as follows:

2.1. Preparation of cowpeas sprout and growol flour

Cowpeas seeds were soaked for 8 h, and then germinated for 36 h. The germinated cowpeas were dried and milled to make flour [5]. The cowpeas sprout flour were prepared for increasing protein of growol. Growol was made of cassava through many step process according to Luwihana [9]. The first step, cassava was peeled, washed, and size reduced. After that cassava was fermented by soaking in water for 5 days. Then the fermented cassava are pressed to remove water, mixed with 30% cowpeas sprout flour, formed, steamed, dried and milled. The dried product was called composite flour that was prepared to in vivo bioassay.

2.2. In vivo bioassay

The *in vivo* bioassay was done to determine the potency of hypoglycemic of composite flour by using 24 *Sprague Dawley* male rats. The experiment sequences of the steps were adaptation of rats for 3 days, divided rats into 4 groups, treated rats for 18 days with the condition of rat and feed treatments, and analysed the blood triglyceride, cholesterol total, High Density Lipoprotein (HDL) cholesterol, Low Density Lipoprotein (LDL) cholesterol and glucose for the treatment of rats on 3rd, 18th days and before treatment as control (0th). The experimental design of this research was randomized complete design with 2 factors. The first factors were rat condition treatments, that were normal rats and diabetic rats which was induced by alloxan injection resulted the type 2 diabetic rats. The second factors were feed treatments, that were standard feed according to AIN–93 [14] and composite flour feed which was prepared by substitution of corn starch in standard feed with the composite flour. The data of this experiments was statistical analysed by Anova (analysis of varian) and DMRT (Duncan Multiple Range
Test). The in vivo bioassay of this research had passed ethical clearance that was approved by Center Research Laboratorium of Gadjah Mada University, Yogyakarta, Indonesia.

3. Result and discussion

3.1. Rat body weight

The data of rat body weight during the experiment are shown in Table 2. The data showed that there were significant different between the body weight of normal rats and diabetic rats, but at the end of experiment the body weight of normal and diabetic rats with composite flour feed treatment showed not significantly different. During the experiments normal rat body weight increased, while diabetic rat body weight decreased especially in standard feed treatment. The body weight of diabetic rats in composite flour feed treatment increased slightly.

| Rats condition | Feed treatment  | 0th days  | 3th days  | 18th days  | %weight gain (+) / %weight loss (-) of rat |
|----------------|-----------------|-----------|-----------|------------|------------------------------------------|
| Normal         | Standard        | 201.36 b  | 204.43 b  | 220.82 b   | +9.66                                    |
|                | Composite flour | 202.03 b  | 205.38 b  | 224.23 b   | +10.99                                   |
| Diabetic       | Standard        | 195.17 a  | 189.24 a  | 179.77 a   | -7.89                                    |
|                | Composite flour | 199.92 b  | 193.19 a  | 203.24 b   | +1.66                                    |

*) The same notation of statistic in the table showed not significantly differences at the same column

The percentage of weight gain and weight loss in in rat body weight on day 18th (end of treatment) to 0th (before treatment) were shown in Table 2 which indicated that in the body weight of diabetic rats with composite flour feed treatment was relatively stable that increased 1.66%. While the body weight of diabetic rats with standard feed treatment decreased 7.89%. This data indicated that the feeding of composite flour was expected to improve the condition of diabetic rats become normal, because of the potential of cowpea sprout protein as functional foods that was contained in composite flour. This phenomena described in the following discussion.

3.2. Glucose

Table 2 showed that blood glucose level of all rats were 73.7–76.9 mg/dL. This indicated that all rats were normal or not diabetic. The glucose level of human diabetic condition was higher than 180 mg/dL, while the glucose level of rat diabetic condition was higher than 109 mg/dL [3]. The glucose level after alloxan injection at 3th days increased significantly and these rats were type 2 diabetics.

| Rats condition | Feed treatment  | 0th days  | 3th days  | 18th days  | 3th days  | 18th days  | 3th days  | 18th days  | %weight gain (+) / %weight loss (-) of rat |
|----------------|-----------------|-----------|-----------|------------|-----------|------------|-----------|------------|------------------------------------------|
| Normal         | Standard        | 73.72 a   | 76.46 a   | 76.78 a    | 73.72 a   | 76.46 a    | 76.78 a   | 73.72 a    | +9.66                                    |
|                | Composite flour | 77.75 a   | 78.34 a   | 78.03 a    | 77.75 a   | 78.34 a    | 78.03 a   | 77.75 a    | +10.99                                   |
| Diabetic       | Standard        | 76.92 a   | 225.00 b  | 230.44 b   | 76.92 a   | 225.00 b   | 230.44 b  | 76.92 a    | -7.89                                    |
|                | Composite flour | 74.04 a   | 224.96 b  | 178.83 ab  | 74.04 a   | 224.96 b   | 178.83 ab | 74.04 a    | +1.66                                    |

*) The same notation of statistic in the table showed not significantly differences at the same column

The potency of hypoglycemic were shown by decreasing of blood glucose level in diabetic rats with composite flour treatment. On 18th days treatment, The blood glucose of the diabetic rats with standard feed increased and they were still diabetee. While the blood glucose of diabetic rats with composite flour feed treatment reduced 20% on 18th days after the treatment, although they are still diabetic. The composite flour might be potential to decrease blood glucose for more than 18 days composite flour
feed treatment. That was indicated that the composite flour that was made of growol and cowpeas sprout flour might be used as functional food to decrease blood glucose for type 2 diabetic therapy.

4. Conclusion

The potency of hypoglycemic was shown by decreasing of blood glucose level in diabetic rats with composite flour treatment. This result indicated that composite flour that was made of composite flour with addition of cowpeas sprout might be used to prevent diabetic. So the composite flour that was made of growol and cowpeas sprout could be potential as functional food.

References

[1] Argmann C and Auwerx J 2006 Insulin secretion: SIRT 4 gets on the act Cell 26 837–9
[2] Eduardo M, Svanberg U, Oliveira, J and Ahrné, L 2013 Effect of cassava flour characteristics on properties of cassava-wheat-maize composite bread types Int. J. Food Sci. 305 407–10
[3] Garrison R 2013 Normal rat blood glucose level http://www.ehow.com/facts_5990203_normal-rat-blood-glucose-level.html[25/9/2012]
[4] Kanetro B, Noor Z, Sutardi and Indrati R 2008 Potency of soybean sprout protein to stimulate insulin secretion of pancreas in normal and diabetic rat Agritech J Teknologi Pertanian 28 50–7
[5] Kanetro B and Dewi S H C 2013 Effect of various local legume sprouts as raw materials of meat analog on the physical (texture), preference and arginine/lysine ratio characteristics Agritech J Teknologi Pertanian 33 1–7
[6] Kanetro B and Luwihana S 2015 Proximate composition and lactic acid bacteria of the best oyek from the treatment of cowpeas (Vigna unguiculata) addition based on its preference Agritech J Teknologi Pertanian 35 261–5
[7] Kanetro B 2015 Hypocholesterolemic properties of protein isolate from cowpeas (Vigna unguiculata) sprout in normal and diabetic rats Procedia Food Sci. 3 112–8
[8] Kanetro B 2018 Amino acid profile of soybean (Glycine max) sprout protein for determining insulin stimulation amino acids Int. Food Res. J. 25 2497–2502
[9] Luwihana S 2011 Change of chemical properties in process of composite flour from cassava Proc. PATPI Conf. (Manado)
[10] Newsholme P, Brennan L and Bender K 2006 Amino acid metabolism, β-cell function, and diabetes Diabetes 55 S 39–47
[11] Newsholme P, Brennan L and Bender K 2007 Amino acid metabolism, insulin secretion, and diabetes Biochem. Soc. Trans. 35 1180–6
[12] Pathak M 2005 Soaked and germinated Glycine max (soybean seeds) is highly effective blood sugar regulator Nat. Prod. Radiance 5 405–9
[13] Pathak M and Martirosyan D M 2011 Immunodetection and quantification of insulin-like antigens in sprouts: development of an efficient functional food Funct. Foods Health Dis. 1 492–507
[14] Reeves P G, Nielsen F H and Fahey G C 1993 AIN-93 purified diets for laboratory rodents: final report of the American institute of nutrition ad hoc writing committee on the reformulation of the ain-76 a rodent diet J Nutr. 123 1939–51
[15] Usuki S, Ito Y, Morikawa K, Kise M, Ariga T, Rivner M and Yu R K 2007 Effect of pre-germinated brown rice intake on diabetic neuropathy in streptozotocin-induced diabetic rats Nutr. Metab. 4 25–31