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Hypoglycemic Activity of Water Soluble Polysaccharides of Yam (Dioscorea hispida Dents) Prepared by Aqueous, Papain, and Tempeh Inoculum Assisted Extractions

Teti Estiasih, Harijono, Weny Bekti Sunarharum, Atina Rahmawati

***Abstract*—**This research studied the hypoglycemic effect of water soluble polysaccharide (WSP) extracted from yam (Dioscorea hispida) tuber by three different methods: aqueous extraction, papain assisted extraction, and tempeh inoculums assisted extraction. The two later extraction methods were aimed to remove WSP binding protein to have more pure WSP. The hypoglycemic activities were evaluated by means in vivo test on alloxan induced hyperglycemic rats, glucose response test (GRT), in situ glucose absorption test using everted sac, and short chain fatty acids (SCFAs) analysis. All yam WSP extracts exhibited ability to decrease blood glucose level in hyperglycemia condition as well as inhibited glucose absorption and SCFA formation. The order of hypoglycemic activity was tempeh inoculums assisted- >papain assisted- >aqueous WSP extracts. GRT and in situ glucose absorption test showed that order of inhibition was papain assisted- >tempeh inoculums assisted- >aqueous WSP extracts. Digesta of caecum of yam WSP extracts oral fed rats had more SCFA than control. Tempeh inoculums assisted WSP extract exhibited the most significant hypoglycemic activity.

***Keywords*—**hypoglycemic activity, papain, tempeh inoculums, water soluble polysaccharides, yam (*Discorea hispida*)

I. Introduction

THE genus *Dioscorea* or yam, which includes 600 to 700 species, is widely distributed throughout the world [1]. *Dioscorea hispida* or locally known as "gadung" is one of the *Dioscoreaceae* family, a family of creep plants that contains several bioactive compounds. *Dioscoreaceae* (*D. alata, D. batatas, D. bulbilfera, D. opposita*) has health beneficial compounds such as dioscorin [2, 3, 4], diosgenin [5, 6, 7], and water soluble polysaccharides (WSP) [8].

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Dioscorin is the major storage protein in yam and functions against angiotensin, which converts enzyme to cause hypertension [9]. Diosgenin is used in making progesterone and other steroid drugs [10]. Recently, the biological activities of the polysaccharides of yam have attracted increasing attention in the biochemical and medical fields [8].

Water soluble polysaccharide of yam is a viscous mucilage containing glycoprotein [1, 11, 12]. Yam contains a large amount of water-soluble mucilage [1]. It has been reported that yam mucilage consists of glycoproteins and polysaccharides such as mannan and cellulose [13, 14].

Water soluble polysaccharides of *Dioscorea batata* consisted of acetylated mannan [8], viscous polysaccharides from wild yam consisted of mannose, arabinose, glucose, galactose, xylose and rhamnose [15] that attributed to soluble dietary fiber, meanwhile polysaccharides of *Dioscorea opposite* Thunb consisted mainly of mannose, and also contained uronic acid [16].

A high dietary fiber intake is emphasized in the recommendations of most diabetes and nutritional associations. It is accepted that viscous and gel-forming properties of soluble dietary fiber inhibit macronutrient absorption and reduce postprandial glucose response that associated with reduced diabetes risk [17]. Colonic fermentation of dietary fiber produces short chain fatty acids (SCFAs) such as acetate, propionate, and butyrate [18]. Various short-chain fatty acids formed during fermentation depended on the specific carbohydrate as substrate, and had differences in physiological effects [19]. Some studies showed that WSP had hypoglycemic effect such as WSP. Nevertheless, there is no study to examine hypoglycemic activity of WSP from *Dioscorea hispida* tuber.

However, WSP of yam usually binds to protein and the existence of this protein is supposed to reduce the hypoglycemic activity. For this reason, we studied three extraction methods, namely aqueous, papain assisted, and tempeh inoculums assisted extraction to obtain crude WSP extract from *Dioscorea hispida*. The two later methods were aimed to partially remove protein from WSP. Papain is a proteolytic enzyme from papaya tree latex and widely used in food processing to hydrolyze protein. Meanwhile, tempeh inoculums consist of proteolytic microbes, *Aspergillus oryzae* and *Rhizopus oligosporus* that are supposed to hydrolyze WSP binding protein of *Dioscorea hispida*.

Tempeh is an Indonesian traditional fermented soybean that involved proteolytic activity of these two molds during fermentation. Although proteolytic activities of papain and tempeh inoculums were not specific, applying both during WSP extraction was supposed to get more pure WSP and have more significant effect on hypoglycemic activities.

II. Materials and Methods

A. Materials

Mature yam *Dioscorea hispida* tuber was obtained from local farmers, commercial crude papain and commercial tempeh inoculums were from local market. Other materials were SCFA standards, sugar standards (Sigma Co.), chemical reagent (analytical grade), alloxan monohydrate (Merck) glucose GOD FS, AIN 93M diet [20], white rats (*Rattus novergicus*) Wistar strain 2-3 month old and body weight of 131-239 g.

B. WSP Extractions

WSP extraction was conducted with three different methods, those were aqueous, papain assisted, and tempeh inoculums assisted extraction [21]. The preparation to obtain tuber slurry was done in similar way. Yam tuber was peeled and blanched by steaming, and then the tuber was crushed with water to get tuber slurry. The tuber slurry was sieved by sieving cloth and filtrate was added by papain for papain assisted extraction method, or tempeh inoculums for tempeh assisted extraction method. Meanwhile, aqueous extraction method was performed without any addition. The filtrate was incubated at 55°C for papain assisted extraction, meanwhile aqueous and tempeh inoculums assisted extractions were performed at ambient temperature. Starch separation was performed by centrifugation. Then, WSP was coagulated by solvent and dried in cabinet dryer. All WSP extracts were analyzed including free sugar composition by HPLC, protein by Kjedahl method, crude fiber, amylose according to AOAC methods [22], relative viscosity at concentration of 100 mg/100 mL at ambient temperature, and yield. Fresh tuber was analyzed for proximate, amylose, and HCN content.

Sugar analysis of polysaccharides was conducted as followed: About 10 g of WSP extracts was added by acetonitrile 80% and stirred until homogeneous. This solution was filtered by filter paper and evaporated to 1 mL of volume, and then filtered by millex 0.45 µm. Analysis was performed using sugar pack column at flow rate of 0.6 mL/min at 65°C, water as eluting solvent with refractive index detector.

C. Hypoglycemic Activity Assay

Hypoglycemic activity assay was referred to method of Ruzaidi et al. [23]. This experiment used 24 white rats (*Rattus novergicus*) strain Wistar. These rats were housed collectively at 20-25°C and were fed by AIN 93-M standard feeding *ad libitum*. Before experiment, the rats were adapted for a week. The treatment was conducted for 4 weeks. The rats were weighed every 3 days, and glucose blood level was analyzed every weeks.

Rats were divided into 4 groups, 3 groups were treated by three types of WSP extracts and one group was control. Each group consisted of 6 rats. The feed was referred to AIN 93 M [20] that consisted of 62.0692% maize starch, 14% casein, 10% sucrose, 4% soybean oil, 5% carboxy methyl cellulose (CMC), 3.5% AIN mineral mix, 0.18% L-cystine, 1.0% AIN vitamin mix, 0.25% choline bitartrate, and 0.0008% TBHQ. Yam WSB was fed orally for 28 weeks with dose of 400 mg/kg body weight. All rat groups were induced intraperitoneally to be hyperglycemia by alloxan 80 mg/g body weight 3 days before treatment. Only rats with blood glucose level of >126 mg/dL were used in this experiment.Every week, blood was taken retro orbital plexus after 16 hours fasting and analyzed for post-prandial blood glucose level. Blood sample was centrifuged at 4000 rpm and the supernatant was analyzed for blood glucose level that referred to glucose oxidase method [24]. This experiment procedure had been approved for ethical clearance from Veterinary Department – University of Brawijaya.

D. Glucose Response Test

Four group (control, treated by aqueous-, papain assisted-, and tempeh inoculums assisted-WSP extracts) of rats consisted of 3 normal rats were adapted to AIN 93-M feed for 3 days. Before blood withdrawn, rats were fasted for 16 hours and each rat was force fed by glucose at dose of 2 g/kg body weight and WSP extracts of 400 mg/kg body weight. This procedure followed method of Xie et al. [25] with some modification. Serum glucose concentration was analyzed at minutes 0, 30, 60, 90, and 120 after oral feeding of glucose and WSP extracts.

E. In Situ Glucose Absorption Test

At day 33th of experiment, rats were anesthetized and gut was withdrawn and reversed. Everted sac was immersed at WSP extract concentration of 10% and glucose concentration of 20%. Glucose absorption test was performed in a tube containing WSP extract, glucose, and everted sac. Physiological salt was poured into inner cavity of everted sac.

The tube was placed at water bath shaker that have temperature of 36°C. Solution from inner everted sac was taken every 10 minutes at minutes 10, 20, 30, and 40. Glucose concentration of solution was analyzed by Nelson-Somogyi method.

F. SCFA Analysis

During surgical, caecum was taken and digesta was withdrawn for SCFA analysis. Digesta was centrifuged at 14000 rpm for 15 minutes. Supernatant of 1 µL was injected into gas chromatography (GC Shimadzu Seri GC 8), with column GP 1200 1% HPP30 on chromosorb waw 2 m in length, column temperature of 130°C, injector and detector temperature of 230°C. Carrier gas was N_2 with pressure of 1.25 kg/cm^2.

G. Data Analysis

Data was analyzed using analysis of variance in nested experimental design, followed by Duncan Multiple Range Test.

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TABLE I
CHARACTERISTICS OF FRESH YAM (*Dioscorea hispida*) AND WATER SOLUBLE POLYSACCHARIDE (WSP) EXTRACTS OF YAM

Components	Fresh yam	Aqueous WSP Extract	Papain Assisted WSP Extract	Tempeh Inoculum Assisted WSP Extract
Yield (% db)	n.a.	9.15	12.02	11.05
Amylose (% db)	0.64	18	32	28
Protein (% db)	1.25	1.97	2.33	1.21
Crude Fiber (% db)	4.04	1.49	1.42	0.72
Free sugar				
• Glucose (ppm)	n.a.	101.10	840.29	1924.62
• Mannose (ppm)	n.a.	109.91	872.14	344.08
Water (% db)	80.23	n.a.	n.a.	n.a.
Ash (% db)	2.70	n.a.	n.a.	n.a.
Fat (% db)	0.57	n.a.	n.a.	n.a.
Carbohydrate (*by difference,* % db)	20.18	n.a.	n.a.	n.a.
Viscosity (cps)	n.a.	0.096	0.094	0.094
HCN (ppm)	19.81	16.06	2.29	6.88

n.a.=not analyzed

III. RESULTS AND DISCUSSIONS

A. Characteristics of Yam WSP Extracts

Characteristics of fresh yam tuber and all WSP extracts were shown at Table 1. WSP extract yield was about 9-12% that higher than the yield of Gembili (*Dioscorea esculenta*) WSP (3-5%) [26]. Enzyme and microbial assisted extraction using papain and tempeh inoculum slightly increased the yield that it was supposed due to partially removing of WSP binding protein.

Amylose content of WSP extracts was higher than fresh yam tuber. Amylose was an impurity in WSP extracts. *Dioscorea* starch has gelatinization temperature of 67-79°C [27] that during incubation at temperature of 55°C at papain asisited ESP extraction, some starch granules were supposed to gelatinize that increase their solubility and some concomitantly coagulated during WSP separation by coagulation. Protein content of WSP extracts was higher than that of fresh yam except tempeh inoculums assisted WSP extract. Protein in yam is a glycoprotein that binds to polysaccharides.

During WSP extraction, protein was concomitantly extracted. It was expected that applying papain and tempeh inoculums during WSP extraction resulted more pure WSP extract including lower protein content.

Papain is a proteolytic enzyme that expected to partially hydrolyze WSP binding protein, however higher protein content in papain assisted extracts compared to fresh yam was supposed to relate to papain contamination of WSP extract.

Meanwhile, tempeh inoculums assisted WSP extract had slightly lower protein content than others due to proteolytic activity of tempeh molds although the decrease of protein content was not significant. Mold growth during fermentation produced biomass that contained protein [28]. The lowest crude fiber was found in tempeh inoculums assisted WSP extract.

Tempeh inoculums beside has proteolytic activity also has amylolytic and cellulolytic activity [29, 20]. Previous study showed that *Rhizopus oligosporus*, one of tempeh inoculums mold, decreased crude fiber in rapeseed cake [31]. Free sugar of WSP extract consisted of glucose and mannose. Fu *et al.* [11] reported that mucilage of *Dioscorea* species of Keelung and Hualien No. 3 comprised of mannose (93.0-95.40%), meanwhile glucose, galactose, arabinose, and xylose were lower than 5%. Viscosity of all of WSP extracts was not significantly different. Slightly lower viscosity of papain assisted and tempeh inoculum assisted WSP extract was supposed due to partially liberation of protein. Cyanide (HCN) content decreased that due to solubility of free cyanide in the water during extraction. The lowest cyanide content was found at papain assisted WSP extract. Applying higher temperature of 55°C during incubation in extraction process might contribute to HCN vaporization.

B. Hypoglycemic Activity of Yam WSP Extracts

Yam WSP extracts had hypoglycemic activity that indicated by decreasing blood glucose level. Sharp decrease in blood glucose level was found in tempeh inoculums assisted WSP extract, followed by papain assisted WSP extract and aqueous extract (Fig. 1).

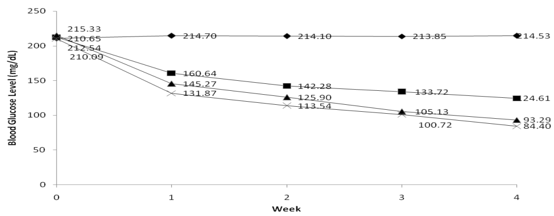
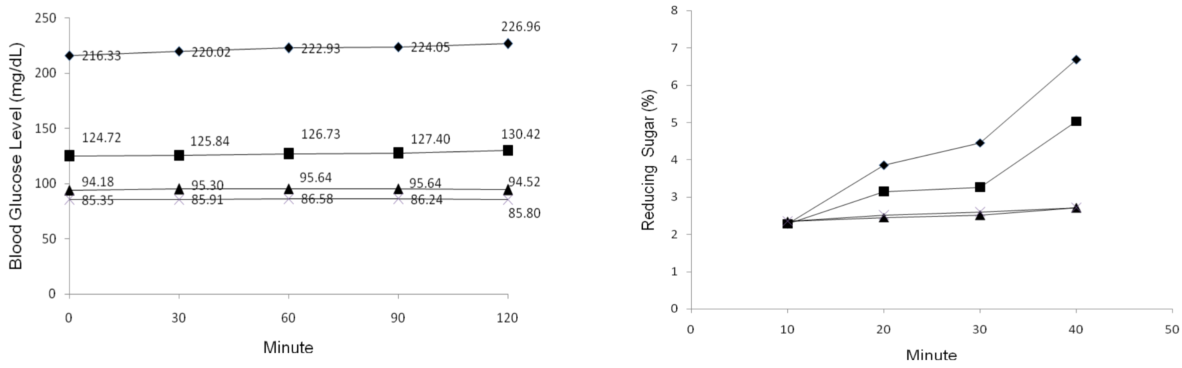


Fig. 1 Blood glucose level of rats treated by aqueous WSP extract (■), papain assisted WSP extract (▲), and tempeh inoculum assisted WSP extract (X) compared to untreated/control group (◆)

After three weeks treatment, hyperglycemic rats had normal blood glucose level for treatment by papain assisted WSP extract and tempeh inoculums assisted WSP extract, meanwhile aqueous WSP extract did not reach normal blood glucose level for 4 weeks treatment.

Dietary fiber could decrease postprandial glucose because their viscous and gel forming properties that inhibits macronutrient absorption [17]. Gel structure was able to entrap glucose and other nutrient that slowed down absorption. However, there is no significant viscosity difference (Table I) of all WSP extracts.



(a) (b)

Fig. 2 Glucose absorption inhibition in glucose response test (a) and *in situ* glucose absorption using everted sac (b). Aqueous WSP extract (■), papain assisted WSP extract (▲), tempeh inoculum assisted WSP extract (X), and untreated/control group (◆)

Viscosity analysis was conducted at room temperature and the condition was different to that found in digestive tract. Other mechanism might be responsible to blood glucose level decline after yam WSP extracts feeding.

The purity of extract might contribute to the ability of yam WSP extract in decreasing blood glucose level. We supposed that actually protein bound WSP in tempeh inoculums assisted WSP extract was the lowest, followed by papain assisted WSP extract. Both were more effective in decreasing blood glucose level than aqueous WSP extract.

It was interesting that tempeh inoculums assisted WSP extract had the highest ability in decreasing blood glucose level. In accordance, fermentation of Chinese yam (*Dioscorea batatas* Decne) flour by *Lactobacillus acidophilus, Streptococcus thermophilus*, and *Bifidobacterium bifidus* improved postprandial glucose [32]. Acidic hydrolysis of water soluble polysaccharides of *Tremella aurentia* decreased glucose plasma level due to formation of bioactive compounds [33].

Bioactive compounds formation such as aglycone isoflavones during tempeh fermentation depended on the substrates [34]. Bioactive compounds in *Dioscorea* were dioscorin [2, 3, 4], diosgenin [5, 6, 7], and WSP [8]. It was supposed that bioactive formation occurred during tempeh assisted WSP extraction that contributed to blood glucose level decline. Yam tuber contains diosgenin, a steroidal saponin that undergoes transformation into sapogenin [35]. Saponin in fenugreek (*Trigonella foenum*) could decrease blood glucose level [36]. Fermentation of *Dioscorea* by red mold *Monascus purpureus* NTU 568 resulted in more potent hypocholesterolaemic effect that supposed due to bioactive formation *monacolin* K [37]. Mc Cue et al. [38] reported that all of the soybean extracts possessed marked anti-amylase activity, with extracts of *R. oligosporus*-bioprocessed soybean having the strongest inhibitory activity, but only slight anti-glucosidase activity.

There was a synergistic effect of tempeh inoculums and soybean as a substrate in hypoglycemic activity. Hence, it was possible that during tempeh inoculums assisted WSP extraction of yam *Dioscorea hispida* some bioactive compounds formed and contributed to hypoglycemic activity. This needed further research.

C. Glucose Response and In Situ Glucose Absorption Inhibition

Glucose response test showed that yam WSP extract was able to inhibit glucose absorption. This test revealed absorption after glucose ingestion. Glucose absorption inhibition was in order of papain assisted WSP extract>tempeh inoculums assisted WSP extract>aqueous WSP extract (Fig. 2a). Sharp rise in glucose blood level after glucose ingestion was found in untreated or control rats group. Similar results were found in *in situ* absorption test by using everted sac (Fig. 2b). Both methods indicated glucose absorption although both had differences. Glucose response test revealed glucose absorption inhibition in digestive tract and it did not indicate the effect of yam WSP extract in long time consumption such as SCFA formation. Meanwhile *in situ* glucose absorption test showed the ability to inhibit glucose absorption pass through intestines.

Papain assisted WSP extract showed the highest inhibition of glucose absorption either in GRT and in situ absorption test (Fig. 2ab). This was supposed due to the purity of WSP extract. More pure WSP extract, impurities that could interference polysaccharides action in glucose absorption inhibition was lower. Papain is a proteolytic enzyme that was supposed, to some extent, partially hydrolyze WSP binding protein. Similar reason was applied to tempeh inoculums assisted WSP extract, although its glucose absorption inhibition was lower compared to papain assisted WSP extract.

It was supposed that glucose absorption inhibition was related to viscosity of WSP extracts, although all extract showed almost similar viscosity. Weak gel formation of yam WSP extract contributed to glucose absorption inhibition. Viscosity in digestive tract of each extract might be different. It was indicated by significant effect of types of extract on glucose absorption inhibition (α=0.05) meanwhile *in situ* glucose absorption inhibition was not affected by WSP extract types. Type of polysaccharides affected inhibition of glucose absorption. Polysaccharides of yam WSP mainly consisted of glucose and mannose or glucomannan. Previously, Chearskul et al. [39] showed that glucomannan taken before performing the oral glucose tolerance test can lower the rise of blood glucose.

Similarly, Vuksan et al. [40] showed that glucomannan fiber added to conventional treatment may ameliorate glycemic control.

Dietary fiber decreases nutrition diffusion for absorption by intestinal mucosa thus decreases blood glucose level. Soluble dietary fiber was able to form gel in the existence of water in intestines. Gel formation slowed down the gastric emptying, fastened transit time, and controlled nutrition absorption. This affected glucose absorption and glycemic index of food products [18]. Previous research [41] showed that intake of diet containing cotton dietary fiber for diabetic patients resulted in lower rise of glucose compared to non cotton dietary fiber diet. Also, dietary fiber shortened transit time and bulk feces weight.

Furthermore, Madar et al. [41] revealed that physical properties of dietary fiber affected its ability in glucose absorption inhibition. Tempeh inoculum assisted WSP extract might be have different physical properties to papain assisted WSP extract. Tempeh inoculum contains *Aspergillus oryzae* and *Rhizopus oligosporus* that has proteolytic activity, as well as amylolytic and cellulolytic activities [29, 30]. Some WSP could be hydrolyzed by cellulolytic activity of tempeh inoculums. Therefore, physical properties of this soluble dietary fiber might be change that caused lower glucose absorption inhibition than papain assisted WSP extract.

D.Short Chain Fatty Acids

Not all the carbohydrate in the diet is digested and absorbed in the small intestine, and a significant portion arrives in the large bowel, where it is fermented by bacteria naturally present [18]. Dietary fiber could influence metabolism and SCFA production in colon [42]. Because fermentation in the hindgut may be elevated several hours after feeding, SCFA may be involved in the longer-term feeling of satiety [43]. Types of WSP extracts produced different composition of caecum SCFA (Fig. 3). In general, caecum of untreated rats had lower SFCA concentration then WSP extracts fed rats except aqueous WSP extract. Papain assisted WSP extract had the lowest SCFA concentration. This extract was supposed to be more pure than tempeh inoculums assisted WSP extract. Tempeh inoculums might be partially hydrolyze yam WSP due to cellulolytic acitivity. Meanwhile, in papain assisted WSP extraction, papain hydrolyze only protein. The purity of WSP extract perhaps affected ability to arrive in large bowel and fermentability in colon.

Certain indigestible oligosaccharides may benefit to gastrointestinal tract health via fermentation and proliferation of desirable bacterial species. Type of oligosaccharides influenced SCFA production [44]. Physicochemical properties of dietary fibers (DF) may influence their fermentation characteristics [45]. The physiological effects of these carbohydrates depend upon several factors including the extent of colonic fermentation and the fermentation products formed [46, 47]. The end products of colonic fermentation are SCFA and gases (CO_2, CH_4, and H_2) [45].

Mannose and glucose were the main sugar coumpounds of yam polysaccharides (Table I). Glucomannan is one of fermentable polysaccharides [48].

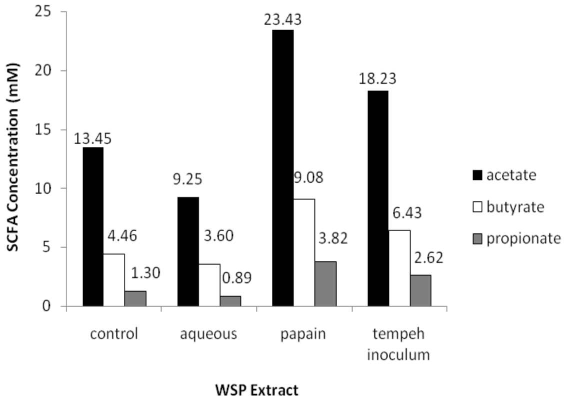


Fig. 3 Short chain fatty acids of caecum digesta of rats fed by WSP extracts

Chen et al. [49] showed that fermentation of konjac glucomannan resulted in greater fecal acetate, propionate and butyrate concentrations and lower fecal pH. Predominant SCFA in caecum digesta was acetate, followed by butyrate and propionate. According to Lunn and Buttriss [18], all of these three SCFAs was predominant SCFA in colon Concentration of each SCFA was vary depending on fermented polysaccharides. In general, acetate was the highest abundance SCFA in digestive tract, and butyrate was the lowest. This research showed that *Dioscorea hispida* or yam WSP produced more butyrate rather than propionate. SCFA production is beneficial to health because of reducing hepatic glucose production [17]. Furthermore, increasing SCFA concentration in vena porta activates hepatic AMPK (*activated protein kinase*). APMK has a function to regulate energy production in cells and metabolic homeostatic regulation [50].

Nevertheles, not only fermentabililty of WSP contributed to blood glucose level reduction, but also some metabolic effects such as insulin sensitivity, hormone secretion modulation in digestive tracts, and other various related metabolism [17].

IV. Conclusions

WSP from *Dioscorea hispida* was hypoglycemic polysaccharides that able to reduce blood glucose level in hyperglycemia condition. Mechanisms of blood glucose level decline were glucose absorption inhibition and SCFA formation.

Extraction methods in WSP extract preparation affected blood glucose level reduction. Tempeh inoculums assisted WSP extract had highest activity in reducing blood glucose level, meanwhile the highest glucose absorption inhibition was found in papain assisted WSP extract. It was supposed that there was a synergism between WSP extract and tempeh inoculums in reducing blood glucose level.

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