Physicochemical characterization of water-soluble polysaccharide of *Pachyrhizus erosus* L. with fermentation assisted extraction method

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Abstract. Water-soluble polysaccharide (WSP) is one of a complex chemical content contained in *Pachyrhizus erosus* L. (PeL). A recent study was examined the effect of FAEM with aqueous and Saccharomyces sp. In this study used a completely randomized design with two factors i.e. water extraction with extracting water addition on the amount of starch used (1:0.5; 1:1; 1:1.5; 1:2) and FAEM with distilled water, *Saccharomyces sp* and *Rhizopus sp*. Interestingly, dextrose equivalent of WSP-PeL (DE = 4) showed increased significant effect of FAEM with *Rhizopus sp* than both distilled water and Saccharomyces sp. Although degree of polymerization and total sugar just showed significant effect, WSP-PeL (DP_{av} = 24) has a good solubility (S_O = 83%) and viscosity (μ = 0.973 cP). These findings suggest that due to the viscous properties of WSP-PeL can inhibits the absorption of excessive macronutrient and decrease blood sugar levels, derived from fermentation WSP-PeL in the colon to produce Saturated-Chain Fatty Acid (SCFA).

1. Introduction

The progressive research of food science and technology has developed rapidly by encouraging the interest in nutraceuticals and functional foods to identify properties and potential applications [29]. The principal reasons for the growth of the functional food market are current population and health trends. The Indonesia National Agency of Drug and Food Control (BPOM RI) has issued basic provisions on functional food as a processed food, safe for consumption that contained one or many bioactive compounds with a specific physiological function and proven to have health benefits [3]. Indonesia as one of the most populous country in the world has abundant natural resources, the various kinds of bioactive components are capable of producing secondary metabolites for functional foods. Forms of secondary metabolites such as antioxidants, antimicrobials, antibiotics, antitumor and anticancer [19].

Bengkoang (*Pachyrhizus erosus* L. (PeL)) as one of horticultural products has grown spread out in Indonesia [23]. PeL is an underutilized crop of family Leguminosae, subfamily Papilionoidea. The underground starchy root of PeL is one of the most popular edible root vegeTables that grow in tropical regions like Indonesia. PeL is brown-skinned, white-fleshed, crispy and juicy with an irregularly globular shape. Both cooked and uncooked PeL can be eaten in many kinds of dishes [12]. The edible of PeL contained fairly large amounts of carbohydrates and crude fibres [13], consists of

82.0 % of water, 14.9 % of carbohydrates, 1.2 % of proteins, 0.1 % of lipids and 1.4 % of crude fibre [20]. Sweet flavour of PeL comes from non-digestible oligosaccharides including inulin as soluble fibres [1, 22]. The soluble fraction is associated with the reduction of cholesterol in blood and the decrease of glucose absorption by the small intestine. Although soluble fibres are less common in foods than insoluble fibres, it is believed to have important effects in the digestive and absorptive processes [5, 21].

Soluble dietary fibre presents a potential prebiotic character [8]. Water-soluble polysaccharide (WSP) is a non-digestible carbohydrate belonging oligosaccharides. WSP is not digested in the small intestine, but reaches the large intestine, where it is fermented by bacteria such as bifidobacteria and lactobacilli, which producing short-chain fatty acids (SCFA) such as acetic, propionic and butyric acids [10]. A propionic acid may affect the glucose metabolism, which able to reduce the level of blood glucose [16; 29; 27]. According to [14], a high intake of fiber is recommended for diabetic patients. Butyric acid is the primary energy source, which able to strengthen the body defense, providing protection against colon cancer [9, 11]. [25] said that the WSP of *Polyporus albicans* mycelial are as immunostimulant and antitumor. This study was carried out to investigate physicochemical characteristic of WSP-PeL with fermentation-assisted extraction method, prepared by aqueous, *Saccharomyces sp* and *Rhizopus spp* assisted extraction.

2. Materials and Methods

2.1. Material

Tubers of yam bean (*Pachyrhizus erosus* L. (PeL)) were identified as elephant varieties and collected from PeL plants grown on Binjai city, the province of North Sumatera, Indonesia. Crude WSP was obtained by various fermentation assisted extraction method (FAEM), prepared by distilled water, *Saccharomyces spp* and *Rhizopus spp* assisted extraction. All chemicals are analytical grade unless otherwise stated.

2.2. Experimental design and data analysis

A completely randomized design with two factors i.e. water extraction with extracting water addition on the amount of starch (P) used (1:0,5; 1:1; 1:1,5; 1:2) and FAEM prepared by distilled water, *Saccharomyces spp* and *Rhizopus spp* assisted extraction. The data obtained were analysed using analysis of variance and continued with Least Significant Range (LSR).

2.3. Preparation of PeL starch

Fresh PeL (150.0 kg) were peeled, washed, cut into small pieces, grated and suspended in distilled water. The suspension was settled down for 6 hours in the refrigerator to separate fibre from starch. The supernatant was collected as the bengkoang crude starch. The crude starch was dried in oven for 12 hours at 40°C, crushed and sieved with 80 mesh to get PeL starch.

2.4. Preparation of WSP-PeL

A WSP-PeL was prepared by water extraction with extracting water addition on the amount of starch used. Respectively, a portion (100.0 g) of the PeL starch added water at ratio (P) in 1:0.5; 1:1; 1:1.5; 1:2 part. The supernatant was taken for each FAEM. FAEM will done with 3 (three) different method (E): extraction without treatment (only with distilled water) fermented, extraction using tape culture (*Saccharomyces sp)* at *0.3*% of supernatant weight and extraction with tempeh culture (*Rhizopus sp)* at 0.3% of supernatant weight for 3 days. Then soaked in 70 % and 96% ethanol to separate between PeL starch and WSP-PeL [31]. Clumps of supernatant is dried and milled to obtain a dry powder of WSP-PeL.

2.5. Total reducing sugar with DNS

Material that has been refined weighed as much as 2-5 g, depending on the reduction of sugar levels and transferred into a 100 ml beaker glass (Pyrex, USA). Added alcohol 80% ± 10-20 mL and stirred using a magnetic stirrer (11-501-7S, 56900308, USA) for 1 hour. Filtered with Whatman filter paper no. 1 in a 250 ml flask. Heated in a water bath for 1 hour to eliminate the alcohol, distilled water up to the mark and stirred. Taken 1 ml and 19 ml of distilled water to Erlenmeyer flash and shaken. Taken 1 ml solution mixture and put into a test tube, added of 3 ml DNS and shaken with a vortex tool (Lab Dancer V, 01-717079, Germany). Heated for 5 minutes and left for 30 minutes. Analysed with a spectrophotometer (Genesys 20, 3SGH318015, USA) at a wavelength of 550 nm to see the absorbance and calculate with standard curves [2].

2.6. Total sugar

Material that has been refined weighed as much as 2-5 g and transferred into a 100 ml beaker glass. Added alcohol 80% ± 10-20 ml and stirred using a magnetic stirrer for 1 hour. Filtered with Whatman paper no. 1 in a 250 ml flask. Heated in a water bath for 1 hour to eliminate the alcohol, distilled water up to the mark and stirred. Taken 1 ml and 19 ml of distilled water to erlenmeyer flash and shaken. Taken 1 mL solution mixture and put into a test tube, added of 0.5 mL phenol 5% and shaken with a vortex tool. Added of 2.5 ml H_2SO_4 concentrated, poured right in the middle of test tube with the perpendicular to the orange colour, left for10 minutes and shaken with a vortex tool. Analysed with a spectrophotometer at a wavelength of 490 nm to see the absorbance and calculate with standard curves [7].

2.7. Dextrose equivalent (DE)

DE is a conversion rate of hydrolysis of starch as measured from the total decline of all sugar produced in hydrolysis of the copper reagent Fehling. This value can be measured by comparing the value of reducing sugar to total sugar produced in hydrolysis [6]. The formula:

$$DE = \frac{\text{reducing sugar}}{\text{Total sugar}} \text{ x } 100 \quad (1)$$

2.8. Degree of polymerization (DP)

DP is an indication of the average value of the molecule monomer unit. It shows the value of glucose units. DP value = 1 and DP dextrose maltose = 2 [30]. The formula:

$$DP = \frac{100}{DE} \quad (2)$$

2.9. Viscosity

Determination of flow time in the viscometer Oswald substance (t2) is performed with 10 ml of the sample taken and inserted into Oswald viscometer. Samples sucked by the pump into the ball to the boundary marks found on the instrument. The sample is allowed to flow down to the extent that there is a mark on the instrument. Recorded the time needed by using a stopwatch. The determination of the density of the substance (d2) is done by taking 10 ml samples was measured weight. The density is the result of dividing the weight by volume of the substance [24]. The formula:

$$\frac{N_{aq}}{n_2} = \frac{d_1 t_1}{d_2 t_2} \quad (3)$$

3. Results and Discussions

3.1. Composition of WSP-PeL

The physicochemical characteristics of WSP-PeL was prepared by water extraction with extracting water addition on the amount of starch used are shown in Table 1 and FAEM with distilled water, *Saccharomyces ssp* and *Rhizopus spp* assisted extraction are shown in Table 2.

Table 1. Characteristics of WSP-PeL was prepared by water extraction with extracting water addition compared with the amount of starch used

Component	Water extraction with extracting water addition on starch			
	P_1 (0,5 part)	P_2 (1 part)	P_3 (1,5 part)	P_4 (2 part)
Reducing sugar (%)	0.098[a]	0.097[b]	0.096[c]	0.095[c]
Total sugar (%)	4.065[a]	4.116[b]	4.156[b]	4.283[c]
Dextrose equivalent	4.065[c]	4.116[b]	4.156[b]	4.283[a]
Degree polymerization	24.609[a]	24.301[ab]	24.065[b]	23.353[c]
Viscosity (cP)	1.004[a]	0.998[ab]	0.990[b]	0.985[b]
Solubility (%)	81.023[b]	81.520[b]	82.600[ab]	83.187[a]

Notes: Notation different letters indicate a significantly different effect on the level of 5%. Each parameter performed 3 times.

Table 2. Characteristics of WSP-PeL was prepared by FAEM with distilled water, tape culture (*Saccharomyces spp)* and tempeh culture *(Rhizopus spp)* assisted extraction

Component	Fermentation assisted extraction method		
	E1 (Distilled water)	E2 *(Saccharomyces ssp)*	E3 *(Rizhopus ssp)*
Reducing sugar (%)	0.097[a]	0.097[ab]	0.096[b]
Total sugar (%)	2.358[a]	2.332[a]	2.296[b]
Dextrose equivalent	4.135[a]	4.153[a]	4.177[a]
Degree polymerization	24.193[a]	24.096[a]	23.956[a]
Viscosity (cP)	1.030[a]	0.980[b]	0.973[c]
Solubility (%)	80.128[c]	82.606[ab]	83.514[a]

Notes: Notation different letters indicate a significantly different effect on the level of 5%. Each parameter performed 3 times.

3.2. Total reducing sugar

As a result on figure 1 and figure 2, it showed that the highest WSP-PeL reducing sugar was P1E1 treatment and the lowest was P4E3 treatment. These indicated that the water extraction of PeL starch caused to increase the solubility of sugar, due to make easier degradation by microorganisms for its growth, so that caused a reducing sugar decreasing [14]. And also, WSP-PeL was fermented by *Rhizopus spp* (tempeh culture), it was can synthesised various types of enzymes that was produced by microorganisms to produce the components that were free of sugar. Accordance with the opinion of [4] and [18] which stated that enzymes were proteins that had catalytic properties, which can degraded macromolecules into low molecular weight component.

3.3. Total sugar

As a result in figure 3 and figure 4, it showed that the highest of total sugar WSP-PeL was P1E1 treatment and the lowest was P4E3 treatment. The increasing of water addition on starch, can made the lower of WSP-PeL total sugar. These were caused by water addition that can dissolved the sugar

component. The water can dissolved the shorter compounds (i.e. monosaccharide, disaccharide and oligosaccharide) [14]. And also the enzymes which be produced by microbe cultures can broke down carbohydrates of the PeL starch and dissolved the compound [17].

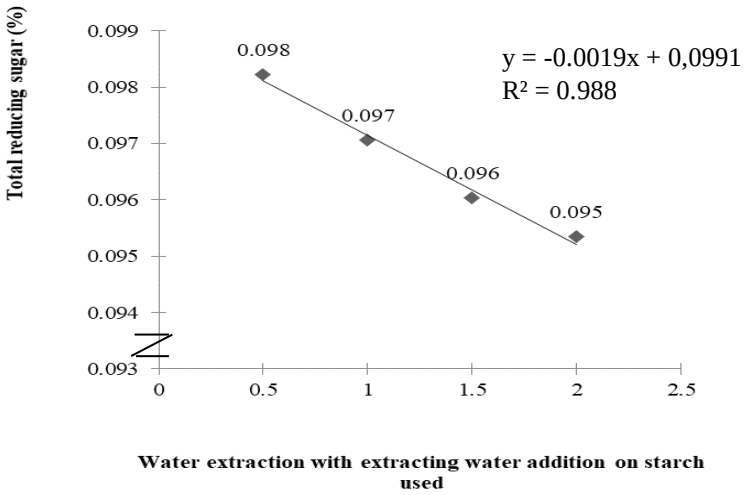

Figure 1. Response a Water Extraction to the PeL Strach of WSP-PeL Reducing Starch

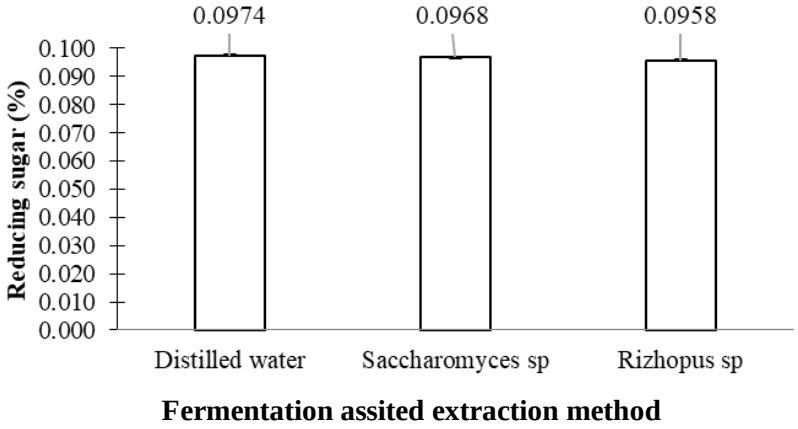

Figure 2. Response a FAEM to the PeL Strach of WSP-PeL Reducing Starch

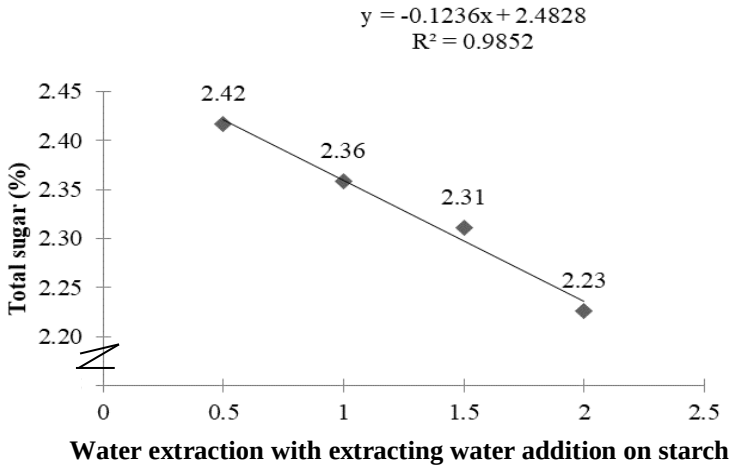

Figure 3. Response a Water Extraction to the PeL Starch of WSP-PeL Total Sugar

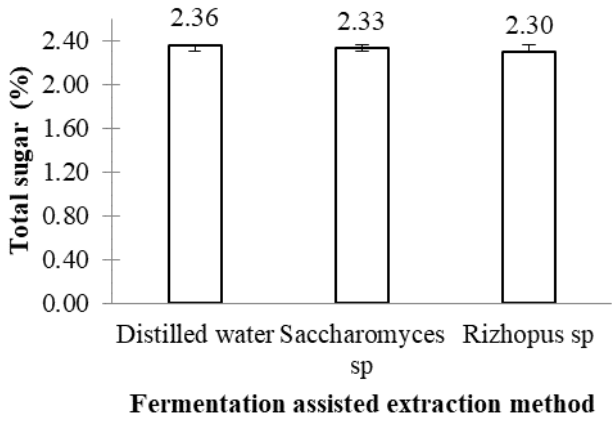

Figure 4. Response a FAEM to the PeL Starch of WSP-PeL Total Sugar

3.4. Dextrose equivalent (DE)

DE was an indication of the total reducing sugars such as D-glucose on a dry weight basis. The addition of more water will lead to the disintegration of polymer saccharides into saccharide monomer units in addition to D-Glucose is D-Fructose. This is in accordance with the opinion of [15] which stated that the Water-Soluble Polysaccharide (WSP) are included in the class of oligosaccharides, which constituent consisting of inulin. Inulin is a polymer of glucose and fructose units linked to each other in the carbohydrate chain [26]. WSP-PeL DE becomes higher than the various types of extraction method using distilled water, "tape" culture (*Saccharomyces spp.)* and *tempeh culture (Rhizopus spp*.) This is because the extraction of fermentation by *Saccharomyces spp* and *Rhizopus spp* can formed various groups of sugar monomers from the starch catalytic reaction processes such as glucose, maltose and fructose. This is in accordance with the opinion of [30] which states that the amount of reducing sugars may be expressed as dextrose equivalent (DE). WSP-PeL DE of PeL is 4, which means it consists of a mixture of several types of sugar.

3.5. Degree of polymerization

The increase of water addition is lead to the disintegration of polymer saccharides into saccharide units in addition to D-Glucose and D-Fructose. Degree of polymerization (DP) is the number of monomer units bound to the carbohydrate structure, so that the amount of addition of water to dissolve most of monomer carbohydrates. This is in accordance with the opinion of [26] that the average DP of inulin greater than 16. WSP which in this case is included in the class inulin constituent oligosaccharides composed of glucose and fructose units.

3.6. Viscosity

The increase of water addition in the extract PeL starch can dissolve the compound produced as a result of the chemical reaction that takes place, so that the particles produced little to resist the flow in the fluid, in accordance with the opinion of [24], which states that the viscosity is one fluid properties which resist the flow or pressure in the liquid.

4. Conclusion

Taken all the results together, the present research has shown that water extraction and FAEM has provides highly significant effect in each parameter of physicochemical characteristic of WSP-PeL analysed. In addition, WSP-PeL can be fermented by bacteria such as bifidobacteria and lactobacilli, which producing SCFA in the large intestine. Because it has a good solubility (S_O = 83%) and viscosity (μ = 0.973 centiPoise) to the viscous properties of WSP-PeL can inhibits the absorption of excessive macronutrient and decrease blood sugar levels. P4E3 treatment (water addition of 2 parts of PeL starch used and extraction method by *Rizhopus sp*) is the best results in these study. However, further studies need tests to determine the potential hypoglycemic effect WSP-PeL as lowering blood sugar levels with mouse model to induce hyperglycemia.

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