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Glucomannan Flour from Porang Tuber with Combination Method of Microwave Assisted Extraction (MAE) and Batchwise Solvent Extraction (BSE) for Diabetic Solutions

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ABSTRACT

In recent years, there has been a considerable increase in the number of diabetes cases. The majority of the time, foods that are advised for diabetics are low in fat, rich in protein, and complex carbs with a high fiber content. Porang tubers are a source of complex carbs and high fiber. Glucomannan is the primary component of porang tubers. Glucomannan can regulate blood sugar levels. Porang's use as a food ingredient has been limited thus far due to its high oxalate content. In this work, glucomannan composition was investigated. Pre-treatment NaCl and combination method of microwave-assisted extraction (MAE) and batchwise solvent extraction (BSE) were applied to achieve purified glucomannan in a shorter amount of time. For MAE–BSE combination, it was found glucomannan content was increased from 23,50% to 92,60%. The quality of glucomannan flour was graded using glucomannan content, moisture, ash, fat, protein, crude fiber and calcium oxalate as the parameters. The result of glucomannan flour was evaluated by the Professional Standard of the People' Republic of China (2002), SNI 01-4270-1996 and SNI 7939:2020 standard procedures. Based on all seven parameters, the glucomannan flour resulting from the MAE-BSE purification methods fit all parameters according to the existing standard, so the sample has potential to be an alternative food ingredient in the treatment of diabetes.

1. Introduction

Diabetes Mellitus (DM) is a disease characterized by insufficient blood glucose control. Type 1 diabetes, type 2 diabetes, maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and steroid-induced diabetes are all subtypes of diabetes. The main subtypes of diabetes are types 1 and 2, which have different pathophysiology, presentation, and management, but both have the potential for hyperglycemia. Diabetes is a leading cause of blindness, heart disease, and kidney failure worldwide, as well as premature death.

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The International Diabetes Federation (IDF) estimates that there are at least 436 million people aged 20-70 years in the world suffering from diabetes in 2019 or equivalent to a prevalence rate of 9.3% of the total population at the same age. If classified by gender, the IDF estimates the prevalence of diabetes in 2019 to be 9% in women and 9.65% in men. Diabetes prevalence is expected to rise to 19.9%, or 111.2 million people aged 65-79 years, as the population ages. The number is expected to rise further, reaching 578 million in 2030 and 700 million in 2045 [1]. Things to control diabetes mellitus are regulation of diet, physical activity, pharmacological treatment/therapy, and involvement of family roles. Dietary arrangements include the content, quantity, and timing of food intake (Type, Amount, Schedule).

Glucomannan is a mannan-family polysaccharide that consists of a polymer of D-mannose and D-glucose [2]. Glucomannan is a water-soluble non-starch polysaccharide known as water-soluble fiber. Glucomannan can lower blood cholesterol and blood sugar levels, lose weight, and affect intestinal activity and immune system function [3]. In addition, several studies have shown that glucomannan has a prebiotic effect in humans and experimental animals [2].

Several methods for extracting and purifying glucomannan have been developed, either mechanically (dry processing) or chemically (wet processing). Because of its simplicity and high efficiency, ethanol extraction is the most commonly used method for purifying glucomannan; however, glucomannan extraction takes a relatively long time [4]. In this method, ethanol acts as an anti-solvent for glucomannan while removing contaminants.

Extraction using a combination method of Microwave Assisted Extraction (MAE) and Batchwise Solvent Extraction (BSE) has been developed. This method combines microwave radiation with heat. The advantage of this method is that it requires a shorter extraction time with a higher extraction yield of the active compound [5]. The MAE method has advantages in terms of time efficiency and solvent requirements so that it can significantly reduce extraction time [6]. Glucomannan extraction using the MAE-BSE combination method is expected to be the method of choice to obtain higher levels of glucomannan and become a more efficient method. This research is included in the category of early research and is relatively at the pilot stage, so the previous implementation has not been carried out and focuses on the implementation that will be carried out, namely by extracting glucomannan from porang tubers with the combination method of Microwave Assisted Extraction (MAE) and Batchwise Solvent Extraction (BSE).

2. Material and Methods

2.1 Material

Porang flour, ethanol food grade, dimethyl carbonate, glucose standard, and sodium chloride were purchased from commercial sources. All reagents and solvents were analytical-grade.

2.2 Pre-Treatment

Porang flour is sifted with an 80-mesh sieve, then 10 grams of the sieved sample soaked in 5% NaCl solution (100 ml volume) for 10 minutes at room temperature to remove calcium oxalate in the flour. Then added ethanol in a ratio of 1:1 to precipitate glucomannan and is stirred with a magnetic stirrer for 5 minutes at 300 rpm. Then the solution is filtered by vacuum pump to separate the filtrate and residue.

2.3 Microwave Assisted Extraction (MAE)

MAE is employed for Glucomannan purification. The variables of this method are solid to solvent ratio 1:6 and the solvent mixture between Ethanol: DMC (50:50);. Ethanol: Water (50:50). After porang flour and solvent were stirred, the mixture was put into a triple neck flask with a specific power (150; 300; 450) and time (30; 60; and 90 min). Spectrofotometer UV-Visible were used to characterize the data.

2.4 Batchwise Solvent Extraction (BSE)

The residue from the microwave-assisted extraction stage then carried out by an extraction process by dissolving the precipitate in a solvent with a ratio of 1:6. Then stirred magnetically at 300 rpm for 5 minutes at room temperature. After 5 minutes, the solution was filtered with a vacuum pump to separate the filtrate and residue. Then the residue re-extracted using solvent. The series of extraction processes with solvent and filtering were repeated up to three times. The final residue obtained is called glucomannan flour. Furthermore, the glucomannan flour was put in an oven at 60°C to reduce the water and ethanol content. Glucomannan flour is declared completely dry if the mass of flour being constant for 3 consecutive weighing. Dried glucomannan flour was allowed to stand in a desiccator until it was 25°C, then grinded with a grinder and stored in a closed container at room temperature.

2.5 Glucomannan Analysis

Analysis of glucomannan using DNS method [7]. A sample of 0.2 grams was stirred in 50 ml of formic acid-NaOH buffer solution (0.1 M) for 4 hours then added formic acid-NaOH buffer to a volume of 100 ml. The mixture was centrifuged at 4000 rpm for 20 minutes. Then 2.5 ml of the glucomannan solution was hydrolyzed with 1 ml of 3M sulfuric acid in a water bath for 90 minutes. The solution was cooled to room temperature, then 1 ml of NaOH solution with a concentration of 6 M was added and diluted to 10 ml using distilled water and homogenized with a vortex. Then the solution and the initial glucomannan solution were pipetted as much as 2 ml each into a test tube, added 1.5 ml of DNS reagent, and homogenized with a vortex. Each homogeneous solution was heated in a water bath for 10 minutes (T = 100 oC). The solution was cooled to room temperature. Then the initial glucomannan solution and the hydrolyzed glucomannan solution were measured absorbance values using a UV-Visible spectrophotometer with a length of 540 nm and compared the results with a standard solution of D-glucose. The content of glucomannan can be calculated by Eq. (1):

$$\%\,\text{Glucomannan} = \frac{5000 f \left(5T - T_0\right)}{m} \qquad 1$$

where;

f : Correction factor of 0.9 (Ratio of BM of glucose and mannan residues with BM of glucose and mannan produced after hydrolysis)

T : The content of glucose in the hydrolyzed glucomannan solution

T_0 : The content of glucose in the initial glucomannan solution

m : Mass of sample

3. Result

Porang flour contains calcium oxalate which is quite high and causes the tongue and throat to feel itchy and hot when consumed. The high content of calcium oxalate crystals in porang flour needs to be reduced or eliminated. In extreme cases, this calcium absorption causes hypocalcemia and paralysis which can be fatal [8]. Daily intake of calcium oxalate in the body should not exceed 70-150 mg per day [9]. Excessive consumption of calcium oxalate will cause crystallization in the kidneys, form kidney stones, and cause other health problems [10]. The process of separating calcium oxalate is carried out by pre-treatment NaCl, a purification process using microwave-assisted extraction and combined with solvent extraction to reduce impurities on the surface of the porang flour granules. The glucomannan purification method used in this study is a modification of the procedure that has been carried out [4].

The purification principle separating glucomannan from components other than glucomannan/impurities according to differences in solubility. This purification stage is divided into three stages, namely treatment with NaCl, extraction with microwave, and maceration with ethanol. The first stage, namely treatment with 100 ml of 5% NaCl for every 10 grams of porang flour with stirring for 10 minutes at room temperature. During the treatment process, salt or NaCl can be ionized in water to become Na+ and Cl-. The more Na+ and Cl- ions contained in the solution, the more bonds with Ca2+ and C2O42- ions can occur, resulting in sodium oxalate (Na2C2O4), which is soluble in water so that the oxalate levels in the treated porang flour decreased because these compounds dissolved in the water and discarded. The reaction is as follows:

$$CaC_2O_4 + 2\,NaCl \rightarrow Na_2C_2O_4 + CaCl_2$$

The presence of stirring during the pre-treatment process will facilitate the low molecular weight calcium oxalate to be released from the surface of the glucomannan granules. After completion of stirring, 100 ml of 96% food grade ethanol was added to precipitate the glucomannan. The second stage is microwave-assisted extraction. In this stage, porang flour is dissolved in a solvent according to a predetermined solvent variation with a solid:liquid ratio (1:6), and extraction is carried out with the help of a microwave according to the specified power and time variations. Microwaves act as energy vectors in materials that can absorb and convert energy into heat by radiation. The greater the intensity of radiation given to the extraction process, the temperature in the extraction process increases. The presence of heat can vaporize the solvent and increase the pressure. The cells in the material will swell and burst. The rupture of the cell wall of the material can make it easier for the target compound to come out and be extracted. Heat and microwaves can indeed increase the solubility and diffusion of active compounds. However, if the temperature is raised too high, it will not significantly affect the extraction yield [11]. The temperature used in this microwave-assisted extraction is 55°C.

The third stage is solvent extraction with a predetermined solvent variation and stirred for 5 minutes. During this solvent extraction process, impurities (starch/starch, nitrogen/protein-containing material, and ash) that were previously trapped between the glucomannan pores will separate from the glucomannan granules. Starch (starch) is easily broken into smaller particles and can be dissolved in ethanol. Ash content (including calcium oxalate), protein, and dyes/pigments will also dissolve in ethanol while glucomannan is insoluble in ethanol [12,13]. Glucomannan extraction is carried out with an antisolvent in the form of ethanol so that only the impurities will dissolve but the glucomannan does not dissolve or expand. The temperature when extraction with ethanol was carried out at room temperature (±25°C) to keep the extraction process running normally and at that

temperature not to damage the structure of the glucomannan compound. After extraction for 5 minutes, the ethanol solution will be dark brown. The color comes from the carotene pigment which has a low molecular weight so that it is in the top layer. The next layer is an impurity component with a larger molecular weight such as starch/starch and ash. And the lowest layer is the component with the largest molecular weight, namely glucomannan.

The filtering process is carried out to separate the filtrate (containing impurities) and glucomannan granules. Filtering is done with the help of a vacuum pump. Solvent extraction was repeated three times so that the impurity dissolution process was more efficient. The more the process is repeated, the more impurities can be separated and the purity of the glucomannan obtained is getting higher. After three repetitions, the granules were filtered and oven-dried at a temperature (±60°C) for 12 hours to reduce the moisture content and the remaining ethanol content. Then grind it with a grinder. The color of the flour produced is brown. This is because the flour undergoes a browning process. In porang, there is a carotene content that reaches 40 mg/kg. Porang tubers also contain polyphenol oxidases (PPO) enzymes and polyphenolic compounds including tannins which cause the flour to brown. Starch content, calcium oxalate, and process temperature also affect the whiteness of porang flour.

The next stage is the analysis of porang flour and glucomannan flour. Analysis of glucomannan content was determined by the DNS method and its absorption was measured at a wavelength of 540 nm using a UV-Visible spectrophotometer. Determination of glucomannan content was carried out by measuring the glucomannan content in the extract and glucomannan hydrolyzate. Measurement of glucomannan levels in glucomannan extract aims to prevent overestimation of glucomannan content due to the presence of free reducing sugars from other sources such as starch that may be present in the test sample. The analysis process begins by making a calibration curve of the comparison compound, namely glucose. Glucose was chosen because it is a monomer of glucomannan which provides a more accurate and precise measurement of glucomannan levels compared to mannose. This is based on the results [7] that the glucose standard provides higher sensitivity than mannose with a more linear correlation coefficient value. Absorbance measurements were carried out at a wavelength of 540 nm because the reddish-orange 3-amino-5-nitrosalicylic acid compound can absorb strongly electromagnetic radiation at a wavelength of 540 nm. The reaction that occurs is that the aldehyde group on glucose is oxidized to a carboxyl group. Meanwhile, DNS as an oxidizing agent was reduced to form 3-amino-5-nitrosalicylate which was reddish-orange/red-brown.

Table 1 shows that the highest glucomannan purity was obtained at a power variation of 450 watts, extraction time of 5 minutes, and the ratio of solvent Ethanol: Water (50:50). The purity of glucomannan decreased from 5 minutes to 10 minutes of extraction time. This happens because the DNS reagent used is very sensitive to light so it can cause the analysis results to deviate. The addition of extraction time did not increase the glucomannan extraction rate, but on the contrary, there was a decreasing trend. This is because the long extraction time can damage the glucomannan compounds from porang tubers. Microwave radiation and heat allow glucomannan to be degraded [5]. In this study, the solvent variation using a mixture of ethanol and dimethyl carbonate (DMC) with a ratio of 50:50 did not succeed in undergoing a methylation reaction. This is because the dimethyl carbonate reagent used is not completely soluble in ethanol so the reaction carried out in the methylation process takes place heterogeneously, which results in the methylation reaction taking place imperfectly. Another cause is the absence of the addition of NaOH which is used as a catalyst for the methylation reaction. Where the addition of sodium hydroxide (NaOH) will make glucomannan in porang flour experience swelling and make glucomannan macromolecules more flexible so that the hydroxyl group on glucomannan becomes more active and more ready to react

with dimethyl carbonate reagent by substitution to form a methoxyl group [14]. Swelling of glucomannan can occur if there is water in the mixture. This is the cause of the extraction of glucomannan using pure food grade ethanol and ethanol: DMC (50:50) in this study did not take place optimally because there was no water coating the glucomannan particles (no swelling). So, when the product is precipitated with anti-solvent, the glucomannan is not completely precipitated. However, if the water composition in the swelling process is too much, it will result in a lower concentration of extracted glucomannan and an increase in the amount of water that coats the glucomannan particles. This causes when the product is precipitated with an anti-solvent, the water molecules that coat the glucomannan molecules may not be completely precipitated, resulting in a decrease in yield and the consumption of many anti-solvents [14]. Therefore, further research is needed in selecting the right solvent ratio to obtain optimal levels of glucomannan.

Table 1
Results of analysis of glucomannan content

Solvent ratio (%)	Power (Watt)	Time (min)	Glucomannan MAE	MAE-BSE
Ethanol: DMC (50:50)	150	5	56,37	59,30
	150	10	49,25	51,17
	150	15	52,01	53,63
	300	5	70,10	72,25
	300	10	52,40	59,01
	300	15	58,49	60,47
	450	5	54,92	79,07
	450	10	48,36	62,36
	450	15	57,76	70,72
Ethanol: Water (50:50)	150	5	57,56	83,55
	150	10	45,22	60,19
	150	15	56,15	79,07
	300	5	63,06	90,05
	300	10	49,51	85,44
	300	15	62,42	86,99
	450	5	81,44	92,60
	450	10	50,52	56,55
	450	15	53,07	60,72
Ethanol (100)	150	5	79,16	80,18
	150	10	61,39	63,34
	150	15	67,73	68,65
	300	5	59,67	81,65
	300	10	37,30	51,61
	300	15	51,50	58,24
	450	5	67,93	72,64
	450	10	54,01	56,39
	450	15	70,13	71,14
Porang flour (raw material)			23,50	

Determination of the best treatment for purified porang flour using a combination method of microwave-assisted extraction and stratified ethanol extraction was based on the parameter of glucomannan content. The expected glucomannan flour is flour with the highest glucomannan content. The best treatment was found in porang flour through microwave-assisted extraction with a power of 450 watts, extraction time of 5 minutes, and solvent variation of ethanol:water (50:50) followed by washing level 3 for 5 minutes each stage with glucomannan content. 92.60%. The best-treated flour is analyzed for calcium oxalate content, protein content, ash content, starch content,

fat content, and crude fiber content and compared with the initial porang flour to determine the level of decline in components other than glucomannan, and compared with commercial glucomannan flour to determine the suitability of the product market.

The increase in glucomannan levels after the pre-treatment process may occur due to reduced starch components in flour due to sifting. Starch with a larger molecular weight than glucomannan will be left in the sieve and glucomannan with a smaller molecular weight will pass along with the components in other fine flours. However, in this study, glucomannan analysis was not carried out after the pre-treatment process, so the increase that occurred could not be quantified. The existence of a microwave-assisted extraction process can increase glucomannan levels by ±34%. This increase is due to radiant heat which makes the cells in the material swell and burst, making it easier for glucomannan to come out and be extracted. The extraction process was continued with multilevel extraction using a solvent by the solvent used in the microwave extraction. The average increase in glucomannan was ±11%. With the results obtained, it is necessary to consider if you will use the MAE-BSE combination method in further research. Some things to consider include the use of a lot of solvents because every 10 gram of solid sample required 60 ml of solvent for MAE and 180 ml of solvent for BSE up to 3 stages; long extraction time; and the increase in glucomannan levels obtained was not high.

Analysis of variance from 27 samples from the MAE-BSE combination experiment was carried out using a Full Factorial Design (FFD) model with a factorial design of 33, namely a design that uses 3 types of factors, namely (power, time, and % solvent ratio) with each factor consisting of 3 levels. This analysis uses glucomannan levels as a response and pays attention to the significance value seen from the p-value and the coefficient of determination R2. The ANOVA results are then summarized in the following Table 2.

Table 2
ANOVA results glucomannan levels MAE-BSE method

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	6	2622.8	437.14	6.41	0.001
Linear	6	2622.8	437.14	6.41	0.001
Power	2	123.1	61.56	0.90	0.421
Time	2	1447.6	723.81	10.62	0.001
Solvent Ratio	2	1052.1	526.05	7.72	0.003
Error	20	1363.0	68.15		
Total	26	3985.8			

The ANOVA results attached in Table 2, it shows the significance level of all the variables used in the glucomannan testing of the MAE-BSE method where the % solvent ratio and extraction time variables have a significant effect on the results of the glucomannan levels obtained (p-value < 0.05). The power variable has no significant effect on glucomannan levels.

Table 3 is linear modeling of the MAE-BSE experiment. This modeling gives an unfavorable coefficient of determination R2 for the response of glucomannan levels (65.80%) so it can be said that this linear modeling is not suitable to describe this MAE-BSE experiment (R2<90%). Furthermore, the coefficient of determination of R2 will affect the residual value which shows the difference between the observed value and the fit value. The residual values of this MAE-BSE experiment are summarized in Figure 1.

Table 3
Model summary ANOVA

S	R-sq	R-sq(adj)	R-sq(pred)
8.25523	65.80%	55.55%	37.68%

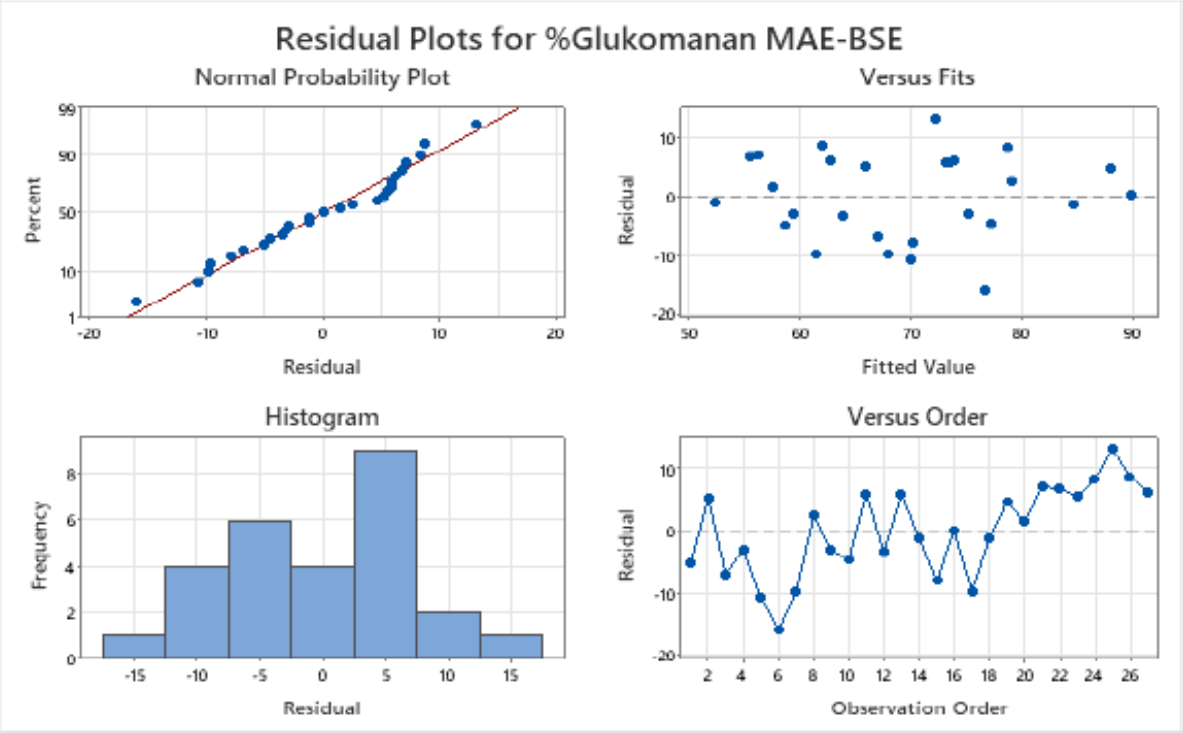

Fig. 1. The residual values of the MAE-BSE experiment

Based on the distribution of the data distribution in Figure 1 that the data is not normally distributed, it can be seen from the normal probability plot that the data is spread around the diagonal line and follows the direction of the diagonal line.

4. Summary

The combination method of Microwave Assisted Extraction (MAE) – Batchwise Solvent Extraction (BSE) at the variation of time and % solvent ratio has a significant effect on glucomannan content in the extract. Purification of glucomannan was carried out by the combination method of Microwave Assisted Extraction – Batchwise Solvent Extraction at a temperature of 55°C and a solid:liquid ratio of 1:6 with variations in power (150, 300, 450 watts), extraction time (5, 10, 15 minutes) and the type of solvent (water, food grade ethanol, and dimethyl carbonate). The best conditions were obtained at 450 watts of power, 5 minutes of extraction time, and solvent food-grade ethanol: water (50:50). Characteristics of glucomannan extracted by the combination method of Microwave Assisted Extraction (MAE) – Batchwise Solvent Extraction (BSE) in the best purification results, which contains glucomannan content of 92.60%.

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