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ONE-STEP ETHANOL EXTRACTION FOR PRODUCING PURIFIED GLUCOMANNAN FLOUR FROM PORANG CHIPS *(AMORPHOPHALLUS ONCOPHYLLUS)*

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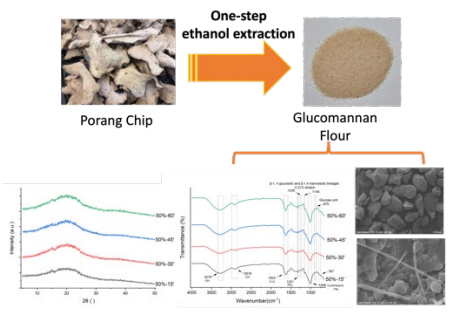
Graphical abstract



Abstract

Porang (Amorphophallus oncophyllus) is unable to be consumed directly due to high calcium oxalate content. Two methods commonly used to reduce calcium oxalate contents are a mechanical method by grinding and sieving and in chemical process by using sodium chloride immersion. Most studies focused on how to increase glucomannan content in porang flour. Glucomannan is a hydrocolloid complex sugar used as dietary fiber. It is utilized as a thickening agent, gel-forming agent, and applied in the food and pharmaceutical industries. In previous studies, purification of glucomannan has been done by milling fresh tuber and extraction with multilevel ethanol concentration. In this study, extraction with ethanol 50% and 70% have been done with a variation of extraction time: 15, 30, 45 and 60 minutes. For preparation, porang chips are ground and sieved into 40 mesh flour. After extraction, glucomannan flour was filtered and dried, then characterized. Glucomannan content, calcium oxalate content, moisture content, ash content, viscosity, morphology, Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) analysis were conducted. The experiment results show that the longer duration of extraction significantly decreased calcium oxalate content. Moisture content ranged from 9.99 – 10.85%. Better ash content was obtained at 50% ethanol, which exhibited under 3% of all immersion duration. The morphology of purified glucomannan flour observed through Scanning Electron Microscopy (SEM) showed a small amount of calcium oxalate as indicated by a needle-like structure. Ethanol concentrations and extraction duration effects on the purity and characterization of glucomannan flour from porang chips have been discussed in detail in this study.

Keywords: Porang, Glucomannan, Ethanol, Extraction, Characterization

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1.0. INTRODUCTION

Indonesia is known as an advanced agricultural country. One of the agricultural products commonly found in Indonesia is corm. In general, these agricultural products are only used to complement food. This is due to the low technology used for processing. One of the high-valued kind of corms that is currently researched and utilized is Amorphophallus Oncophyllus, also known as "Porang." Amorphophallus Oncophyllus species belongs to the Araceae family (taros). Porang is usually exported as raw materials in the presence of chips. It contains carbohydrates, fats, proteins, minerals, vitamins, and dietary fiber. However, only several areas in Indonesia cultivate this plant [1].

Some examples of products derived from porang corms are raw materials for glue and other adhesives and can also be used in beauty & health products, namely as konjac sponges & capsule shells. In the food industry, one that is widely used is the Konjac glucomannan (KGM). Konjac Glucomannan (KGM) is extracted from *Amorphophallus Oncophyllus,* which has a significant health benefit. It is low in calories so that it can be helpful as a healthy diet food [2]. Furthermore, this plant has

high economic potential, so developing these products is crucial. In Indonesia, this corm still needs to be widely processed into processed products due to the need for more research on producing porang flour to meet SNI (Indonesian National Standard) requirements [3]. The optimum processing of porang corms into crude flour to obtain flour with high glucomannan content, low calcium oxalate content and low production costs need to be developed so that farmers can process themselves and get a higher selling value.

Recently, various methods have been developed for extracting KGM (Konjac Glucomannan) in porang corms. The commonly used method uses ethanol as a solvent; pure porang flour (PKF) is macerated with an ethanol solution and then filtered and dried to a constant weight [4]. In prior studies, fresh raw corms were milled and extracted five and seven times at various multilevel concentrations using ethanol to perform glucomannan purification [5]. Porang tubers are unable to be directly consumed due to high calcium oxalate content, around 1.98% [6]. Calcium oxalate is a crystalline compound which is insoluble in water. If this compound is highly consumed and exceeds 71 mg/100g, kidney failure is inevitable [7]. Therefore, developing an improved method to decrease calcium oxalate content is necessary [8]. Two methods commonly used to reduce calcium oxalate contents are mechanical and chemical. Mechanical method is including grinding and sieving and is possible with cyclone-assisted to separate calcium oxalate due to its low mass density [9]. Chemically, crude porang flour is macerated using sodium chloride (NaCl) to initiate ionization reaction and leads to decreasing calcium oxalate content, which has been previously used by Rofi'ana et al. (2018) [10].

Thus, the objective of this study is to observe the effect of ethanol concentrations and extraction times on the purity and characterization of glucomannan flour from porang chips with one-step extraction. The characterizations, including the glucomannan content, Calcium Oxalate, moisture content, ash content, viscosity, microstructure, Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) results, were investigated in the present work.

2.0 METHODOLOGY

2.1 Materials

The raw material used is dried porang chips obtained from Madiun from East Java farmers, food grade ethanol (96%) as solvent. Reagent with analytical grade (AR) used, such as 3.5-dinitro salicylic acid (DNS), NaOH, $NaHSO_3$, H_2SO_4, d-glucose, potassium sodium tartrate, phenol crystals, formic acid, and other chemicals were purchased from Sigma Aldrich Inc. Germany. The properties of crude porang flour from porang chips used in this study are presented in Table 1.

Table 1 Characteristics of Crude Porang Flour

Properties	Content	Standard*
Glucomannan (%)	55.635	60
Calcium Oxalate (%)	0.065	0.04
Moisture Content (MC) (%)	10.38	13
Ash Content (%)	8.854	5
Viscosity (m.Pas)	400	14000

*Common Flour 2nd Grade Standard of Professional Standard of The People's Republic of China for Konjac Flour

2.2 Preparation of Crude Porang Flour

Porang chips were dried at 60°C, ground, and sieved into 40 mesh flour.

2.3 Ethanol Treatment

Crude porang flour was stirred in ethanol 50% and 70% (v/v) with a ratio of 1 gram: 15 ml at room temperature (30 °C) each for 15, 30, 45, and 60 minutes followed by filtration to obtain Purified Glucomannan Flour. Then, the flour was dried in an oven at 60 °C for 24 hours.

2.4 Determination of Glucomannan Content

0.2 grams of Purified Porang flour was stirred electromagnetically into 50 ml formic acid-sodium hydroxide buffer solution (0.1 M) at room temperature for 4 hours. Then, the solution was diluted with formic acid-sodium hydroxide to 100 ml; then centrifuged at 4000 rpm for 20 min. The 5 ml supernatant solution was homogenized with H_2SO_4 (3 M) and then heated in a water bath for 90 minutes. Then, the solution was added with NaOH (6 M) and diluted to 25 ml with DI water to form a hydrolysate solution [11]. Glucomannan content is calculated by Eq. (1):

$$\text{Glucomannan Content } (\%) = \frac{\varepsilon\,(5T - T_0)\times 50}{m\times(1-w)\times 1000}\times 100 \quad (1)$$

where ε = correction factor (0,9), T = glucose content of KGM hydrolysate (mg), T0 = glucose content of Konjac Glucomannan sample solution (mg), and m = mass of glucomannan flour (0,2 g).

2.5 Determination of Calcium Oxalate Content

Two grams of glucomannan flour were homogenized in a mixture of 190 mL DI water and 10 mL HCl (6 M), then heated at 100 °C for 1 hour. Then, DI water was added to make the solution become 250 mL. The solution was filtered to discard the filtrate; then, the solution was divided into 2 parts of 125 ml each. Titration was performed using NH_4OH 25% (v/v) and methyl red indicator followed by heating in a water bath to 86 – 90 °C. Then, the solution was cooled and filtered. The solution was reheated to 90 °C added 10 ml of $CaCl_2$ 5% (w/v) while stirring, then cooling and allowing the solution to stand overnight at 5 °C. The centrifugation was done to the solution with 2500 rpm for 5 min, and the decantation method was performed to obtain a precipitate. A 10 ml H_2SO_4 20% (v/v) was added to each precipitate to dissolve it. The precipitate was combined and dissolved up to 300 ml with DI water. Taking 125 ml of each solution, perform a permanganometric titration using $KmnO_4$ (0.05 M) [3]. Calcium oxalate content (mg/100 g) was calculated using **Eq.2.**

$$\text{Ca} - \text{Oxalate Content } (\text{mg}/100\ \text{gr}) = \frac{V_{KmnO_4}\times 0.00225\times Df}{m\times 5}\times 10^5 \quad (2)$$

where V_{KmnO_4} : $KmnO_4$ volume, N_{KmnO_4} : $KmnO_4$ standard normality, 0,00225 = 1 cm^3 $KmnO_4$ 0,05 (mol/L) solution equivalent to 0,00225 grams oxalic acid anhydrate, Df : dilution factor, 5 : $KmnO_4$ redox number

2.6 Viscosity

Porang flour (1%) was dissolved in deionized water with constant stirring at 150 RPM and room temperature (30 °C) uninterrupted for 1 hour. Then, the first determination of viscosity was recorded using a viscometer NDJ-8S with the #4 rotator at 12 RPM. The viscosity measurement is every 0.5 h until the viscosity shows the maximum value, then drops. The average value of maximum viscosity is taken as the viscosity value of the samples [11].

2.7 Moisture Content (MC)

Moisture content was performed using the AOAC method by comparing the weight using oven drying (105°C) for 24 hours [12].

2.8 Ash Content

Ash content was performed using the AOAC method by comparing the weight using oven drying (500°C) for 4 hours [13]

2.9 FTIR Spectra

Fourier Transform IR Spectra were performed using an FTIR Spectrophotometer (Agilent Cary 630, United States). Powder samples were analyzed at room temperature in the 600 – 4000 cm^{-1} [14]. All data were baseline corrected by using Origin Software 2023b.

2.10 Microscopy analysis (SEM)

The morphology of the porang flour was obtained with a scanning electron microscope (Hitachi, flexSEM 1000, Japan). Surface morphology was observed at a voltage of 15 kV at 150 and 5000 magnifications.

2.11 X-Ray Diffraction (XRD)

The structural properties of porang flour were characterized by an X-ray diffractometer in the 2θ range of 5 – 90°. Sample diffraction peaks were analyzed, and the degree of crystallinity was estimated using Origin Software 2023b.

3.0 RESULTS AND DISCUSSION

3.1. Glucomannan Extraction

Crude porang flour has significant calcium oxalate content but lower in glucomannan and viscosity according to the standard. Glucomannan has a bigger particle size and mass density compared to calcium oxalate. Ethanol was chosen as a solvent due to its ability to dissolve impurities in glucomannan flour, while unable to dissolve glucomannan [4]. In addition, its ethanol is volatile, colorless, and an organic solvent. The extraction process with ethanol was carried out with a ratio of 1 gr: 15 ml at room temperature (RT) with ethanol 50% and 70% (v/v). The flour will be stirred in ethanol for 15, 30, 45, and 60 minutes each. This experiment data of purified glucomannan flour is shown in Table 2. The method using ethanol extraction has also been carried out in the study of Saputro, Lefiyanti, and Mastuti (2014), which used ethanol concentrations of 40%, 50%, and 60%; it concludes the more concentrated ethanol solvent used, the better the glucomannan content obtained [4], [5]. This result differs from previous studies, where the best glucomannan content was obtained from the shortest duration (15 minutes) of maceration using ethanol 50%, to mention 76.2167%. With the relatively decreasing trend, due to ethanol being more volatile in comparison to deionized (DI) water is attributed to higher solvent loss over a longer extraction time and higher concentration resulting in a less efficient extraction. The purified flour was observed to have a 26.32 – 36.99% higher glucomannan content after being processed. In this research, longer ethanol extraction and duration resulted in relatively higher removal of calcium oxalate.

The ethanol's color changes from clear to yellow in the ethanol extraction process. This is because ethanol can remove fine powder impurities in glucomannan particles such as oxalate, starch, protein, ash, etc. Longer contact time between flour and solvent yielded more impurity compounds released and dissolved by ethanol. The moisture content on purified glucomannan flour overall was not significantly different, ranging from 9.99 – 10.85%. However, it is slightly higher than crude porang flour; these occurrences may happen in various storage setups. The quality and nutritional value of food is affected by ash content. In practice, ash content is related to the mineral content of a material. The ash content at high temperatures indicates pollutants, namely inorganic substances such as sand, soil, and others. The mineral content influences the ash content in the flour. The density also influences the increase in ash content. The purification process of porang flour is conducted at room temperature, which can be a factor in the browning reaction. This is due to PPO enzymes and tannins, which are phenolic compounds. The oxidation reaction of phenolic compounds into quinones will polymerize into melanin pigment with a dark color. Sodium bisulfite with a concentration of 2% is used as a bleaching agent to prevent the enzymatic browning process [15]. Adding Sodium Bisulfite to flour samples can prevent the browning reaction so that the flour samples have a bright yellow-to-white color.

Table 2 Characteristics of Purified Glucomannan Flour

Ethanol (%)	Extraction time (min)	Glucomannan (%)	Ca-Oxalate (%)	MC (%)	Ash Content (%)
50	15	76.217	0.032	10.850	1.514
	30	72.323	0.026	10.100	1.954
	45	75.298	0.015	10.540	1.667
	60	71.560	0.022	9.960	2.263
70	15	75.682	0.046	10.220	3.408
	30	75.113	0.019	10.320	3.157
	45	73.032	0.014	10.170	2.955
	60	70.282	0.022	9.990	2.457
Standard		90	0.03	10	3

*Purified flour Top Grade Standard of Professional Standard of The People's Republic of China for Konjac Flour

3.2. Viscosity

The viscosity of purified glucomannan flour is presented in Table 3. The viscosity in 70% ethanol with 15 minutes of extraction is the highest, 5450 mPa.s. The viscosity obtained before and after treatment fluctuated compared to the glucomannan contents obtained. The results are much smaller than the minimum

standard of glucomannan flour, which is 14,000 mPa.s. [11]. The resulting product flour has a very runny structure and does not form a gel as expected [16]. Despite being hydrophilic, glucomannan does not dissolve well in water [5]. The viscosity value is affected by glucomannan content in the flour. Higher glucomannan content results in higher viscosity.

Table 3 Viscosity of Purified Glucomannan Flour

Ethanol (%)	Extraction time (min)	Viscosity (mPa.s)
50	15	810
	30	1430
	45	2049
	60	1820
70	15	5450
	30	2080
	45	2160
	60	2860

3.3. Morphology

To understand the morphology of porang flour and purified glucomannan flour, it was observed using a Scanning Electron Microscope (SEM) and was presented in Figures 1-3. The results showed a needle pattern structure at 5000x magnification represented as calcium oxalate. Calcium oxalate is crystalline compound and it is insoluble in water [17]. The needle structure was significantly smaller after treatment, and the glucomannan granule surface structure became smoother. This indicates that ethanol extraction can reduce calcium oxalate content and increase glucomannan content in porang flour [11].

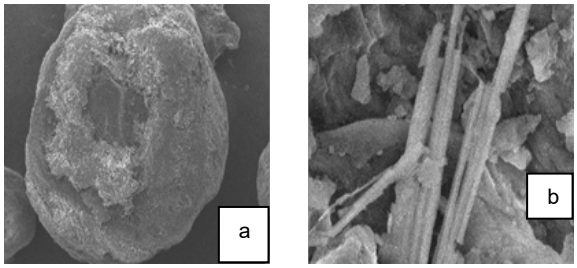

Figure 1 SEM Images of (a) Glucomannan and (b) Calcium Oxalate in Crude Porang Flour at 150x and 5000x Magnification

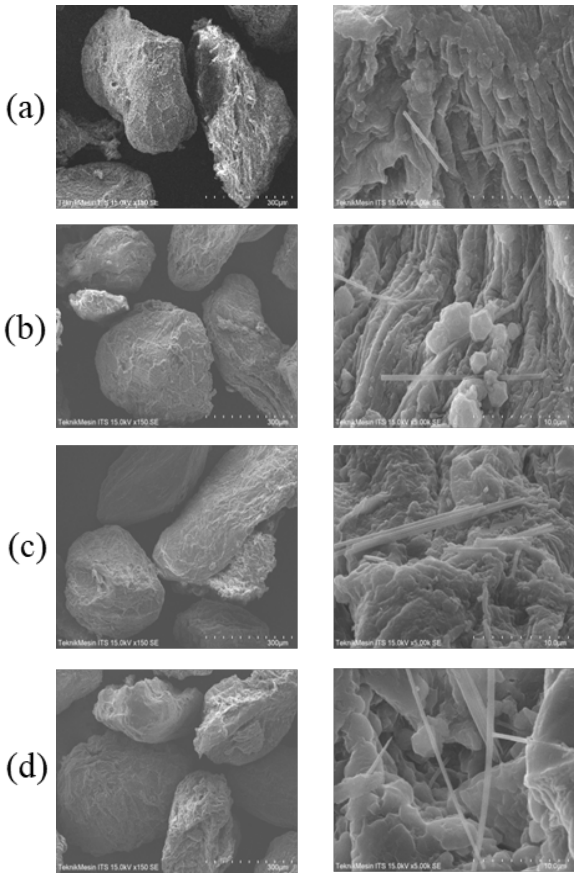

Figure 2 SEM Images of Purified Glucomannan Flour 50% Ethanol Extraction (a) 15 minutes, (b) 30 minutes, (c) 45 minutes, and (d) 60 minutes at 150x and 5000x magnifications

3.4. FTIR Analysis

The functional groups of a substance are analyzed using Fourier Transform Infrared Spectroscopy by reviewing the peaks from the readings of specific functional groups. In addition, it is also possible to analyze quantitatively by reviewing the absorption strength of compounds at specific wavelengths. Figure 4 compares the functional groups in 50% and 70% ethanol extraction treatment. Absorption peaks characteristic of glucomannan compounds were compared between crude and purifided flour, it is observed as a stretching vibration such as O-H group at 3278 – 3291 cm^{-1}, C-H at 2918 – 2922 cm^{-1}, C=C at 1623 cm^{-1}, cyclohexane ring vibration at 1004 – 1006 cm^{-1}, -glycosidic and -manosidic compounds at 760.3 cm^{-1} which is a combination of the structure of glucose and mannose [18]. Purified glucomannan flour showed a reducing peak of -OH and C-O-C stretch compared to crude flour, with 50% ethanol exhibited higher absorption peak than ethanol 70%.

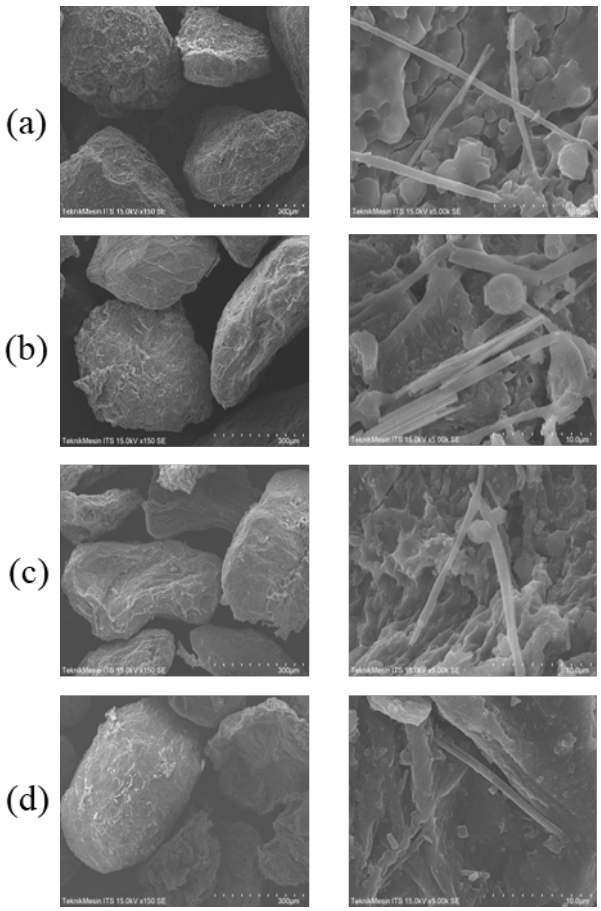

Figure 3 SEM Images of Purified Glucomannan Flour 50% Ethanol Extraction (a) 15 minutes, (b) 30 minutes, (c) 45 minutes, and (d) 60 minutes at 150x and 5000x magnifications

3.5. XRD Analysis

The XRD pattern of all purified glucomannan compared to crude flour showed a similar pattern which attributed to broad peak at 2θ = 16–25° as shown in Figure 5, indicating an amorphous structure with a small crystallinity [5]. It shows an extremely weak and looser molecular interaction. Increasing ethanol concentration relatively elevated peak diffraction. Glucomannan comprises two monosaccharides with acetyl groups on the chain segments, which led to the molecule chain having an insufficient steric regularity [19]. This result is in accordance with Yanuriati, 2017 [10], which reported that native glucomannan had a low crystallinity. Increasing ethanol concentration relatively elevated peak diffraction, which indicated lower glucomannan content had a more crystalline phase [17, 20, 21]. Li et al. [22] reported that glucomannan crystallinity increased due to inter and intramolecular hydrogen bonds showed by the water activity value of 0.25, indicating the activated water molecules can be neglected in terms of number. When the intermolecular hydrogen bonds of glucomannan become more assertive, it is hard to be milled [23].

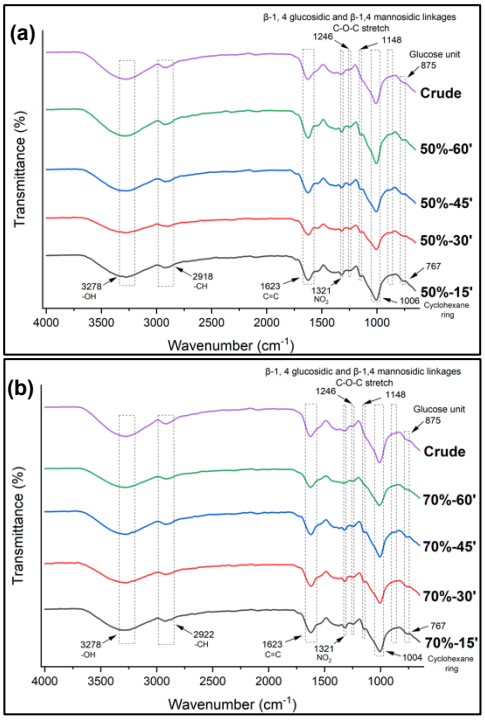

Figure 4 FTIR Spectra of Purified Glucomannan Flour (a) 50% Ethanol Extraction and (b) 70% Ethanol Extraction

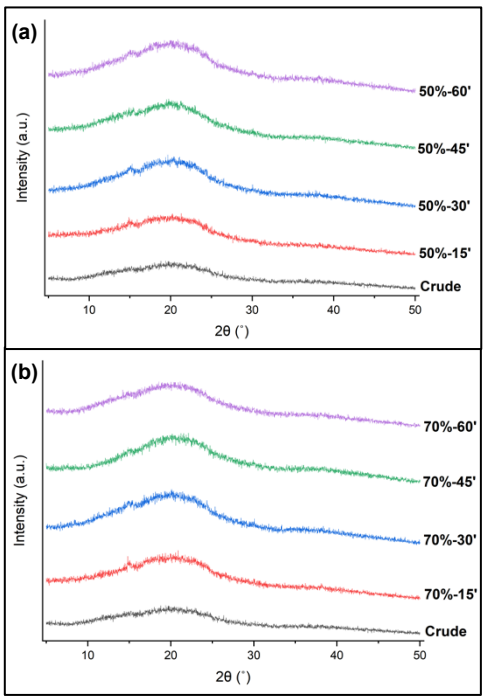

Figure 5 X-ray Spectrum of Purified Glucomannan Flour (a) 50% Ethanol Extraction and (b) 70% Ethanol Extraction

4.0 CONCLUSION

The extraction method from porang chips using ethanol solvent successfully produced a purified glucomannan flour with the best treatment up to 76.2167%, significantly increasing 26.32 –

36.99% of glucomannan content. Moisture content is in the range of 9.99 – 10.85%. It decreased in calcium oxalate content relevance with the increasing extraction time. Ash content for 50% ethanol is better than 70%, under 3% of ash contents for all variables in 50% ethanol. A small amount of Calcium oxalate was found in purified glucomannan flour from SEM analysis indicated by a needle structure. XRD analysis showed a broadly similar pattern, indicating all purified glucomannan flour was in an amorphous state.

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