Physicochemical Properties of Crude and Purified of Glucomannan Flours

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Abstract

Konjac glucomannan (KGM) is a versatile polysaccharide extracted from *Amorphophallus* species tubers. Commonly, extracted crude porang flour still has some impurities which limits the application of utilizing the material. High purity of processed glucomannan flour could satisfy the demand of growing awareness of healthy diet and industrial scale production. In this study, the physicochemical properties of purified glucomannan flour compared with crude porang flour were investigated. Thermal stability of the purified flour was increased indicated by higher degradation temperature, which was revealed by Thermogravimetric Analysis (TGA). The structural characteristics were investigated by FTIR and X-ray Diffraction (XRD) analysis. In addition, Scanning Electron Microscopy (SEM) was conducted to study the morphology and grain size of crude and purified flours. The results revealed that purified glucomannan flour has better thermal stability, higher crystallinity, smaller granule size and significantly higher viscosity compared to crude flour.

Keywords: Characterization; Glucomannan; Physical; Porang; Structure

1. Introduction

Amorphophallus oncophyllus, belonging to the *Araceae* family (taros), is a plant that produces a variety of compounds, including carbohydrates, fats, proteins, minerals, vitamins, and dietary fiber. “Konjac” has become a common term related to the plant [1]. High potential compound which extracted from this plant, konjac glucomannan (KGM) has been utilized as a food additive and exported as an industrial raw material, despite its limited cultivation and production [2]. KGM is a natural polymer which consists of D-glucose and D-mannose in a 1.6:1 molar ratio (depending on the variety), linked together with 1.4-glycosidic bonds [3]. It is a non-ionic polysaccharide which has an exceptional ability to absorb and retain water, making it a valuable ingredient in various industries. Water-holding capability is frequently utilized as a gelling agent, texturizer, and thickener in food and pharmaceutical products [4], [5]. KGM has several health benefits, including being low in calories, making it a suitable component of a healthy diet. Growing awareness of the health benefits of konjac glucomannan (KGM) leads to an increasing demand for producing this material on an industrial scale [6]. The plant has significant economic potential, highlighting the importance of developing these products. In Indonesia itself, “porang” or konjac bulb exports have shown a considerable amount, it is reported that 8,570 tons of porang which worth US$19,645,62 have been exported which shared approximately 20% of the market demand. The primary destination countries for supplying include Malaysia, China, Japan, and Thailand [7], [8]. Nonetheless, the value of these exports is relatively low due to the fact that the porang is typically sold in tuber and chip forms [9]. In addition, according to the data provided by Volza Grow Global, in 2023, Indonesia imported a substantial quantity of glucomannan, primarily sourced from China, totaling 162 tons [10]. This phenomenon indicated Indonesia is heavily reliant on imported processed products due to the limited number of manufacturers in the country capable of producing high-grade commercial glucomannan flour. The distinct disparity between supply and demand reflexed a potential substantial growth in the porang and glucomannan flour export market, particularly for farmers and manufacturers who can capitalize and utilize independently on the existing demand and increase their production to meet the needs of the international market.

Based on our best knowledge, limited study has been conducted to analyze the physical and chemical properties for high-grade commercial glucomannan flour derived from local Indonesian porang products. Therefore, this research aims to study the physicochemical properties of crude porang and purified glucomannan flours, which can serve as a benchmark for further developing glucomannan research and presenting the competitive quality of local products.

2. Materials and Method

2.1. Materials

Raw yellow porang chips were obtained from Porang farmers, Madiun, Indonesia. Porang chips were dried, grounded and sieved into 40 mesh size and named as crude flour. Purified glucomannan flour was obtained from PT. Raja Porang, Indonesia and used as received without further treatment with only sieved with the same size as crude flour. All the chemicals used in this study were purchased from Sigma Aldrich Inc. Germany including 3.5-dinitro salicylic acid (DNS), sodium hydroxide (NaOH), sodium bisulfite ($NaHSO_3$), sulfuric acid 98% (H_2SO_4), d-glucose, potassium sodium tartrate, phenol crystals, and formic acid used without further purification.

2.2 Analysis Methods

2.2.1 Glucomannan

Glucomannan content was analyzed using the colorimetry method utilizing 3,5-Dinitro salicylic acid (DNS) as main reagent under publishing by the Professional Standard of People Republic of China for Konjac Flour [11]. The first step was performing reagent and buffer solution preparation. The reagent was prepared by combining solution A (0.7 g phenol, 1.5 ml of NaOH 10%, 0.7 gr $NaHSO_3$, 7 ml DI water) and solution B (88 ml of 1% 3,5-DNS solution) then furher stored in enclosed dark bottle. Buffer solution was prepared by combining 1 ml of formic acid, 0.25 gr NaOH, and further dissolved with DI water to 250 ml solution. After that, the callibration curves was conducted by making 1 mg/ml d-glucose standard and sequencelly transfered 0.4, 0.8, 1.2, 1.6 and 2.0 ml into 25 ml of volumetric flask, with using DI water as blank solution. Then, 2 ml of DI water was added following by 1.5 ml of 3.5-DNS to each flask. Then, each mixture was heated for 5 minutes in waterbath followed by subsequent cooling to room temperature and dilluted by DI water to 25 ml and homogenized well by proper shaking. Absorbance was determined at 550 nm to exhibit callibration curve plot with glucose content (mg) against absorbance (A). Sample preparation analysis was performed at room temperature. Started with a 0.2-gram sample of porang flour stirred for 4 hours in a 50 ml formic acid-sodium hydroxide buffer solution (0.1 M). The solution was subsequently diluted to 100 ml using the formic acid-sodium hydroxide buffer and centrifuged at 4000 rpm for 20 minutes. The 5 ml supernatant solution was then homogenized with 3 M H_2SO_4 and heated in a water bath at boiling temperature for 90 minutes. Finally, the solution was treated with 6 M NaOH and diluted with deionized water to a total volume of 25 ml, resulting in the formation of a hydrolysate solution. Glucomannan content was obtained by the Eq. 1:

$$\text{Glucomannan Content (\%)} = \frac{\varepsilon\,(5\mathrm{T}-\mathrm{T}_0)\times 50}{\mathrm{m}\times(1-\mathrm{w})\times 1000}\times 100 \quad (1)$$

Where

ε : the ratio of the molecular weight of the glucose and mannan residues in the glucomannan to the molecular weight of the glucose and mannan produced after hydrolysis (0.9)

T : hydrolysate content (mg)

T_0 : glucose content (mg)

w : moisture content

m : sample mass (g)

2.2.2 Calcium Oxalate

Calcium oxalate content was analyzed using the titration method as published by the Indonesian National Standard for porang chips [12]. A 2-gram sample of glucomannan flour was homogenized in a mixture of 190 mL deionized water and 10 mL hydrochloric acid (6 M) and heated at 100°C for 1 hour. The solution was then diluted with deionized water to a total volume of 250 mL. The solution was filtered to discard the filtrate, and the resulting solution was divided into two equal parts of 125 mL each. Titration was performed using ammonium hydroxide (25% v/v) and methyl red indicator, followed by heating in a water bath at 86-90°C. The solution was then cooled, filtered, and reheated to 90°C. Calcium chloride (5% w/v) was added while stirring, and the solution was cooled and allowed to stand overnight at 5°C. The solution was centrifuged at 2500 rpm for 5 minutes, and the decantation method was used to obtain a precipitate. A 10 mL solution of sulfuric acid (20% v/v) was added to each precipitate to dissolve it, and the

precipitates were combined and dissolved in deionized water to a total volume of 300 mL. From each solution, 125 mL was taken and subjected to permanganometric titration using potassium permanganate (0.05 M). Calcium oxalate content was calculated by the Eq. 2:

$$\text{Ca} - \text{Oxalate (mg/100g)} = \frac{V_{KMnO4} \times 0.00225 \times Df}{m \times 5} \times 10^5 \quad (2)$$

where

$VKmnO_4$: $KmnO_4$ volume

N_{KmnO4} : $KmnO_4$ standard normality, 0,00225 = Correction factor of $KmnO_4$ and oxalic acid anhydrate

Df : dilution factor

5 : $KmnO_4$ redox number.

2.2.3 Viscosity

1% porang flour solution was prepared by dissolving the flour in deionized water under continuous stirring at 150 RPM and room temperature (30°C) for a duration of 1 hour. The initial viscosity of the solution was measured using a viscometer (NDJ-8S). The viscosity was subsequently measured at regular intervals of 0.5 hours until the maximum value was recorded [11].

2.2.4 *Moisture Content (MC)*

MC was measured based on the gravimetric method [13]. Dry flour powders were weighed as initial weight, then the samples were dried at 105 °C for 24 h to achieve constant weight.

2.2.5 Ash content

The ash content of the samples was determined using the gravimetric method, which involves comparing the initial and final weights of the samples after furnace drying at a temperature of 500°C for a duration of 4 hours [14].

2.3 Characterization

2.3.1 FTIR (Fourier Transmission Infrared)

The functional group analysis of crude porang and purified glucomannan flour were analyzed using FTIR Spectrophotometer Agilent Cary 630 and MicroLab software (Agilent Technologies, Inc, USA) at room temperature (30 °C) and wavelength range of 650 – 4000 cm^{-1} with step of 2 cm^{-1}. Each sample was directly deposited in a diamond sampling window followed by pressing the sample press tip.

2.3.2 TGA (Thermogravimetric Analysis)

The thermal stability of crude porang and purified glucomannan flour was analyzed using TGA550 (Shimadzu Corporation, Kyoto, Japan) type in air atmosphere at 25 – 600 °C with heat rate of 20 °C/min.

2.3.3 XRD (X-ray Diffraction)

The crystallinity properties of edible films were examined using XRD (D8 Advance, Bruker, Karlsruhe, Germany) at 25 ± 1◦C equipped with Cu-Kα radiation wavelength at 0.1542 nm. The measurement data used a range of 2θ = 5 – 50° at scan rate of 10° min^{-1}. Crystallinity index was calculated using Origin Software 2023b and obtained by Eq. (3):

$$\text{Crystallinity Index (\%)} = \frac{\text{Crystalline peak area}}{\text{Total area}} \quad (3)$$

2.3.4 SEM (Scanning Electron Microscopy)

The flour powders were examined using a Scanning Electron Microscope (Hitachi, FlexSEM 1000, Japan) at accelerated voltage of 15 kV at magnifications of x50, x150, and x5000, respectively.

3. Results and Discussion

3.1. Compositions

The significance of porang flour purification is depicted in Table 1. The glucomannan content is the primary indicator of the quality of porang flour. Based on the classification with the Chinese Ministry of Agriculture guide on konjac flour, crude flour was categorized as a high content (40 – 70%) [11]. However, impurities such as calcium oxalate still considered a high amount for raw material (> 40 mg/100g) [12], which require special attention due to their potential health risks if consumed in long term period and above daily allowance (50 mg/ kg) [15], including skin irritation to kidney failure [16]–[18]. In commercial purified glucomannan flour, calcium oxalate content significantly decreased up to 94.67%. This event affected the higher glucomannan content yielded, which in accordance with previous findings by Chairiyah et al., mentioned that calcium oxalate content has significant relationship [19]. The purification process also affects the significantly rocketed viscosity property, owing to highly reduced calcium oxalate which has much lower solubility in water compared with glucomannan [20], [21]. The viscosity value of the purified flour meets the required standard with 30% more viscous. This elevated viscosity was achieved with a relatively short mixing time and low concentration (1%), which is satisfactory for thickeners in industrial applications. Previous study revealed that the maximum concentration possible is 3% to get the gelation process in order to achieve optimal homogenization [22]. Another finding revealed that KGM could be considered as the additional ingredient for juice thickener in lower concentration [23]. Higher viscosity means thicker fluid consistency, which the glucomannan has stronger molecular forces and internal friction between the fluid and resulted a slower fluid flow [24], [25]. Moisture content indicates the presence of water in a material, which is one of the crucial parameters in order to maintain food shelf life [26]. Moisture content in crude flour is slightly higher than purified ones, owing to the chips raw material that has not undergone advanced drying processes. Ash content measured the mineral or inorganic residue that remains resulting from the combustion of organic constituents [27], [28]. It reflects the impurity compounds present in the material, which can be observed the purified flour has improved with much lower value. This phenomenon has been demonstrated in previous studies that the purification process of glucomannan flour effectively removes the inorganic matter and impurities [18], [29].

Table 1. Composition Comparation of Crude and Purified Glucomannan Flours

Properties	Crude	Purified	Standard
Glucomannan (%)	55.64	90.60	90*
Ca-Oxalate (mg/100g)	65	3.46	30**
Viscosity (m.Pas)	400	41050	32000*
MC (%)	10.38	10.09	10*
Ash content (%)	8.85	1.33	3*

* Professional Standard of the Konjac flour, 2002

** Indonesian National Standard for Porang Chips, 2020

3.2 FTIR Analysis

Functional groups of glucose and mannose in crude and purified glucomannan flour were characterized by infrared spectroscopy analysis, which have almost identical characteristics with slight shifts in peaks position. In Figure 1, broad peak at 3500 – 3200 cm^{-1} was attributed to stretching vibration of inter and intra-molecular O-H bonds [30]. Glucomannan is dominated by a broad hydroxyl group with high intensity, confirming the hydrophilic nature of glucomannan. The absorption peak observed in the range of 2923 - 2850 cm^{-1} was attributed to the stretching vibration of alkane groups, specifically C-H_2 and C-H. These findings have been confirmed in the previous study, where the methyl group was revealed at 2920 cm^{-1} [31]. The absorption band at 1638 cm^{-1} corresponded with C=O bond correlated with O-H. This carbonyl group proved the occurrence of β-1,4 linked glucose and mannose of glucomannan [1]. The acetyl group in the form of C-O group was indicated at 1321 cm^{-1} [18]. In the fingerprint region (800 – 1200 cm^{-1}), specific characteristics of glucomannan were revealed, including absorption peaks at 1149 and 1243 cm^{-1} indicative of the presence of β-1,4 glycosidic and β-1,4 mannosidic bonds, corresponding to C-O-C functional groups [32] .

Additionally, a prominent band observed at 1010 cm^{-1} which was associated with the stretching vibration of C-O-C functional bond, while bands at 874 and 805 cm^{-1} corresponded to other C-H bonds.

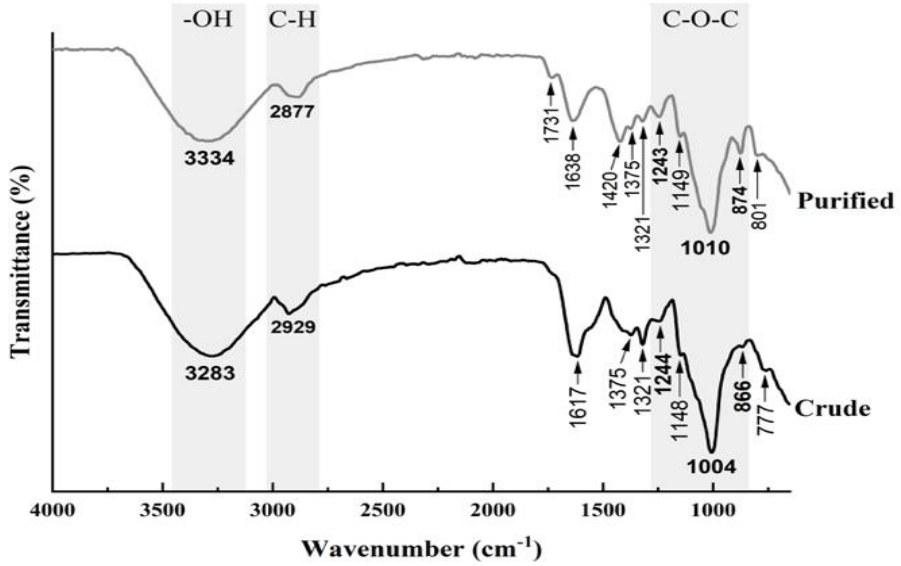

Figure 1. FTIR Spectra of Crude and Purified Glucomannan Flour

3.3 TGA Analysis

Thermal stability of crude and purified glucomannan flour was investigated by conducting thermogravimetric analysis. In Figure 2, thermal events and corresponding weight loss were revealed. The initial degradation phase was happening in the range of 100 – 250°C. Due to the inherent hydrophilic nature of glucomannan, the thermograms showed a constant weight loss which was assigned to the removal of moisture content [33], [34]. Thermogravimetric analysis revealed higher moisture degradation of crude porang flour (16.72%) than purified glucomannan flour (12.79%). This event is consistent with its higher moisture content in composition. The purified glucomannan flour has conducted an advanced drying process to maintain the quality of commercial product. The second degradation region occurred between 250 – 350 °C, where significant weight loss was observed, i.e. 53.94% 57.9% for crude and purified glucomannan flour, respectively. This condition indicated the decomposition of intermolecular side chains such as saccharide rings [35]. The third phase of degradation occurred at 400 – 600 °C, which represented the decomposition of polysaccharide crystalline composites [36], [37]. Interestingly, the derivative curve of purified glucomannan flour exhibited a higher peak (Td = 276 °C) than crude porang flour (Td = 262 °C), demonstrating the improved thermal stability of purified glucomannan flour.

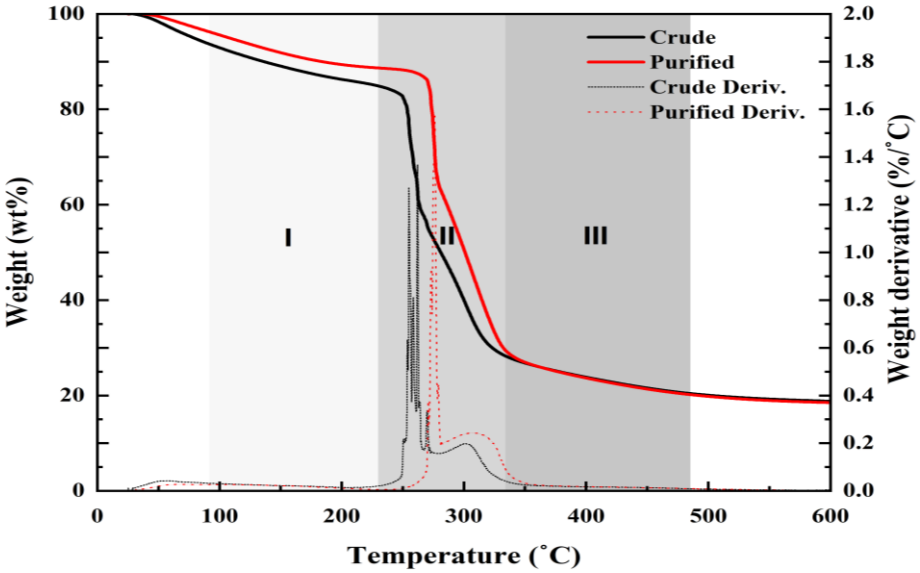

Figure 2. TGA thermograms and Weight Derivative for the Crude and Purified Glucomannan Flour

3.4 XRD Analysis

The XRD (X-Ray Diffraction) patterns of all purified glucomannan samples showed a similar amorphous structure compared to the crude flour, as evidenced by the broad peak at 2θ = 16 – 25° (Figure 3). This broad peak indicates a predominantly amorphous structure with a small degree of crystallinity, suggesting extremely weak and loose molecular interactions within the glucomannan structure. Interestingly, purified flour showed a significant increase in peak diffraction intensity at 19.51° and leads to elevated crystallinity index, with a value of 27.95% with the addition of weaker peak at 14.45°. Whereas the crude flour had a crystallinity index of 23.03% and highest peak diffraction was observed at 19.68° followed by weaker peak at 15.21° and concluded the amorphous structure [38]. Crystallinity index is the ratio area between crystalline peaks and total area of amorphous and crystalline in a compound. This event is consistent with the study by Yanuriati et al., which reported that purified flour has the highest crystallinity compared to crude flour native glucomannan [29].

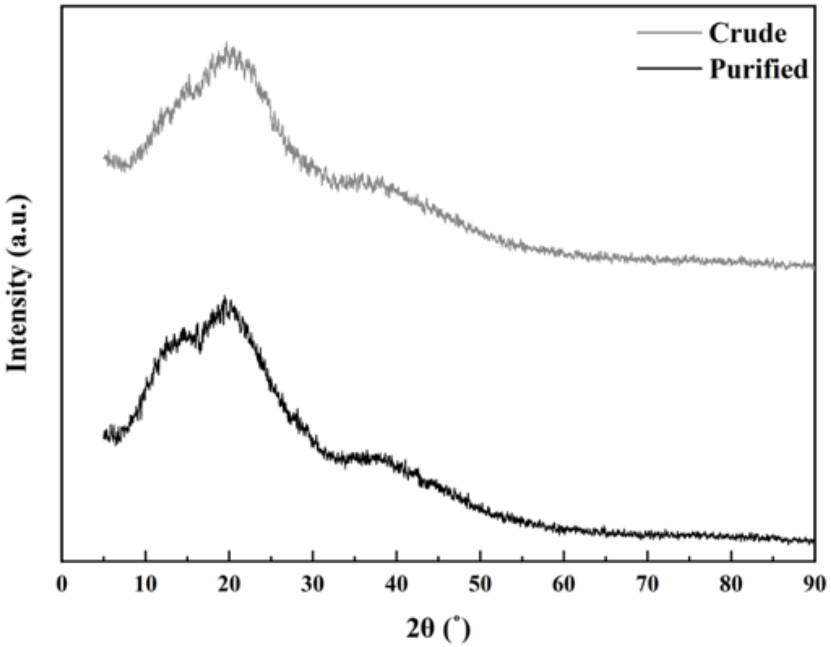

Figure 3. X-Ray Diffraction of Crude and Purified Glucomannan Flour

3.5 SEM Analysis

The morphology of crude and purified glucomannan flour was observed using SEM analysis and presented in Figure 4. Dispersity of distribution showed notable change in size and granule gap. Despite using the same sieving mesh size (40 mesh), smaller granules of purified flour were apparent with an average size of 519 μm than crude flour of 622 μm. From the 50x magnification, it was revealed that crude flour has more gaps and irregular structure instead of purified flour with a smaller cavity and a denser number of granules. This event proved the higher crystallinity of higher purity samples, owing to its more compact structure and solid intermolecular bonds [29], [39]. A needle-like structure was observed at 5000x magnification in the crude flour, which was identified as calcium oxalate. Purified glucomannan flour showed no indication of needle-like structure instead exhibited a more smooth and compact surface structure. This result suggested that extremely low calcium oxalate content has been proved, which leads to high glucomannan content.

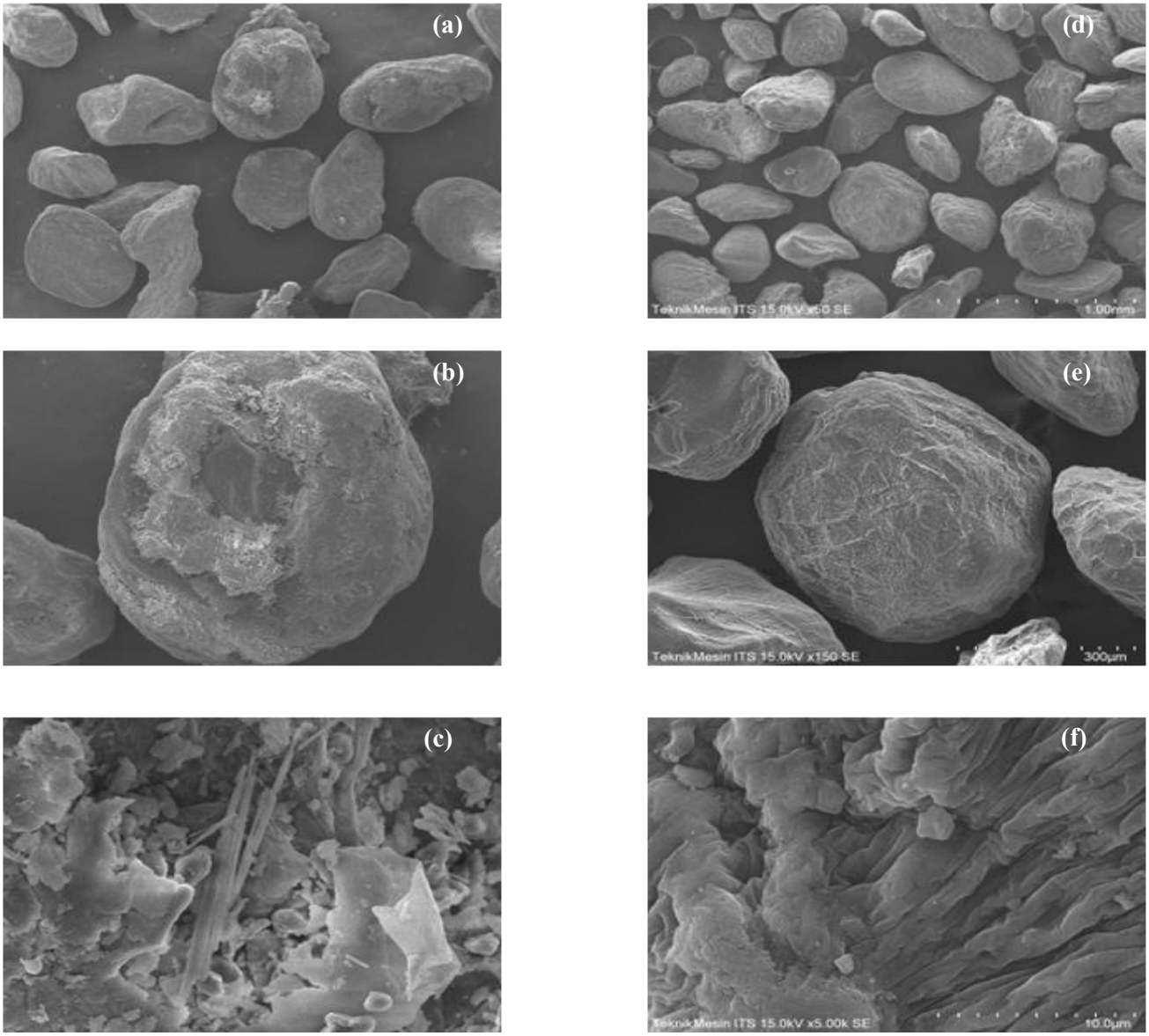

Figure 4. SEM Picture of (a) – (c) Crude and (d) – (f) Purified Glucomannan Flour at 50x, 150x, and 5000x Magnifications

4. Conclusions

The physicochemical comparation of crude and purified glucomannan flour has been investigated. Purification of commercial standard increased glucomannan purity of 90.6% and lowered the calcium oxalate content up to 94.67% Morphological observations revealed the disappearance of the needle compound with a denser and compact structure. As a result, the viscosity increased significantly, and there was a minor rise in crystallinity. Functional group analysis showed similar characteristics, with a minor shift in wavelength.

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