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ORIGINAL RESEARCH

THE UTILIZATION OF ETHANOL AS ANTISOLVENT TO ENHANCE THE GLUCOMANNAN CONTENT OF TUBER OF AMORPHOPHALLUS MUELLERI BLUME

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Abstract

Porang (Amorphophallus muelleri Blume) is widely found in Indonesia. The high Glucomannan (GM) content in Porang (Amorphophallus muelleri Blume) compared to other tubers becomes the main advantage of Porang. Glucomannan is a low-calorie fibre polysaccharide with a β-1,4 bond composed of D-glucose and D-mannose. However, its Calcium Oxalate (CaC2O4) content is an issue and should be minimized when consumed. The research focused on increasing the GM content of raw Porang tuber donated from PT ABS., Bogor. Initially, the raw material was roughly sliced and soaked in NaCl solution (10% or 15 %-w), washed with gently warm water (approx. 40 °C), and air dried to result in Crude Konjac Flour (CKF). Further purification of CKF will produce Konjac Glucomannan (KGM). The KGM has a higher content of glucomannan. Ethanol (EtOH) was used as an antisolvent in the purification step. The final product of KGM was analyzed regarding GM, calcium oxide, moisture, and ash content. A 15%-w of NaCl solution followed by warm water washing could reduce the 59% content of Calcium Oxalate. Meanwhile, further purification using 50% EtOH increased 627.85% GM content from their initial value in Porang tubers. Hence, the treatment combination of NaCl-warm water washing and EtOH purification maximally removes calcium oxide and increases the GM content in the KGM.

KEYWORDS:

Ethanol, Glucomannan Konjac, NaCl, Purification, Porang

1 | INTRODUCTION

Sources of food in Indonesia are very abundant. Based on the Food Security Agency data, there are 77 types of food plants as sources of carbohydrates, 75 types of sources of oil or fat, 228 types of vegetables, 26 types of nuts, 110 types of spices, up to 389 types of fruits in Indonesia. Even though carbohydrate sources such as sago, corn, and tubers are promising, Indonesian people still rely too much on rice commodities, so other commodities are not given much attention.

Porang tubers (Amorphophallus sp.) are included in the Araceae family. Porang has a strategic value to be developed. Therefore, encourage the Indonesian government to increase exports of Porang commodities by establishing it as one of the commodities included in the Ministry of Agriculture program, namely the Triple Exports Movement or GRATIEKS (Agricultural Research and Development Agency, 2021). Porang tubers contain carbohydrates, fibre, fat, protein, and minerals like others. Compared to other tubers, the advantage of Porang tubers is their glucomannan (GM) content. The levels of GM in Porang tubers vary due to the influence of harvest age, the type of Porang, and the conditions of the cultivation environment.

Until now, exports of Porang tubers have been limited (mostly) to raw materials (tubers) or semi-finished materials such as Porang chips with a low selling value. Therefore, to increase the added value of Porang tuber, it is necessary to carry out further processing processes, enhancing the GM content and no longer exporting in the form of raw materials.

Glucomannan is a water-soluble polysaccharide with -1,4 bonds composed of D-glucose and D-mannose. Glucomannan is a low-calorie fibre that can help lower cholesterol levels and weight loss programs[1]. Glucomannan has been used since ancient times as a food ingredient and medicine in China and Japan, and its use is increasingly widespread in the pharmaceutical, cosmetic, and several chemical fields[2]. Glucomannan can be used as flour, yam cake, shirataki, jelly, thickening agents in the food industry, and a filler and tablet binder, stabilizer, and drug delivery system for the pharmaceutical industry. Glucomannan flour, also known as Konjac Glucomannan (KGM), is one of the preparations of GM, which is beneficial for people with diabetes due to its low glycemic index and beneficial for people with high cholesterol[3]. Porang tubers can have a more economical value as a food source when processed into KGM flour. Before processing it into KGM, Porang tuber is generally sliced, washed, and oven-dried, resulting in Porang chips. The Porang chips usually have lower Calcium Oxalate content due to the washing process. The quality of Porang chips is regulated based on SNI 7939:2020. The Porang chips are classified into three different classes. Porang chips, class I, have the highest GM content, ca. >35%, and calcium oxide is at max. 30 mg/100 g. Higher content of GM, ca. >85%, is classified as KGM. The NY/T 494-2002 standard by ROC (Republic of China) regulates the classification of KGM. The best quality of KGM should be content at min—90% of GM. At the same time, GM flour with 60-85% of GM content is termed Crude Konjac Fluor (CKF).

Several purification processes, such as mechanical, chemical, enzymatic, or combination, can increase the GM content. In addition, attention should be given to calcium oxide. Calcium Oxalate can cause itching and kidney problems if consumed excessively and continuously[4]. Soaking the tubers in water for hours can remove this oxalate content. Moreover, a salt solution for soaking reduces Calcium Oxalate levels in tubers[5]. Therefore, this study aims to process Porang tubers into KGM using mechanical and chemical methods to increase GM and reduce Calcium Oxalate levels.

2 | PREVIOUS RESEARCHES

Researchers already tried several methods to enhance the GM content from the Porang flour. However, none of the methods integrate all the efforts from Porang tubers (Amorphophallus sp.). A combination of soaking the tuber with warm water and NaCl solution (5%, 10%, and 15% of NaCl) for an hour was tried by Nurul and Nafi'ah[6]. A higher concentration of NaCl solution will remove more Calcium Oxalate and increase the GM content. A solution of 15% NaCl removes 91.6% of Calcium Oxalate and increases the GM content to 21.5%. Using 15% NaCl solution for soaking the sliced tuber only slightly 3% increases the GM content.

However, a salty flavour is obtained when a washing step is not done appropriately after soaking. Due to the low GM content, the final product is called Crude Kojac Flour (CKF).

Moreover, Ernawati et al.[7] purified GM from CKF using Ethanol (EtOH) as a solvent in many concentration variances, ca. 60, 75, 80, and 90%-v. It is known that EtOH 90%-v increases the GM content up to 58.20%, while its oxalate content is not reported. Other researchers purify GM from chips Porang as a starting material, such as Pasaribu et al.[8]. Instead of using a NaCl solution and warm water, Pasaribu et al. (2019) chose to use natrium bisulfite (NaHSO3) combined with EtOH. EtOH of 30, 40, and 50%-v were tried for four hours. Combining 2% NaHSO3 and 50%-v of EtOH increases 51,31% of GM content. Hence, it finally reached 83,96% of GM. Unfortunately, the residue of NaHSO3 is detected in the final product of Konjac GM (KGM). In contrast, the oxalate content is not reported. Ethanol is an appropriate solvent to purify the GM, while oxalate is another case. Therefore, Nurlela et al.[9] combine EtOH and NaHSO3 to either increase the GM or remove the oxalate from the Porang tuber. The sliced Porang tuber is soaked at NaHSO3 for 20 minutes, oven-dried, and pulverized to obtain the CKF. Subsequently, the CKF was purified with EtOH 40%-v for an hour, repeated with EtOH 60%-v and 80%-v simultaneously; 3 hours total. The other options are purified three times with EtOH 60-v. Though it has been extracted 3x, the highest GM is only 62.2%. Those below the standard value of GM content for KGM, ca. >85%[10]. Lastly, the combination of the soaking process and EtOH purification was done by Wardani et al.[11]. Soaking with warm water, 40 oC, and 15% NaCl solution for an hour, oven-dried, pulverized, and finally purified for 30 minutes in EtOH, 60% produced CKF with 38.53% of GM content. Therefore, this research proposes an integrated process covering physical and chemical processes of purifying the GM from Porang tuber to reach the quality of KGM.

3 | MATERIAL AND METHOD

3.1 | Material

The materials used in this study were Porang tubers obtained from PT ABS (Bogor, Indonesia). Aquadest was commercially purchased while other chemicals such as Ethanol (EtOH) 96%, Sodium Chloride, Phenylhydrazine, Sodium Hydroxide, Acetic Acid, Aceton, Hydrochloric Acid, Nitric Acid, Potassium Chloride, Sulfuric Acid, Potassium Permanganate are all in analytical grade from Merck (Darmstadt, Germany)—standard glass apparatus, as well as a set of reflux apparatus equipped with magnetic and hotplate-stirrer (Cimarec, Thermo Fisher Scientific, England), were also used. The local Sukolilo, Surabaya, Indonesia market purchased a commercial potato slicer. At the same time, hand food blenders (mitochiba, mitoelectric, Indonesia) were commercially available in the local market.

3.2 | Methods

The overall scheme of the experiment works is illustrated in Fig. 1 below.

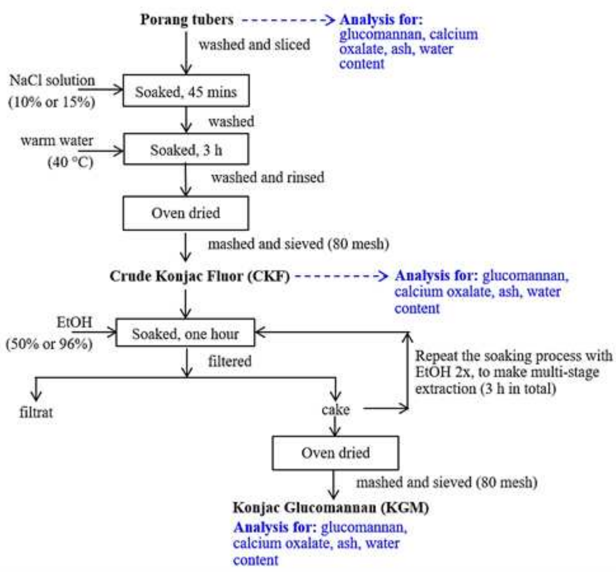

FIGURE 1 Process diagram of overall experiments.

Process of making Crude Konjac Flour

Glucomannan fluor was made by washing and cutting Porang tubers using a commercial potato slicer, soaking in NaCl solution (10% or 15%) for 45 minutes, and finally soaking in 40 oC water for 3 hours. After that, the tubers were dried in an oven. Dried tubers were then mashed with a hand blender and sieved with a size of 80 mesh. The obtained fine size of Porang fluor is later termed Crude Konjac Fluor (CKF).

Glucomannan purification process

The grounded CKF in 80 mesh was soaked using 50% EtOH with a single purification step for 3 hours or three purification steps for 1 hour each, hence a total of 3 hours. The final solution (CKF and EtOH mixture) was filtered and dried in an oven. Meanwhile, 96% EtOH is also used as the solvent for comparison with single-step purification.

Sample Analysis

The resulting samples were analyzed for GM, calcium oxide, moisture, and ash. The GM content was analyzed using the phenyl hydrazine method following the SNI 7939-2020, Calcium Oxalate content using the Permanganometry titration method (SNI 7939-2020), moisture content using oven (SNI 7939-2013), and ash content using furnace (SNI 7939-2013).

4 | RESULTS AND DISCUSSION

Raw materials, i.e., Porang tubers and Porang chips, are donated from PT. ABS. have specifications as shown in Table 1 . The Porang tubers subsequently proceeded as described in subsection 3.2.

TABLE 1 Raw materials specifications

	Raw materials	
	Tubers	Chips
Glukomannan (%)	11.93	65.35
Calcium oxalate (mg/100 g)	75.56	94.48
Water (%)	79.26	10.15
Ash (%)	6.63	13.21

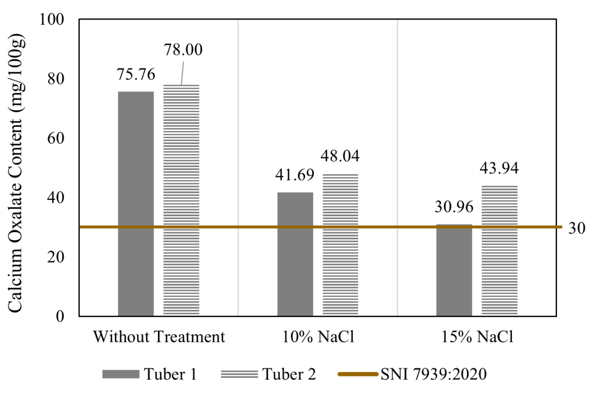

FIGURE 2 Calcium Oxalate Content in Porang Bulbs Soaked with 10% and 15% NaCl

4.1 | Pretreatment of The Porang Tubers

Porang tubers contain Calcium Oxalate crystals. Calcium Oxalate in Porang tubers generally has a needle crystal form (rafida) and druse, which can cause itching and irritation[12]. Based on the Indonesian National Standard (SNI 7939:2020) for Porang chips, the maximum permitted level of calcium oxide for quality I is 30 mg/100g. Excessive consumption of Calcium Oxalate can cause health problems such as precipitation in the kidneys and the formation of stones in the urinary [4]. Therefore, Calcium Oxalate's content in Porang

tubers must be minimized. Immersing the sliced Porang tubes in a NaCl solution (10 or 15%) and soaking them in warm water are strategies to minimize the calcium oxide. The decrease of calcium oxide contact after the treatment is shown in Fig. 2 .

It is seen (Fig. 1) that treatment with 10% NaCl solution only decreases the Calcium Oxalate to 44.87 mg/100 g. This value is still above the minimum allowable limit of Calcium Oxalate (SNI 7939:2020), ca. 30 mg/100g. In contrast, a 15% NaCl solution may reach the minimum allowable limit of Calcium Oxalate. Hence, a higher NaCl solution (ca. 15%) removes 59.02 % and 50.38% of calcium oxide from fresh Porang tuber and Porang chips, respectively. A reaction may be present between the calcium oxide and NaCl solution, causing a decrease in the calcium oxide content. NaCl was ionized in water. Sodium ions bind to oxide ions to form sodium oxide. Meanwhile, chloride ions bind to calcium ions, forming a white precipitate of calcium chloride, easily soluble in water (Eq. 1)[11].

$$NaCl + CaC_2O_4 \rightarrow Na_2C_2O_4 + CaCl_2 \quad (1)$$

NaCl solution in 15% concentration gave the best results for removing Calcium Oxalate ca. 51%. The higher the NaCl concentration, the more Na+ and Cl- ions were contained in the solution. Subsequently, more Sodium Oxalate bonds were produced and soluble in water. At the same time, 10% of the NaCl solution only removed Calcium Oxalate 41.20%. Finally, warm water soaking could reduce and wash out calcium oxide[6]. Unfortunately, attention should be given to the side effects of soaking in a high NaCl solution, one of which is to produce salty flour.

As well as NaCl solution, soaking the Porang tubers in warm water may decrease calcium oxide content. Soaking the tubers in warm water (38-48°C) for more or less than 4 hours can reduce the components that can cause itching without causing starch gelatinization[13]. The warm water soaking, after NaCl, also aims to reduce the salty taste that may occur due to the NaCl soaking process. Attention may arise during the water soaking. There was a physical change in the Porang tubers and the solution used for soaking. After soaking, Porang tubers have a gel-like layer covering the surface due to the dissolved GM in the solution. The high concentration of NaCl (15%) will open the structure of the tuber; therefore, GM will quickly come out from the structure to the solution. Glucomannan dissolves in water and forms a stable gel[14]. Hence, a double soaking strategy with warm water and NaCl solution or vice versa is not recommended. Though reducing the Oxalate content, the reduction of undesirable GM content also happened.

4.2 | Quality Content of Glucomannan Fluor after Treatment with Ethanol

TABLE 2 Glucomannan Fluor from Porang Tubers with Different Purification Strategies

Purification Strategies	Glucomannan (%)	Calcium Oxalate (mg/100 g)	Moisture (%)	Ash (%)
Reference standard				
SNI 7939:2020	35.00	30	12.00	4.00
NY/T 494-2002	85.00	ND*	10.00	3.00
Samples				
Without Any Treatment	12.18±0.36	76.78±1.73	76.83±3.43	8.01±1.94
10% NaCl+Single Stage EtOH	64.27±1.40	29.64±3.51	13.49±4.30	4.17±1.48
10% NaCl+Multiple Stage EtOH	81.61±0.66	26.62±7.62	9.18±1.19	4.11±0.97
15% NaCl+Single Stage EtOH	66.29±2.34	27.56±6.24	14.27±0.10	4.14±0.07
15% NaCl+Multiple Stage EtOH	87.10±0.39	17.54±3.05	9.09±0.34	2.10±0.54
10% NaCl+96% EtOH	51.13	37.39	6.13	2.71
15% NaCl+96% EtOH	51.68	40.99	8.12	2.35

**N.D = not determined; **single and multiple purification stages were done using EtOH 50%-v.*

Glucomannan Content

Glucomannan is expected to be the main component in GM flour, with the highest content among other components. Glucomannan is a polysaccharide composed of D-Glucose and D-Manose in the form of fibre naturally and is soluble in water, low in calories, translucent, and has an odor-like jelly [15]. Immersion of the Porang tubers with EtOH can affect the quality of GM flour, including the contents of GM itself, calcium oxide, water, and ash. Increasing the GM content in Porang tubers in this research was done by soaking them in 50% or 96% Ethanol (EtOH) (Table 2).

Ethanol was used as a solvent to reduce the impurity compounds found on the surface of the Porang flour granules. Glucomannan has a non-linear (β-1,4) bond between mannan and water-insoluble glucan. Glucomannan is a tricky grain to crush[16]; its particles are more prominent than other particles in the tuber[17], soluble in water, and form a gel[18]. Based on their chemical structure, they can potentially have many intramolecular hydrogen bonds between their molecules[19]. When GM meets water, intermolecular hydrogen bonds occur, increase the solubility of GM in water, and form a gel[20].

In contrast, GM is not soluble in EtOH[21]. Therefore, a proper ratio of EtOH to GM is needed as an anti-solvent without causing any gelling of GM[11]. Without EtOH as an anti-solvent, GM will dissolve in water and cannot be separated from other components to be removed, such as ash and calcium oxide. Purifying the CKF with EtOH will minimize impurities and increase the GM content. More purification steps will maximize EtOH in dissolving impurities on the GM granules' surface[22] and increase the GM purity.

It is seen that multiple purification steps with EtOH increase the GM (Table 2). Ethanol removes fine micro powder and impurities from the surface, such as ash, oxalate, starch, and protein, which are still contained in GM particles. Ethanol concentration of 50% has a higher polarity than absolute EtOH (ca. 96% EtOH) due to the containment of water; moreover, 50% EtOH dissolves more polar impurities. The higher the concentration of EtOH, the lower their polarity. The consequence is that non-polar components may dissolve. More purification steps maximize the EtOH in dissolving impurities on the surface of the GM granules[22].

While 50% EtOH was used as an antisolvent in the purification step, no thickening was observed when Porang tubers were soaked using NaCl solution, i.e., either 10% and 15% concentration and warm water. Physical changes are observed in the colour change of the ethanol solution; it becomes cloudy right after filtering, indicating that some substances dissolved and carried in the EtOH.

Calcium Oxalate Content

Ethanol purification steps increase the GM content; in contrast, they lower the Calcium Oxalate content (Table 2). It is seen from Table 1 that single steps of EtOH purification just lowered slightly below the standard of SNI 7939:2020, ca. 30 mg Calcium Oxalate/100 g of Porang tuber. In addition, multiple purification steps, i.e., three steps, can reach 17.54±3.05 mg Calcium Oxalate/100 g with the combination of 15% NaCl as a pretreatment process. A low content of Calcium Oxalate in the final product, i.e., konjac glucomannan (KGM), may be removed by EtOH. As an antisolvent, EtOH removes the attached impurities and Calcium Oxalate in the CKF[23]. Hence, the calcium oxide content decreased (Table 2).

Different steps of purification also affect the Calcium Oxalate content. More purification steps will prolong the contact time between Crude Konjac Flour (CKF) or Porang flour with EtOH. Therefore, more impurities and calcium oxide are removed, lowering the calcium oxide content. In addition, stirring also affects purification; it facilitates the release of impurity components from the surface of the Porang flour, as well as ash, oxalate, starch, protein, and others[11]. However, the stirring effects are not studied in this research.

Moisture and Ash Content

The moisture content expresses the age-storage of the KGM. Low moisture content is desirable, extending shelf-life. High water content can affect microbial growth and enzyme activity and cause damage to foodstuffs due to spoilage [24]. The lowest moisture is KGM with a combination treatment of 10% NaCl and 96% EtOH, i.e., 6.13% (Tabel 1). The value fulfilled both requirements of SNI 7939:2020 and NY/T 494-2002. All the multistep purification also resulted in moisture content below 10%, as requested by NY/T 494-2002 standard.

At the same time, only KGM with a combination treatment of 15% NaCl with multiple steps of purification and KGM with purification using 96% Ethanol meet both the standards of Porang chips in SNI and NY/T 494-2002, respectively 4 and 3%. High ash content in flour can affect the colour of the flour. High ash content can make the colour of the flour brown[24]. A low ash level may be achieved by grinding the KGM grain. Indicates that a combination of physical and chemical treatment is needed to reach the physical-chemical properties of KGM as required by both standards of SNI 7939:2020 and NY/T 494-2002.

5 | CONCLUSION

Combining treatments using 15% NaCl-warm water washing followed by multistep purification using 50% Ethanol gave the best results, which fulfilled both requirements' standards of SNI 7939:2020 and NY/T 494-2002. Resulting in 87.10±0.39% GM, 17.54±3.05 mg Calcium Oxalate/100 g, 9.09±0.34% of moisture, and 2.10±0.54 of ash. A combination of physical and chemical treatment is needed to reach the physical-chemical properties of KGM as required by both standards of SNI 7939:2020 and NY/T 494-2002

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CREDIT

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