Effect of harvest timing and length of storage time on glucomannan content in porang tubers

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Abstract. Amorphophallus muelleri Blume synonym of A. oncophyllus (porang) is one of the species of porang that grows in Indonesia, especially in East Java. It has a high economic value because the tubers contain glucomannan. Earlier work reported that harvest timing and length of storage affected secondary metabolite containing on plants. The study objective was to determine the glucomannan content of porang at 1, 2 months before dormant, at dormant and length of storage time (1 and 2 months after dormant) at room temperature. Glucomannan was isolated from fresh tubers to easier removal of the impurities and obtain the high purity of glucomannan. The highest obtained glucomannan was determined its functional groups using Fourier Transform Infrared (FTIR). The highest glucomannan content was derived in tubers taken at dormant compared to before and after dormant. FTIR spectra confirmed the presence of functional groups that compose the glucomannan compound.

1. Introduction
Amorphophallus muelleri Blume Synonyms of A. oncophyllus (porang/iles-iles kuning) is a type of porang that grows in Indonesia, especially in Java, which has the highest glucomannan content compared to other types that grow in Indonesia [1]. Porang has high economic value because the tubers contain glucomannan. Glucomannan is a polysaccharide consisting of D-glucose and D-mannose units. Glucomannan is a water-soluble dietary fiber, strong hydrocolloid and low calorie which is widely used in the food and non-food industries such as in the cosmetics and health products industries [2]. The benefits of glucomannan in health have been extensively studied, including cholesterol reduction [3-6], normalizing triglyceride levels in the blood. [7], controlling blood sugar levels [8], improves intestinal performance [9], reduces postprandial glycemia and cardiovascular risk [10].

Generally, purification of glucomannan is conducted on porang which has been dried and made flour. Purification is conducted mechanically and chemically using ethanol. Washing of porang flour with ethanol will dissolve other compounds except for glucomannan because ethanol has a high polarity [11]. The drying process will cause other components that affect glucomannan impurities to attach firmly to the surface of the glucomannan granules so that it will be difficult to remove and reduce the purity of glucomannan [12]. To get high purity, glucomannan was isolated from fresh porang tubers. From that study obtained more than 90% glucomannan purity using repeated extraction methods (7 times) using 50% ethanol [12].

Glucomannan content varies depending on the part of the tuber and the period of plant growth [13], plant type, plant age, length of time after harvest [14-17]. Amorphophallus konjac at the beginning of
growth had a lower glucomannan content than when it was dormancy [18-19]. Sometimes to meet high
market demand, porang are harvested before entering dormancy, usually 1 or 2 months before dormant.
Conversely, when the demand is low, porang that have been harvested are stored or processed into chips
and flour. Length of storage affects the glucomannan content. The purpose of this study was to determine
the glucomannan content of porang at 1, 2 months before dormant, at dormant and length of storage
time (1 and 2 months after dormant) at room temperature to propose the right time of either harvesting
or storing the porang tubers in order to get the high glucomannan content. Also to see the effectiveness
of the direct glucomannan isolation method from fresh tuber using ethanol.

2. Materials and Methods
The primary materials used in this study were three years old fresh porang tubers from the species
Amorphophallus muelleri Blume synonyms of A. oncophyllus (porang/iles-iles kuning) that were
harvested 2 months and 1 month before dormant, during dormancy, and had been stored for 1 and 2
months after harvest at room temperature. These porang tubers were obtained from the Bogor
Agricultural University experimental garden, Bogor, Indonesia. Ethanol 96%, aquadest, and other
chemicals were analytical grade from Merck Co, Tedia, etc.

2.1. Isolation of Glucomannan from Porang Fresh Tuber
Glucomannan was directly isolated from fresh tubers of porang. This method was modified from
Yanuriati [12]. The tubers were peeled, then sliced and shredded. Then milled in 40% ethanol around
1500 rpm for 1 hour, filtered and pressed. These milling, filtering, and pressing processes were repeated
in 60% dan 80% ethanol. The ratio of tuber (gram) and ethanol (ml) 1: 7. The obtained glucomannan
granules were dried in the oven at 40°C. Then weighed and characterized the purity and physicochemical
properties, including proximate, yield, glucomannan content, viscosity, visual color difference, and
polarization plane rotation. The result was compared to commercial glucomannan.

2.2. Physicochemical Properties
2.2.1. Chemical Compositions. The content of moisture, ash, lipid, protein, carbohydrate, crude fiber
were determined according to AOAC methods [20]. The starch was analyzed qualitatively by staining
 glucomannan granules using I-KI [21].

2.2.2. Glucomannan Content. Glucomannan content was analyzed using a 3,5-DNS method. Reagent
3,5-Dinitro Salicylic Acid resulted in a different yellow color in the samples, then analyzed using a UV-
VIS spectrophotometer [22]. Reagent 3.5-Dinitro Salicylic Acid was made by mixing solution A (0.7 g
phenol; 1.5 ml of 10% sodium hydroxide; 5 ml distilled water; and 0.7 g sodium bisulfite) with solution
B (22.5 g potassium sodium tartrate; 30 ml of 10% sodium hydroxide; 88 ml dinitro salicylic acid 1%).
Glucomannan extract was made by dissolving 0.2 g in 100 ml buffer solution (formic acid-sodium
hydroxide), stirred for 4 hours, then centrifuged at 4000 rpm for 20 minutes, the supernatant was
 glucomannan extract.
The next step was to made glucomannan hydrolysate. 5 ml of glucomannan extract mixed with 2.5
ml of 3M sulfuric acid, heated in boiling water bath for 1.5 hours. The solution allowed to cool to room
temperature then added 2.5 ml of 6 M NaOH, then diluted with distilled water to 25 ml volumetric flask.
Both the glucomannan extract and hydrolysate (2 ml) were added 3.5 DNS reagent (1.5 ml). Absorbance
was then measured at 550 nm used UV-VIS spectrophotometer. D-glucose standard solution (1 mg/ml)
was diluted to 0.20%, 0.40%, 0.80%, 1.20%, and 1.60% using deionized water (DI). About 1.5 ml of
1% 3.5 DNS solution was added to 2 ml of the sugar standards. Each mixture was heated for 5 min in a
boiling water bath and cooled to room temperature before being diluted to 25 ml with DI water in a
volumetric flask. Absorbance was then measured at 550 nm, and a plot of the measured absorbance
against the glucose content (mg) was constructed. The glucomannan content was calculated by the
following equation:
3. **Results and Discussion**

3.1. **Physicochemical Properties**

Glucomannan was extracted from fresh tubers, to obtain high purity glucomannan. Generally, glucomannan was purified on porang which has been dried and made flour. The drying process would cause other components except glucomannan stuck firmly to the surface of glucomannan granules so it would be difficult to remove and reduce the purity of glucomannan [12]. The chemical composition of the glucomannan extract showed in Table 1.
Table 1. Chemical composition of the glucomannan extract

| Chemical Composition (%) | 2 months before dormant | 1 month before dormant | During dormancy | 1 month after dormant | 2 months after dormant |
|--------------------------|-------------------------|------------------------|-----------------|----------------------|-----------------------|
| Moisture                 | 12.02                   | 12.76                  | 11.57           | 11.41                | 9.92                  |
| Ash                      | 2.10                    | 1.93                   | 1.51            | 0.72                 | 0.74                  |
| Lipid                    | 0.42                    | 0.49                   | 0.77            | 0.24                 | 0.14                  |
| Protein                  | 13.16                   | 12.97                  | 13.92           | 3.78                 | 3.43                  |
| Carbohydrate             | 72.12                   | 72.19                  | 71.27           | 68.26                | 85.71                 |
| Crude fiber              | 2.58                    | 2.44                   | 2.30            | 1.38                 | 0.89                  |
| Starch (I$_2$-KI test)   | -                       | -                      | -               | -                    | -                     |
| Yield                    | 24.20                   | 26.24                  | 69.36           | 42.68                | 47.60                 |

- = no color (negative)

One of the most critical proximate components is water content. The water content contained in foodstuffs can affect the quality and storability of these foodstuffs. High water content in food can cause some damage including microbial growth, browning reactions, and fat hydrolysis. The minimum water level limit where microorganisms can still grow is around 14-15% [25]. From the test results obtained all glucomannan extracts have water content below 14%, which ranges from 9-12%.

The yield is one of the parameters that determine the efficiency of a method. The yield obtained from the extraction results is quite good, the highest reaches 75%. However, the yield obtained was quite low in the tubers sample 2 months and 1 month before dormant, because the work was less neat and not effective.

Table 1 showed that the most significant content of porang tuber was carbohydrate, ranging from 70-85% dry weight. Carbohydrates found in porang are starch, fiber, and glucomannan. Components that have high economic value are glucomannan. Glucomannan is a polysaccharide composed of D-glucose and D-mannose units. The results of the analysis by hydrolysis of acetylation on glucomannan produced a trisaccharide consisting of two D-mannose and one D-glucose so that in one molecule glucomannan contained D-mannose of 67% and D-glucose of 33% [14, 26].

Direct glucomannan isolation method from fresh porang tuber using ethanol p.a was effective in producing glucomannan with high purity. There is a small percentage value of impurity compounds such as ash, fat, crude fiber. Meanwhile, the protein content is quite diverse, because some proteins are not too soluble in ethanol p.a and tend to settle. So, it is filtered together with glucomannan during the filtration process. Starch analyzed qualitatively using I-KI. The presence of dark blue color after staining indicated high starch content of glucomannan [13]. The result showed a yellowish color indicating that the extract did not contain or only contained little starch. During the stirring process, impurities such as ash, fat, protein, starch, and fiber that were found around glucomannan granules in fresh tubers were easier to remove than if glucomannan purification was conducted on drying porang chips and flour. The process of drying porang chips makes the impurity compounds stick more firmly.

Glucomannan content varies depending on the part of the tuber and the period of plant growth [15], the type of plant, the age of the plant, the length of time after harvest [14,16-18]. Porang is generally harvested when entering the dry season (May-June). At that time, plants enter a period of rest or dormancy. However, sometimes to meet high market demand, porang are harvested before entering dormancy. Amorphophallus konjac at the beginning of growth has a lower glucomannan content than when the plant dormancy [18-19]. This is because glucomannan was used as an energy source for leaf growth. After growth reaches its maximum, glucomannan is no longer used for metabolic processes, but more accumulated in the tubers until the plant reaches the dormancy phase. This is appropriate with the results in Table 2, the glucomannan content of 2 months tuber and 1-month tuber before the dormancy period is lower compared to the tuber when dormant.
Conversely, when the demand is low, porang that have been harvested are stored. The high water content in porang is 70-85%, porang tubers are easily damaged by fungi or mold [14]. Therefore, generally, porang tubers are dried into chips or further processed into porang flour. However, the purity of glucomannan obtained is lower than if glucomannan is extracted directly from fresh tubers. The test results in Table 2, obtained glucomannan of 1 month and 2 months after dormant lower than during dormancy. After the dormancy phase, the content of glucomannan in the idioblast gradually decreases during the embryonic stage: from the formation of buds to the appearance of leaf strands [27]. During the initial bud development, glucomannan in idioblast decreases and changes gradually to low-density material, which results in low glucomannan expression than that produced at the dormancy stage.

| Table 2. Glucomannan content of the glucomannan extract |
|--------------------------------------------------------|
| Glucomannan Content (%) ± SD                           |
| 2 months before dormant      | 44.04 ± 0.03 |
| 1 month before dormant      | 83.42 ± 0.10 |
| During dormancy              | 90.0 ± 1.44  |
| 1 month after dormant        | 76.91 ± 1.29 |
| 2 months after dormant       | 53.58 ± 0.49 |

In this study, the tubers used in each monthly test were different tubers, because the sampling used was random. The difference in glucomannan content was also thought to be due to the different photosynthesis rate due to the influence of genetic factors and environmental factors such as water availability, availability of CO₂, the impact of light and temperature [28]. Tuber weights tested were varied. Tuber weight is one of the factors that influence glucomannan content. Earlier work reported that 11% of tuber weight variation was influenced by glucomannan content and 89% was influenced by other factors, such as carbohydrates and water content [29]. The results were statistically analyzed by one way ANOVA test. The F value was higher than the F critical value at the sign. The test showed that there was a difference of glucomannan content significantly at their time of harvest.

3.2. Calcium oxalate
Insoluble oxalate compound in Porang is calcium oxalate. The presence of calcium oxalate is as a defense against herbivores. But, a high concentration of calcium oxalate can cause irritation and itching. The glucomannan extract of the dormant tuber contains 1.35% (w/w) of calcium oxalate, its value was quite small compared to glucomannan extraction from the flour. Isolation of glucomannan from the flour produced glucomannan with 2.11% of calcium oxalate [30]. The extraction process with multilevel ethanol could increase glucomannan levels and reduce levels of calcium oxalate [31-32].

3.3. Color
The color of glucomannan extract varied as shown in Figure 1. The extraction of tubers before dormant were darker in color because the preparation of the sample before extraction was too long so that the tubers had browned. This became an evaluation for the next extraction, of the tubers at dormant and after dormant.
3.4. Characterization of glucomannan

The extract that has the highest glucomannan content was isolated from tuber at dormant, characterized by FTIR to determine the glucomannan constituent, functional groups. In FTIR data spectra in Figure 2, there is a broadband at the 3432 cm\(^{-1}\) wave number which is the absorption of OH groups on glucose [33-34] and mannose compounds, the composites of glucomannan, absorption bands in the range 2850-2925 cm\(^{-1}\) show the vibration of asymmetry stretch and symmetry of alkane, CH groups [34], CH\(_2\), [35-36] and CH\(_3\) [33]. Whereas the shift of C=O [34;37] and C-O groups [33] of acetyl groups were absorbed in 1878 cm\(^{-1}\) and 1323 cm\(^{-1}\). Furthermore, absorption bands at 1444 cm\(^{-1}\) and 1383 cm\(^{-1}\) showed asymmetric bending vibrations and symmetries of CH, CH\(_2\), and CH\(_3\). C-O-C stretch vibration was shown in the 1158 cm\(^{-1}\) band. The C-O group on the alcohol bond could be seen in the uptake of the 1027 cm\(^{-1}\) band, and the \(\beta\)-pyranose mannose and glucose bonds are shown in the absorption band of 890 cm\(^{-1}\) [38].
4. Conclusions
Glucomannan content varies depending on the age of the plant and the length of time after harvest. The highest glucomannan content was obtained in tubers taken at dormant compared to 1, 2 months before and 1, 2 months after dormant. After growth reaches its maximum, glucomannan is no longer used for metabolic processes, but more accumulated in the tubers until the plant reaches the dormancy phase. After the dormancy phase, the content of glucomannan in the idioblast gradually decreases during the embryonic stage: from the formation of buds to the appearance of leaf strands. Direct glucomannan isolation method from fresh porang tuber using ethanol p.a was effective in producing glucomannan with high purity. There is a small percentage value of impurity compounds such as ash, fat, crude fiber, starch, and calcium oxalate. FTIR spectra confirmed the presence of functional groups that compose the glucomannan compound.

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