LC-MS analysis of carbohydrate components in Porang tubers (*Amorphophallus muelleri* Blume) from the second and the third growth period

D Gusmalawati$^{1,2,3,*}$, E L Arumingtyas$^{2,3}$, R Azrianingsih$^{2,3}$ and R Mastuti$^2$

$^1$Biology Department, Faculty of Mathematics and Natural Sciences, Tanjungpura University, West Kalimantan, Indonesia
$^2$Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, East Java, Indonesia
$^3$Indonesian Porang Research and Development Center (P4I), Brawijaya University, East Java, Indonesia

*e-mail: dwi_gusmalawati@yahoo.com*

**Abstract.** The presence of carbohydrates, especially glucomannan, causes porang tuber to be important in some industries including food, cosmetics, medicines, and other industries. Porang has four growing periods (GP) in its life cycle. The GP1 until GP3 is the vegetative phase and GP4 is the generative phase. The tubers used as industrial materials generally were derived from GP2 and GP3 plants. The study aimed to analyse carbohydrate components in porang tubers from GP2 and GP3 plants using Liquid Chromatography Mass Spectrometry (LC-MS) method. The tubers produced from plants at GP2 and GP3 were extracted by maceration method using methanol solvent (pa) for 1 h. Liquid extracts obtained was further identified for the levels of carbohydrate components using LC-MS technique. The carbohydrate components identified with LC-MS were selected for quantifications. LC-MS analysis showed that both GP2 and GP3 tubers contained 67 types of chemical compounds. In the GP2 tubers 8 types of carbohydrate components were detected. Whereas in the GP3 tuber in addition to the 8 types of carbohydrate components that are the same as in the GP2 tuber there is also 1 other type of component, namely trehalose. Glucomannan is the highest levels of the carbohydrate component in GP2 and GP3. However, glucomannan levels in GP2 tuber (58,845.54µg/g) were lower than those in GP3 tuber (99,436.85µg/g). Therefore, GP3 tubers are better than GP2 to be used as industrial materials.

1. **Introduction**

Porang (*Amorphophallus muelleri* Blume) is classified into the Araceae family, a tuberous herb with high glucomannan levels [1-3]. Glucomannan is a carbohydrate composed of glucose and mannose monosaccharides, useful in the food, health, cosmetics, and other industries [4, 5]. Glucomannan is very well used for diet programs, lower cholesterol, prevent heart disease, and reduce high blood pressure. In addition, it can also be used for industrial purposes such as paper, textile, rubber, paint, artificial leather, plastics, film coatings, glues, mineral purification, and water purification [6-9]. The many benefits of glucomannan cause porang tubers have important economic value, so the demand for industrial needs is increasing [10, 11].
Porang plants have a different life cycle than plants in general. The length of the life cycle from seedbed to fruiting and lodging requires 38-48 months or consists of four growing periods (GP). The growth period of one to three is a vegetative phase which produces petioles, leaves and tubers with each phase lasting 5-6 months. In GP4, tubers produced from GP3 undergo a generative phase, which is the flowering process that produces fruit and seeds. This generative phase lasts for 8-9 months and after that the plant dies [12-15].

Porang tuber harvesting is generally carried out in the dry season (April-July), when the plant enters a dormant period, i.e. tubers from GP3 because it has heavier weights than tubers from GP2. Porang tubers suitable for industrial use are tubers from GP2 and GP3, because the glucomannan content in both tubers is more than 41.8%. Nevertheless, GP3 tubers have the highest levels of glucomannan compared to GP1, GP2, and GP4 [2, 16-18].

Utilization of porang tubers as industrial material at this time considers more carbohydrate level, especially glucomannan, so research on glucomannan in several growing periods has been carried out. However, other carbohydrate components have not been studied. In fact, other carbohydrate components such as glucose, xylose, arabinose, rhamnose, trehalose, and galactose also have important benefits in the industrial field. Glucomannan can be used as an ingredient in the cosmetics, food and health industries because it has high viscosity. Mannan functions as a food thickener and thickener. Glucose, xylose, and galactose as energy sources, prevent and repair disease. Mannose can cure worm infections. Rhamnose and trehalose are good for health and cosmetics, while arabinose is an antioxidant [18-22]. The purpose of this study was to identify carbohydrate components in porang tubers from GP2 and GP3 plants with LC-MS. Carbohydrate components that have been identified are then chosen to be quantified, so that tuber age or harvest time can be known to get the best quality tubers in industrial requirements.

2. Material and Methods

2.1. Description of Samples and Determination of Harvest Time
The samples used in this study were porang tubers derived from plants of the second (GP2) and the third (GP3) growing period, one tuber each. This tuber was obtained from the porang garden in Bantur District, Malang Regency, and East Java Province. Harvesting of tubers was carried out in May 2019, when the plants have fallen, i.e. the stems and leaves have dried up and were separated from the tubers.

2.2. Extraction of Carbohydrate Components
Every 0.5 g of fresh porang tubers was crushed in 17.5 mL of methanol; 7.5 mL distilled water was added and allowed to macerate at room temperature for about 1 hour by continuous shaking in the shaker. The extract was filtered through Whatman No. 1 filter paper, and the solids retained macerated with methanol-water (3 x 25 mL) as mentioned above. The filtrate was collected and transferred to a 100 mL volumetric flask. Next, the filtrate was evaporated using a rotary evaporator to separate the methanol solvent, so that half the volume of the previous solution was obtained. The extract solution was centrifuged at 5000 rpm for 20 minutes. Then, the supernatant was taken and analyzed using LC-MS [23].

2.3. LC-MS Analysis of Carbohydrate Components
The carbohydrate components in porang tubers were identified using SHIMADZU analytical LCMS system. LC-MS setting was supported by LC-MS Solution for Windows. LC parameters were set using one pump with a flow rate: 0.8 mL / min and a pressure of 15 kgf / cm². Carbohydrate levels were examined in UV light at wavelengths of 254 and 190 nm, for 1 sec response time, AUX range 2 AU / V, D2 lamp, mode + polarity for UV detection settings and used 100 minutes stop time for LC acquisition time data setting. For MS parameter, the interface parameter was set at 250 °C, 250 CDL, nebulizer gas flow 1.5 ml / min, and block temperature 400°C. While MS parameter was set at scan
type mode, scan acquisition mode +, 0.5 second interval, detector gain 1.5 kV, start at 50 m / z, final at 500 m / z, scan speed 1000, +4.5 kV voltage probe high and 25 V CDL voltage [23-25].

3. Result and Discussion

LC-MS analysis on Porang tubers from GP2 and GP3 each showed 67 compounds marked by the number of peaks in the chromatogram (figure 1). The peaks on the chromatogram were formed according to the retention time (RT) of each compound. Peak height expressed the total ion chromatogram (TIC). Tubers harvested from GP2 plants have fewer carbohydrate components than tubers from GP3 plants. Tubers from GP2 had 8 carbohydrate components (xylose, arabinose, rhamnose, glucose, galactose, mannose, mannan, and glucomannan) (figure 1A). Tubers from GP3 had 9 carbohydrate components (xylose, arabinose, rhamnose, glucose, galactose, mannose, trehalose, mannan, and glucomannan) (figure 1B). Carbohydrate component detected in both tubers, eight carbohydrate components in GP2 were the same as carbohydrate component detected in GP3. Eight carbohydrate components have the same retention time, namely xylose, arabinose, rhamnose, glucose, galactose, mannose, mannan, and glucomannan, whereas trehalose was only detected in GP3 which certainly has a different retention time. Based on the total ion chromatogram (TIC), all carbohydrate components in the tubers from two different growth periods have differences in the chromatogram uptake response. Glucomannan was a carbohydrate component that has the highest TIC in tubers from both of the growing period. However, glucomannan TIC on GP2 was lower than GP3.

Carbohydrate components detected in GP2 and GP3 tubers were subsequently quantified (figure 2). The highest carbohydrate component in both tubers was glucomannan. However, glucomannan in GP3 tubers was higher than GP2. While the lowest carbohydrate component was arabinose, but arabinose in GP2 tubers was higher than GP3 tubers. The levels of other carbohydrate components such as xylose, arabinose, rhamnose, glucose, galactose, and mannan in GP2 tubers were higher than in GP3, whereas for GP3 mannan and glucomannan tubers were higher than in GP2. The order of carbohydrate components based on the levels from highest to low in GP2 was glucomannan, manose, mannan, rhamnose, xylose, galactose, glucose, and arabinose, whereas in GP3 were glucomannan, mannan, galactose, glucose, manose, xylose, trehalose, glucose, and glucose arabinose.

The difference in carbohydrate components and their levels in porang tubers were thought to be due to the age of the tubers or harvest time. Based on its development, the new GP2 tubers experience 2 growing periods and GP3 has 3 growing periods in the vegetative phase. This difference causes the synthesis and accumulation of carbohydrate components of the two tubers was different. So that the carbohydrate component of GP3 tubers was thought to have been synthesized and accumulated optimally. Glucomannan deposition in idioblasts follows a pattern of temporal regulation, with increased expression at the end of the vegetative cycle, i.e. when the plant falls and has passed the perfect vegetative phase [26]. The carbohydrate component of potato tubers harvested from plants was 4 weeks less (7 carbohydrate components) than the tubers harvested at 10 weeks (15 carbohydrate components). Trehalose was detected in tubers aged 10 weeks. This difference was influenced by environmental, developmental, and plant genetic factors [19]. Besides the age or tuber harvest time, the difference in carbohydrate component in both porang tubers was suspected because the weight of tubers harvested in GP2 was smaller than in GP3 tubers. The formation of food reserves, such as carbohydrates in plants can increase the weight of tubers, so that tubers that have greater weight have high levels of glucomannan [2, 27].

Previous research on glucomannan levels in porang tubers showed that tubers from GP2 and GP3 third growing periods had different glucomannan levels. Glucomannan tuber level in the second growth period was 46-48%, while GP3 tubers were 47-55% [2, 13]. The levels of glucomannan in the Amorphophallus plant are affected by several factors namely: plant type and age of harvest, parts that are grinded, tools used rotation speed of the grinding tool and the grinding time replication, and age of plants, and how to process those [28-30]. Glucomannan levels in several different studies: ±35%, ±41%, and range from 35-55%. Glucomannan levels in Amorphophallus muelleri are higher than Amorphophallus variabilis [3, 26].
Figure 1. Chromatogram result of porang tubers from different growth periods. A. Tuber from GP2, B. Tubers from GP3. TIC: total ion chromatogram, RT: retention time, a. Xylose, b. Arabinose, c. Rhamnose, d. Glucose, e. Galactose, f. Mannose, g. Mannan, h. Glucomannan, i. Trehalose
Research on carbohydrate components and their levels in these porang tubers can provide information about the harvest time or age of the tubers used for industrial purposes. GP3 tubers were tubers that have more components and have more glucosminan level than GP2 tubers. But to find out more details about the synthesis and accumulation of carbohydrate components, it is necessary to examine the carbohydrate component of porang tubers before and after harvest in both growth periods.

4. Conclusion
Porang tubers from GP2 and GP3 plants have been analysed by LC-MS technique and clearly showed the presence of 67 compounds in each growing period. Eight carbohydrate components were identified in GP2 tubers and 9 were identified in GP3 tubers. Besides trehalose, the 8 carbohydrate components identified in GP3 tubers were the same as the carbohydrate components identified in GP2 tubers (xylose, arabinose, rhamnose, glucose, galactose, mannose, mannan, and glucosimannan). Glucosimannan levels in GP3 tubers were higher than GP2 tubers. These results are important to determine the age of the tubers used as industrial material, tuber harvested from GP3 plant is better than GP2.

5. Acknowledgements
This research was supported by the 2017 Domestic Postgraduate Education Scholarship (BPP-DN), the Ministry of Research, Technology and Higher Education as well as the Indonesian Porang Research and Development Centre (P4I) University of Brawijaya, Malang, Indonesia.

References
[1] Yuzammi 2000 A taxonomic revision of the terrestrial and aquatic Aroids (Araceae) in Java School of Biological Science Faculty of Life Science University of New South Wales Thesis 34-35.
[2] Sumarwoto 2005 Iles-Iles (Amorphophallus muelleri Blume); Description and other properties J. Biodiversitas 6 185-190.
[3] Jansen PCM, Wilk CVD, and Hetterscheid WLA 1996 Amorphophallus Blume Exdecaisne In Flach, M. and F. Rumawas (Eds.) Prosea: Plant Resources Of South- East Asia 9 34.
[4] Hidayat R 2013 Porang production technology as cash crop in several industrial forest plant
commodities East Java "Veteran" National Development University 32-34.

[5] Alonso S 2008 Glucomannan, a promising polysaccharides for biopharmaceutical purposes Eur Jp Harm Biophar 2 453-62.

[6] Koswara S 2013 Processing of porang tubers (Iles-iles) Department of Food Science and Technology and Seafast Center LPPM IPB Bogor 2-4.

[7] Pitojo S 2007 Cultivation series: Suweg: Alternative food ingredients, low in calories Canisius Publisher Yogyakarta 6-7.

[8] Zhang YQ, Xie BJ, and Gan K 2005 Advance in the application of konjac glucomannan and its derivatives Carbohydrate Polymer 60 27-31.

[9] Vuksan V, Sievenpiper JL, Owen R, Swilley JA, Spadafora P, Jenkins DJA, Vidgen E, Brigenthi F, Josse RG, Leiter LA, Xu Z, and Novokmet R 2000 Beneficial effects of viscous dietary fiber from konjac-mannan in subject with the insulin resistance syndrome Diabetes Care 23 9-14.

[10] Rokhmah DN and Supriadi DN 2015 Prospect of developing iles-iles (Amorphophallus muelleri Blume) as an effort to diversify food in Indonesia Sirinov 3 1–10.

[11] Perhutani 2007 Starfruit and porang cultivation to improve the welfare of the community in and around the forest www.dephut.go.id Accessed November 10, 2018.

[12] Gusmalawati D 2013 Generative organ development structure and growth power of porang (Amorphophallus muelleri Blume) Brawijaya University Postgraduate Program Malang Thesis 8-10.

[13] Indriyani S 2011 Porang growth patterns (Amorphophallus muelleri Blume) and environmental influences on oxalate and glucomannan content Airlangga University Postgraduate Program Surabaya Dissertation 34-36.

[14] Budiman and Arisoesilaninggish E 2012 Predictive model of Amorphophallus muelleri growt in some agroforestry in East Java by multiple regression analysis Biodiversitas 13 18-22.

[15] Sumarwoto and Widodo W 2008 Growth and yield of elephant food yam (Amorphophallus muelleri Blume) first growing period at various doses of N and K fertilizers Agrivita 30 67-74.

[16] Astanto K 2008 Potential iles-iles tubers as annual savings Bul. Palawija 15 15-20

[17] Indonesian Porang Research and Development Center (P4I) 2013 Dissemination module: cultivation and development of porang (Amorphophallus muelleri Blume) as one of the potential local raw materials Brawijaya University Malang 7-9.

[18] Sumarwoto 2007 Review: Mannan content in the iles-iles plant (Amorphophallus muelleri Blume) Bioteknologi 4 28-32.

[19] Roessner U, Wagner C, Kopka J, Trehewey RN and Willmitzer L 2000 Simultaneous analysis of metabolites in potato tuber by gas chromatography-mass spectrometry The Plant Journal 23 131-142

[20] Outsuki T 1968 Studies on reserve carbohydrates of flour Amorphophallus species, with special reference to mannan Botanical Magazine Tokyo 81119-126.

[21] Syaefullah M 1990 Study of glucomannan characteristics and "indigenous" iles-iles (Amorphophallus oncophyllus) sources with variations in the drying process and soaking doses Postgraduate Program of IPB Bogor Thesis 45-47.

[22] Suprihatin T 2019 Potential of Turmeric (Curcuma longa L.) Powder in improving ovary aging in wistar (rattus norvegicus) premenopausal white rats Biology Doctoral Program Biology Department FMIPA Brawijaya University Malang Dissertation 41-42

[23] Nguyen D, Yu J, Mho S, Lee H, Lee W, Yee S, Lee G, and Paik M 2013 Method optimization for rapid measurement of carbohydrates in plasma by liquid chromatography tandem mass spectrometry Bull. Korean Chem. Soc. 34 1571-1574.

[24] Parasuraman S, Anish R, Balamurugan S, Muralidharan S, Kumar KJ, and Vijayan V 2014 An overview of liquid chromatography-mass spectroscopy instrumentation Pharmaceutical Methods 5 47-55.
[25] Rogatsky E, Jayatillake H, Goswami G, Tomuta V, and Stein D 2005 Sensitive LC-MS quantitative analysis of carbohydrates by cs-attachment american society for mass spectrometry J. Am. Soc. Mass Spectr. 16 1805-1811.

[26] M. J. Chua, Trevor, H. K. Chan and T. C. Baldwin. 2013. Temporal and spatial regulation of glucomannan deposition and mobilization in corms of Amorphophallus Konjac (Araceae) American Journal of Botany 100 337–345.

[27] Lestari BL 2011 Kajian ZPT Atonik dalam berbagai konsentrasi dan interval penyemprotan terhadap produktivitas tanaman bawang merah (Allium ascolanicum L.) Rekayasa 40 33-37.

[28] Haryani K and Hargono 2008 Proses pengolahan iles-iles (Amorphophallus sp.) menjadi glukomannan sebagai gelling agent pengganti boraks Momentum 4 38-41.

[29] Suhirman S, Yuliani S, Imanuel E, and Laksmanahardja MP 1995 Advanced processing research and diversification of iles-iles crops [Report on Industrial Plant Research Results] Bogor: BALITRO.