

EKSTRAKSI GLUKOMANAN DARI TEPUNG PORANG (*Amorphophallus muelleri* Blume) dengan Etanol

Extraction of Glucomannan from porang (*Amorphophallus muelleri* Blume) flour using Ethanol

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ABSTRAK

Iles-iles kuning (*Amorphophallus muelleri* Blume) adalah sumber potensial glukomanan, suatu senyawa polisakarida yang memiliki beberapa sifat khusus yang sering digunakan di berbagai bidang industri, farmasi, dan makanan. Kualitas glukomanan yang diproduksi di dalam negeri masih belum dapat menyamai kualitas glukomanan impor. Penelitian ini bertujuan untuk mengetahui pengaruh perbedaan metode ekstraksi glukomanan dari tepung iles-iles kuning menggunakan etanol untuk mendapatkan kadar glukomanan yang tinggi dengan kualitas yang lebih baik. Glukomanan yang baik memiliki viskositas tinggi dan kandungan air, abu, protein, lemak, dan pati yang rendah. Ekstraksi tepung iles-iles kuning menggunakan etanol konsentrasi bertingkat (40, 60, dan 80%) mampu menghasilkan kadar glukomanan lebih tinggi dan kualitas lebih baik daripada etanol 60% dengan tiga kali ulangan. Ekstraksi menggunakan etanol bertingkat mampu meningkatkan kadar glukomanan dari 16,43% menjadi 62,2%. Spektra Fourier Transform Infra Red (FTIR) dari glukomanan hasil ekstraksi menunjukkan adanya gugus-gugus penyusun senyawa glukomanan (O-H, C=O, C-O, C-H) seperti yang ditunjukkan juga pada spektra glukomanan komersil.

Kata Kunci: *Amorphophallus muelleri* Blume, tepung iles-iles, glukomanan, ekstraksi konsentrasi bertingkat, etanol.

ABSTRACT

Iles-iles kuning (Amorphophallus muelleri Blume) is a potential source of glucomannan, a polysaccharide compound that has several special properties which often used in various fields of industry, pharmacy, and food. The quality of glucomannan produced domestically still cannot match the quality of imported glucomannan. This study aims to determine the effect of difference extraction methods of glucomannan from iles-iles kuning flour using ethanol to obtain high contain of glucomannan with better quality. Good quality of glucomannan has high viscosity and contains small amount of water, ash, protein, fat, and starch. Extraction of iles-iles kuning flour using multilevel concentration of ethanol (40, 60, and 80%) was able to produce higher glucomannan and better quality than ethanol 60% with three times of extraction. Extraction using multilevel ethanol was able to improve glucomannan content from 16,43% to 62,2%. Fourier Transform Infra Red (FTIR) spectra of extracted glucomannan showed the functional groups composing the glucomannan compound (O-H, C=O, C-O, C-H) similar to the spectra of commercial glucomannan.

Keywords: *Amorphophallus muelleri* Blume, iles-iles flour, glucomannan, multilevel concentration extraction, ethanol.

INTRODUCTION

The tuber of iles-iles kuning (*Amorphophallus muelleri* Blume) is a very potential source of glucomannan because it contains high glucomannan (Yanuriati *et al.*, 2017). Glucomannan is a polysaccharide consisting of units of D-glucose and D-mannose (Saputro *et al.*, 2014). Glucomannan has been used since ancient times as food and medicine in China and Japan, and even now its use is increasingly widespread in the pharmaceutical, cosmetic and some chemical fields. Koswara (2013) said that iles-iles

contain 5-60% of glucomannan, and iles-iles in Indonesia generally have glucomannan content of around 14-35%.

Iles-iles kuning is a potential source of glucomannan but the quality of glucomannan produced domestically still cannot match the quality of imported glucomannan. This can be caused by the quality of glucomannan produced in the country is still far from the standard that should be used in various fields. According to Mulyono (2010), increasing the quality of glucomannan flour can be done by multilevel extraction.

Table 1. Composition of Iles-Iles Kuning Flour Based on Literature

Component	Iles-Iles Kuning Flour before Treatment	Commercial Glucomannan Flour
Moisture (%)	8,71	8,25
Ash (%)	4,47	0,37
Starch (%)	3,90	0,27
Protein (%)	2,34	0,63
Fat (%)	3,25	0,79
Viscosity (c.Ps)	3313	11000
Glucomannan (%)	43,99	92,51

(Source : Widjanarko *et al.*, 2014)

Experiment of glucomannan extraction from iles-iles kuning flour has been widely carried out in Indonesia. Faridah & Widjanarko (2013) using multilevel concentration of ethanol for glucomannan extraction, i.e. first extraction with 40% ethanol, then the precipitated sample was extracted again with ethanol 60%, and finally extracted with 80% ethanol. The extraction with multilevel concentration of ethanol resulted 79.26% of glucomannan. Furthermore, Saputro *et al.* (2014) compared extraction using ethanol solvents with different concentrations, which was 40, 50, and 60%. The highest content of glucomannan was produced from ethanol

60%, which was 64.22%. According to Yanuriati *et al.* (2017), glucomannan content will be higher if the extraction is repeated. Fithri (2017) has also researched extraction of iles-iles kuning flour with water, then washed with 1-propanol and resulted 64.49% of glucomannan content. However, 1-propanol is not recommended because the price is more expensive than ethanol. In addition, ethanol is safe solvent if the processed product are applied for food and health.

In this study, glucomannan was extracted from iles-iles kuning flour using ethanol with two different methods. The first method is extraction using ethanol with multilevel

concentration that is extracted with ethanol concentration of 40% (stage I), then proceed with extraction using ethanol 60% (stage II), and finally extracted with ethanol 80% (stage III), respectively one-time repetition in each stage. Next, the second method is extraction using ethanol 60% repetitively for three times (fixed concentration).

MATERIAL AND METHODS

Equipment and Material

The primary materials used in this study were three years old iles-iles kuning tubers from the species *Amorphophallus muelleri* Blume. The tubers were obtained from the Bogor Agricultural University experimental garden, Bogor, Indonesia. Ethanol 96%, aquadest, sodium metabisulphite, H₂SO₄, NaOH, HCl, hexane, D-glucose standard, 3,5 Dinitrosalicylic acid, boric acid, formic acid-NaOH buffer, lugol reagent, potassium phosphate buffer, and other chemicals were analytical grade from Merck, Smart Lab, etc. The equipments used in this study were spectrophotometer UV-Visible Shimadzu, Fourier Transform Infra Red Perkin Elmer Spectrum Two, viscometer Brookfield DV-III, magnetic stirrer, centrifuge, oven, and glass ware.

Methods

Preparation of iles-iles flour

The tubers were washed first, then peeled and sliced into \pm 2 mm. The chips were washed using water and soaked in 2000 ppm

of sodium metabisulphite solution for 20 minutes. The chips were dried in the oven for 16 hours at 65°C, then grinded using a disk mill. Flour was sieved by sieve sized 60 mesh and stored in a dry container (Aryanti & Abidin, 2015).

Extraction of Glucomannan

The first method is extraction of glucomannan with multilevel concentration of ethanol. Iles-iles kuning flour was put into ethanol 40% with ratio 1 gram flour /15 mL of solvent and stirred for 1 hour then filtered with cotton cloth, then the sample was extracted again with ethanol 60%, and 80% with the same treatment. The precipitate was dried in oven at 45°C for 12 hours. Sample was grinded and sieved in 60 mesh-sized. The second method is extraction of glucomannan using ethanol 60%, the ratio was 1 gram flour/15 mL solvent and extracted for 1 hour. The sample was filtered with cotton cloth. The extraction was conducted for three times repetitively. The precipitate was dried in oven at 45°C for 12 hours. Sample was grinded and sieved in 60 mesh-sized.

Physicochemical properties: colour and viscosity

The colour of glucomannan is directly observed visually. The viscosity determined using viscometer, sample was taken about 0.2 g and dissolved in 10 mL hot water. About 90 mL water added to the solution and the viscosity was measured.

Glucomannan content

The determination of glucomannan content was carried out using the colorimetric method with 3,5-Dinitrosalicylic acid reagents (Chua *et al.*, 2012). Glucomannan extract was made by dissolving 0.2 g sample in NaOH-formic acid buffer solution (0.1 mol/L, 100 mL) and stirred for 4 hours. This solution was centrifuged at 4000 rpm for 30 minutes. The supernatant was glucomannan extract.

Glucomannan hydrolysate was made by mixing 5 mL glucomannan extract with 2.5 mL H₂SO₄ 3M shaken with vortex and heated for 90 minutes in water bath, then the solution allowed to cool in room temperature and added 2.5 mL NaOH 6 M. The solution was added with deionized water up to 25 mL, and the solution formed was glucomannan hydrolysate. Glucomannan extract and hydrolysate were measured by colorimetric method, while deionized water was used as a blank.

The standard of D-glucose (1 mg/mL) was diluted to a solution of 0.10, 0.2, 0.30, 0.40, and 0.50 mg/mL with deionized water. About 2 mL of standard solution was added with 3.5 DNS 1% (1.5 mL), then heated for 5 minutes in boiling water, and added deionized water to 25 mL after it was cooled. The treatment of glucomannan extract and glucomannan hydrolysate was the same as the D-glucose standard. These solutions are readily absorbed in wavelength of 550 nm. Glucomannan content was calculated by :

$$\% \text{ Glucomannan} = \frac{5000 \times f(5T - T_0)}{m} \times \frac{100}{100 - w}$$

where f = correction factor, T = glucose content of glucomannan hydrolysate (mg), T_0 = glucose content of glucomannan extract (mg), m = mass of glucomannan (200 mg) and w = water content of glucomannan

Chemical composition

Moisture content was determined by weight difference after drying of samples, following the official method of AOAC (2005). Ash was determined gravimetrically (AOAC, 2005). Protein content was calculated from the nitrogen content (N%: 6.25) analyzed by Kjeldahl method (AOAC, 2005). Fat content was determined using a Soxhlet apparatus according to SNI 06-6989.10-2004. Crude fiber was determined using acid-base extraction according to SNI 01-2891-1992. The starch was analyzed qualitatively by staining glucomannan granules using I₂-KI.

Characterization using Fourier Transform Infra Red (FTIR)

Specific functional groups of glucomannan was determined using FTIR-UATR. Spectrum sample was read in the range 4000-400 cm⁻¹.

RESULT AND DISCUSSION

Preparation of Iles-Iles Kuning Flour

Iles-iles kuning tubers soaked using sodium metabisulfite 2000 ppm to prevent browning process. Browning is caused by the presence of carotene content, the enzyme polyphenol oxidase (PPO) and polyphenolic compounds including tannins (Zhao *et al.*,

2010). Starch, calcium oxalate, and temperature also affect the brightness of flour (Sumarwoto, 2007). To prevent browning, in this study iles-iles kuning chips soaked first using sodium metabisulfite.



Figure 1. Iles-Iles Kuning Chips after Soaked in Sodium Metabisulfite.

Chips that have been soaked with sodium metabisulfite did not brown (Figure 1). Sodium metabisulfite is a reducing compound, sulfite ions in this compound work to inhibit non-enzymatic browning because the carbonyl group will react with sulfite (Lubis *et al.*, 2004). According to Wedzicha *et al.* (1984), sulphur (IV) oxospecies could inhibit browning or Maillard reactions during storage on

vegetables. In Dwiyono's research (2014), soaking iles-iles kuning tubers in sodium metabisulfite solution increase the brightness without reducing glucomannan content.

Converting iles-iles kuning tubers into flour is to reduce high water content. According to Koswara (2013), the water content of iles-iles tubers is relatively high, between 70-80%. This situation causes during storage of glucomannan will be damaged by enzyme activity. In addition, the minimum water content limit at which microorganisms can still grow is around 14-15% (Fardiaz, 1989).

Colour

The color of glucomannan extract showed in Figure 2. It can be seen that the yellow color of the samples produced after extraction was fainter than iles-iles kuning flour. Meanwhile, the glucomannan extract from method I was faded more than method II. Iles-iles kuning tubers have carotene content of around 40 mg/kg, and the compound caused iles-iles kuning tubers have yellow color (Wootton *et al.*, 1993).

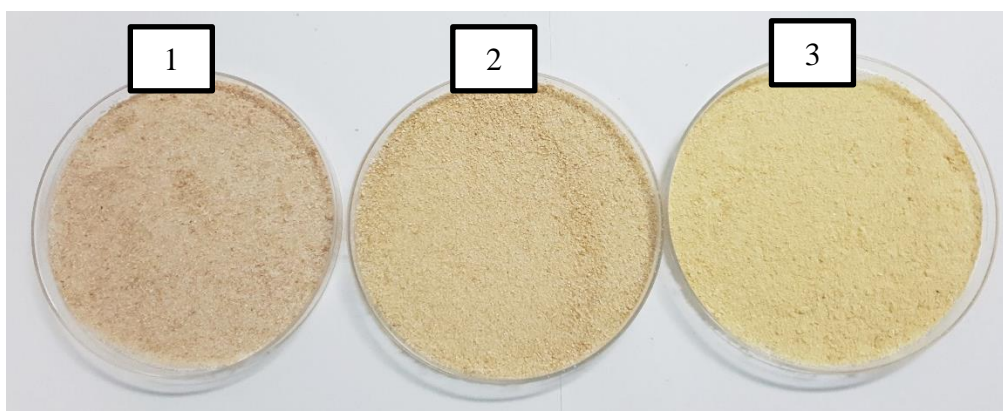


Figure 2. Difference Colour of Glucomannan Extract; (1) Extraction Using Multilevel Concentration of Ethanol; (2) Extraction Using Fixed Concentration of Ethanol; (3) Iles-iles Kuning Flour

Carotene is a carotenoids, which are soluble in fat or organic solvents but insoluble in water. Therefore, the higher concentration of ethanol used, the more soluble the carotene. Ethanol concentration in method I which was higher than ethanol concentration in method II causes the carotene contained in flour to dissolve more so that the color of glucomannan extracted by method I becomes brighter.

Composition of glucomannan

Extraction of iles-iles kuning flour was carried out using ethanol with two different methods, the first method was extraction using multilevel concentration of ethanol (40, 60, 80%) and the second method was using fixed concentration of ethanol 60% for three times repetitively. The comparison of physicochemical composition of glucomannan showed in **Table 2**.

Table 2. Composition of Glucomannan Extract

Parameter	Iles-Iles Kuning Flour before treatment	Sample of Extraction Method I	Sampel of Extraction Method II
Glucomannan (%)	16,43	62,2	43,02
Viscosity (cP.s)	-	4024,14	549,88
Moisture (%)	8,32	10,30	10,63
Ash (%)	3,37	0,52	1,39
Protein (%)	7,13	4,86	5,96
Fat (%)	0,3	<0,0001	<0,0001
Crude Fiber (%)	3,54	3,97	3,69
Starch*	+++	+	++

+ = blue colour

Iles-iles kuning flour extraction using multilevel concentration of ethanol resulted higher glucomannan content than extraction using ethanol with fixed concentration. This is caused by more impurities dissolved in extraction method I than method II. These impurities have different solubility in ethanol. Polarity of solution is determined from its dipole moment. Dipole moment of water is 1.84 Debye, greater than ethanol which is about 1.69 Debye. The polarity of ethanol 40% is higher than ethanol 60% and 80%, because 40% ethanol consists of 40% of ethanol and 60% of water. Ethanol 40% would dissolve more polar compounds, such as protein and

sugar (Xu *et al.*, 2014). Ethanol 60% dissolves compounds that are insoluble in ethanol 40%, like starch (Nurlela *et al.*, 2019). Ethanol 80% has a lower polarity so that it would dissolve compounds with lower polarity as well as fat, calcium oxalate, and ash (Kurniawati & Widjanarko, 2010). This method can maximize the extraction of glucomannan and glucomannan with high purity can be produced. According to Widjanarko and Megawati (2015), the low value of glucomannan yields can be caused by imperfect coagulation or precipitation processes. The process of coagulation are affected by three factors, heating, stirring, and

the addition of electrolytes. According to Nindita *et al.* (2012), the extraction time that is not optimal can cause the flour not to be extracted properly.

In this study, viscosity of method I and II were 4024.14 cP and 549.88 cP, respectively. According to Faridah (2016), glucomannan has a very high molecular weight (> 300 kDA) which can produce a very thick liquid. The viscosity of the extracted glucomannan was closely related to the glucomannan content contained in the flour. Higher glucomannan content would increase the viscosity of the flour. Glucomannan is able to absorb water and expand until it reaches 138-200% of the initial weight of flour and occurs quickly. Its ability to absorb water affected the viscosity (Kurniawati & Widjanarko, 2010). The value of viscosity from this study is still low compared to the value of viscosity from the study of Widjanarko *et al.* (2011), which is about 9833 cP.s. It can be caused by the change in amorphous form of glucomannan during extraction process using ethanol. The amorphous form changes from easily dissolved in water to crystalline form that is difficult to dissolve in water, so the viscosity value decreases (Kato & Matzuda, 1969). The low viscosity value can also be caused by the large particle size, making it difficult to smooth it with a blender or pestle because of its very hard texture (Mulyono, 2010).

Moisture content is one of the most important characteristics in food because water can affect the appearance, texture, and taste of food ingredients. High moisture

content can damage the freshness of food because it is easier for bacteria, mold and yeast to grow (Yusuf *et al.*, 2016). The minimum moisture level limit where microorganisms can still grow is around 14-15% (Fardiaz, 1989). From the test results obtained all glucomannan extracts have water content below 14%, which ranges from 8-11%.

Compounds that attach to glucomannan such as ash, fat, protein, and starch are considered impurities because they can affect the special characteristic of glucomannan. Starch that is attached to glucomannan decrease the strength of the gel formed of glucomannan. The presence of starch in glucomannan also results in decreasing the viscosity of glucomannan solution (Fadilah, 2017). **Table 2** showed the decrease in impurity content after extraction using ethanol. The impurities content in glucomannan extract using multilevel concentration of ethanol (method I) was lower than using ethanol with fixed concentration (method II). The impurities present in the extracted glucomannan flour have different polarity, so that multilevel concentration of ethanol would be more effective in separating them. The presence of dark blue color after staining indicated high starch content of glucomannan. Lugol's solution (KI-I₂) would not detect simple sugars such as glucose or fructose (Libretext, 2019). The result showed yellowish blue indicating that the glucomannan only contained little starch.

Characterization of Glucomannan

The extract glucomannan characterized by FTIR to determine the functional groups of

glucomannan. Figure 3 showed the spectrum of glucomannan samples.

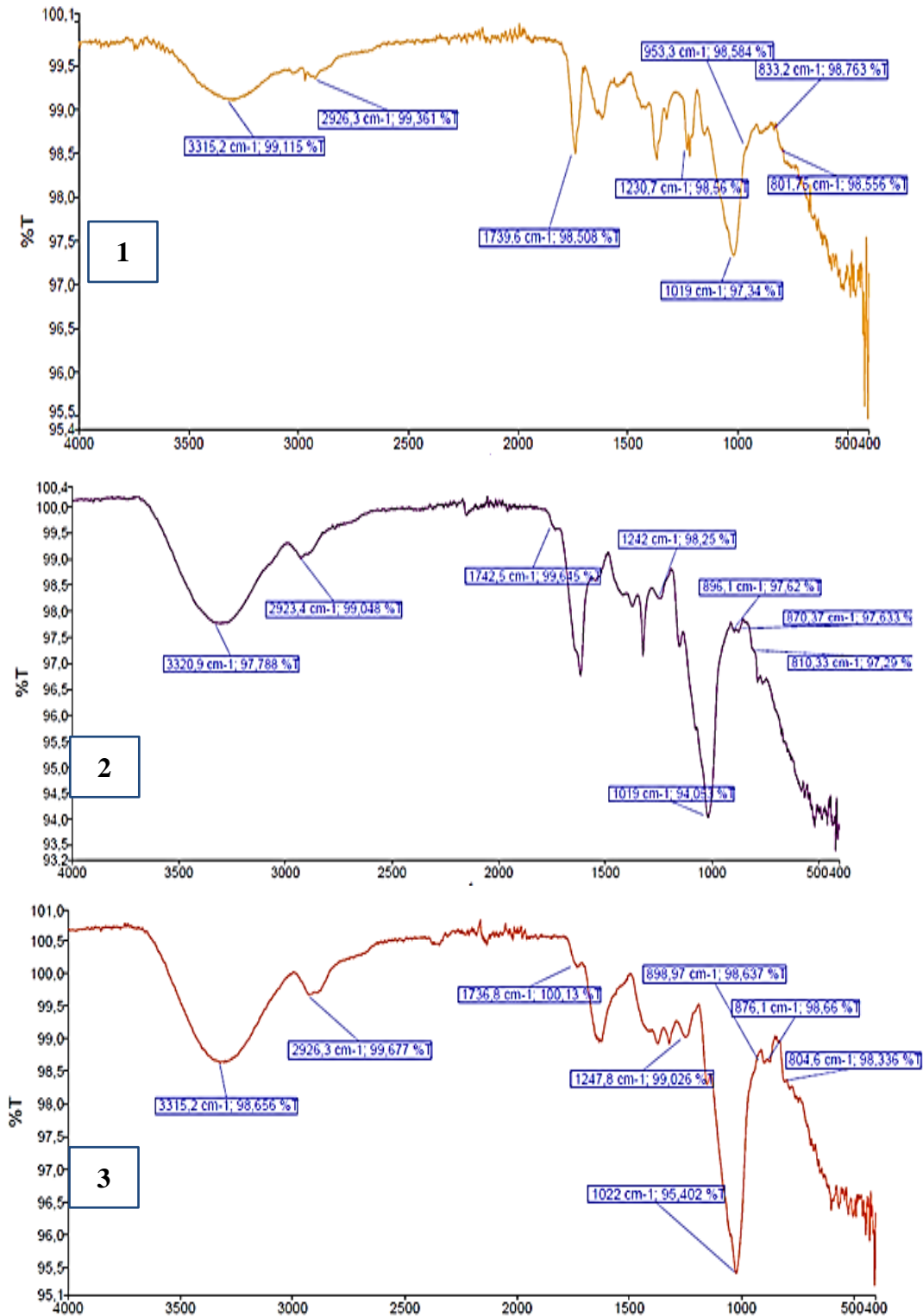


Figure 3. FTIR Spectra of Extract Glucomannan; (1) Method I; (2) Method II; (3) Commercial Glucomannan

Glucomannan extracted from method I and method II showed O-H at peak of 3315 cm^{-1} and 3320 cm^{-1} respectively. The peak at 3000-3700 cm^{-1} showed the O-H group on the glucomannan structure. This was consistent with the statement of Zhang *et al.* (2001) stating that the glucomannan spectrum is dominated by spectral bands related to the stretching vibrations of O-H and water groups in the range of 3396 cm^{-1} . The peak at wave number 2926 cm^{-1} and 2923 cm^{-1} were showed the C-H group. The carbonyl group on acetyl was clearly shown in wave number about 1739 cm^{-1} and 1736 cm^{-1} in the glucomannan flour extracted by method I and method II. Sastrohamidjojo (1992) stated that the absorption of carbonyl group bonds (C=O) was at wavenumber 1850-1630 cm^{-1} . The C-O group from ether can be seen at wavenumber 1230 cm^{-1} and 1247 cm^{-1} , where the group will give results at wave number 1260-1200 cm^{-1} . The absorption band at wavenumber 1019-1016 cm^{-1} indicated the presence of COC functional groups and this was supported by the research of Setiawati *et al.* (2017) that the COC function groups were present in wavenumbers of 1020 cm^{-1} , as well as the opinion of Sastrohamidjojo (1992) which stated that COC bond uptake give absorption in the range of wave numbers 1300-1000 cm^{-1} .

The glucomannan spectrum from the extraction method I and method II were almost similar to the commercial glucomannan spectrum, although there was a slight shift. This could be caused by a number of

impurities present in the extracted glucomannan in this study. Functional group in protein, starch, fat, etc. could disturbed glucomannan spectrum readings. The peaks intensity of FTIR spectra of method II was more similar to commercial glucomannan. However, the commercial glucomannan that we used does not provide information on its glucomannan levels. Thus, it could not be assumed that glucomannan extracted by method II was purer than method I. The result of the proximate test (Table 2) showed the impurities (ash, protein, starch) of glucomannan extracted by method I less than method II, as well as glucomannan content and viscosity of glucomannan extracted by method I was higher than method II that means method I can produce glucomannan with higher levels with better quality.

CONCLUSIONS

From this study, the highest glucomannan content (62.20%) was obtained from *iles-iles* kuning flour by extraction with multilevel concentration of ethanol 40, 60, 80% (method I). While the extraction method with fixed concentration of ethanol 60% (method II), resulting in 43.02% of glucomannan content. Visual observation of flour color, viscosity, and the proximate test showed that method I gave better results than method II. Determination of functional groups using FTIR from extracted glucomannan flour produce similar absorptions bands to commercial glucomannan.

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