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ORIGINAL RESEARCH PAPER

APPLICATION OF RESPONSE SURFACE METHOD ON PURIFICATION OF GLUCOMANNAN FROM *AMORPHOPHALLUS ONCOPHYLLUS* BY USING 2-PROPANOL

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Abstract: Glucomannan purification methods affect the properties of mannan and have influence to the scope of glucomannan applications. Combination between ethanol solution and thermal treatment is commonly method applied to purify glucomannan obtained from *Amorphophallus sp.* However, 2-propanol was reported to be more effective in removing glucomannan impurities including the starch and the carotenes. The objective of this research was to study the effect of 2-propanol concentration, temperature and time as well as their interaction on purification of glucomannan obtained from *Amorphophallus oncophyllus* by using response surface methods. The relevant parameters (glucomannan content, starch content, degree of whiteness, yield) were investigated in order to establish mathematical model. The results showed that the linear models were reliable to predict the responses ($R^2 \geq 0.926$). Temperature was a significant variable for the all responses. This purification method improved the absorbance of the functional groups at ~2900 cm^{-1} of stretching of C-H vibration and ~1730 cm^{-1} of acetyl group which is responsible on glucomannan solubility. However, this method reduced the absorbance of the functional groups at ~1650, ~1070, ~1020 and 900 - 800 cm^{-1} of absorbed water or protein, C-O alcohol and β-glucosidic and β-mannosidic linkages, respectively. The morphology observation revealed that this purification method achieves separation and removal of impurities which encapsulated the glucomannan and subsequently released the glucomannan granules. The highest content of glucomannan obtained in this study was 72.30 % when 90 % 2-propanol at 75 °C for 180 min was used for purification.

Keywords: *Amorphophallus oncophyllus, extraction, glucomannan, 2-propanol, purification, response surface methods*

INTRODUCTION

Glucomannan has been involved in many traditional Asian foods such as noodle, tofu, jelly and other foods over centuries. Glucomannan is water soluble and is one of the most viscous compounds, due to its high water-absorbing capacity. Glucomannan has been approved as a food additive by the United States Food and Drug Administration (FDA) since 1994 [1]. Due to the unique rheological and gelling properties of glucomannan, this compound is widely employed as emulsifiers and stabilizers in food products. The current usage of glucomannan in the West is to be used as material for dietary supplements and nutraceuticals as well as food additives. Despite the variety of glucomannan sources, the most widely known of the sources is *Amorphophallus konjac* K. Koch ex N.E.Br tuber which commonly found in Japan and China [2].

Konjac glucomannan (KGM) is a linear heteropolysaccharide composed of β-1,4-linked D-mannose and D-glucose monomers with 1 to 1.6 of glucose/mannose ratio and certain short side branches at the C-3 position of the mannoses through β-1,6-glucosyl units. The degree of branching is estimated at approximately 3 branches for every residue. Acetyl groups along the KGM backbone on average every 9-19 sugar units randomly present at the C-6 position [1 – 3]. Professional standard of RRC sets minimum 70 % glucomannan content for top grade common konjac flour [4] while European community requests 75 % for konajc gum [5]. There are numerous impurities found in crude tuber flour including starch, cellulose and nitrogen-containing materials and carotenes. These impurities encapsulated the glucomanan granules [6].

Purification steps of glucomannan from the tuber played an important role in studying glucomannan because it influences the scope of applications of the glucomannan. Therefore, it is important to develop the purification method which not only produce glucomannan with high purity but also convenient and efficient. Ethanol solution commonly applied to remove the impurities of glucomannan granules. To improve purification process, the solution was combined with thermal treatment [7]. Ohashi *et al*. [6] also reported a comparable performance of ethanol and 2-propanol (isopropanol - IPA) in glucomannan purification. Among C1-4 lower alcohols, IPA is preferred as coagulating agent of glucomannan. Amorphophallus tuber contains ~40 mg carotenes/kg fresh weight. Ethanol was effective in purify glucomannan but it did not dissolve the carotenes so well. Moreover, IPA is suitable as a food-processing aid and more effective to remove undesirable components of glucomannan including carotenes compared to ethanol [8].

Being a member of the philodendron (arum) family, the *Amorphophallus oncophyllus* was reported to contain 64 % of glucomannan [9]. Ethanol solution was commonly used to purify glucomannan of *A. oncophyllus* [6, 7, 10]. Although there were some studies reported the extraction and purification of glucomannan of the tuber, to the best of our knowledge there was a little information on isolation of the glucomannan by using IPA solution combined with temperature and time. Hence, the aim of this study was to investigate the effect of interaction of parameters namely purification temperature, IPA concentration and time on purification of glucomannan of *A. oncophyllus* by using response surface methods (RSM).

RSM consists of a group of mathematical and statistical procedure that can be used to study the relationship between responses and number of independent variables. RSM is a popular and an effective optimization technique for analyzing complex processes,

which is widely used in various extraction processes. This method is efficient and easier to manage experiments in comparison with other methods. The main advantage of RSM is the use of smaller number of experiments to evaluate multiple parameters and their interaction [11].

MATERIALS AND METHODS

Purification of glucomannan

10 g of crude *A. oncophyllus* flour (60 - 80 µm) were dispersed in a magnetically stirred (200 rpm) 2-propanol (IPA) solution. The detail variables of the extraction conditions are presented in Table 1. The sol was vacuum filtered and the obtained cake was dried at 50 °C for 24 h. After drying, the sample was used for further analysis. The crude flour contained 58.16 % of glucomannan and 8.92 % of starch.

Purified flour determination

All purified flours were analyzed for the determination of glucomannan and starch content, degree of whiteness (DoW) and yield. The glucomannan content was determined based on the method of Chua et al. [2]. Starch content was determined as explained by Rahayu [9]. The DoW analysis was accomplished by Konica Minolta Chroma meters CR-400. The sample with highest glucomannan content was analysed by SEM using FEI Inspect S50 device and FTIR using IR Prestige Shimadzu device methods, in order to determine the morphology and the functional groups. The peaks were assigned by comparison with the literature data.

Experimental design and statistical analyses

A 2^3 factorial design consisted of 12 experimental points including four replicates run at centre point (Table 1), was adopted to determine the effect of three independent variables, namely temperature (T), time (t) and IPA solution (S). It was also used to determine the individual and the interaction effects between them. The variables were coded according to the equation (1). The coding of variables is important because it provides dimensionless coordinate system, where variables for lower, higher, and center level are represented as -1, +1, and 0, respectively. The coded value was calculated using the equation (1) [12].

$$X = \frac{x_i - x_0}{\Delta x} \quad (1)$$

where X is the coded value, x_i is the corresponding actual value, x_o is the actual value in the center of the domain, and Δx is the increment of xi corresponding to a variation of 1 unit of X. The uncoded and coded values for the three variables are presented at Table 1. The coded values of the test variables were used to determine combination effect of temperature (X_1), time (X_2) and solvent concentration (X_3).

Table 1. *Experimental points in term of coded and uncoded of independent variables together with the observed and the predicted of the responses*

Variables						Responses							
T[a] [°C]		t[b] [min]		S[c] [%]		GM[d] [%]		Starch [%]		Yield [%]		DoW[e]	
(x_1) [f]	(X_1) [g]	(x_2) [f]	(X_2) [g]	(x_3) [f]	(X_3) [g]	Obs [h]	Pred [i]	Obs [h]	Pred [i]	Obs [h]	Pred [i]	Obs [h]	Pred [i]
45	-1	120	-1	60	-1	67.30	67.27	8.51	8.39	89.3	89.43	55.10	55.03
45	-1	120	-1	90	1	70.18	70.15	7.92	7.8	87.9	88.04	54.05	53.98
45	-1	180	1	60	-1	70.29	70.26	7.35	7.23	83.6	83.74	55.05	54.98
45	-1	180	1	90	1	71.10	71.07	6.59	5.47	86.2	86.34	56.28	56.21
75	1	120	-1	60	-1	70.48	70.45	5.12	5.00	85.3	85.44	57.24	57.16
75	1	120	-1	90	1	71.85	71.82	4.23	4.11	76.1	76.24	58.96	58.89
75	1	180	1	60	-1	72.07	72.04	4.94	4.82	84.2	84.34	57.82	57.74
75	1	180	1	90	1	72.3	72.27	4.36	4.24	76.1	76.24	58.54	58.46
60	0	150	0	75	0	70.67	70.67	5.72	5.88	82.7	83.73	56.04	56.56
60	0	150	0	75	0	70.28	70.67	5.28	5.88	85.1	83.73	57.28	56.56
60	0	150	0	75	0	71.10	70.67	5.88	5.88	85.3	83.73	55.59	56.56
60	0	150	0	75	0	70.43	70.67	5.70	5.88	82.9	83.73	56.70	56.56

[a] - temperature; [b] - time; [c] - solvent concentration; [d] - glucomannan, [e] - degree of whiteness, [f] - uncoded; [g] - coded; [h] - observed; [i] – predicted

Glucomannan concentration, starch content, yield and DoW were selected as the responses (Y) of the experiment. The data of two-level factorial design of three variables were fitted to a polynomial model (2) as follow:

$$Y = b_o + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_2 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 \qquad (2)$$

where Y is the response variable, b_1, b_2 and b_3 account for the main effect of the variable X_1, X_2, and X_3, while b_{12}, b_{13}, b_{23} and b_{123} represent the second order and third order interaction terms. The independent term b_o represents the response at zero level of every variable ($X_1 = X_2 = X_3 = 0$) which is at the center of the design [12].

The data processing and calculations were carried out using STATISTICA 9.0 to estimate the coefficients of the regression equations. The equations were validated using analysis of variance (ANOVA) to determine the significance of each term in the fitted equations and to estimate the goodness of fit in each case. The best model with no significant lack-of fit ($p > 0.05$) and the highest correlation coefficient (R^2) was selected. The equation (2) was used to plot the response surfaces for the variables studied. The surface plots were generated as a function of two independent variables, with the third variable kept at the zero level.

RESULTS AND DISCUSSION

Model fitting

The 12 design experimental points in term of coded and uncoded independent variables together with observed and predicted responses are presented at Table 1. Experimental data of the 12 design points showed the result of glucomannan content, starch, yield and DoW varied in the range of 67.3 - 72.3 %, 4.23 - 8.50 %, 76.10 - 89.43 % and 54.05 - 58.96, respectively. The maximum glucomannan content (72.3 %) was obtained

at extraction using 90 % IPA at 75 °C for 180 min. Meanwhile, the lowest starch (4.23 %), the lightest color (58.96) and the highest yield (76.1 %) were obtained at the same combination condition using 90 % IPA, at 75 °C for 120 min.

The effect or parameter estimates on the responses are shown in Table 2. Temperature variable was statistically significant ($p < 0.05$) and showed as the largest parameter estimated. Moreover, this variable was the most important variable for all the responses. The results indicated that the glucomannan content, starch, DoW and yield were mainly controlled by temperature. In addition, temperature had a positive impact on glucomannan content but showed a contrary effect on starch and yield. All individual variables affected glucomannan and starch content significantly, while yield was significantly influenced by temperature and time.

Table 2. *The effect and coefficient of independent variables on the responses*

Factors	Glucomannan			Starch			Yield			Degree of whiteness		
	Coeff[a]	Effect	*p*	Coeff[a]	Effect	*p*	Coeff[a]	Effect	*p*	Coeff[a]	Effect	*p*
Mean/ intercept	**70.76**	**70.76**	**0.00**	**5.88**	**5.88**	**0.00**	**83.72**	**83.72**	**0.00**	**56.55**	**56.55**	**0.00**
(1) T[b]	**0.98**	**1.96**	**0.00**	**-1.34**	**-2.68**	**0.00**	**-3.16**	**-6.34**	**0.00**	**1.51**	**3.02**	**0.00**
(2) t[c]	**0.66**	**1.32**	**0.00**	**-0.44**	**-0.88**	**0.03**	**-2.01**	**-4.03**	**0.01**	0.33	0.66	0.24
(3) S[d]	**0.74**	**1.49**	**0.00**	**-0.48**	**-0.96**	**0.02**	-1.06	-2.13	0.07	0.29	0.58	0.28
1 by 2	-0.26	-0.52	0.08	0.43	0.86	0.03	**-2.31**	**-4.63**	**0.01**	0.28	0.57	0.29
1 by 3	-0.23	-0.47	0.10	**0.11**	**0.22**	**0.44**	0.79	1.58	0.15	-0.25	-0.51	0.35
2 by 3	**-0.40**	**-0.80**	**0.02**	-0.11	-0.22	0.45	0.64	1.28	0.22	0.16	0.32	0.54
1*2*3	0.112	0.23	0.36	0.18	0.37	0.23	-0.36	-0.73	0.46	-0.41	-0.82	0.16

[a] - coefficient; [b] - temperature; [c] - time; [d] - solvent concentration; **bold** = significant value

The combination of time-solvent concentration was statistically significant in the range of glucomannan content studied. Meanwhile, the combination of temperature-solvent concentration and temperature-time were statistically significant for starch and yield respectively. Interestingly, DoW only affected significantly by temperature.

Quality of the models fitness was evaluated by analysis of variance (ANOVA) (data were not shown). Response with $p<0.05$ indicated a very high significance for the corresponding coefficients. Moreover, the results of ANOVA indicated that the lack of fit was clearly not significant for glucomannan content, yield, starch and DoW models ($p = 0.751$, 0.661, 0.061 and 0.650, respectively). This indicated that the models could represent the observation data. The mathematical models representing glucomannan content, starch, yield and DoW as a function of the coded test variables are expressed as in Table 3.

Table 3. *The mathematical models representing glucomannan content, starch, yield and degree of whiteness as a function of the coded test variables as well as the R^2 and $R^2_{adjusted}$*

Mathematical models	R^2	R^2_{adj}
Glucomannan content [%] = $70.670 + 0.979X_1 + 0.661X_2 + 0.744X_3 - 0.401X_2X_3$	0.978	0.940
Starch [%] = $5.883 - 1.340X_1 - 0.443X_2 - 0.478X_3 + 0.43X_1X_3$	0.973	0.927
Yield [%] = $83.725 - 3.162X_1 - 2.012X_2 - 2.312X_1X_2$	0.965	0.904
Degree of whiteness = $56.55 + 1.508X_1$	0.926	0.798

R^2 (regression coefficient) values as showed in Table 3 represent how well the model fits to the experimental data. By applying a regression analysis to the experimental data, it was obtained that the range of R^2 value of the models was 92.6 % (DoW) to 97.8 % (glucomannan) (Table 3). This implied that 92.6 - 97.8 % of the variations of dependent variables could be explained by the fitted model. This meant that the models were reliable to make a prediction for the responses based on the variables.
For a good statistical model, R^2_{adj} should be close to R^2. Except for the DoW, the difference values between R^2 and R^2_{adj} are relatively small ($\leq$ 7.2 %) which implied that only less than 7.2 % of the total variation were not explained by the model. This meant that both values were in reasonable agreement. It also indicated that the observed and the predicted values were in a high degree of correlation.
Figure 1 is plotting of correlation of temperature and time on the glucomannan content, starch, DoW and yield respectively, all at zero level of the solvent concentration variable. Various shapes of the contour plots indicated different of interaction between the variables and the responses.

Influence of temperature

Figure 1 shows a response surface plotting corresponding to join effect of temperature, time and solvent concentration on glucomannan content - Figure 1a), starch - Figure 1b), DoW - Figure 1c) and yield - Figure 1d). All responses showed that the effect of temperature was more prominent than other variable. The glucomannan content kept increase in the range studied, as shown in Figure 1a) and did not shown the level off. Hence, the optimum of glucomannan purification condition did not found in the range variables studied. The highest glucomannan improvement (24.3 %) was observed using 90 % IPA at 75 °C and 180 min.
Xu et al. [7] reported that improving temperature purification glucomannan of Amorphophallus konjac higher than 78 °C did not result in higher glucomannan content significantly. Hence, it was not recommended to purify glucomannan higher than 78 °C. Moreover, Tatirat et al. [13] found clear film formation when purified glucomannan at 85° and 95 °C. They suggested that these temperatures were higher than the exothermic transition temperature of glucomannan which attributed to disorder transition in molecular chain. Hence, these temperatures would not be suggested for glucomannan purification process.
This positive correlation between temperature and glucomannan content was consistent with previous report. Xu et al. [7] reported that temperature effect was favorable to improve glucomannan purity from 74.13 to 90.63 % by using 40 % ethanol at temperature 68 °C. Although they found the glucomannan content was higher than that of this study, however, the total improvement of glucomannan content between Xu et al. [7] and this study was not significantly difference. In fact, purification of this study was conducted in shorter time (180 min) than that of Xu et al. [7] (240 min). This suggested that purification glucomannan by using IPA solution performed slightly better than did ethanol solution.

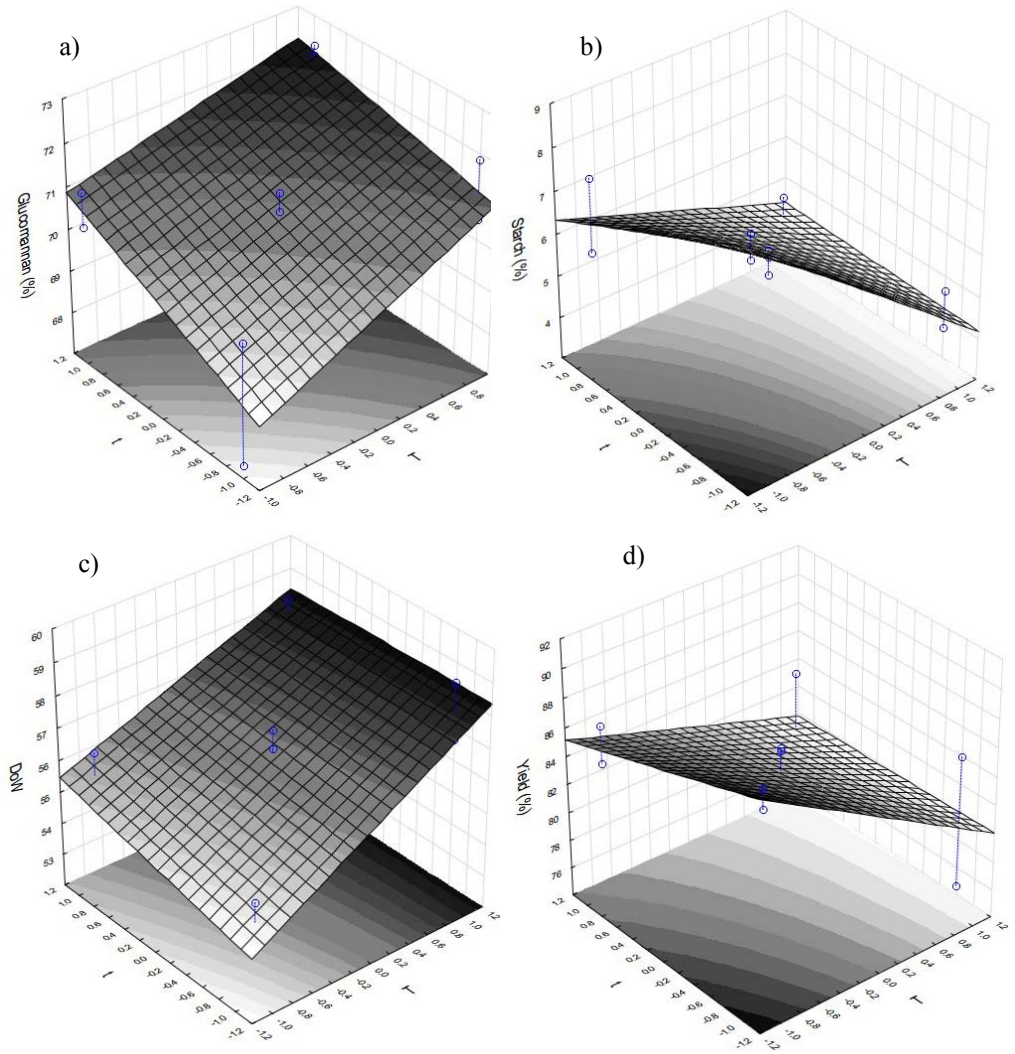

Figure 1. *The response surface plotting of temperature and time effect on glucomannan (a), starch (b), DoW (c) and yield (d), all on zero level of solvent concentration*

A negative correlation between temperature and either starch or yield was observed in this study. The starch and yield reduced to 4.2 % and 76.1 % over purification, respectively. Ohashi *et al.* [6] reported that numerous impurities present in crude konjac flour principally starches, cellulose and nitrogen-containing material. These impurities encapsulate the glucomannan granules; hence, these impurities need to be removed to obtain high purity. The more impurities were removed, the lower the yield obtained.

By using ethanol solution, Xu *et al.* [7] reported the effect of temperature on removing the impurities of glucomannan. They found the optimal temperature to remove the impurities was 68 °C, in which soluble sugar and starch were almost all removed. Moreover, nearly 95 % and 80 % of protein and ash were eliminated respectively. This result was confirmed that the improvement of glucomannan content was contributed by removing the impurities during the purification process. Although applying different

solvents in the attempts to purify glucomannan, Xu *et al*. [7], Tatirat *et al.* [13] and this study were in agreement that temperature affected glucomannan extraction significantly. The distinction of the starch content between this study and Xu *et al.* [7] could be due to the difference in purification method, solvent efficacy to removed impurities and the nature of the tuber raw materials. Ohashi *et al.* [6] reported that both IPA and ethanol have similar effect in removing impurities of *A oncophyllus*.
Temperature showed a significant contribution on the yield. As discussed in previous parts, increasing temperature could remove more impurities which in turn reduced the yield. The result of this study on the yield was not in line with Tatirat and Charoenrein [14] which reported insignificant effect of temperature on yield.
This research showed that DoW only affected significantly by temperature. The lightest color of purified glucomannan was observed using 90 % IPA at 75 °C for 120 min. In this condition, the lightest value improved from 53.06 to 58.96. *Amorphophallus* tuber contains up to 40 mg carotene/kg which could participate on the brownies of the flour and the carotenes dissolved significantly in IPA during glucomannan purification [8].

Influence of time

Time was a significant variable for the glucomannan content and starch but not for the yield and DoW. Figure 1(a) shows glucomannan content increased significantly. The effect of time on improving glucomannan content was more prominent in the initial of purification process. This result was in agreement with Rahayu [9] who found the presence of join effect of time and solvent concentration on glucomannan purification. Similar trend regarding positive correlation between time and glucomannan content was also reported by Widjanarko et al. [10] and Rahayu [9] in glucomannan ultrasound-assisted extraction (UAE). Since the rise of glucomannan content was obtained due to removing the impurities, additional contact time gave more chance for the flour to contact with solvent and dissolved more impurities. Widjanarko et al. [15] found a quadratic effect of time on glucomannan purification by using UAE. This effect was not observed in this study.
Our result showed that time did not contribute a significant effect on DoW -Figure 1c). Widjanarko et al. [15] reported that the longer the extraction time was beneficial for removing all impurities on the surface of glucomannan granules therefore maximize the score of DoW. This discrepancy could be due to the difference in purification method. This study used the conventional extraction method, while Widjanarko et al. [15] applied UAE method. UAE is known to be more effective and more efficient than conventional method [16]. The help of ultrasound could make the effect of time become more prominence on the purification process.
Although time was not a significant variable on yield, however, the more impurities released resulted in the lower yield - Figure 1d). Similar effect was reported by Widjanarko et al. [10] who found the total yield was not significantly affected by time during purification of glucomannan by UAE.

Influence of solvent concentration

Although not as prominent as temperature and time, the solvent concentration was a significant variable for all responses studied except for DoW. The presence of small part

of water in the solvent helped to remove the water soluble impurities of the flour. However, too much water leads the crude flour to absorb more water. Rahayu [9] reported concentration of glucomannan shows a positive effect on the viscosity. Moreover, at concentration of glucomannan below 0.55 %, the hydrosol behaves approximate Newtonian fluid, and at higher concentration, it behaves pseudoplastically [17]. Widjanarko et al. [10] also reported that glucomannan granules of the crude *A. oncophyllus* flour swollen bigger in dilute ethanol solution than in the higher concentrated ethanol solution. Hence, it was likely that the viscosity and swelling ability of the flour could inhibit contact between the solvent and other insoluble impurities of the crude flour. As a result, there was not much more impurities could be able to be removed to the solvent and still intact coating the glucomannan granule in higher IPA concentration.

Our study was in contrary with Mulyono [18] who found higher glucomannan content was obtained in less ethanol concentration than the higher ethanol concentration ones. The different result between this study and Mulyono's [18] could be contributed by the nature of the tuber. Mulyono [18] used the crude flour which contained 31 % of glucomannan. This concentration was only a half part of the glucomannan content found in the crude flour of this study. It meant that many impurities were found in Mulyono's sample, in which the impurities probably were dominated by the more water soluble ones. As a result, low concentration of ethanol solvent was more suitable in Mulyono sample's purification.

FTIR spectrum

Comparison of FTIR spectra between the crude flour, the sample purified using 90 % IPA solution at 75 °C for 180 min and the commercial glucomannan (Patrick Holford, glucomannan content 98 %) in the wavelength range of 4000 - 400 cm^{-1} is presented in Figure 2. In general, the spectra of both samples demonstrated similar peak ranges of wavelength but difference in intensity of the absorbance. Basically, the spectra were in agreement with data of Chua et al. [2] and Nguyen et al. [1].

The samples have similar absorbance intensity at a wide band of 3000 - 3700 cm^{-1} which attributed to O-H stretching vibration of the glucomannan and ~2900 cm^{-1} which assigned to -CH_2- stretching vibration. The glucomannan purification by using IPA solution seemed to affect slightly on the later functional group. Commercial glucomannan showed a superior absorbance level at ~2900 and ~2200 cm^{-1}.

The crude flour and the purified samples demonstrated comparable peaks in the range 2400 - 400 cm^{-1}. Some peaks that observed in 1000 - 2000 cm^{-1} were carbonyl of acetyl groups (~1726 cm^{-1}), absorbed water or protein (~1650 cm^{-1}), angular deformation of C-H (~1410 and 1370 cm^{-1}), C-O ether bond (1150 cm^{-1}) and C-O alcohol bond (stretching at ~1070 and ~1020 cm^{-1}). The absorbance intensities of the purified sample were lower than those of the crude flour at ~1070 cm^{-1}. This could indicate that the purification by using IPA solution reduce functional group in this wavelength. Moreover, the purification also reduced absorbance values of functional groups at 1000 - 400 cm^{-1}, were assigned to β-glucosidic and β-mannosidic linkages which observed at 900 - 800 cm^{-1} [2].

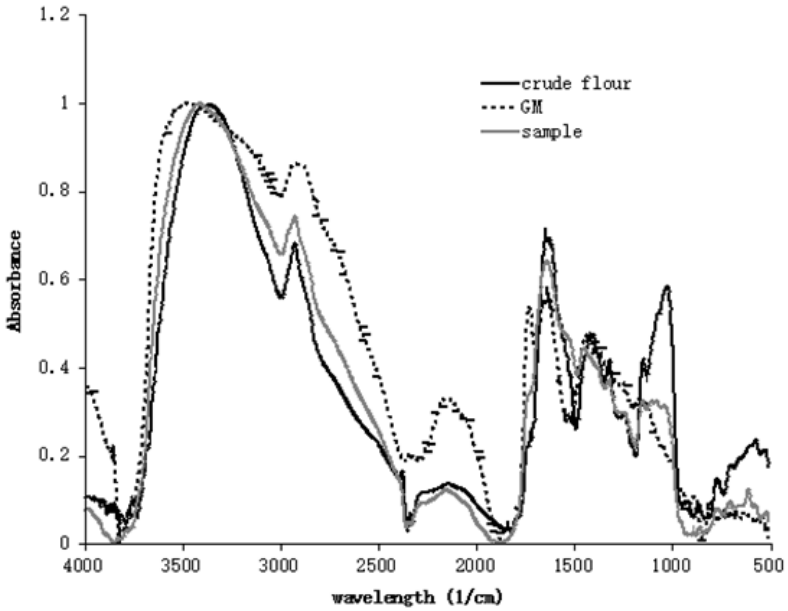

Figure 2. *Comparison of FTIR spectra of the crude flour (black line), the purified sample (using 90 % IPA solution, at 75 °C for 180 min)(grey line) and the commercial glucomannan (GM)(black dash)*

Morphology

Figure 3 describes SEM images of the crude flour and the sample purified using 90 % IPA solution at 75 °C for 180 min to observe morphology of the samples. The Figure 3 obviously shows that the crude flour have a course and cluttering surface, and surrounding by many crumb particles - Figure 3a) and c). It was suggested that the cluttering particles were impurities that coated the glucomannan granular, including protein, starch and soluble sugar. This result was in agreement with Tatirat and Charoenrein [14].

Meanwhile, the surface of purified flour demonstrated a smother surface with fewer crumbs, suggested that the purification with combination between temperature, time and concentration of IPA solution removed some impurities that enclosed the glucomannan granules. Figure 3b) shows the surface of the purified flour demonstrated layers that had been peeled and scrubbed.

Furthermore, the result showed that either the crude or purified flour had bigger particle size than the commercial glucomannan granules (figure not shown). This difference could be contributed by different grinding method. The flour of this sample was ground by house-hold blender resulted in bigger and not uniform size, while the commercial glucomannan was normally ground with high powerful blender resulted in smaller and more homogeneous particle size [13].

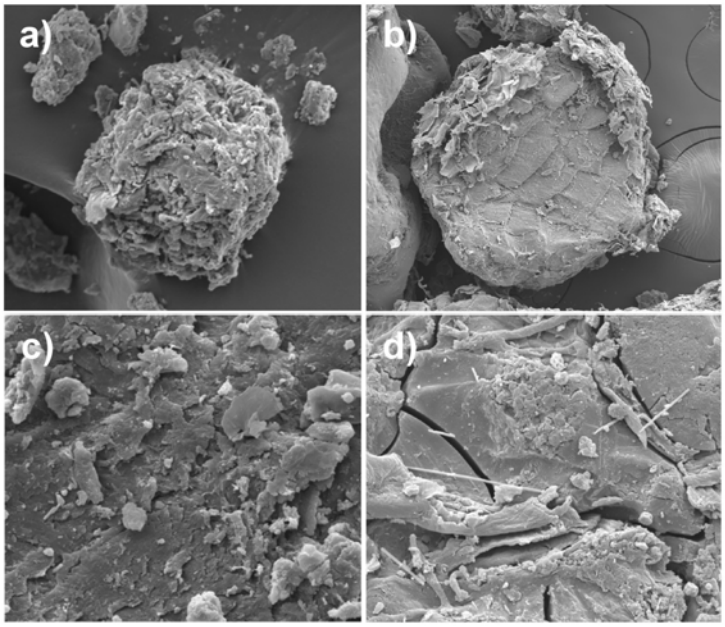

Figure 3. *Comparison of SEM images of the crude flour (left) and the purified sample (right), magnification: 450x - a) and b); 5000x - c) and d)*

CONCLUSION

The interaction effects of temperature, time and IPA concentration on purification glucomannan obtained from *A. oncophyllus* were studied using responses surface method. The results showed that the linear models were reliable to make predictions of the responses ($R^2 \geq 0.926$) in the range studied. Temperature was a significant variable for the all responses. Interaction variables were significant only for the starch content and the yield. Combination of the independent variables seemed to improve absorbance of the functional groups at ~2900 cm^{-1} which attributed to asymmetric stretching of C-H 2900 cm^{-1} and ~1730 cm^{-1} of acetyl group which is responsible on glucomannan solubility, but reduced level of the functional groups at ~1650, ~1070 - ~1020 and 900-800 cm^{-1} of absorbed water or protein, C-O alcohol and β-glucosidic and β-mannosidic linkages, respectively. The SEM analysis revealed that this purification method achieves separation and removal of impurities which encapsulated the glucomannan and subsequently released the glucomannan granules. The highest content of glucomannan obtained in this study was 72.30 % when 90 % 2-propanol at 75 °C for 180 min was used for purification.

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