

Optimization of Multilevel Ethanol Leaching Process of *Porang* Flour (*Amorphophallus muelleri*) Using Response Surface Methodology

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Abstract— Porang flour is a processed product derived from porang tuber (*Amorphophallus oncophyllus*), contains polysaccharide hydrocolloid such as glucomannan and it can be used as gelatinization, viscosity, filming, emulsifier and stabilizer agent. Porang flour has low content of glucomannan, contributing to its dark brown color and contains high level of calcium oxalate which can cause itching, irritation and other health effects. In this study, the multilevel ethanol optimization and maceration method were used to extract the glucomannan content from porang flour by the leaching process. Response surface methodology (RSM), Central Composite Design (CCD) on three independent variables leaching time (X_1), stirring speed (X_2) and ratio of solvent to flour (X_3) with response of glucomannan content and calcium oxalate were used as a tools to study the optimum responses. Responses model were quadratic with optimal condition as follows: leaching time was 4 h 6 min and 18 sec, stirring speed was at 443.45 rpm and ratio of solvent to flour was 8.92 ml/g. Under these conditions, the experimental yield of glucomannan content and calcium oxalate were 79.19 % and 0.08 %, respectively, which were very close to the predicted values of 79.26 % and 0.07 %, respectively.

Keywords— Response Surface Methodology; Glucomannan; Calcium Oxalate; Optimization; Porang Flour.

I. INTRODUCTION

Porang flour is a processed product from porang tuber (*Amorphophallus oncophyllus*) produced. Using stamp mill and contains about 67.5% (Faridah, 2011). This is much lower than the normal glucomannan level needed which is about $\geq 90\%$. Thus, there is a need to conduct studies on how to enhance the recovery rates of glucomannan in porang tuber.

Glucomannan is a soluble dietary fiber that has strong hydrocolloid properties and calorie content. Due to its glucomannan content, porang flour have potential role as a major export commodity in food, non-food and health-purposing manufactured-food (Kohyama *et al.*, 1993). Glucomannan, due its high fiber contents, can be used as a food additive.

In Indonesia, the main problems on the development of porang flour were due to the fact that the after processed flour has low levels of glucomannan and high presence of calcium oxalate. It is a well known facts that calcium oxalate causes itching, skin irritation, crystallization in the kidney and other health's effects. Therefore, it has not been applied in the manufacturing of food product in Indonesia. So, it is

clear that improving the glucomannan and reducing the calcium oxalate in porang flour is very important to enable it to be used in food manufacturing. In this study, recovery of glucomannan was conducted by the addition of porang flour to ethanol solution or leaching by multilevel ethanol using RSM.

Water and organic solvent as media, which have miscible properties, can be use in purifying the porang flour. Ethanol solvent has volatile properties, colorless, and non-toxic to human life (Sugiyama *et al.*, 1971; Widjanarko *et al.*, 2011). Ethanol will not propagate porang flour expansion. To date, there is a dearth of information on the method for the recovery of porang flour with high glucomannan content. The objectives of this research were to optimize the time, the stirring speed and the best ratio between multilevel ethanol to porang flour, in order to produce low level calcium oxalate and high level of glucomannan content.

II. MATERIALS AND METHODS

A. Materials

Porang or local name for conjac bulb, native to Indonesia, with diameter ranged 15-25 cm, height ranged 5-10 cm and

weight ranged 500-2500 g were obtained from Sumber Bendo Village, Saradan Subdistrict, Madiun Regency, Indonesia. The chemicals with purity were NaOH, formic acid, concentrated HCl (37%), concentrated H₂SO₄ (95%), CaCl₂, ether, metil red indicator, phenolphthaline (pp) indicator, NH₄OH, Kjeldahl tablet, boric acid 30%, sodium sulfate, dinitrosalicylic acid (DNS).

B. Extraction methods

Porang flour (25 g) was weighed and transferred into sterile beaker glass. To extract the porang flour, 200 mL of ethanol 40% and 50 mL hydrogen peroxide 0.5% were added to the mixture and stirred using homogenizer based on the design described in Table 2. Porang flour was filtered, and the deposit was rinsed using ethanol 60% and was subjected to the mixture. The solution of porang flour was filtered from deposit and separated from filtrate. The deposit was rinsed using ethanol 80%, and then filtered using Whatman paper 41 (length : 35 mm, width : 5mm). The deposit was dehydrated by drying in oven at 40°C for 12 h to produce porang flour from maceration extraction.

C. Analysis methods

Water content was determined by weight difference after drying of samples, following the official method of AOAC (1995). Fat content was determined using a Soxhlet apparatus according to AOAC (1995). Protein content was calculated from the nitrogen content (N% : 6.25) analyzed by Kjeldahl method. Ash was determined gravimetrically (AOAC, 1995). Starch content as described by Zapata *et al.* (2004). Yield was calculated based on the weight of the crude porang flour to the total weight of porang used. Calcium oxalate was determined as described by Ukpabi and Ejidoh (1989) and glucomannan assay was conducted as described by Peying *et al.* (2002). Scanning electron microscope (SEM) (*Instruction Manual FEI type InspectS50*) was used to observe microscopically on rough porang flour and leaching optimization process of porang flour was carried out using maceration method.

D. Experimental design

The improving of glucomannan content and reducing of calcium oxalate content by multi level ethanol were optimized using RSM, employing the CCD. The range and center point values of three independent variables were as presented in Table 1 were based on the results of preliminary experiments on the extraction of porang flour as described under the extraction method section in this study (Widjanarko *et al.*, 2011). Leaching time (X1), stirring speed (X2), and ratio of solvent ethanol to flour (X3) were chosen for independent variables

All treatments comprised of 20 processes of leaching, in which each process condition followed the central composite design. Analysis of the experimental design and calculation of predicted data were carried out using Design expert Software version 7.1 to estimate the responses. The behavior of the system was explained by the following quadratic equation:

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=2}^3 \beta_{ij} X_i X_j + \epsilon \quad (1)$$

where Y is the observation response, β_0 is the intercept, β_i is linear coefficients, β_{ii} quadratic coefficients, β_{ij} is treatment interaction coefficients, X_i is treatment code for “i” factor and X_j is treatment code for “j” factor.

TABLE I
INDEPENDENT VARIABLES AND THEIR LEVELS USED FOR CCD

Independent variables	Levels				
	-1,68	-1	0	+1	+1,68
Leaching time (h) (X1)	2,32 63,64	3 200	4 400	5 600	5,68 736,36
Stirring speed (rpm) (X2)	1:4.64	1:6	1:8	1:10	1:11.36
Ratio of solvent to flour (X3)					

TABLE II
CENTRAL COMPOSITE ARRANGEMENT FOR INDEPENDENT VARIABLES AND THEIR RESPONSE

No	Flour parameters			Response	
	Leaching time (h)	Stirring speed (rpm)	Ratio of solvent to flour (v/b)	Glucomannan (%)	Ca-Ox (%)
1	5	600	1:10	69,37	0,09
2	4	400	1:8	79,08	0,07
3	5,68	400	1:8	65,11	0,12
4	3	200	1:10	76,92	0,14
5	3	600	1:6	62,97	0,12
6	4	400	1:8	79,11	0,07
7	4	400	1:8	81,07	0,08
8	5	200	1:6	65,04	0,1
9	4	400	1:4.64	75,58	0,13
10	5	600	1:6	70,14	0,1
11	4	63,64	1:8	62,73	0,15
12	4	400	1:11.36	79,43	0,11
13	4	400	1:8	79,02	0,1
14	2,32	400	1:8	62,14	0,17
15	3	200	1:6	68,58	0,14
16	5	200	1:10	67,98	0,1
17	4	400	1:8	78,07	0,07
18	4	400	1:8	76,21	0,07
19	3	600	1:10	70,87	0,12
20	4	736,36	1:8	65,73	0,08

E. Statistical analyses

The responses obtained from each set of experimental design (Table 1) were subjected to multiple non-linear

regressions using the Design Expert software (Version 7.1, Stat-Ease Inc., Minneapolis, MN). The quality of the fit of the polynomial model equation was expressed by the coefficient of determination, R² and the significances of the regression coefficient were checked by F-test and p-value.

III. RESULTS AND DISCUSSION

A. Fitting the model

Response surface methodology in this research was used to determine the appropriate model for predicting the responses of glucomannan and calcium oxalate content. Montgomery (2001) noted that model analysis are used to determine appropriate model in RSM methodology, which are subsequently used to predict the response for independent variable. The evaluated model included linear, 2FI (interaction), quadratic or cubic. The selection of model was conducted based on: P-value model, lack of fit test, and model summary statistics (R², Adjusted R² and std dev. (Table 3) (Bradley, 2007; Bezerra *et al.*, 2008).

TABLE III
ANALYSIS OF VARIANCE (ANOVA) FOR FITTED QUADRATIC POLYNOMIAL MODEL

Source	Sum of squares	d.f	Means square	F - value	p-value Prob > F
Glucmannan					
Model	776,83	9	86,3140	21,3394	< 0,0001
Linier	45,59	3	15,20	0,32	0,8143
2FI	68,08	3	22,69	0,42	0,7422
Quadratic	663,16	3	221,05	54,65	< 0,0001
Qubic	23,58	4	5,89	2,10	0,1995
Residual	40,4482	10	4,0448		
Lack of Fit	27,8410	5	5,5682	2,2083	0,2025
Pure error	12,6072	5	2,5214		
Cor total	817,2746	19			
R ² =0,9505, Adjusted R ² = 0,9060, std. dev. = 2,01					
Ca-Oxalate					
Model	41,7075	9	4,6342	52,4315	< 0.0001
Linier	5,555E-003	3	1,852E-003	2,72	0,0791
2FI	1,375E-004	3	4,583E-005	0,055	0,9821
Quadratic	8,422E-003	3	2,807E-003	12,00	0,0012
Qubic	7,945E-004	4	1,986E-004	0,77	0,5816
Residual	0,00234	10	0,000234		
Lack of Fit	0,001607	5	0,000321	2,19134	0,2047
Pure error	0,000733	5	0,000147		
Cor total	0,016455	19			
R ² =0,8578, Adjusted R ² = 0,7298, std. dev. = 1,36					

The selection of the model based on sequential model sum of square showed that the significant and advised model was

quadratic (Table 3). When the p-value is less than 0.05, it shows that this variable is significant. When the p-value is less than 0.001, it shows that the variable is highly significant and that means this variable had a greater influence than other variables (Cai *et al.*, 2007). The other model suggested based on sequential model sum of square was quadratic, P < 0.0001 at glucomannan and P < 0.0012 at calcium oxalate, indicating that the fitness of this model was highly significant. While based on lack of fit tests at glucomannan and calcium oxalate content were P > 0,2025 and P > 0.1056 respectively, which implied an insignificant difference relative to the pure error and a good fitness of the model. Analysis of variance from quadratic response surface showed that quadratic model has a significant effect to response.

Quadratic model has a least standard deviation compared with other models such as the determination coefficient (R²= 0,9505), Adj. R² of 0.9060 to improve the glucomannan content and R²= 0,9505, Adj. R²= 0.7298 to reduce the calcium oxalate content. It means that variables of leaching time, stirring speed and ratio of solvent to flour were significantly different to response variance of 90.6% and 72.98%, whilst the rests were 9.4% and 27.02% influenced to other factors which was not variables considered in this research. Widjanarko *et al.* (2011) noted that leaching time and the level of leaching play a role to improve the glucomannan content and reduce the calcium oxalate.

A value R² greater than 0,75 indicates the aptness of the model is significant. The optimization of the extract of steviosides from stevia rebaudiana leaves using RSM have a value R²=0.98, P-value = 0.0074 and lack of fit (P>0.05) showed that quadratic model was significant (Puri *et al.*, 2012). Silva *et al.* (2007) research optimization of extraction of phenolics from *Inga edulis* leaves using RSM have a value R²= 0.85 for total phenolic, R²= 0.87 for total flavonoid and 0.96 for total flavonoid with lack of fit (P>0.05) showed that this model is significant.

B. Response surface and influence of variables on yield

Quadratic equation can be used to predict the responses from several levels. Quadratic equation obtained:

to improve the glucomannan content is:

$$Y_1 = -46.64 + 43.22 X_1^2 + 0.06 X_2^2 + 5.75 X_3^2 + 0.01 X_1 X_2 - 0.88 X_1 X_3 - 1.30 \times 10^{-3} X_2 X_3 - 5.10 X_1^2 - 1.22 \times 10^{-4} X_2^2 - 0.05 X_3^2$$

to reduce the calcium oxalate is:

$$Y_2 = 0.74 - 0.18 X_1 - 3.15 \times 10^{-4} X_2 - 1.83 \times 10^{-3} X_3 + 1.88 \times 10^{-5} X_1 X_2 - 2.5 \times 10^{-5} X_1 X_3 - 1.25 \times 10^{-7} X_2 X_3 + 0.02 X_1^2 + 2.55 \times 10^{-7} X_2^2 + 4.78 \times 10^{-6} X_3^2$$

This research has 3 factors of variables, therefore there were 3 response graphs which describe the relationship between leaching time, stirring speed and ratio of solvent to flour. The effect of leaching time, stirring speed and ratio of solvent to flour which quadratic to response of improving the glucomannan and reducing the calcium oxalate were shown in Figure 1.

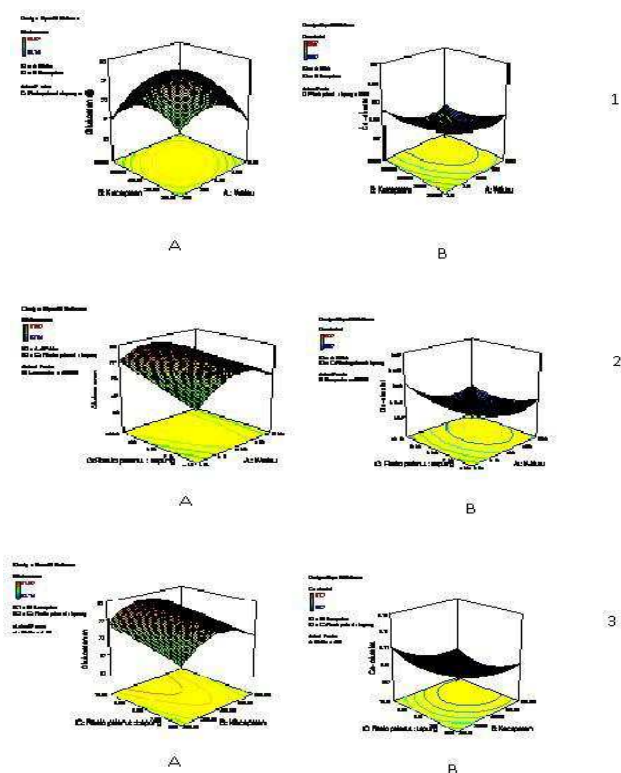


Fig. 1. The surface plots of the yield of glucomannan content (A) and calcium oxalate content (B) from porang flour as affected by leaching time, stirring speed and solvent/flour ratio. Where (1) is leaching time and stirring speed; (2) is leaching time and solvent/flour ratio; (3) is stirring speed and solvent/flour ratio

1) *Effect of leaching time to glucomannan content and calcium oxalate of porang flour:* Leaching process was carried out using 3 different ethanol concentrations (40% ethanol for the first stage, 60% ethanol for the second stage and 80% ethanol for the third stage) and the difference in the concentrations of ethanol used may allow a distinctive component to be dissolved based on the polarity. At higher concentration of ethanol used, the level of polarity will be decreasing, therefore allowing the non-polar components to be dissolved.

Shimizu and Shimara (2004) reported that 80% ethanol may cause distinctive component from porang flour to be dissolved and such ethanol concentration may reduce content of starch, protein, fat, oxalate, ash and calcium oxalate. While Ramadhan and Phaza (2007) also noted that porang flour leaching using ethanol could separate non-glucomannan compound as ethanol has a high polarity and is able to dissolve several compounds, such as resin, fat, oil, fatty acid, carbohydrate and other organic compounds, preventing the glucomannan granule from expanding and dissolving into the solution.

Maceration method extraction time is appropriately required to ensure the distribution of extracted compounds from solid component to its solvent. Leaching time can play a role in the leaching process, therefore the optimal leaching time can be achieved to produce flour which contains high glucomannan content, low calcium oxalate and has light porang flour color.

Increasing leaching time that exceeds the optimal leaching time can lead to decreasing glucomannan content

and improving calcium oxalate content. However, its decreasing and increasing were not the same as the amount obtained using optimal leaching time. Kato (1970) reported that the degraded glucomannan will produce oligosaccharides such as manosa, glucose, mannobiosa, manosil glucose, manotriosa, manobiosil glucose, manotetraosa, manotriosil glucose and manotetraosil glucose. Claver *et al.* (2010) also reported that leaching time can lead to the degradation of dissolved glucomannan. The extraction of Chinese sorghum flour produces a low polysaccharide throughout the duration of the leaching time used. Caili *et al.* (2006) noted that the increase in the optimization of extraction time from xyloglucan produces less xyloglucan.

2) *Effect of stirring speed to glucomannan content and calcium oxalate of porang flour:* The faster stirring speed of leaching can stimulate the loss of distinctive compounds, therefore producing porang flour with high glucomannan content and low calcium oxalate. Glucomannan is located inside a cell which envelopes the distinctive compounds, such as starch, protein and calcium oxalate. The leaching process using stirring can help to increase ethanol's ability to dissolve compounds on the cell surface and also to release distinctive components out of the ethanol.

Keil (2007) noted that mechanical effects can increase the penetration from liquid to cell membrane walls, support the release of cell components, and increase mass transfer. Stirring speed can also accelerate the extraction process or purification of glucomannan as reported by Povey (1988), who found that extraction has a mechanical effect to enhance stronger liquid penetration energy into cell tissue and increase the penetration of ethanol into the cell, therefore oxalate can be released easily.

Mason *et al.* (1996) and Paniwnyk *et al.* (2009) noted that mechanical effects increase the penetration of liquid into the wall of the cell membrane, supporting the release of cell components, and improve mass transfer. Caili *et al.* (2006) also suggested that the contribution of mechanical effects on the extraction of xyloglucan with physical phenomena, such as diffusion through cell walls and rinsing all broken cell contents.

Sarkosh (2012) noted that stirring or agitation on the extraction process can increase efficiency and shorten the time of extraction. The stirring process can enlarge the surface leaching area of extracted material with solvent to increase efficiency and may lead to an increase in the temperature of extraction. The increase in extraction temperature may affect the extraction efficiency in several extraction processes. Metz and Ganor (2001) reported that the extraction efficiency increases at a stirring speed of 800 rpm, and it will decrease at a stirring speed of 1000 rpm. And Sarkosh (2012) also noted that the increasing of stirring speed may lead to intensity pounding between particles in the materials getting higher.

3) *Effect of different ratio of solvent to flour to glucomannan content and calcium oxalate of porang flour:* Glucomannan response increased and calcium oxalate decreased as the ratio of solvent increased. When ethanol

solvent applied increase, the distinctive component and non-glucomannan compounds also increase which can be dissolved by ethanol. The increasing of glucomannan and the decreasing of calcium oxalate on each leaching were directly proportional with the decreasing of distinction compounds such as non-glucomannan.

In general, a larger volume of solvent can dissolve constituents more effectively leading to improved extraction of rutin and quercetin from *Euonymus alatus* (Thunb.) (Yang and Zhang, 2008). Gamse (2002) also reported that when the number of solvent used increase, the dissolve solid increasing due to the dispersion of the particle, as higher solvent ratio will increase the mass transfer. Qing *et al.* (2009) noted that the penetration during mass transfer was affected by concentration gradient between solid and liquid. The higher solvent ratio will produce porang flour which contained high glucomannan.

Increase of solvent ratio above optimal response point can lead to decrease in the glucomannan content. Susanto (1999) reported that the comparison between number of sample and extracted liquid affected the efficiency of extraction. Yang and Zhang (2008) reported that an increasing in extraction of rutin and quercetin with an increasing in the ratio of solvent : sample weight, especially when the ratio increased from 20 to 40 ml/g and will decrease after the ratio increased from 40 to 60 ml/g. There were also similar findings reported by Claver *et al.* (2010), on the lower recovery polysaccharides extraction from Chinese sorghum powder when solvent ratio and time increased.

4) Response optimal point and verification: Stationary point value obtained from canonical analysis was carried out under optimal conditions: leaching time of 4 h 6 min and 18 sec, stirring speed 443.45 rpm and ratio of solvent to flour 8.92 ml/g (Table 3). The response of glucomannan content and calcium oxalate content under optimal conditions were 79.19% and 0.075%, respectively. These condition were optimal to obtained glucomannan and the lower of calcium oxalate on porang flour using maceration method.

Verification was performed by comparing response analysis value from the research to response value calculated by Design Expert. Accuracy level of glucomannan and calcium oxalate were 99.91% and 97.33%, respectively, whilst the difference value were 0.09% and 2.66%,

respectively, obtained from realexperiments, demonstrating the validation of the RSM model (Table 4).

The difference of predicted value was significant ($p > 0.05$) obtained from realexperiments which was demonstrated in the validation of the RSM model. The result of analysis confirmed that the response model was adequate for reflecting the expected optimization and the model was satisfactory and accurate.

5) Physicochemical analysis of optimal porang flour

Leaching optimization process of maceration method may increase glucomannan and decrease calcium oxalate content of porang flour. As mentioned in Table 4, several trends were observed. An increase in glucomannan content was found in leaching optimization process of porang flour using maceration method from 67.02% to 79.19% and also a decrease in calcium oxalate was found from 0.28% to 0.07%. This difference was further evidenced as seen in the microstructure using scanning electron microscopy (SEM) at magnification 100 x as shown in Figure 2. Microstructure of non-glucomannan from leaching at optimized process of porang flour using maceration method showed that granules surface of non-glucomannan decreased compared with rough porang flour due to impurities of glucomannan. This phenomenon showed that there were synchronization between data observed visually (Figure 2) and quantitatively (Table 5).

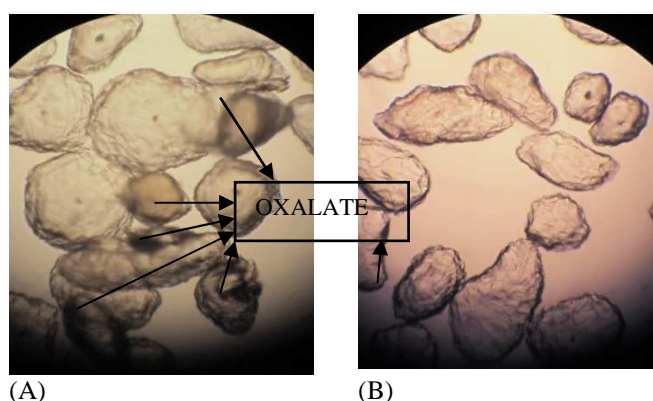


Fig. 2. Microstructure of porang flour (A) before leaching, (B) leaching optimization process using maceration method at magnification 100x

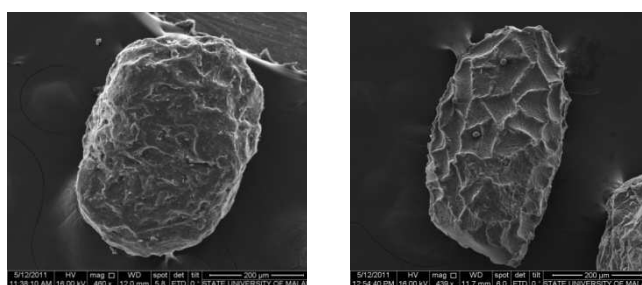
TABLE IV
SELECTED OPTIMAL POINT FROM DESIGN EXPERT MODE

	Leaching time (h)	Stirring speed (rpm)	Solvent/flour ratio	Glucomannan (%)	Ca-oxalate (%)	Desirability
Prediction	4.13	434.45	8.92:1	79.19	0,075	0,93
Actual	4.13	434.45	8.92:1	79.26	0,073	-
Accuracy level (%)				99.91	97.33	
Difference value (%)				0.09	2.66	

TABLE 5
PHYSICO-CHEMICAL PROPERTIES OF ROUGH PORANG FLOUR
AND OPTIMIZATION OF MACERATION LEACHING OF PORANG
FLOUR

Parameter	Rough porang flour	
	Flour optimization (%)	Optimization of maceration leaching (%)
Moisture	12.17	12.54
Ash content	2.63	0.38
Starch content	2.7	1.26
Protein content	2.35	0.78
Fat content	1.49	0.45
Oxalate content	0.28	0.073
Glucomannan content	67.019	79.187
White degree	50.75*	53.19*
Viscosity	6300**	9633.33**
Yield	62.71	89.83

Leaching at optimized conditions of porang flour using maceration method produced clear glucomannan granule and form of scales as shown in Figure 3. Takigami (2000) reported that the surface of glucomannan granule has a light colour, clean and more visible in the scales after leaching process.



(A) (B)
Fig. 3. Scanning electrom microscopy (SEM) observation on (A) rough porang flour; (B) Leaching optimization process of porang flour using maceration method

The other components of porang flour were decreased with the increased of glucomannan content such as starch, protein, fat and ash. A similar process was observed for calcium oxalate due to its solubility properties to ethanol solution. Leaching time, stirring speed and ratio of optimal solvent played important role to increase solubility of starch, protein, fat and ash in several concentration of ethanol solvent. Shimizu and Shimahara (2004) reported that ethanol solvent may lead to the decreasing amount of distinctive compounds such as starch, protein, fat, oxalate and ash

compared with rough porang flour which produce high glucomannan content.

IV. CONCLUSION

The response surface methodology was used to estimate and optimize the experimental variables : leaching time (h), stirring speed (rpm) and ratio of solvent of flour (ml/g). All variables were the important factors which affect leaching process, on the response model values ($P < 0,0001$) for glucomannan content and calcium oxalate content. Response model was quadratic with optimal condition as follows : leaching time of 4 h 6 min and 18 sec, stirring speed of 443.45 rpm and ratio of solvent to flour 8.92 ml/g enhance the experimental yield of glucomannan content and calcium oxalate.

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