Comperation of Porang Flour (morphophallus muelleri) Purification Method : Conventional Maceration (gradient ethanol leaching) and Ultrasonic Maceration Method using Response Surface Methodology

Anni Faridah

Faculty of Tourism and hospitality, Padang State University, Padang, West Sumatra 25131, Indonesia
E-mail: faridah.anni@gmail.com

Abstract— **Porang flour (Amorphophallus muelleri) contained fairly high of glucomannan and slightly orange white colour whereas also contained oxalates which are toxic to human. This toxic property caused unacceptable for food applications. This systematic study was undertaken to compare two of the optimizations method of flour porang purification i.e. using various of ethanol concentration washing (conventional maceration) and ultrasonic maceration method to obtain high glucomannan levels and high viscosity with low calcium oxalate and bright white colour of flour. Response surface methodology (RSM), Central Composite Design (CCD) on three independent variables (leaching time (X1), stirring speed (X2) and the ratio of solvent to flour (X3)) for the conventional maceration and two independent variables (time leaching (X1) and and the ratio of solvent to flour (X2)) for ultrasonic maceration, has been done. Response analyzed on glucomannan levels, viscosity, concentration of calcium oxalate, and brightness. Response has a quadratic model except the brightness of the colours of the linear model in both maceration. The ultrasonic maceration obtained faster washing time i.e. 3 hours, 53 minutes and 18 compare than the conventional maceration and used more less solvent and get a high levels of glucomannan response values , viscosity values , well white brightness colour and a low levels of oxalic acid value.**

Keywords— **porang flour; RSM; ethanol; maceration; ultrasonic**

I. INTRODUCTION

Glucomannan is a stongly hidrocolloid water-soluble polysaccharide, can establish a gel, high viscosity property , low in calories, so it has high potential to be developed in the food and non food industry [1]. Purification the porang flour using mechanical method resulted 67.02% of glucomannan and 0.398% of calcium oxalate [2]. Glucomannan application on food products can be made by purifying the glucomannan that can produce high level of glucomannan, high viscosity, reduced calcium oxalate (almost free), obtaining bright white colour flour which may elevate the economic value of the porang flour itself. Glucomannan purification can be optimized by use chemicals by using conventional leaching maceration and ultrasonic maceration technique.

Leaching can be done by adding some chemicals that can dissolve or precipitate any impurities or undesirable compounds except glucomannan which exist in porang flour. The solvent selection was base on its properties such as selectivity, recovery easiness, coefficients, and its chemical activity [3]. Porang flour can be extracted using water miscible solvent like ethanol that could dissolved in water,

but will not cause glucomannan to swell. The use of water miscible solvents will not make glucomannan to expand and its impurities will be easy to separate [4]. The ethanol washing process can dissolve the non-glucomannan compounds due to its high polarity is fit to dissolved resins, fats, oils, fatty acids, carbohydrates and other organic compounds [5].

Porang flour laundering mechanism by using ethanol solution can be assumed as a leaching process (solid – liquid extraction), with retrieval some soluble compounds in the solid surface by using liguid material (solvent) [5]. In this leaching process, the soluble compounds trapped in solids and move through the pores of the solid. Solute diffuses out from the solid surface of particles and move into the lining around the solids, then to the solution.

Smirnova rt al [6] reported that glucomannan extraction method by using alcohol precipitation is intended to produce a high purity of glucomannan. High purity of konjak flour has been purified by alcohol precipitation that wash the konjac flour out from soluble starch. After leaching process, yield of rendemen contained high levels of gum and unwanted compounds including sulfur dioxide can be reduced [7].

Conventional leaching maceration method was different from ultrasonic maceration. Ultrasonic leaching will increase the permeability of cell walls, forming cavitation (holes) and improve mechanical stress. The mechanical effect of ultrasound led to increase the penetration of the solvent into the cells and improves mass transfer [8] [9], thus this mechanism will make the obtained result of ultrasonic maceration aplication to the cell [10]. Leaching ultrasonic maceration will be faster, but expensive compared to conventional leaching maceration.

The main advantage of using ultrasonic leaching are more time-efficiency and improved yield [10], [11], [9]. [12] also concluded that using fast, non-destructive, accurate, automated of ultrasonic measurement can be applied into the laboratory even in on-line measurement. Ultrasonic extraction requires less time with high productivity, reduce the amount of solvent and lower operational costs [13]. [14] mentioned that ultrasonic produces cell disruption, particle size reduction, shortening time and increase efficiently. [15] acquired that ultrasonic extraction rate was faster (34 minutes) than the conventional method during the extraction of pectin from grapefruit peel. This study aimed to compare conventional and ultrasonic maceration method on leaching optimization of flour porang by using an ethanol solution to obtain high level of glucomannan, high viscosity, bright white colour of the flours and low content of calcium oxalate.

II. MATERIALS AND METHODS

A. Material, Solvents and Reagents.

Porang flour that has been used was the result of bulbs porang flouring optimization [2]. Porang (local name of konjac bulb) is native to Indonesian, size is range from 15-25 cm in diameter, 5-10 cm in height and 500-2500 g in weight. It was obtained from Bendo Village, Saradan Subdistrict, Regency Madiun, Indonesia. The chemicals pro analysis (p.a) purity were used: NaOH, formic acid, concentrated HCl (37%), concentrated H2SO4 (95%), CaCl2, ether, methyl red indicator, phenolphetaline (pp) indicator, NH4OH, Kjeldahl tablets, boric acid 30%, sodium sulfate and dinitrosalycilicacid (DNS).
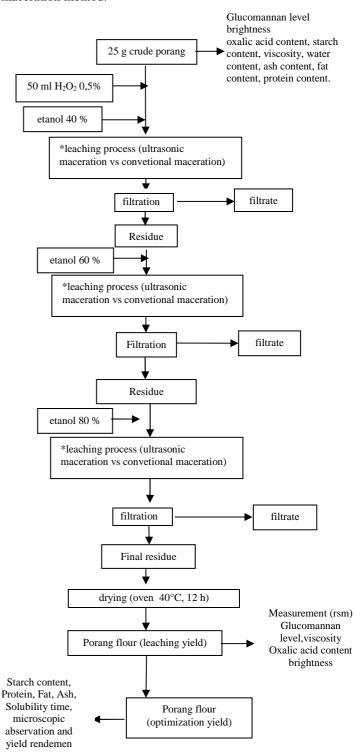
B. Extraction methods

Research design on both maseration method was the same. The difference was during application of homogenizer or stirrer with certain speed in the conventional method while ultrasonic maceration, vibrating horn system (amplitude of 300 dB) and a frequency of 60 kHz was used. The procedures, flow charts and observations applied can be seen in Figure 1.

C. Analysis methods

Water content was determined by weight difference after drying of samples, following the official method of [17]. Fat content was determined using a Soxhlet apparatus according to [17]. Protein content was calculated from the nitrogen content (N% : 6.25) analyzed by Kjeldahl method. Ash was determined gravimetrically [17]. Starch content as calculated as described by [18]. 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 [20] and glucomannan assay was conducted as described by [21]. Scanning electron microscope (SEM) (*Instruction Manual FEI type Inspect S50*) was used to observe microscopically on rough porang flour and leaching optimization process of porang flour was carried out using maceration method.

Note:*The conventional maceration using a homogenizer according to the research design (Table I) and ultrasonic maceration method using vibrating horn system (60 kHz, 300 decibels) the research design (Table II)

Fig. 1. Flow chart of porang flour laundering using conventional maceration and ultrasonic maceration (Modified from [16])

The improved of glucomannan content and reduced of calcium oxalate content using by various (multi level) ethanol concentration were optimized using RSM, employing the CCD. The range and center point values of three independent variables were 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 [4]. Leaching time (X_1), stirring speed (X_2), and ratio of solvent ethanol toflour (X_3) were chosen for independent variables

Independent variables	Levels				
	-1,68	**-1**	**0**	**+1**	**+1,68**
Leachig time (h) (X_1)	2,32	3	4	5	5,68
Stirring speed (rpm) (X_2)	63,64	200	400	600	736,36
Ratio of solvent to flour (X_3)	1:4.64	1:6	1:8	1:10	1:11.36

Free variable	Level of free variable				
	-1,414	**-1**	**0**	**+1**	**+1,414**
Contact time (minutes) (X_1)	10,86	15	25	35	39,14
Ratio (sovent : flour) (X_2)	5,17:1	6:1	8:1	10:1	10,83:1

D. Statistical analyses

The responses obtained from each set of experimental design (Table 1 and 2) were subjected to multiple non-linear regressions using the Design Expert software (Version7.1, Stat-Ease Inc., Minneapolis, MN). The quality of the fit of the polynomialmodel equation was expressed by the coefficient of determination, R_2 and the significances of the regression coefficient were checked by F-test and p-value.

III. RESULT AND DISCUSSIONS

A. Fitting the model

Response model has sequence value (sequential model of sum of squares) p <5% and quadratic. If the values of response model in order to p <5%, so the quadratic model has a significant response. Response of brightness, p value = <5% , it's a linear model. [22] also get a sequential model of sum of squares value of p = 0.0013 (0.13%), which mentioned that the chances of a model error of less than 5%, or quadratic model has a real influence on the response. [23] informed that if the value of p <0.05, the model was significant and has a greater influence for response compare with the other models. [24] also mentioned that the smaller of p value, the model will be more significant. Model selection that based on sequential sum of squares, indicated the significant model and recommended for all three responses was a quadratic (P <0.0001). This showed, that the chances of a model error, less than 5%, or quadratic model has a real influence on the response. This applied to both of the optimized porang flour (conventional and ultrasonic maceration). However, the response model of brightness was a linear model (Table III). Quadratic model of all of three response and linear for brightness has chosen due to its value

of p <5% which was better in according to the statement of [24], the significantly quadratic polynomial models on optimizing of ultrasonic leaching of water-soluble polysaccharide from Boletus edulis mycelia using RSM with a p value of 0.0003.

	Purification	
	Conventional (4 response)	Ultrasonic (4 Response)
MODEL	Quadratic, Linear (colour)	Quadratic, Linear (colour)
Value of p Model	< 0,003	< 0,02
Value p Lack of fit	> 0,05	> 0,05
Value of p R^2	> 0,81	> 0,85
Value of p Adj R^2	> 0,75	> 0,75

According to lack of fit test, recommended model on both macerations were quadratic for glucomannan levels, viscosity, concentration of oxalic acid and linear for brightness. For a comparison with similar technique, [25] mentioned that the model will be significant if lack of fit test values > 0.05.

The R2 value, that close to 1, illustrates the high correlation between the observed and predicted values [26]. [27] mentioned that the value of R2 = 0.58 was quite good and acquire an acceptable models. [28] informed that the value of R2 > 0.75 indicates the model accuration. The R2 value on both optimization of flour porang results was > 0.75 (Table III). This means that the variable such as washing time, stirring speed and the ratio of solvent: flour have affected to the diversity of responses for glucomannan levels, viscosity, concentration of oxalic acid and brightness.

B. Response optimal point and verification

Figure 2 shows that time variable, stirrer speed and the ratio of solvent's provided the optimum point to the interaction response. In this study, the expected result was the porang flour that will has higher levels of glucomannan with maximum viscosity values, low oxalate levels and good acceptable of brightness. The optimum solution point obtained from Design Expert computational results are shown in Tables IV and V.

Conventional maceration rensponse   Conventional maceration contour

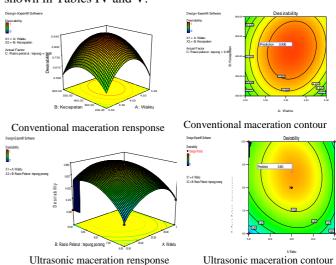Ultrasonic maceration rensponse   Ultrasonic maceration contour

Fig. 2. Surface and Contour responese of Variable Optimum Point towards Interaction Response

Canonical analysis to quadratic polynomial models were used to determine the shape and the curve of response surface, as well as the location of a stationary point or the optimum point of the surface response and to determine whether the response will be minimum or maximum [29]. The actual value for a stationary point obtained from canonical analysis were 4.16 hours for contact time, 434 rpm for stirring speed and 9.35: 1 for the ratio of solvent: flour of (Table IV), for the results of conventional and ultrasonic maceration is 25.10 minutes. While the point optimum of solvent ratio variables: porang flour is 8.65: 1 or in solution property is 216.25 ml (Table V).

Table IV also shows the predicted response of glucomannan levels, viscosity, oxalic acid and the brightness

on a optimum conditions of conventional maceration respectively for 79.38%, 9701.09 cPs, 0.076%, and 53.94. This condition was the best condition to obtain the highest levels of glucomannan and viscosity, low levels of oxalic acid and the quite well brightness of porang conventional laundering maceration flour. Thus, for the results of the optimization of ultrasonic maceration, optimum solution point variables, obtained the optimum response values of glucomannan levels (85.74%), oxalic acid content (0.044%), the viscosity (13970.7 cPs), and 59.78 of brightness. The optimum solution point obtained from Design Expert computational results that are shown in Table V.

TABLE IV.
OPTIMUM SOLUTIONS POINT OF SELECTED CONVENTIONAL MACERATION DESIGN EXPERT CALCULATION RESULTS

Source	Washing time (h)	Stirring speed (rpm)	Ratio (solvent: flour)	GM content (%)	Viscosity (cPs)	Oxalat acid (%)	Brightness	Desirability	Ket.
Prediction	4,16	434,04	9,35:1	79,38	9701,09	0,076	53,94	0,936	Selected
Verification	4,16	434	9,35:1	80,17	9733,33	0,077	53,95	-	-
The difference				0,79	32,24	0,001	0,01	-	-
The diversity value (%)				0,99	1,36	1,31	0,89		

source : [21]

TABLE V
OPTIMUM SOLUTIONS POINT OF SELECTED ULTRASONIC MACERATION DESIGN EXPERT CALCULATION RESULTS

source	Time (minutes)	Ratios : f (v/b)	GM content (%)	Axalic acid (%)	viscosity (cPs)	Brightness	Desirability
Design	25,10	8,65 : 1	85,74	0,044	13970,7	59,78	0,843
Verification	25,10	8,65 : 1	84,37	0,045	13750	60,38	-
The difference			1,37	0,001	220,7	1,39	-
The diversity value (%)			1,62	2,22	1,6	2,33	-

The verification of optimum results should be done as an evidence that the optimum point solutions that provided by the independent variables. Design Expert program surely gives a response results in accordance with the optimum response that has been determined by the program and optimal absolutely. Verification step was done by comparing the value of the research response analysis with response value calculation results Design Expert software. The percentage of differences in the value of each response was not wide and calculation results value of the verification almost closed to Design Expert calculation. The difference of predictive value with the research result was not more than 5%, that indicates the model was quite appropriate for the extraction process [30].

Washing optimization of porang flour with ultrasonic maceration were fast (25 minutes, 6 seconds), less solvent (8.65) and generate of glucomannan levels, viscosity and brightness also reduce of calcium oxalate (Table VI). [31] reported the effect of mechanical ultrasonic extraction, due ultrasonic energy waves cause fluid penetration force more

powerful to the inside of the extracted cells compared with classical extraction methods. [10] emphasized that the main advantage of the use of ultrasonic extraction are an efficiency, faster extraction times and improved results. Ultrasonic leaching requires less time with high productivity, that can reduce the amount of solvent and lower operational costs [14]. [15] informed that the ultrasound produces cell disruption, particle size reduction, shortening the time and more efficiently. [16] acquire ultrasonic extraction rate several times faster (30 minutes) rather than maceration (24 hours) on the extraction of steroids and triterpenoids of Chresta spp. Ultrasonic extraction method is better than the conventional methods of extraction and ultrasonic vibrating horn is better than an ultrasonic bath [32]

TABLE VI
THE DIFFERENCES VALUES OF VARIABLES AND RESPONSE OF CONVENTIONAL AND ULTRASONIC MACERATION OPTIMIZATION LAUNDERING

Method	Washing time (minutes)	Solvent ratio	Glucomannan (%)	Viskosity (c Ps)	Calcium oxalate (%)	Brightness
Conventional	246	9,35	80,17	9733	0,109	53,94
Ultrasonic	25,1	8,65	84,37	13750	0,064	60,38

C. Physicochemical analysis of optimized porang flour

Significantly increased levels of glucomannan from 67.02% became 80.17% (conventional) and 84.37% (ultrasonic) is shown on Table VII. Ethanol washed and the use of ultrasonic can dissolve more impurities compounds and non glucomannan components higher than conventional maceration. It also can be seen in Figure 3 that observation

with a light microscope by a magnification of 100x and reinforced by SEM-EDAX (Figure 4). Observations on conventional porang flour starch surface showed that non glucomannan granules were more than ultrasonic maceration porang flour.

Conventional porang flour's viscosity (9733,33 cPs) was lower than ultrasonic maceration (13750 cPs). High and low viscosity of porang flour were closely related to levels of contained glucomannan. The washing process also can increase glucomannan levels and decline the starch, protein, fat, ash contents and calcium oxalate contrations. [33] mentioned that the purification process of konjac flour by washing with ethanol is very effective in reducing impurity components that located on the surface s glucomannan granule. The viscosity increasement was associated with the increase glucomannan. Washing process with ethanol and ultrasonic maceration, high levels of glucomannan will lead the viscosity elevation. [34] concluded that glucomannan has a high molecular weight (> 300 kDa), where it can form a thick solution pseudoplastis. This is also supported by a statement of [16], that glucomannan in water at room temperature will give high viscosity and form a gel when added lime.

TABLE VII
PHYSICO-CHEMICAL ANALYSIS OF OPTIMIZED PORANG FLOUR

Parameter	Sample	Data	
		Conventional maseration**	Ultrasonic maceration
Calcium oxalate (%) (%)	0,398	0,109	0,064
Glucomannan (%)	67,02	80,17	84,37
Water content (%)	12,17	14,11	8,99
Ash content (%)	2,63	0,331	0,32
Starch content (%)	2,70	1,25	0,53
Protein (%)	2,35	0,87	0,26
Fat (%)	1,49	0,47	0,53
Brightness	50,76*	53,93*	60,38*
Viskosity (c.Ps)	6300	9733,33	13750
Rendemen (%)	62,71	89,87	77,41
Solubility rate (sec)	1205	812	772
Residue of H_2O_2 (%)	-	0,012	0,007215

Description: * use no unit, where a value of 100 is assumed as pure white. ** [21]

The optimization process of conventional maceration method for reducing calcium oxalate also shown an interesting result. Decreased levels of calcium oxalate from 0.398% into 0.109% (conventional) and 0.064% (ultrasonic) was due to the property of the miscible non polar components that contacted with high concentrations of ethanol with stirring and cavitation support. This reduction of contained calcium oxalate caused by its solubility in

ethanol. [35] stated that the oxalic acid is widely precipitated in the light fraction (tobiko) of porang flour that containing impurities compounds (calcium oxalate, starch, protein) and with a small particle size (less than 1x10-2 mm), that would be lost with ethanol washery.

Optimized porang flour by ultrasonic maceration has brightness value (60,38) higher than conventional (53,93) and control sample (50,76). The enhancement of brightness due to sonolisis process on ultrasonic maceration. According to [9], ultrasonic can cause sonolisis in water, resulting in H + and radical HO⁻, thus generated the hydrogen peroxide. Hydrogen peroxide can oxidize the color pigments in porang flour which will provide brighter flour color. While [36] explained that hydrogen peroxide has the ability to form reactive free radicals that react with organic molecules and oxidize macromolecules, and break it down into smaller molecules. The longer of washing time, the longer of the sonolisis process and the oxidation process will be better, thus the degree of white flour also enhancing.

Ultrasonic process also helped to eliminate impurities compounds that cause the dark colour of flour. Effects of ultrasonic cavitation will generate fractures and help break down the cell walls and increase the penetration of ethanol into the cell [37]; [38], so that the impurities that exist on the surface granules glucomannan can be detached, diffuse out of the cell surface, and easily dissolved in ethanol. The longer with the washing time, more impurity components were separated from the granules glucomannan, that make brighter surface of flour.

Residual of hydrogen peroxide in conventional and ultrasonic optimized porang flour were 0, 012% and 0.007215% (120 ppm and 72.15 ppm) respectively (Table VII). This residual is in safe consideration for consumption because it does not exceed the maximum limit hydrogen peroxide residues on food. [39] reported that FDA allowed the maximum of hydrogen peroxide residue in food is amount of 500 ppm

Optimized porang flour solubility rate by using ultrasonic maceration flour was faster (772 sec) than the conventional (812 sec) and control sample (1205 sec). The purity of glucomannan was indicated to enhance the rate of flour dissolution. (Table VII).

Microscopic observations of the granules showed that the deployment of impurities in ultrasonic maceration optimization was less than the conventional maceration due to the effectivity of ethanol solvent in agreement with [40].

Observation then continued by analyzing the granules element in a field of 20 μm plain using SEM-EDAX as shown in Figure 4. Figure 3 and 4 (a) indicates that the ultrasonic maceration or convetional maceration has ability to clean the impurities that covered of glucomannan granule surface. Figure 4 (b) is the spectrum of elements forming granules consisting of carbon, oxygen, potassium, calcium and chlorine with their composition as can be seen in Table VIII. Ultrasonic maceration method obtained cleaner granules compare with conventional maceration and samples, that make the granule surface texture become more rough and bumpy. While the high imputiry of flour will looked contain needle-shaped crystals due to presence of calcium oxalate (Figure 5). [33] and [41] also found oxalic acid crystals in the konjac flour with needle shaped.
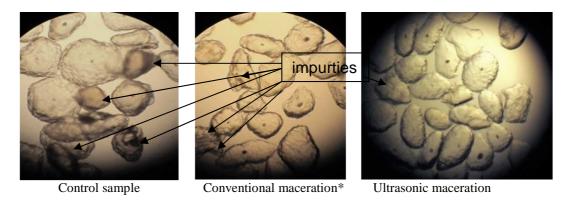
Control sample   Conventional maceration*   Ultrasonic maceration

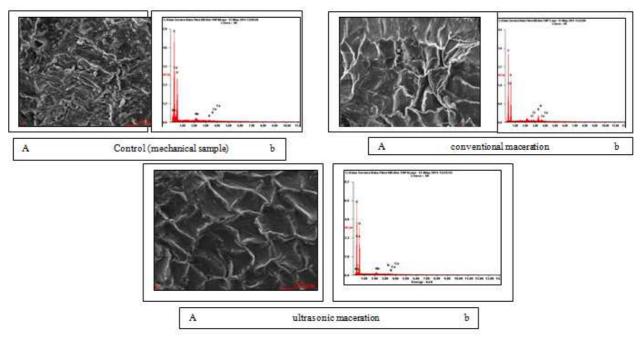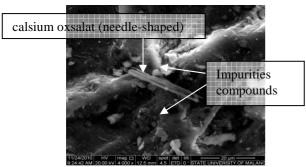Fig.3 Light microscope magnification of 100 x observation of porang flour * [21]

Fig. 4 Glucomannan garanule size at 20 μm (a) Spectrum Shaping Elements granules (b). Porang flour optimization results purification

TABLE VIII
COMPOSITION OF FORMING ELEMENTS OF PORANFG FLOUR ON FIGURE 4.

Mechanical (control sample)		Conventional maceration	
Elements	%	Elements	%
C	56,75	C	61,94
O	39,79	O	36,01
Cl	0,30	Cl	1,44
K	2,48	K	0,38
Ca	0,68	Ca	0,23

Ultrasonic maceration	
Elements	%
C	57,50
O	40,75
Cl	1,24
K	0,35
Ca	0,16

Glucomannan forming elements are carbon, oxygen and hydrogen. The hydrogen was not detected by using EDAX analysis. Heigh levels of glucomannan, make the element carbon and oxygen also higher, while other elements become lower (Table VIII). The calcium that suspected as   a sharper of the calcium oxalate allegedly lower in connection with higher levels of glucomannan. The percentage levels of calcium and oxalate in the mehanical control sample , conventional and ultrasonic maceration respectively are (0.398% and 0.68%),(0.109% and 0.23%) and (0.064% and 0, 16%) with similiarity tandency. Glucomannan granule is very strong compared to the other components of the granules. This phenomenon due to the ultrasonic cavitation, the expansion cycle can lead aincreasing of pressure and temperatures, which can accelerates the purification process of glucomannan

4000x magnification, wide field of observation of 20 μm

Fig. 5 SEM observation of needle-shaped oxalic acid crystals

IV. CONCLUSIONS

Purification process using ethanol with conventional maceration can increase glucomannan levels (16.4%), viscosity value (32.7%), brightness (5.9%) and reduce other components including calcium oxalate levels as 263.6%. The washing time, stirring speed and the ratio of solvent affected to the response of glucomannan levels, viscosity, concentration of oxalic acid and brightness. Optimal conditions were obtained, namely the washing time is 4.16 hours or 246 minutes, stirring speed of 434.04 rpm and solvent ratio of 9.35. In optimum conditions, the response glucomannan levels, viscosity, concentration of calcium oxalate and brightness of consecutive prediction results are 79.38%, 9701.09 cPs, 0.108% and 53.94 while the results of verification are 80.17% respectively, 9733.33 c.Ps, 0.109% and 53.95.

Purifiation by ultrasonic maceration method also shows that the washing time and the ratio of solvent: flour affected to the response of glucomannan levels, viscosity, oxalic acid levels and brightness. Optimization conditions obtained, the washing time is 25 minutes 6 seconds and the ratio of solvent: 8.65 porang flour. In optimum conditions the response of glucomannan levels is 85.74%, 0.044% of oxalate levels, 13970.7 cPs the value of viscosity, and 59.78 of brightness. For the verification results, the levels of glucomannan, viscosity, concentration of calcium oxalate and brightness respectively are 84.37%, 13750 c.Ps, 0.064%, and 60.38.

Next observation is required to study that ultrasonic maceration method is faster by using less solvent and higher levels of response values of glucomannan, viscosity and good acceptance of brightness with lower levels of oxalic acid value compare with conventional maceration method.

For future study, it is recommended tha a scale up research for covential maceration needed to be examine due to easy application for a big amount of the product, more over, advance experiment for ultrasonic methode also need to explore to hold down the high cost of ultrasonic machine procurement.

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