**Optimization of fast maceration extraction of polished yellow konjac (Amorphophallus muelleri Blume) flour by Box-Behnken response surface methodology**

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**Abstract**

Polished yellow konjac (Amorphophallus muelleri Blume) flour (PYKF) from the centrifugal milling process still contains a relatively high level of impurities that may be harmful to health, especially calcium oxalate. The extraction process using ethanol is needed to reduce the amount of impurities surrounding the glucomannan granules and hence to improve the purity of the latter. This study was aimed at determining the effects of process conditions including ethanol concentration, solvent to flour ratio and extraction time on the physicochemical properties of purified yellow konjac flour (PrYKF) using Response Surface Methodology. The desired characteristics of PrYKF were low calcium oxalate content and a high degree of whiteness (DoW), viscosity, and glucomannan content. The calcium oxalate content, viscosity, and glucomannan content of PrYKF were affected by ethanol concentration, solvent to flour ratio, and extraction time. In contrast, the DoW was only affected by ethanol concentration. The prediction of the optimum condition by Design-Expert was found at 46.36% of ethanol concentration, 8.33 mL/g of solvent to flour ratio, and 63.01 mins. Under these conditions, the characteristics were as follows: 0.12±0.00% w.b. calcium oxalate, 68.76±0.00 DoW, 29012±0.00 cP viscosity, and 84.59±0.00% d.b. glucomannan content. The actual verification was closely related (p > 0.05) compared to the Design-Expert software prediction by paired t-test. The fast maceration is a successful method to improve the characteristics of PYKF to PrYKF in a shorter time.

1. **Introduction**

Yellow konjac is an Indonesian native plant belonging to the Amorphophallus genus and muelleri Blume species (Santosa et al., 2003; Widjanarko et al., 2014), that is found in forest areas, particularly in East Java and East Nusa Tenggara Province (Santosa et al., 2003; Santosa and Sugiyama, 2016). Yellow konjac has the highest glucomannan content among Amorphophallus species found in Indonesia (Ohtsuki, 1967). The glucomannan content in the yellow konjac tuber (YKT) is 32 to 55%, depending on the plant growth period, tuber age, and size (Sumarwoto, 2005). Glucomannan is a water-soluble dietary fiber, that extends and forms a gel, making it very commonly available in both the food and non-food sectors (Zhang et al., 2005). Several studies reported that glucomannan reduces blood plasma cholesterol, enhances carbohydrate metabolism, bowel movement, and colon ecology (Chua et al., 2010), and is potential as a colon cancer drug carrier (Zhang et al., 2014). Generally, glucomannan is used as a gelling agent, stabilizer, thickener, fat substitute, and suspension in food products (Zhang et al., 2005; Jiménez-Colmenero et al., 2013; Abo-Srea et al., 2017). However, YKT also contains calcium oxalate, which causes itching, skin irritation, kidney problems, or other health problems when directly consumed (Faridah and Widjanarko, 2013). The dry method is commonly used to distinguish glucomannan and non-glucomannan components in yellow konjac. The process was started
by cutting and drying tuber to make yellow konjac chips, then grinding using mechanical milling, followed by air separation and polishing process (Witoyo et al., 2020; Witoyo et al., 2021). However, this process is challenging to produce yellow konjac flour (YKF) with international standards for konjac flour (Liu et al., 2002). Therefore, enhancing their degree of whiteness (DoW), viscosity, glucomannan content, and reducing the impurities of YKF is essential to do.

The maceration method was commonly used for YKF extraction by using ethanol or propanol as the solvents. The research was started by Sugiyama et al. (1972), who immersed the konjac flour in ethanol at a concentration of 50% and 80% for three days in each concentration, following Ogasawara et al. (1987). Ogasawara and his colleague performed a similar procedure using a 50% ethanol concentration for seven days, followed by three days with an ethanol concentration of 80%. In Indonesia, glucomannan extraction was pioneered by Faridah and Widjanarko (2013). Faridah and her colleague conducted CCD-RSM optimization of glucomannan extraction using multilevel leaching ethanol – pair with H$_2$O$_2$, with the ethanol concentration of 40-80% and the extraction time of 3-5 hours with a solvent to flour ratio of 6:1 -10:1 mL/g, so that the total time was 12 -15 hours. This process produced purified yellow konjac (PrYKF) with a high content of glucomannan and low calcium oxalate. Wardhani et al. (2016) developed the glucomannan extraction process from *porang* flour using the CCD-RSM with three factors (temperature, extraction time, and 2-propanol concentration). They recommended the extraction temperature of 75°C, for 3 hrs and 2-propanol concentration of 90% as the best conditions to produce PrYKF. However, the maceration method developed by previous researchers had many shortcomings, such as long extraction time, and required a lot of energy and solvent, as well as a high extraction temperature, which implies that it was not economical and challenging to scale up from the pilot plant to industrial scale.

Moreover, exploring the application of the different ethanol concentrations during extraction and their effect on YKF's physicochemical properties is limited. Impaprasert et al. (2013) reported that ethanol concentration as an anti-swelling agent during the extraction affected the *A. muelleri* flour properties. Guidan et al. (2008) reported that the ethanol concentration and extraction time affected the glucomannan content and konjac micro flour's viscosity. Based on these facts, this study optimized ethanol concentration, solvent to flour ratio, and extraction time for extraction PYKF in order to produce PrYKF in a shorter time.

2. Materials and methods

The yellow konjac flour (YKF) used in this study presents the optimum quality characteristics of polished yellow konjac flour (PYKF) as defined in our previous work. The PYKF had the following characteristics: 0.56% w.b. calcium oxalate, 20208 cP viscosity, 62.16 DoW, and 68.18% d.b. glucomannan content (Witoyo et al., 2021). Merck KgaA (Darmstadt, Germany) provided all analytical chemical reagents used without further purification.

2.1 Extraction process

The extraction process of PYKF was performed according to the method described by Faridah and Widjanarko (2013) with minor modifications. In the first stage, around 30 g of PYKF were weighed and transferred into a glass beaker and added with 0.05% (w/v) sodium metabisulfite in ethanol (40 – 60% concentration with 6:1 - 10:1 mL/g solvent to flour ratio) to the mixture. The mixture was agitated with a magnetic stirrer at a speed of 440 rpm for 40 – 80 mins. Then the PYKF was filtered and the first residue was obtained. In the second stage, the first residue was rinsed again with the same ethanol concentration as in the first stage (concentration 40 – 60% with 6:1 - 10:1 mL/g of solvent to flour ratio, but without the addition of sodium metabisulfite), followed by agitation at a speed of 440 rpm for 40 – 80 mins, and filtered again to obtain the second residue. The second residue was rewashed using the same procedure as in the second stage to obtain the final residue, followed by drying in a tray dryer at 45±5°C for 5 hrs. The dried purified yellow konjac flour (PrYKF) was stored for further analysis.

2.2 Calcium oxalate

The volumetric method was used to determine calcium oxalate, as described by Iwuoha and Kalu (1995). A sample of 2 g yellow konjac flour, 10 mL HCl, and 190 mL distilled water were combined and heated for 1 h at 100°C. Then, the solution was cooled and diluted to 250 mL with distilled water in a volumetric flask. After being filtered, the mixture was split into two parts. Each filtrate portion received four drops of methyl red indicator and NH$_4$OH until the colour turned yellow. After that, the mixture was reheated until it reached 90°C, cooled, and filtered again. Before adding 10 mL of 5% CaCl$_2$, each final filtrate was reheated until it reached 90°C. The mixture was kept at 5°C overnight. Each filtrate was centrifuged at 8000 rpm for 15 mins to obtain the pellets, followed by 10 mL of 20% H$_2$SO$_4$ to dissolve the pellets. After that, the two filtrate portions were combined and diluted to 250 mL with distilled water in a volumetric flask, then heated to nearly boiling before titrating with 0.05 M standardized KMnO$_4$ to achieve a
pink colour that lasted 30 s. Equation 1 was used to calculate calcium oxalate content (%) in yellow konjac flour.

**Calcium Oxalate Content (%) = \frac{TyV_{me}×D_{t}×10^{6}}{ME×M_{e}×10^{8}} (1)**

Where \( T \) is the volume of KMnO\(_4\) used for titration (mL), \( V_{me} \) is the equivalent mass volume (1 cm\(^3\) 0.05M KMnO\(_4\) equivalent to 0.0025 g anhydrous oxalic acid), \( D_{t} \) is a dilution factor, \( ME \) is the molar equivalent of KMnO\(_4\) solution (0.05), and \( M_{e} \) is the mass of the yellow konjac flour (g).

### 2.3 Degree of whiteness

Minolta CR-10 Chromameter (Minolta, Japan) was used for monitoring the surface of yellow konjac flour and expressing it as Hunter Value. Equation 2 was used to calculate the degree of whiteness (DoW) of yellow konjac flour from the measured Hunter value (\( L, a, \) and \( b \)) (Witoyo et al., 2019).

\[ \text{DoW} = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \] (2)

**2.4 Viscosity**

An NDJ-1 rotational viscometer was used to measure the sol of yellow konjac flour at room temperature with a spindle of 4 and a stirring speed of 12 rpm (Witoyo et al., 2020). Before measurement, the 1% w/v sol of yellow konjac flour was prepared by mixing in a constant temperature water bath (75°C) (Memmert, Germany) with a constant agitation speed (150 rpm) until it became perfectly hydrated (according to Liu et al., 2002 with minor modifications).

### 2.5 Glucomannan content

The glucomannan content of yellow konjac flour was determined by a spectrophotometric method, as described by Chua et al. (2012) with modifications. A sample of 200 mg of yellow konjac flour was dissolved in 50 mL of NaOH-formic acid buffer and mixed overnight at room temperature. The mixture was dissolved in a volumetric flask with a NaOH-formic acid buffer to 100 mL and centrifuged for 15 mins at 4000 rpm to obtain glucomannan extract. Then, 5 mL of glucomannan extract were combined with 2.5 mL of 3 M sulfuric acid and then immersed for 90 mins in the water bath to make the glucomannan hydrolysate. The mixture was cooled, and a solution of 2.5 mL of 6 M sodium hydroxide (NaOH) was added. In a volumetric flask, the mixture was diluted to 25 mL using distilled water. The glucomannan extract and hydrolysate were measured by the spectrophotometer at 540 nm. The glucose content (mg) in both samples was calculated from the standard linear regression equation for D-glucose and distilled water used as control. Equation 3 was used to calculate the glucomannan content (% d.b.).

\[ \text{GM content (% d.b.)} = \frac{0.9 \times (55 - T_{o}) \times f}{m \times (100 - w)} \times 100 \] (3)

Where \( T \) is glucose content in yellow konjac flour hydrolysate (mg), \( T_{o} \) is glucose content in yellow konjac flour extract (mg), \( m \) is the mass of yellow konjac flour (mg), and \( w \) is moisture content in yellow konjac flour (%), and \( f \) is dilution factor.

### 2.6 Experimental design and statistical analysis

The Box Behnken Design - Response Surface Methodology (BBD-RSM) was chosen for the glucomannan extraction process from PYKF. The present study's central point is based on the preliminary study, i.e., the ethanol concentration of 50%, solvent to flour ratio of 8:1 mL/g, and extraction time of 60 mins. The temperature and number of extractions were set at room temperature (28±2°C) and three times, respectively. The range of independent variables used in this study is shown in Table 1. Design Expert 13 trial versions (State Ease, Inc. Minneapolis, MN, USA) were used to analyze physicochemical properties of purified yellow konjac flour (PrYKF) based on the multivariate principle. The calculation of non-linear models of the observed system using the quadratic equation is shown in Equation 4 (Wang et al., 2019).

\[ Y = \beta_{0} + \sum_{i=1}^{k} \beta_{i} X_{i} + \sum_{i=1}^{k} \beta_{ii} X_{i}^{2} + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} X_{i} X_{j} + \epsilon \] (4)

Where \( \epsilon \), is a random error, \( k \) is the number of independent parameters, \( X_{i} \) and \( X_{j} \) are the coded independent variables, \( \beta_{0}, \beta_{i}, \beta_{ii}, \) and \( \beta_{ij} \) are the regression coefficient of the intercept, linearity, quadratic, and interaction of the treatment, respectively. \( Y \) is the observed response (calcium oxalate, DoW, viscosity, and glucomannan content). The p-value of the model, \( R^{2} \), adjusted \( R^{2} \), lack of fit value, and adequate precision were used to select the appropriate model of each response. The accuracy of the optimum extraction condition was evaluated by comparing the software prediction and actual laboratory research (verification) in 3 replications using paired T-test using Minitab 17 (Stat View, USA).

| Table 1. The range of wet extraction process |
|--------------------------------------------|
| Independent Factor and Unit | Ethanol Concentration (%) | Solvent to Flour (S/F) ratio (mL/g) | Extraction time (mins) |
|-----------------------------|--------------------------|----------------------------------|---------------------|
| Low (-1)                    | 40                       | 6                                | 40                  |
| Centre (0)                  | 50                       | 8                                | 60                  |
| High (+1)                   | 60                       | 10                               | 80                  |

### 3. Results and discussion

#### 3.1 Fitting model and statistical analysis

The selection of the model used in this study was

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based on the following criteria: p-value of models, R^2, adjusted R^2, lack of fit value and adequate precision, calculated by the Design Expert Software 13 trial version. The responses data of purified yellow konjac flour (PrYKF), the quadratic coefficient regression, and the second-order polynomial fit for all responses are presented in Table 2, Table 3, and Table 4, respectively. The evaluation result of the model showed that all responses (calcium oxalate, DoW, viscosity, and glucomannan content) were aligned with the quadratic model with the p-value ≤ 0.0012, R^2 > 0.94, Adjusted-R^2 > 0.87, lack of fit value > 0.05, and the adequate precision > 4. These data proved that the quadratic model was adequate for predicting all the observed responses of PrYKF in this study. Faridah (2016) and Wang et al. (2019) noted that the higher R^2 and adjusted R^2 imply that the gained model was appropriate and adequate to describe the data for all responses. Faridah and Widjanarko (2013) and Faridah (2016) explained that a p-value < 0.05 indicates that the developed model has a significant effect on the observed response, and the lack of fit value ≥ 0.05 indicates an insignificant difference compared to a pure error. The adequate precision is measured as a ratio of signal to noise. An adequate precision ratio larger than 4 is desirable and assumed as an adequate signal.

3.2 Calcium oxalate

The calcium oxalate content in PrYKF varied from 0.12 - 0.31% w.b. The values obtained in this study were higher than the research data reported by Widjanarko et al. (2014) (0.03-0.09%) and Faridah and Widjanarko (2013) (0.08-0.17%) using the UAE and multilevel maceration methods, respectively. However, data from this study were similar to data reported by Hermanto et al. (2019), which are 0.12-0.26%. The quadratic effects of ethanol concentration, solvent to flour ratio, and extraction time on calcium oxalate content are illustrated in Figure 1A. Figure 1 (A1) shows that the calcium oxalate decreased when the ethanol concentration and the solvent to flour ratio were in the range of 40-50% and 6-8 mL/g, respectively, at a fixed extraction time (60 mins). When the ethanol concentration and the solvent to flour ratio exceeded 50% and 8 mL/g, respectively, the calcium oxalate increased. Moreover, a minimum calcium oxalate content was obtained at an ethanol concentration of 50% and an extraction time of 60 mins at a fixed solvent to flour ratio (8 mL/g). However, the calcium oxalate increased when ethanol concentration and extraction time were higher (Figure 1 (A2)).

Figure 1 (A3) indicates that calcium oxalate decreased when the solvent to flour ratio and the extraction time ranged from 6-8 mL/g and 40-60 mins, respectively. The calcium oxalate increased when the solvent to flour ratio and extraction time exceeded 8 mL/g and 60 mins, respectively. Chua et al. (2012) reported that 50% of ethanol effectively dissolved impurities in

| Std | Ethanol concentration (%) | Solvent to flour ratio (mL/g) | Extraction time (mins) | Calcium oxalate (% w.b.) | DoW | Viscosity (cP) | Glucomannan (% d.b.) |
|-----|---------------------------|-------------------------------|------------------------|--------------------------|-----|---------------|---------------------|
| 1   | 40                        | 6                             | 60                     | 0.19                     | 68.04| 27000.00      | 81.04               |
| 2   | 60                        | 6                             | 60                     | 0.31                     | 64.20| 23975.50      | 76.02               |
| 3   | 40                        | 10                            | 60                     | 0.16                     | 68.57| 27000.00      | 82.91               |
| 4   | 60                        | 10                            | 60                     | 0.25                     | 64.36| 24687.50      | 77.87               |
| 5   | 40                        | 8                             | 40                     | 0.21                     | 68.17| 26500.00      | 80.06               |
| 6   | 60                        | 8                             | 40                     | 0.31                     | 63.57| 23062.50      | 77.82               |
| 7   | 40                        | 8                             | 80                     | 0.17                     | 68.17| 27125.00      | 80.77               |
| 8   | 60                        | 8                             | 80                     | 0.28                     | 64.62| 24062.50      | 77.48               |
| 9   | 50                        | 6                             | 40                     | 0.23                     | 68.36| 26125.00      | 80.52               |
| 10  | 50                        | 10                            | 40                     | 0.28                     | 68.12| 26625.00      | 80.78               |
| 11  | 50                        | 6                             | 80                     | 0.20                     | 67.66| 26812.50      | 78.18               |
| 12  | 50                        | 10                            | 80                     | 0.16                     | 68.32| 26125.00      | 82.32               |
| 13  | 50                        | 8                             | 60                     | 0.13                     | 67.95| 29562.50      | 85.18               |
| 14  | 50                        | 8                             | 60                     | 0.16                     | 68.41| 28437.50      | 83.33               |
| 15  | 50                        | 8                             | 60                     | 0.14                     | 68.62| 29312.50      | 84.60               |
| 16  | 50                        | 8                             | 60                     | 0.14                     | 68.21| 28125.00      | 84.57               |
| 17  | 50                        | 8                             | 60                     | 0.12                     | 68.11| 28562.50      | 83.21               |

Pred.: software prediction, ver.: verification.
Values with different superscripts within the same row are significantly different.
Table 3. Regression coefficient table for all responses of PrYKF

| Responses      | a         | A       | B       | C       | AB      | AC      | BC      | A²      | B²     | C²     |
|----------------|-----------|---------|---------|---------|---------|---------|---------|---------|--------|--------|
| Calcium        | +0.1385   | +0.0525 | -0.0100 | -0.0279 | +0.0034 | -0.0202 | +0.0573 | +0.0347 | +0.0468|
| DoW            | +68.26    | -2.02   | +0.1398 | +0.0713 | +0.0933 | +0.2627 | +0.2255 | -1.98   | +0.0073 | -0.1512|
| Viscosity      | +2880.00  | -1479.62| +65.56  | -226.56 | +178.00 | +93.75  | -296.87 | -2184.31| -949.94| -1428.19|
| Glucomannan    | +84.18    | -1.95   | +1.01   | -0.0549 | -0.0074 | -0.2634 | +0.9692 | -3.07   | -1.65  | -2.08  |

*p<0.01, **0.01≤p<0.05, ns p>0.05,
Y = a + Ax₁ + Bx₂ + Cx₃ + ABx₁x₂ + ACx₁x₃ + BCx₂x₃ + A²x₁² + B²x₂² + C²x₃²
Where a = intercept, A = ethanol concentration, B = solvent to flour ratio, C = extraction time, AB = interaction ethanol concentration and solvent to flour ratio, AC = interaction ethanol concentration and extraction time, BC = interaction solvent to flour ratio and extraction time, A² = ethanol concentration quadratic, B² = solvent to flour ratio quadratic, C² = extraction time quadratic.

Table 4. The second-order polynomial fit of the quadratic model for all responses of PrYKF

| Responses      | Significance model | R²    | Adjusted R² | Lack of fit | Adequate Precision |
|----------------|--------------------|-------|-------------|-------------|--------------------|
| Calcium Oxalate| 0.0012*            | 0.9454| 0.8753      | 0.2190      | 10.4151            |
| DoW            | <0.0001*           | 0.9886| 0.9739      | 0.3419      | 21.6363            |
| Viscosity      | 0.0003*            | 0.9642| 0.9181      | 0.7172      | 13.1498            |
| Glucomannan    | 0.0007*            | 0.9538| 0.8944      | 0.4032      | 11.0100            |

*significant, ns not significant (p>0.05)

Figure 1. Quadratic Response Surface of independent variables for calcium oxalate (A) and DoW (B)

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konjac flour. The optimum ethanol concentration as an anti-swelling agent in the extraction process of *Amorphophallus muelleri* flour from Thailand was 50% ethanol. Moreover, ethanol concentrations higher than 50% caused the deposition of both polysaccharides and impurities (Impaprasert et al., 2013). Faridah and Widjanarko (2013) noted that the extraction time, stirring speed, and solvent to flour ratio of 3-4 hrs, 200-400 rpm, and 6-8 mL/g, respectively, were able to best reduce calcium oxalate concentration. Widjanarko et al. (2014) noted that calcium oxalate decreased quadratically with increasing extraction time. The extraction time ranges from 10.86-39.14 mins and the solvent to flour ratio from 5.17-10.83 mL/g, respectively, when extracting glucomannan from *porang* flour using ultrasonic methods.

### 3.3 Degree of whiteness

The DoW of PrYKF ranged between 63.57-68.62. This result was higher than the research results reported by Wardhani et al. (2016) (54.05-58.96) and lower than in the study by Impaprasert et al. (2013). The degree of whiteness (DoW) of PrYKF is affected by independent variables, as depicted in Figure 1B. In the present study, the ethanol concentration (Figure 1 B1 and Figure 1 B2) had negatively affected the DoW. The increase in the concentration of ethanol causes a decrease in the DoW of PrYKF. Using high concentrations of ethanol during the extraction process can destroy some materials in glucomannan granules, increasing the presence of certain impurities (Impaprasert et al., 2013). Impaprasert et al. (2013) also reported that 50-90% ethanol concentration as an anti-swelling agent has a lower whiteness index in comparison with konjac flour produced using 10-30% ethanol concentration.

Figures 1 B1 and B3 show that the solvent to flour ratio and extraction time did not significantly affect the DoW of PrYKF. This agrees with the results of Wardhani et al. (2016) who reported that the increase in extraction time and the ratio of isopropanol and flour did not affect the degree of whiteness of the YKF. Moreover, Widjanarko et al. (2014) noted that the increase in the solvent to flour ratio in the extraction of YKF by the ultrasonic method had no significant effect on the degree of whiteness. However, the extraction time had a significant effect on the degree of whiteness of YKF. The authors reported that all impurity components on the surface of glucomannan granules could be reduced by the prolonged extraction time and resulted in the maximum of DoW in PrYKF. Different extraction methods might cause different results from those obtained by the Widjanarko et al. (2014) study. Widjanarko et al. (2014) used the UAE method, whereas the conventional maceration method was used in the present study. The UAE method for extraction is more efficient and effective than the conventional method (Wang et al., 2019).

### 3.4 Viscosity

The viscosity of PrYKF ranged from 23062.50 - 29562.50 cP. These values were higher than those reported by Faridah (2016) (9733.33 - 13750 cP) and Impaprasert et al. (2014) (3539.20 - 17321.33 cP). The values obtained in this study were almost identical to those reported by Yanuriati et al. (2017) (24650 - 27940 cP) and lower than Shimizu and Shimahara's patent (Shimizu and Shimahara, 1973) (64300 - 67500 cP). However, some results met the first-grade purified konjac flour standards issued by the Chinese Government, which require a minimum viscosity of 28000 mPas (Liu et al., 2002). The quadratic response surface of viscosity based on ethanol concentration, solvent to flour ratio, and extraction time is shown in Figure 2A. The effect of ethanol concentration and solvent on the flour ratio on viscosity is shown in Figure 2 (A1). The use of ethanol concentration and solvent to flour ratio ranged from 40-50% and 6-8 mL/g at a fixed extraction time (60 mins) improved the viscosity of PrYKF. However, with the increase in the value of these parameters, the viscosity decreased significantly.

Figure 2 (A2) illustrates the effect of ethanol concentration and extraction time on viscosity at a fixed solvent to flour ratio (8 mL/g). The maximum viscosity was reached at an ethanol concentration of 50% and an extraction time of 60 mins, but above these values, the viscosity decreased significantly. The effect of the solvent to flour ratio and the extraction time on fixed ethanol concentration (50%) on viscosity is shown in Figure 2 (A3). The viscosity of PrYKF increased with an increase in the solvent to flour ratio, and extraction times ranged from 6-8 mL/g and 40-60 mins, respectively. When the solvent to flour ratio and the extraction time exceeded 8 mL/g and 60 mins, the viscosity decreased. Impaprasert et al. (2013) reported that the highest viscosity of *Amorphophallus muelleri* Blume flour was obtained at an ethanol concentration of 50% and tended to be stable when ethanol concentrations increase. Shimizu and Shimahara (1973) reported using an ethanol concentration of 30-80% to dissolve certain impurities, such as starch, fat, protein, oxalate, and ash, based on the "like-dislike" principle. Widjanarko et al. (2014) noted that the increase of extraction time and solvent to flour ratio to 25 mins and 8 mL/g would dramatically increase viscosity and decrease sharply over its conditions. An increase in the extraction time and the solvent to flour ratio allow the solvents to interact with glucomannan.
So, the impurities that cover them are removed leading to an improvement of glucomannan's purity, and an increase in viscosity (Faridah, 2016). Nevertheless, prolonging contact time and increasing solvent to flour ratio beyond above mentioned optimum range lead to glucomannan degradation. As a result, its purity declines and the viscosity decreases (Widjanarko et al., 2014).

3.5 Glucomannan content

Glucomannan content of PrYKF ranged from 76.02–85.18% d.b. These results were higher than those reported by Faridah and Widjanarko (2013) (62.14 – 79.43% d.b.) and lower than the data reported by Hermanto et al. (2019) (80.79 - 87.79% d.b.). In general, some results of the glucomannan content in PrYKF almost met the first-grade standard for purified konjac flour (PKF), with minimum glucomannan content of 85% d.b. (Liu et al., 2002). The effect of the extraction parameters on glucomannan content is shown in Figure 2B. Based on the 3D response surface of the effects of the concentration of ethanol and the solvent to flour ratio as illustrated in Figure 2B1, the glucomannan content increased when the ethanol concentration and the solvent to flour ratio were in the range of 40-50%, and 8-10 mL/g, respectively, at a fixed extraction time (60 mins). When the ethanol concentration and solvent to flour ratio exceeded 50% and 8 mL/g, respectively, glucomannan content decreased. When the ethanol concentration and extraction time exceeded 50%, and 60 mins, glucomannan content may rise gradually and then decrease (Figure 2 (B2)).

Figure 2 (B3) shows that the glucomannan increased when the solvent to flour ratio and extraction time were in the range of 6-8 mL/g and 40-60 mins, respectively. When the solvent to flour ratio and extraction time of YKF exceeded 8 mL/g and 60 mins, the glucomannan content decreased. Impaprasert et al. (2013) reported that...
10-50% of ethanol concentration increased the glucomannan content of konjac flour from Thailand. However, the glucomannan content decreased when the ethanol concentrations exceeded 50% during the extraction process, and this condition might be due to the accumulation of impurities in the mixture solution. Guidan et al. (2008) reported that glucomannan content in konjac micro flour decreased with ethanol concentration higher than 50% during the extraction process. Furthermore, the best extraction time for the extraction of konjac flour into konjac micro flour with the highest glucomannan content is 60 mins. Widjanarko et al. (2014) and Faridah and Widjanarko (2013) reported that increasing the solvent to flour ratio and leaching period until the optimal point during the multiple leaching process can increase the glucomannan content and decrease the calcium oxalate content of YKF. Wardhani et al. (2016) explained that the increase in contact time allows the solvent to dissolve impurities covering granules and thus increase glucomannan purity. However, increasing the solvent to flour ratio and extraction time exceeded the optimum point during the extraction process resulting in glucomannan degradation (Faridah and Widjanarko, 2013; Widjanarko et al., 2014).

3.5 Response surface optimization and verification

The optimum conditions for producing the PrYKF with low calcium oxalate and high viscosity, DoW, and glucomannan content were predicted as follows: ethanol concentration of 46.36%, solvent to flour ratio of 8.33 mL/g, and extraction time of 63.01 mins. Under these conditions, the desirability value representing the closeness of a response to its ideal value was 0.955 on the 0-1 scale. The characteristics of PrYKF obtained under these conditions were as follows: 0.12±0.00% w.b. calcium oxalate, 68.76±0.00 DoW, 29012±0.00 cP viscosity, and 84.59±0.00% d.b. glucomannan. The verification with three replications under the optimum conditions resulted in following values: calcium oxalate: 0.14±0.01% w.b., DoW: 68.41±0.40, viscosity: 28667±557 cP, and glucomannan content: 83.26±1.71% d.b. The verification confirmed that the results agreed with the software predictions, with a p-value >0.05 (not significantly different) for all observed responses based on paired t-test (Table 2). Its results reflect that the BBD-RSM approach was appropriate and successful in predicting all PrYKF responses. The desirability value of 0.955 indicated that the Design-Expert software's extraction condition prediction had accuracy and validity equal to 95.50%.

4. Conclusion

The calcium oxalate, viscosity, and glucomannan content of PrYKF were affected by all the extraction factors (ethanol concentration, solvent to flour ratio, and extraction time). In contrast, the degree of whiteness was only affected by ethanol concentration. The quadratic model represented all the responses of PrYKF. The ethanol concentration of 46.36%, solvent to flour ratio of 8.33 mL/g, and 63.01 mins extraction time were found as the best extraction conditions for producing PrYKF, with characteristics as follows: 0.14±0.01% calcium oxalate, 68.41±0.40 DoW, 28667±557 cP viscosity, and 83.26±1.71% d.b. glucomannan content. The short maceration was a successful method to improve the characteristics of PYKF and obtain PrYKF in a shorter time.

Conflict of interest

All of the authors declared no conflict of interest in this study

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