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Optimization of the fermentation process in the manufacture of modified porang flour using the biologically modified cassava flour starter

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Abstract

One method that can be developed to improve the quality of porang flour is through a fermentation process using the biologically modified cassava flour (Bimo CF) starter, which consists of a carrier material and an active ingredient of lactic acid bacteria. This research was conducted with the aim of studying the optimum concentration of Bimo CF starter and the fermentation time used in processing porang flour. The research was conducted using the response surface methodology (RSM) with a 3-level factorial design. The lower and upper limits for Bimo CF starter concentrations were 0.1 and 0.5%, and 12 and 24 h for fermentation time, respectively. The results of the research showed that the responses that are significantly influenced by the applied independent variables include: protein, fat, starch, crude fiber, calcium oxalate, glucomannan, and yield. Whereas the response of moisture, ash, and whiteness levels was not significantly affected. Optimization results showed that the concentration of Bimo CF starter and the optimum fermentation time were 0.5% and 20 h, respectively, with a level of desirability of 0.91%. The verification results showed that the calcium oxalate levels were far below the predicted value, which was half of the predicted value. Likewise, the glucomannan content showed a value far above the predicted value. This indicates that the optimum fermentation process obtained from this study can be developed to produce modified porang flour with low calcium oxalate and high glucomannan.

1. Introduction

Currently, the Indonesian government is promoting a national porang planting program in several regions to meet the demand for chips and porang flour in both the domestic and foreign markets. The readiness of porang tuber processing technology in the nation should support the Indonesian government's program to increase porang plant production. One form of processing that has the opportunity to be developed is porang flour. The obstacle faced in the processing of porang flour is the presence of an itchy feeling in the flour produced due to the high content of calcium oxalate. In addition, porang flour produced today has a low glucomannan content and a dark brown color (Faridah and Widjanarko, 2013). Various attempts were made by previous researchers to reduce calcium oxalate levels in porang flour, including soaking it with various acid solutions. The results of Wardani and Handrianto (2019) reported that calcium oxalate levels in porang flour can be reduced by soaking in a solution of starfruit juice, lime, and vinegar with optimal concentrations of 7.5 and 20%, respectively. The decrease in calcium oxalate levels was 62.68% for starfruit, 65.94% for lime, and 90.27% for acetic acid.

One method that can be developed to improve the quality of porang flour is the fermentation process. Making porang flour through a tuber fermentation process can be done through spontaneous fermentation

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or fermentation using a starter. Spontaneous fermentation is carried out with the help of microbes, which naturally reproduce because their living environment is suitable for their growth (Sopandi and Wardah, 2014). Fermentation using a starter is carried out by means of microbes that are deliberately added in the form of a starter that grows and reproduces, actively changing the fermented food. One of the starters that has been applied to tuber fermentation to produce flour is the Bimo CF starter.

The Bimo CF starter is a seed for fermentation in the process of making biologically modified cassava flour. This starter consists of a carrier and an active ingredient of lactic acid bacteria. Bimo CF starter is made from raw flour, added with a certain concentration of nutrient enrichment ingredients to increase the effectiveness and stability of lactic acid bacteria (Misgiyarta and Suismono, 2014). Bimo CF starter is a seed in the form of flour that is used for fermenting cassava in the form of chips. The Bimo CF starter uses active ingredients from various lactic acid bacteria that are safe for food, enriched with nutrients, and made with technology that results in high stability and effectiveness of the starter (Misgiyarta *et al*., 2009). Due to its success in producing high-quality modified cassava flour, the Bimo CF starter was developed for use in the manufacture of other flours such as modified taro flour, modified sweet potato flour, and modified breadfruit flour (Purba *et al*., 2014; Histifarina and Rachman, 2016; Astuti *et al*., 2017).

The use of Bimo CF starter in the fermentation process for making porang flour has never been done, where the characteristics of porang tubers are similar to those of taro and other tubers, so the successful application of this Bimo CF starter is to produce porang flour of quite high quality. This study examined the optimization of the porang tuber fermentation process using the Bimo CF starter to manufacture modified porang flour. The optimized fermentation process is determined by the concentration of the Bimo CF starter and the fermentation time used.

2. Materials and methods

2.1 Materials

The materials used in the manufacture of porang flour include fresh porang tubers, water, and Bimo CF starter. While the materials used in testing the quality of modified porang flour include Luff Schoorl solution, 20% KI, 4 N H_2SO_4, 1.25% H_2SO_4, 3.25% NaOH, 96% ethanol, 0.1 N $Na_2S_2O_3$, 1% starch indicator, diethyl ether, and 0.1 N $KMnO_4$.

2.2 Procedure and research design

The research used the response surface methodology (RSM) with a 3-Level factorial design. The independent variables determined in this study were Bimo CF starter concentration (X_1) and fermentation time (X_2). The lower limit and upper limit for the Bimo CF starter concentration variable were 0.10 and 0.50%, while the fermentation time variable was set at 12 h as the lower limit and 24 h as the upper limit. The center point of each factor was determined three times to replicate it, so that 13 experimental units were obtained. The optimum fermentation process verification was carried out three times.

2.3 Procedure of porang flour processing

The manufacture of porang flour used in this study refers to the method used by Yuliana *et al*. (2020) with modifications to starter concentration and fermentation time. The process of making porang flour includes: porang tubers that have been selected and meet quality standards were cleaned and washed to remove physical contaminants such as soil, then a soaking process was carried out in warm water (40°C) with the aim of opening the pores of the tuber tissue so as to facilitate the process of reducing the calcium oxalate content. Porang tubers that have been soaked in warm water were drained using a colander basket for 15 min. Porang tubers that have been cleaned and drained were then reduced in size by shrinking (1-1.5 mm in size). Size reduction is also part of efforts to reduce the calcium oxalate content and facilitate drying. The fermentation process was carried out by adding the Bimo CF starter and adjusting the fermentation time according to the treatment. Washing was done using ordinary water after the porang chips had undergone a fermentation process. This washing aims to reduce calcium oxalate content and clean the rest of the starter. After that, the draining process was carried out for 15 min with the aim of removing the water content of the washing results and facilitating the drying process. After draining, the drying process was carried out using a cabinet dryer at a temperature of 60°C for 24 h. The dried porang chips were ground using a blender to produce modified porang flour. The resulting modified porang flour was then sieved using an 80-mesh sieve (Indonesian National Standards, 2011) to produce finely modified porang flour.

2.4 Observation parameters

The responses observed as dependent variables in this study were moisture, ash, protein, fat, starch, crude fiber, calcium oxalate, glucomannan, whiteness degree, and yield.

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2.4.1 Moisture

The moisture content was calculated based on the weight lost during heating in the oven at 130±3°C, following the AOAC Official Method 925.10 (AOAC INTERNATIONAL, 2005). The empty crucible and lid are heated in the oven at a temperature of 130±3°C for approximately 1 h and cooled in a desiccator for 20 to 30 min, then weighed with an analytical balance (crucible and lid) (W0). A sample of 2 g is put into a crucible, closed, and weighed (W1). The crucible containing the sample was heated in an open state by placing the lid next to the crucible in the oven at a temperature of 130±3°C for 1 h after the oven temperature reached 130±3°C. The heated crucible was closed while still in the oven, then immediately transferred to the desiccator and cooled for 20 to 30 min so that the temperature was the same as the room temperature and then weighed (W2). The test was carried out in duplicate. The moisture content in the sample is calculated using Equation 1.

$$\text{Moisture (\%)} = \left(\frac{W_1\text{-}W_2}{W_1\text{-}W_0}\right) \times 100\% \quad (1)$$

2.4.2 Ash

Ash was calculated based on the weight of ash formed during combustion in the furnace at 550°C until white ash was formed, following the AOAC Official Method 923.03 (AOAC INTERNATIONAL, 2005). The crucible was heated in a furnace at a temperature of 550±5°C for approximately 1 h, cooled in a desiccator to equal room temperature, and then weighed using an analytical balance (W0). A total of 3 g of samples were put into a crucible and weighed (W1). The sample's crucible was placed in a furnace at a temperature of 550±5°C until white ash was formed and a fixed weight was obtained. The crucible was immediately transferred into the desiccator and cooled until the temperature equals the room temperature, then weighed (W2). The test was carried out in a duplicate. The ash in the sample was calculated using Equation 2.

$$\text{Ash (\%)} = \left(\frac{W_2\text{-}W_0}{W_1\text{-}W_0}\right) \times 100\% \quad (2)$$

2.4.3 Protein

Approximately 2 g of sample homogenate was carefully weighed on weighing paper. Then, it was folded, and put into the Kjeldahl flask, following the AOAC Official Method 992.23 (AOAC INTERNATIONAL, 2005). Two catalyst tablets and several boiling stones were added. Then, 15 mL of concentrated H_2SO_4 (95-97%) and 3 mL of H_2O_2 were added slowly, and the mixture was left for 10 min in an acid chamber. Next, the destruction process was carried out at a temperature of 410°C for 2 h or until the solution was clear, then let it sit until it reached room temperature and 50-75 mL of distilled water was added. After that, Erlenmeyer flask was prepared to contain 25 mL of 4% H_3BO_3 solution containing indicators as a distillate container, and then the Kjeldahl flask containing the destruction results was installed on a series of steam distillation devices. 50-75 mL of sodium hydroxide-thiosulfate solution is added. Next, distillation was carried out, and the distillate was stored in the Erlenmeyer flask until the volume reached a minimum of 150 mL (the distillate would turn yellow). Then, the distillate results were titrated with HCl 0.2 N, which had been standardized until the color changed from green to natural gray. Blank work was carried out, such as sample stages, and sample testing was carried out in duplicate. The protein content in the sample was calculated using Equation 3.

$$\text{Protein (\%)} = \frac{(V_A\text{-}V_B)\,\text{HCl} \times \text{N HCl} \times 14.007 \times 6.5}{\text{Sample weight (g)} \times 1000} \times 100\% \quad (3)$$

Where V_A = HCl volume for sample titration, V_B = HCl volume for blank titration, N = Normality of standard HCl used

2.4.4 Fat

The fat content was determined following the AOAC Official Method 920.85 (AOAC INTERNATIONAL, 2005). A total of 3 g of porang flour (W1) was weighed in a 250 mL beaker. A total of 20 mL of HCl pro analysis, 30 mL of water, and a few boiling stones were added. Then, the beaker glass was covered with a watch glass and boiled for 15-20 min. After that, the watch glass was rinsed with hot water. Then, a funnel and filter paper were prepared. The solution was filtered in a hot state and rinsed with hot water until the pH was neutral or equal to the pH of the rinse water. Then the filter paper that had been filled with porang flour samples was dried in an oven at a temperature of 105°C for 10 min.

The empty flask (W2) was weighed and the filter paper containing a sample of porang flour was placed into the fat sleeve. Approximately 50 mL of diethyl ether was added into a flask. Then, the fat sleeve was inserted into the Soxhlet extractor, and the Soxhlet circuit was installed correctly. Extraction was carried out with an extraction cycle of about 5 min/cycle for 3-4 h. Then, the diethyl ether solvent in the flask was evaporated until it was dry. The fat-filled spherical flask was placed in a 105°C oven for 2 h to remove any remaining diethyl ether and moisture. Next, the fat flask was cooled in a desiccator for 30 min. The fat flask was weighed (W3) to a constant weight. The test was carried out in duplicate. The crude fat content of the sample was calculated using Equation 4.

$$\text{Fat (\%)} = \frac{W_3\text{-}W_2}{W_1} \times 100\% \quad (4)$$

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2.4.5 Starch

A total of 0.1 g of the sample was weighed in a 250 mL Erlenmeyer flask, 50 mL of water, and 5 mL of 25% HCl were added, then heated at 100°C for 3 h (Ifmaily, 2018). Once cooled, the suspension was neutralized with 25% NaOH to pH 7. Transferred quantitatively into a 100 mL volumetric flask, then filled to the mark with water. This solution was then filtered back with filter paper. A total of 25 mL of this filtrate is added to 25 mL of Luff Schoorl's solution in an Erlenmeyer flask. A blank was also made, namely 25 mL of Luff Schoorl solution with 25 mL of aquadest. The Erlenmeyer flask was connected to a reverse cooler and then brought to a boil. The boiling of the solution was maintained for 10 min. Next, it was quickly cooled, 15 mL of 20% KI was added, and 25 mL of 25% H_2SO_4 was carefully added. Then cover and put in a dark place for 30 min. The released iodine was titrated with a 0.1 N $Na_2S_2O_3$ solution using a starch indicator of 2-3 mL. To clarify the color change at the end of the titration, starch should be added at the end of the titration. The starch content was calculated using Equation 5.

$$\text{Starch (\%)}=\frac{\text{mg glucose}\times \text{FP}\times 0.9}{\text{mg sample}}\times 100\% \quad (5)$$

where mg glucose = Luff Schoorl table number, based on the difference in titration ml, FP = volume of the titration filtrate

2.4.6 Crude fiber

Crude fiber is the part that cannot be hydrolyzed by sulfuric acid (1.25% H_2SO_4) and sodium hydroxide (3.25% NaOH). The crude fiber is determined following the AOAC Official Method 920.86 (AOAC INTERNATIONAL, 2005). The portion was calculated gravimetrically. A total of 2 g to 4 g of sample (W) was weighed and put into a 500 mL Erlenmeyer flask. Then, in an upright cooler, add 50 mL of H_2SO_4 1.25% solution and boil for 30 min. Approximately 50 mL of 3.25% NaOH was added and then refluxed for 30 min using a condenser. Samples in the hot state were filtered with a Buchner funnel containing filter paper that had been dried, and the weight was known. The sediment contained in the filter paper was washed sequentially using hot H_2SO_4 1.25%, hot water, and ethanol 96%. The filter paper and its contents were lifted, then put in an oven and dried at a temperature of 105°C, then cooled and weighed until the weight remained (W1). If it turns out that the crude fiber content is greater than 1%, then the filter paper and its contents are ashed and then weighed until the weight is fixed (W2). The test was carried out in duplicate. The crude fiber content in the sample was calculated using Equation 6.

$$\text{Crude Fiber (\%)}=\left(\frac{W_1-W_2}{W}\right)\times 100\% \quad (6)$$

2.4.7 Calcium oxalate

Calcium oxalate testing begins with a heating stage (Maulina *et al*., 2012). At this stage, 2 g of porang flour was suspended in 190 mL of distilled water, which was put into a 250 mL Erlenmeyer flask, then 10 mL of 6 M HCl solution was added. The suspension was heated at 100°C for 1 h, followed by cooling, and then water was added up to 250 ml before filtration. After the heating process, it was continued to the permanganate titration stage. At this stage, the amount of filtrate of 125 mL produced from the heating stage was diluted to 300 mL. Then, 125 mL was heated until it was almost boiling, then titrated with a $KMnO_4$ 0.05 M solution until it changed color to almost disappear pink color, which lasted for 30 s. Equation 7 can calculate the calcium oxalate content.

$$\text{Calcium oxalate (\%)}=\frac{(V_A-V_B)KMnO_4\times N\ KMnO_4\times 45}{W\times 100}\times 100\% \quad (7)$$

Where: V_A = $KMnO_4$ volume for sample titration, V_B = $KMnO_4$ volume for blank titration, W = Sample weight

2.4.8 Glucomannan

A total of 1 g of porang flour and 0.1 g of aluminum sulfate were put into 100 mL of aquadest DM at a temperature of 70°C while stirring for 35 min (Hadi and Kurniawan, 2020). The remaining deposits were centrifuged at a speed of 2000 rpm for 1 h. Supernatants were taken and then mixed with ethanol in a 1:1 (v/v) ratio while stirring until a precipitate was formed. The resulting sediment was filtered using filter paper, dried at 55°C for 6 h, and then weighed.

2.4.9. Whiteness degree

The whiteness testing was carried out using a chromameter (Pasca *et al*., 2022). The measurement was carried out by placing the porang flour sample on a black container and then leveling the surface of the flour sample with a spatula. The flour sample was then analyzed by placing a measuring head on it. The porang flour samples were then identified with L*, a*, and b* Hunter values. Calculation of white degrees using Equation 8.

$$\text{Whiteness degree} = 100 - [(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2} \quad (8)$$

2.4.10 Yield

The yield measurement was carried out by using the equation to compare the dry weight of the resulting porang flour with the weight of fresh porang tubers before peeling.

$$\text{Yield}=\frac{\text{Weight of porang flour (g)}}{\text{Weight of porang tubers (g)}}\times 100\% \quad (9)$$

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2.5 Data analysis

The research data were analyzed using Design Expert 13 software (Stat-Ease) to determine the optimum concentration of Bimo CF starter and fermentation time and verify the optimum fermentation process.

3. Results and discussion

3.1 Optimization of the fermentation process

Based on Table 1, it was evident that the factors applied had a significant impact on seven responses. This showed that the fermentation process using the Bimo CF starter has a considerable influence on the characteristics of the modified porang flour. Nainggolan *et al*. (2019) have reported that the fermentation process in the manufacture of fermented cassava flour affects the physical and chemical properties of the resulting flour. Likewise, the lactic acid bacteria (LAB) fermentation process in the manufacture of modified gadung (*Dioscorea hispida* Dennst) flour affects the chemical composition of the flour (Setiarto and Widhyastuti, 2016). Based on the model's significant value, lack of fit, and adequate precision (Table 1), there are five responses used to optimize the fermentation process in making modified porang flour.

$$\text{Protein } (Y_3) = 10.96 - 0.49X_1 - 0.57X_2 + 1.25X_1^2 + 0.02X_2^2 - 0.05X_1X_2 \quad (10)$$

The results of the analysis of variance of the quadratic model of the protein content response showed that only the fermentation time factor had a significant effect, while the concentration of starter and the interaction between factors had no significant effect. The quadratic effect of fermentation time has a positive effect on increasing the protein content of modified porang flour (Equation 10), which means that at a long fermentation time (24 h), the protein content of modified porang flour tends to increase. In this study, protein content tended to decrease from 12 to 18 h of fermentation and increase over 18 h of fermentation. The phenomenon of the effect of fermentation time on the protein content of modified porang flour is the same as that which occurred in the process of making modified taro flour. In the manufacture of modified taro flour, there was a decrease in protein content during fermentation using Bimo CF if the fermentation time was less than or equal to 14 h, but there was an increase in protein content if it was more than 14 h compared to the initial protein content of taro. This is because during fermentation, the lactic acid bacteria present in Bimo CF produce protease enzymes that will convert proteins into amino acids, which then become lactic acid, resulting in a decrease in protein. However, after 14 h of fermentation, there was an increase in protein because the number of microbes in the fermentation process increased. The longer the fermentation time, the more the number of microbes will increase, so that the dissolved protein content will increase. This increase in the amount of protein was due to the increasing number of microbes that act as single-cell proteins, that is, proteins obtained from microorganisms (Astuti and Setyawati, 2016).

$$\text{Fat } (Y_4) = 8.59 - 0.26X_1 - 0.49X_2 + 1.38X_1^2 + (8.62*10^{-3})X_2^2 - 0.04X_1X_2 \quad (11)$$

The quadratic model of the fat content showed that the fermentation time factor had a significant effect on the fat content both linearly and quadratically, while the Bimo CF starter concentration factor and the interactions between factors had no significant effect. Based on Equation 11, it was shown that the effect of fermentation time is linearly more dominant than quadratic. This was based on the coefficient of fermentation time, which was linearly higher than quadratic. Thus, based on this mathematical equation, the effect of fermentation time was inversely proportional to the fat content of modified porang flour, where the longer the fermentation time, the lower the fat content of modified porang flour. The results of this study were the same as those found in the manufacture of modified taro flour, which went through a fermentation process where the fat content of the flour decreased as the fermentation time increased. This is because during the fermentation process, fat dissolution occurs and is wasted during washing (Siletty *et al*.,

Table 1. The analysis of variance for the response of the chemical and physical properties of modified porang flour.

Response	ANOVA						
	Significant Model	Lack of Fit	Mean	Std. Dev	Adj R^2 Model	Pred. R^2 Model	Adeq. Precision
Moisture (Y_1)	NA	0.80	12.82	0.40	0.00	-0.17	3.03
Ash (Y_2)	NA	0.62	5.05	0.22	0.00	-0.17	NA
Protein (Y_3)	0.00	0.36	5.62	0.10	0.92	0.75	14.08
Fat (Y_4)	0.00	0.05	2.48	0.18	0.95	0.84	19.67
Starch (Y_5)	0.00	0.46	5.49	0.73	0.87	0.67	12.75
Crude fiber (Y_6)	0.00	0.00	5.27	0.05	0.88	0.34	13.30
Calcium oxalate (Y_7)	0.00	0.82	1.21	0.03	0.88	0.80	10.05
Glucomannan (Y_8)	0.00	0.72	14.62	0.33	0.98	0.96	26.50
White degrees (Y_9)	0.07	0.29	59.55	1.16	0.38	-0.28	7.07
Yields (Y_{10})	0.00	0.00	10.30	0.15	0.77	-0.25	7.26

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$$\text{Starch } (Y_5) = 25.08 - 17.53X_1 - 1.53X_2 + 17.22X_1^2 + 0.03X_2^2 + 0.27X_1X_2 \quad (12)$$

The quadratic model for the response to starch content also showed that the fermentation time factor had a significant effect on the response to the starch content of modified porang flour, while the concentration of the Bimo CF starter and the interactions between the factors had no significant effect. Based on this mathematical Equation 12, an increase in the fermentation time factor tends to decrease the starch content of modified porang flour. Microbes that break down starch into simple sugars are to blame for the decrease in starch content of modified porang flour due to the fermentation process. The amylase activity contained in the porang tubers is optimized to hydrolyze starch into simpler components (Nainggolan *et al*., 2019). Thus, the longer the fermentation time, the higher the process of breaking down starch into simple sugars, which causes the starch content of modified porang flour to decrease. The decrease in starch content of modified porang flour due to the fermentation process can be associated with the possibility of transforming starch into glucose as a carbon source for protein or fat synthesis. Furthermore, glucose is converted into end products such as volatile fatty acids and neutral compounds (Gunawan *et al*., 2019).

$$\text{Crude fiber } (Y_6) = 6.66 - 1.34X_1 - 0.11X_2 + 1.19X_1^2 + (1.74*10^{-3})X_2^2 + 0.04X_1X_2 \quad (13)$$

The crude fiber content of modified porang flour decreased with increasing fermentation times (Equation 13). The decrease in crude fiber content can be caused by the hydrolysis of carbohydrates. LAB can hydrolyze fiber into simple monosaccharides (glucose) (Wang *et al*., 2021). During the fermentation, LAB produces metabolites such as lactic acid and acetic acid, which cause the environment to be quite acidic. These conditions produce microbes with high cellulase activity, degrading cellulose and hemicellulose and increasing dissolved polysaccharides. Cellulose, hemicellulose, and lignin are dietary fiber components in the insoluble dietary fiber group, which underlie the increase in soluble dietary fiber after fermentation (Yonata *et al*., 2022).

$$\text{Calcium oxalate } (Y_7) = 2.31 + 0.14X_1 - 0.13X_2 - 0.11X_1^2 + (3.49*10^{-3})X_2^2 - (8.33*10^{-3})X_1X_2 \quad (14)$$

The results of the analysis of response data for calcium oxalate showed that the quadratic fermentation time factor has a significant effect on calcium oxalate (Equation 14). This showed that increasing the fermentation time quadratically would affect the increase in calcium oxalate. The results showed that increasing the fermentation time to the median value tended to decrease the calcium oxalate of modified porang flour, while the fermentation time above the median value tended to increase the calcium oxalate (Figure 1). This indicates that the optimum point of the fermentation time to obtain low levels of calcium oxalate in the modified porang flour was around the mean of the fermentation time. The decrease in calcium oxalate in modified porang flour with a fermentation time of 18 h was the same as that reported by Ferdian and Perdana (2021) where modified porang flour produced by the wet milling method experienced a decrease in calcium oxalate content until the fermentation time was 18 h and increased again in the 24 h fermentation time. The decrease in calcium oxalate in modified porang flour was caused by damage to the cell walls of porang tubers by microbial activity during the fermentation process, so much of the calcium oxalate dissolved during washing after the fermentation process.

$$\text{Glucomannan } (Y_8) = -21.17 + 4.28X_1 + 3.68X_2 - 2.93X_1^2 - 0.09X_2^2 - 0.11X_1X_2 \quad (15)$$

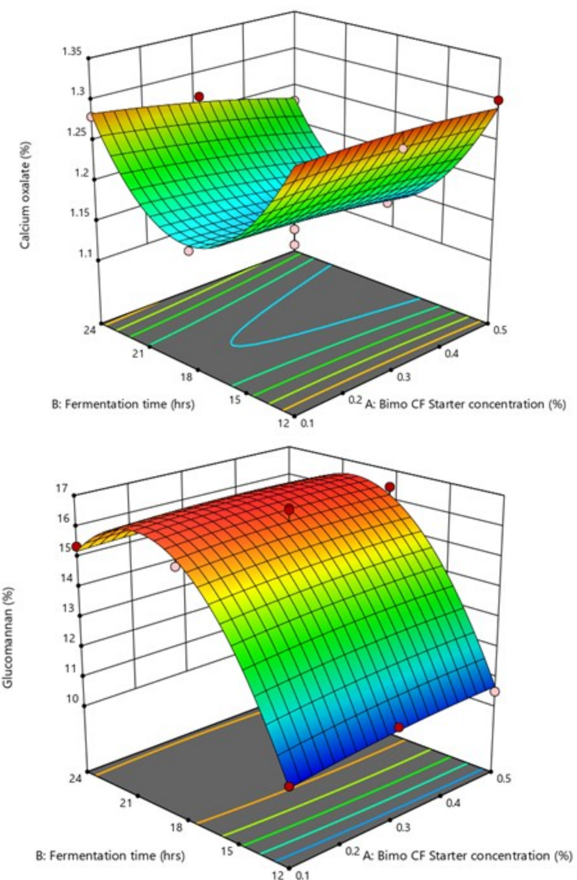

Figure 1. Surface plot of the response effect of Bimo CF starter concentration and fermentation time on the calcium oxalate and glucomannan content of modified porang flour.

The glucomannan content response showed that the treatment of fermentation time both linearly and quadratically, has a significant effect on the glucomannan content, where increasing the fermentation time linearly will increase the glucomannan content of the modified porang flour, while increasing the fermentation time quadratically will decrease the

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glucomannan content of the resulting porang flour (Equation 15). This showed that the fermentation time has reached the optimum point for increasing the glucomannan content of modified porang flour. In this study, the increase in glucomannan occurred during fermentation from 12 h to 18 h, and at 24 h of fermentation, the glucomannan content of modified porang flour tends to decrease (Figure 1). In porang tuber fermentation using *Lactobacillus bulgaricus* bacteria with a fermentation time of 12 h, there was an increase in glucomannan because microorganisms began to degrade starch. This is because during fermentation, microbial activity occurs, which causes starch degradation accompanied by the formation of simple sugars, which are used as energy in growth and activity. Starch decomposition can release glucomannan granules, which cause concentrations to increase (Nur'aini *et al*., 2021).

The analysis of the yield response data showed that the quadratic treatment of the fermentation time had a significant effect on the yield of the modified porang flour (Equation 16). Increasing the fermentation time quadratically will increase the yield of modified porang flour. This was likely due to the fact that the longer the fermentation time, the more decomposition by LAB microbes in the Bimo CF starter increases, which has an impact on increasing the yield.

$$\text{Yields } (Y_{10}) = 15.08 - 0.56X_1 - 0.53X_2 + 0.85X_1^2 + 0.02X_2^2 - 0.02X_1X_2 \quad (16)$$

Optimization of the Bimo CF starter concentration and the fermentation time was carried out based on consideration of the response, which had a significant effect by setting a target close to the flour quality standard. Based on this, the response used as the basis for optimization is set at a minimum value for the responses of protein, fat, and calcium oxalate. While starch is set at the target of 4% and glucomannan levels are set at the maximum value. For the responses taken into consideration in optimizing, the degree of importance is determined for each of the five, and the other three responses are each. The results of the optimization analysis showed that the Bimo CF starter concentration and the fermentation time in the manufacture of modified porang flour were 0.5% and 20 h, respectively, with a desirability value of 0.91. This means that 91% of the predicted value of the program simulation results follows the target of the desired chemical and physical properties of modified porang flour. The optimum of the Bimo CF starter concentration and fermentation time will produce modified porang flour with a predicted value of the chemical and physical properties of modified porang flour, i.e., protein 5.24%, fat 1.94%, carbohydrate 74.48%, starch 4.30%, dietary fiber 5.24%, calcium oxalate 1.14%, glucomannan 16.54%, and yield 10.02%.

3.2 Verification of optimum fermentation process

Based on the results of verification of the Bimo CF starter concentration and fermentation time optimum in the manufacture of modified porang flour (Table 2), it showed that some of the response values are below the lowest predictive interval values at the 95% confidence level, and there are also response values that are above the highest predicted interval values at the 95% confidence level. Responses below the lowest prediction interval value are as expected and still in the standard range of flour quality. The water content of porang flour has met the SNI No 7939:2020 porang flakes quality standard, which is ≤13%, and can be classified as first-class quality (Indonesian National Standards, 2020).

The interesting thing from the results of this study was that after verification, the calcium oxalate levels were far below the predicted value, which was half of the predicted value. Likewise, the results of the verification showed the glucomannan content of porang flour had a value far above the predicted value of glucomannan. The levels of calcium oxalate from this study were higher than those from earlier studies, where porang flour produced using a maceration extraction process was 0.11 - 0.14% (Faridah, 2016; Witoyo *et al*., 2022), but they were lower than porang flour produced using a

Table 2. The verification of Bimo CF starter concentration and fermentation time optimum in the manufacture of modified porang flour.

Response	Predicted Mean	Std Dev	n	SE Pred	95% PI low	Data Mean	95% PI high
Moisture (Y_1)	13.05	0.42	3	0.33	12.30	9.51	13.80
Ash (Y_2)	5.05	0.22	3	0.14	4.74	4.53	5.36
Protein (Y_3)	5.24	0.09	3	0.09	5.03	4.65	5.44
Fat (Y_4)	1.94	0.18	3	0.16	1.56	3.74	2.33
Starch (Y_5)	4.29	0.73	3	0.65	2.77	2.15	5.82
Crude fiber (Y_6)	5.24	0.05	3	0.05	5.13	7.72	5.35
Calcium oxalate (Y_7)	1.14	0.02	3	0.02	1.09	0.66	1.19
Glucomannan (Y_8)	16.54	0.33	3	0.29	15.85	39.68	17.24
White degrees (Y_9)	59.84	1.16	3	0.91	57.79	64.47	61.89
Yields (Y_{10})	10.02	0.15	3	0.13	9.71	10.87	10.34

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fermentation process, which is 0.83% (Sulastri *et al*., 2021). Likewise, the glucomannan levels produced in this study were quite high and close to the levels of glucomannan in modified porang flour through the LAB fermentation process, especially 44-52% (Ferdian and Perdana, 2021). The high level of glucomannan found in porang flour indicates the high quality of this flour because the benefits of porang flour are mainly due to its glucomannan content (Handayani *et al*., 2020). This showed that the optimum fermentation process obtained from this research can be developed for producing flour with low calcium oxalate and a high glucomannan content.

4. Conclusion

Bimo CF starter concentration and fermentation time in manufacturing modified porang flour affect the physical and chemical properties. Optimization results showed that the concentration of Bimo CF starter and the optimum fermentation time were 0.5% and 20 h, respectively. With a level of desirability of 0.91%. The verification results showed that the calcium oxalate levels were far below the predicted value, which was half of the predicted value. Likewise, the glucomannan content showed a value far above the predicted value. This indicates that the optimum fermentation process obtained from this study can be developed to produce modified porang flour with low calcium oxalate and high glucomannan.

Conflict of interest

The authors declare no conflict of interest.

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