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4 Optimization of temperature and drying time of indigenous cocktail yeast mold culture using response surface methodology (RSM)

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Abstract

The study was conducted to obtain an optimal combination of time and temperature of the drying process of indigenous cocktail yeast mold culture using RSM. The cocktail yeast mold culture was dried using an oven. The cocktail cultures contain *Penicillium citrinum*, *Aspergillus niger*, *Acremonium strictum*, and *Candida famata*, namely AC (Amylolytic Culture). The Response Surface Methods (RSM) with Design-Expert® 7.00 software, namely Mixture design with D-optimal was performed. The drying time was between 24-48 hrs and the drying temperature was between 40-50°C. The total of 16 formulas of the combination of drying time and temperature for processing the dried cultures were produced by RSM. The response chosen was total viability of mold and yeast, water content, water activity, and pH. The result of optimization and verification was obtained by the model: $\text{pH (AC)} = -0.058A - 1.56 \times 10^{-003}B + 7.13$, where A = drying temperature (°C), B = drying time (hr). The AC optimization was achieved at a combination of drying temperatures and time of 50°C for 48 hrs. Desirability values were 0.729. The optimum formula for AC has viability of total yeast mold of 7.39×10^6 CFU/g, moisture content of 5.62%, a_w 0.303, and pH 4.18.

1. Introduction

Fermented corn flour made from local white corn *anoman* variety has been done. The results of Farasara *et al.* (2014) showed that the fermentation process changed the characteristics of corn flour produced compared to its natural flour. Likewise, the results of research by Rahmawati, Maulani and Saputra (2018), the use of different types of starter produces different characteristics of flour. The use of indigenous microorganisms in the form of fresh starter cultures is not easy to carry and keep it. To overcome this and facilitate the fermentation process, Rahmawati *et al.* (2017) made a dried cocktail of starter culture that consists of indigenous yeast and mold. A dried starter culture is easier to carry and keep it. A good starter culture will produce a good product. To produce optimal products, it is needed to optimize the process of making dried culture as well, especially the process of making dried indigenous cocktail starter culture. The cocktail starter culture is made using an oven as a dryer. The

temperature and drying time affected the viability of microorganisms. This will affect the quality of the resulting cocktail starter culture. Based on this, the research was conducted to obtain the optimum combination of time and temperature that will produce the best quality of dry indigenous cocktail starter culture. Optimization of the process of making starter culture was carried out by the Response Surface Methodology (RSM) method with Design-Expert® 7.00 software, namely Mixture design with D-optimal.

RSM is a statistical and mathematical technique used for the development, improvement, and optimization of production processes by estimating the relationship between independent variables and the results (responses) observed so that obtaining optimum information about independent variables influences the response. This method has long been used in biological research and food technology applications, especially in the stages of the process and formulation optimization (Myers *et al.* 2009). In making dried cocktail starter

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culture, the viability of microorganisms is very important so that the fermentation process runs optimally. Besides the water content and water activity will affect the long shelf life. Based on this, the response chosen in determining the optimization of dried indigenous cocktail yeast mold culture is the viability of microorganisms (total mold and yeast), moisture content, water activity, and pH.

This study aims to obtain the optimum temperature and drying time in making indigenous cocktail yeast mold culture, namely AC with the response specified above. The AC cocktail cultures contain *Penicillium citrinum*, *Aspergillus niger*, *Acremonium strictum*, and *Candida famata*. In addition, this study aims to determine the parameters of the optimum process of drying the indigenous cocktail yeast mold culture using an oven with RSM. The determination of the optimum formula is based on the results of the formula response measurement. The indigenous cocktail yeast mold culture is expected to contain the yeast and mold with the optimum number because it will be used to ferment local white corn grits.

2. Materials and methods

2.1 Microorganisms

Microorganisms used as a starter culture prepared were *P. citrinum*, *A. niger*, *A. strictum*, and *C. famata*, namely AC. The microorganisms used were previously isolated and identified from spontaneous fermentation of corn grits (Rahmawati et al., 2013).

2.2 Culture preparation and enumeration

A loop of each mold was streaked onto fresh Potato Dextrose Agar (PDA) slant and then incubated at 30°C for five days. After five days, molds were harvested by scraping, suspended in 10 mL sterile water and appropriately diluted for enumeration using hemacytometer. Yeast culture was prepared as above, but incubation was carried out at 30°C for two days. Yeast enumeration was also carried out using hemacytometer (Farasara et al., 2014).

2.3 The process of making AC-indigenous cocktail yeast mold culture

AC-indigenous cocktail yeast mold culture consists of indigenous amyolytic yeast and mold culture from Rahmawati et al. (2013). Growing media used in making cocktail yeast and mold culture is corn flour (Rahmawati et al. 2017). The drying process in making cocktail yeast mold culture is carried out based on the formulation suggested by RSM.

The technique of making cocktail yeast mold culture includes the stages: sterilizing corn flour, then put it into a sterile basin and adding sterile distilled water as much as 2/3 of the total weight of corn flour. Prepared culture suspensions (AC) containing 10⁶ CFU/mL per microorganism, then piped as much as 10% of the amount of water used. After that, all stir until homogeneous and put ± 17 grams in each petri dish. Petri dishes were then incubated at 30°C for 5 days. Furthermore, the dough is dried using an oven with a range of 40-50°C for 24-48 hours. The dried AC-indigenous cocktail yeast mold culture is made powder using a blender that has been sprayed with 70% alcohol. The AC-indigenous cocktail yeast mold culture powder is packed in plastic clips with silica gel and then tested for viability (total number of yeast molds), water activity, water content and pH.

2.4 Optimization of the drying process of making AC-indigenous cocktail yeast mold culture using the RSM method

Optimization of the drying process of making AC-indigenous cocktail yeast mold culture was carried out by the Response Surface Methodology (RSM) method through the Design Expert® 7.00 (DX7) statistical application. The experimental design was made with the aim of obtaining a combination of several components with an optimum response (Myers et al., 2009). The mixture design used is D-optimal. The independent variables in the experiment are drying time (h) and drying temperature (°C). The drying time is between 24-48 hrs and the drying temperature is 40-50°C (Rahmawati et al., 2017) (Table 1). After the RSM issued a suggestion on the optimum process for making AC-indigenous cocktail yeast mold culture was made.

Table 1. Independent variables and the level used in the experiments

Independent variables	Limits	
	Lower (-1)	Upper (+1)
Temperatures (°C)	40	50
Times (h)	24	48

2.5 AC-indigenous cocktail yeast mold culture analysis

The product produced is tested for the response. The responses observed were viability (CFU/g), moisture content (thermo-gravimetry) (AOAC 2006), water activity (Aw meter Rotronic Hygrolab) (AOAC 2006), and degree of acidity (pH meter Orion Thermo-Scientific) (AOAC 2006). Of the four responses, viability is determined to be maximized with a scale of interest of 5 (very important). Where water content, water activity and pH values are minimized with a scale of interest of 3 (important). This is because the main value expected is the total viability of the molds

produced optimally so that the yeast will be effective if used during the fermentation process.

2.6 Statistical analysis

Data that has been obtained is then processed using Design Expert software. The results obtained will be translated into the model of the response function equation to the independent variable chosen for the response and interaction between responses. In the final stage of optimization, the program will recommend an optimal combination of processes. Optimal conditions are chosen by comparing the value of the desirability of each solution. The selected combination is the one with the highest desirability value.

The model also showed precision adequacy values (Adeq. Precision > 4).

3.2 Yeast mold viability

The AC indigenous cocktail yeast mold culture had a viability value ranged from log 4.2041 - log 8.1139 CFU/g. Figure 1 shows that the viability of yeast molds tends to decrease with higher temperatures and longer drying time. Data can be seen in Table 3. This is because the higher the temperature and drying time can cause mold yeast to die. However, in general, the viability of yeast molds in the AC indigenous cocktail yeast mold culture is relatively high. Pitt and Hocking (2009) stated that *Penicillium*, *Rhizopus*, *Aspergillus* grew optimally at temperatures of 35-37°C. The viability of total yeast mold in the AC dried culture is an important parameter in determining the culture quality because it will act as a starter culture in the white corn grits fermentation process. Rahmawati et al. (2017) found that AC-starter culture has been drying by oven at 40°C for 48 hrs, has yeast mold availability as log 7.66 CFU/g. Besides that, the results are in line with Oliveira et al. (2002) where the minimum number of microorganisms for the starter is 10⁶ CFU/mL. Yuliana and Rizal (2006) stated that the ideal conditions for using a starter culture as an inoculum is equal to 2.1x10⁷ CFU/g. As a comparison, the CC indigenous cocktail yeast mold culture has a viability

3. Results and discussion

3.1 Optimization of drying process parameter and verification of the model

Table 2 shows that the model can describe the relationship of drying process parameters (drying temperature and time) to viability, moisture content, water activity, and pH value, which are linear and quadratic models. The significance value of the model, lack of fit, and determination coefficient (predicted R-squared, adjusted R-squared) indicate that there was a match between the distribution of data and the model.

Table 2. The math model of drying process parameter as a response of the AC indigenous cocktail yeast mold culture

Parameters	Math model	Significance model	Lack of fit	Adj R ² model*	Pred R ² model*	Adeq precision*
Viability (CFU/g)	Quadratic	0.0272	0.0415	0.5099	0.3083	5.041
Water content (%)	Linear	0.0031	0.0375	0.5255	0.4423	7.68
Water activity (a _w)	Linear	0.0427	0.3158	0.2897	0.1237	4.622
Degree of acidity (pH)	Linear	0.0032	0.2709	0.5234	0.3883	6.721

*Information: Adj= Adjusted; Pred= Predicted; Adeq= Adequate

Table 3. The viability, water content, a_w and pH responses for AC indigenous cocktail yeast mold culture as the result of RSM optimization

Number	Independent variables		Viability (Log CFU/g)*	Water content (%)*	a _w content*	pH value*
	Drying temperature (°C)	Drying time (h)				
1	40	24	8.11 ^{abc}	7.33 ^q	0.464 ^k	4.57 ^{fg}
2	40	24	7.30 ^{abc}	6.44 ^q	0.463 ^k	5.05 ^{fg}
3	40	36	8.04 ^{abc}	7.21 ^p	0.427 ^{ijk}	4.68 ^{ef}
4	40	48	7.97 ^{abc}	7.31 ^q	0.391 ^{ghij}	4.94 ^g
5	40	48	7.61 ^{abc}	7.31 ^q	0.426 ^{ghij}	4.96 ^g
6	43	27	6.78 ^a	5.63 ^e	0.312 ^{bcdef}	4.27 ^{abc}
7	44	37	6.87 ^a	6.24 ^j	0.439 ^{jk}	4.19 ^{ab}
8	45	48	6.58 ^a	6.93 ^o	0.336 ^{ef}	4.27 ^{abc}
9	46	33	5.18 ^a	5.07 ^c	0.284 ^{abcde}	4.50 ^{de}
10	47	24	5.50 ^{ab}	5.57 ^d	0.319 ^{bcdef}	4.69 ^{cd}
11	47	24	4.84 ^a	5.93 ^d	0.309 ^{bcdef}	4.43 ^{cd}
12	48	39	4.20 ^a	4.37 ^a	0.262 ^{abc}	4.48 ^g
13	50	30	6.45 ^a	5.95 ^h	0.296 ^{fghi}	4.22 ^{abc}
14	50	30	7.28 ^a	5.78 ^h	0.451 ^{fghi}	4.22 ^{abc}
15	50	48	7.15 ^a	5.72 ^f	0.334 ^{def}	4.16 ^{ab}
16	50	48	6.77 ^a	5.72 ^f	0.298 ^{def}	4.12 ^{ab}

1 Samples means with same superscripts in the same column are not significantly at $\alpha = 0.05$

value ranged from log 6.23 to log 8.43 CFU/g (Rahmawati et al., 2018)

The model chosen by the program for the appropriate viability response was quadratic with an R^2 value of 0.5099 (Figure 1). The viability response model (AC) has an F value of 4.12 with a p-value (Prob>F) was 0.0272 whereby the model was significant at $\alpha < 0.05$. However, the ANOVA results did not show that the drying temperature and time significantly affected the viability response with a significant lack of fit value which was smaller than 0.05 (0.0415). The value of lack of fit is bad, the result is significant because it describes the suitability of the model with the response (Keshani et al., 2010).

The AC-indigenous cocktail yeast mold culture consists of more than one microorganism. So, the activity of AC during the fermentation process varies, because the optimum conditions for growth during incubation for each microorganism vary and maybe there was competition for nutrients by microorganisms varies.

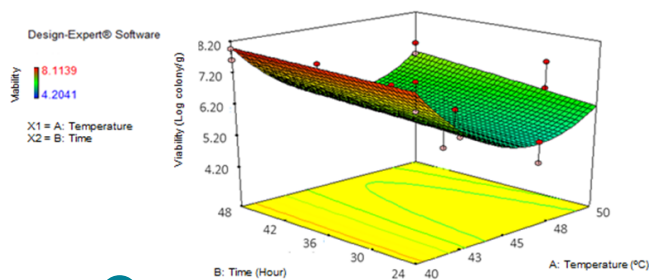


Figure 1. A 3D Graphical combination between temperature and drying time to the viability of AC starter.

3.3 Water content

The moisture content of AC-indigenous cocktail yeast mold culture ranges from 4.37 - 7.33%. Figure 2 shows that the percentage of moisture content tends to decrease with higher temperatures and longer drying time. Rasulu et al. (2012) stated that the water content value is influenced by drying process because the drying process facilitates water evaporation. The moisture content is relatively low because during the fermentation process starch degradation occurs in corn flour accompanied by the formation of simple sugars and release of water. Degradation of starch by microbes caused a decrease in the ability of materials to retain water because of the loss of hydroxyl groups which play a role in absorbing water. Water content determines the shelf life of the product to be stored because it is a marker of the availability of water in food for living microorganisms (Barbosa-Canovas et al., 2007). It means products that have lower water content will have a longer shelf life.

The model chosen by the program for the appropriate moisture content response was linear with an R^2 value of 0.5255 (Figure 2). This model response has a F value 9.31 with p-value (Prob>F) was 0.0031 which is significant at $\alpha = 0.05$. However, the ANOVA results did not show that the drying temperature and time significantly affected the water content response with a significant lack of fit value which was smaller than 0.05 (0.0375). A significant lack of fit values illustrates model not suitability with responses.

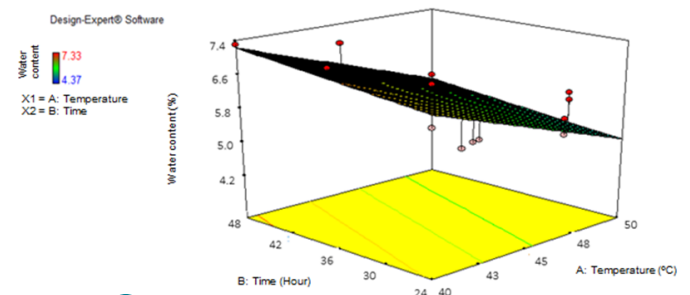


Figure 2. A 3D graphical combination between temperature and drying time to the moisture content of AC starter

3.4 Water activity

The a_w value for AC-indigenous cocktail yeast mold culture ranges from 0.262 - 0.464. Figure 3 shows that the a_w value tends to decrease with higher temperatures and longer drying time. This result was in line with Rahmawati et al. (2017) that AC starter culture has a_w value is 0.450. Water activity (a_w) is a parameter that shows the amount of free water in a product. Free water in food is needed by microbial growth for nutrient processes, media for enzymatic reactions and synthesis of cellular components (Rahayu and Nurwitri, 2012). The product that has a lower a_w value will have a longer shelf life because microorganisms can only live in certain a_w conditions. In general, yeast molds can live at certain minimum a_w values. *Aspergillus* lives at a minimum of a_w 0.78, *Penicillium* 0.88, and yeast can generally live at a_w around 0.88-0.94. The minimum water activity for mold growth is 0.80, while yeast can grow at a minimum of 0.88 a_w . The AC-culture has lower a_w value. Based on the food stability map as a function of water activity this AC-culture will be degraded by lipid oxidation, non-enzymatic browning, and enzymatic reaction (Barbosa-Canovas et al., 2007).

The model chosen by the program for the corresponding a_w response is linear (Figure 3). This model showed that the response was affected by drying temperature and time, not by the interaction. The RSM equation or model for optimization of water activity for AC-indigenous cocktail yeast mold culture is as follows:

$$a_w(\text{AC}) = -0.011A - 5.12 \times 10^{-004}B + 0.87$$

Where a_w = water activity; A = drying temperature ($^{\circ}\text{C}$); and B = drying time (hr).

This model has a F value 4.06 with p-value (Prob>F) is smaller than 0.05 (0.0427). It showed that the model was significant at $\alpha = 0.05$, which means it can describe that the a_w response quite well. The ANOVA results also showed that the drying temperature and time had a significant effect on a_w response with insignificant lack of fit values which were greater than 0.05 (0.3158). The value of lack of fit is greater than 0.05, which indicated that it was not significant describing the model mismatch with the response (Keshani *et al.*, 2010). The equation showed that the effect of temperature and drying time is inversely proportional to water activity. That is, the drying temperature and time higher can cause a lower water activity.

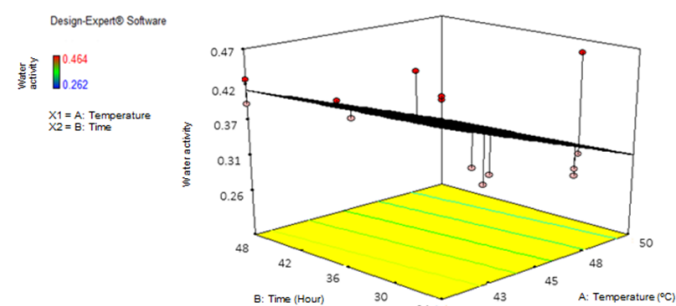


Figure 3. A 3D graphical combination between temperature and drying time to the a_w (water activity) of AC starter

3.5 pH value

The pH value of AC-indigenous cocktail yeast mold culture ranges from 4.12-5.05. Figure 4 shows that the pH value tends to decrease with a higher temperature and a longer drying time. The pH value or acidity level shows the active hydrogen ion concentration. The pH value is used to determine the various types of microbes that may grow in products where each microbe has a specific growth pH. This pH value is in line with previous studies, namely indigenous yeast mold yeast at pH 4-5 (Rahmawati *et al.*, 2017). The results of the Pratama *et al.* (2013) showed that the pH values of starter culture for bread, tempe, and *Lactobacillus plantarum* are 4.37, 3.43, and 3.93 respectively in 96 hrs fermentation. The pH value affects the microorganism's growth and life. Each type of microbe has an optimum pH and pH range for its growth. In general, mold and yeast can grow in a wider pH range than bacteria (Rahayu and Nurwitri, 2012). While molds have a very wide pH growth range, which is between 2.0 to 8.5, yeast has a pH range of growth between 4.0 to 4.5 and will not grow well in alkaline environments (Muchtadi and Sugiyono, 2013). During fermentation, a group of microorganisms is capable of fermenting nutrients contained in food to convert some or all of the food

components into fermented products, such as lactic acid, ethanol, CO_2 , or other organic acids. The accumulation of organic acids caused the pH to decrease during incubation. According to Kartohardjono *et al.* (2007), that CO_2 gas contributes to reducing the pH value.

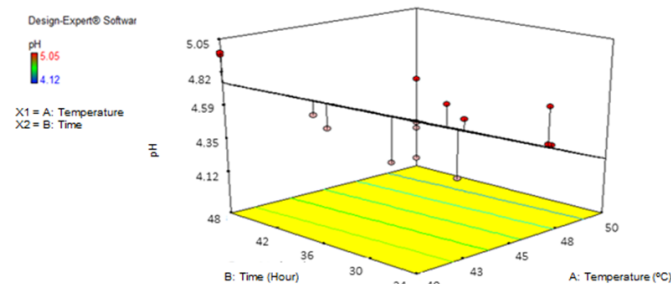


Figure 4. A 3D graphical combination between temperature and drying time to the pH value of AC starter

The selected model by the program for the pH response in the appropriate AC-cocktail culture is a linear model with an R^2 value of 0.5869. This model is significant with a value of $p < 0.05$ (Figure 4). The RSM equation or model for optimization of pH for AC-indigenous cocktail yeast mold culture is as follows:

$$\text{pH (AC)} = -0.058A - 1.56 \times 10^{-003}B + 7.13444$$

Where A = drying temperature ($^{\circ}\text{C}$); and B = drying time (hr)

The ANOVA showed that the drying temperature and time had a significant effect on the pH response of AC-indigenous cocktail yeast mold culture with an insignificant lack of fit values which were greater than 0.05 (0.2709). These results showed that the obtained model has a match with the linear design. The value of lack of fit is good, the result is not significant because it describes the suitability of the model with the response (Keshani *et al.* 2010).

During the fermentation process yeast activity will produce acids such as lactic acid, acetic acid and ethanol and CO_2 which reduce the pH value (Corsetti and Settanni, 2007). In addition, acid production also affects the aroma of the final product. Yeast is more resistant to acidic conditions than mold. Halm *et al.* (2004) reported that yeast has a high tolerance for lactic acid. Even *Candida crusei* found in corn fermentation for ogi production can stimulate the growth of *Lactobacillus plantarum*. The decrease in the pH value of AC yeast is caused by the activity of molds and amylolytic yeasts that hydrolyze amylose into sugar which then becomes organic acid.

Yeast used as a starter culture is also reported to produce various enzymes, for example *Kodamae ohmeri* produces phytase enzymes in grains (Li *et al.*, 2008) and

Table 4. The ANOVA test results in the optimum formulation response of drying AC culture

Response	Actual	Prediction	SE mean	95% CI low	95% CI high
Viability (log colony/g)	6.4771	6.8687	0.54	5.66	8.08
Water content (%)	6.02	5.62	0.31	4.96	6.28
a _w	0.343	0.303	0.031	0.24	0.37
pH	4.17	4.18	0.11	3.95	4.41

lipase (Bussamara *et al.*, 2010); *Candida famata* produces glucoamylase (Mohammed, 2007) and lipase and protease enzymes (Wojtatowicz *et al.*, 2001); whereas *Candida krusei* has lipolytic, esterase and amylolytic activity which contributes to the final flavor of food products. Yeast which has lipolytic activity acts as a fatty acid precursor and contributes significantly to the flavor of the final product. Amylolytic yeast can cut complex compounds from starch and oligosaccharides into simple sugars that can improve the nutritional quality of the material, because it becomes easier to digest and plays an important role in the aroma, flavor, taste and structure of the final product (Omemu *et al.*, 2007).

Numerical optimization results obtained from a solution of maize flour drying formula with desirability value for AC 0.729. Desirability value is a parameter that showed the best optimization results with a range of values 0–1. The closer it is to 1, the solution recommended by the program is able to fulfill the desires according to predetermined criteria (Myers *et al.*, 2009). The results of the ANOVA test for the four responses are presented in Table 4.

Table 4 shows that the actual value obtained from the four responses on the AC line is still in the range of 95% confidence intervals (CI). The suitability between predictions and measurement results shows that the model used is verified and quite consistent. The graph of the relationship between the results of verification between the combination of temperature and duration of drying with AC viability is shown in Figure 3, where the graph of the relationship with moisture content is presented in Figure 4. The combination of corn flour drying formula chosen by the program for AC was 50°C for 48 hours.

4. Conclusion

AC-indigenous cocktail yeast mold culture is expected to contain indigenous yeast molds with optimum quality characters that will be used to ferment local white corn grits. The viability, water content, a_w, and pH responses were measured to optimize 16 formulas. The optimization and verification resulted the following model:

$$\text{pH (AC)} = -0.058A - 1.56 \times 10^{-003}B + 7.13$$

Where A = drying temperature (°C), B = drying time (h). The AC optimization was achieved at a combination of drying temperatures of 50°C for 48 hrs. Desirability values are 0.729. The optimum formula for AC has viability of 7.39×10^6 CFU/g, moisture content of 5.62%, a_w 0.303, and pH 4.18.

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