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FR-2019-247

Food Research <foodresearch.my@outlook.com> Sun, Jul 7, 2019 at 5:38 PM
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Dear Dr. Rahmawati,
Thank you for submitting your manuscript for consideration for publication in Food Research.
Best regards
Son Radu
Chief Editor

From: Rahmawati, ST, M.Si. Dr. <rahmafarasara@usahid.ac.id>
Sent: Sunday, 7 July, 2019 12:09 AM
To: foodresearch.my@outlook.com
Cc: Rahmawati Farasara; ddsaputra87@gmail.com; dashcbdk@gmail.com; yanti rm
Subject: Manuscript Submission

Dear Prof. Son Radu, PhD.
Chief Editor
Food Research

I hereby submit a new manuscript entitle "DRYING PROCESS OPTIMIZATION OF 'INDIGENOUS COCKTAIL YEAST MOLD' CULTURE USING RESPONSE SURFACE METHODOLOGY (RSM)" for consideration by the Food Research. I confirm that this work is original and has not been published elsewhere nor is it currently under consideration for publication elsewhere.
I enclose herewith cover letter, manuscript submission form filled, ms word manuscript, proof read of manuscript, and the plagiarism check.
I hope this manuscript can be published in Food Research journal.
Thank you for your kindness.

Best regards,

Dr.Rahmawati.

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Date : 7nd July 2019

Manuscript ID : FR-2019-247

Please return by : 7nd August 2019

Title of Manuscript : DRYING PROCESS OPTIMIZATION OF "INDIGENOUS COCKTAIL YEAST MOLD" CULTURE USING RESPONSE SURFACE METHODOLOGY (RSM)

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Evaluation Criteria	Grade				
	A (Excellent)	B	C	D	E (Worst)
1. Appropriateness of Contents			x		
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4. Research Methodology			x		
5. Data Analysis				x	
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(REVIEWER'S SECTION) REVIEWER'S COMMENTS/SUGGESTIONS		(AUTHOR'S SECTION) AUTHOR'S ACTION/RESPONSE *NOTE FOR AUTHOR: Please state your response to the reviewer's comments/suggestion below
1.	**Title** …OPTIMIZATION OF "INDIGENOUS COCKTAIL YEAST MOLD" CULTURE… Quotient mark in the title should be deleted	
2.	**Abstract** It is better to write "AC2" in a complete form to give an understanding to reader, since an abstract might appears separately form the manuscript Abstract is lack of conclusion and suggestion	
3.	**Keywords** *Min. 3 and Max. 6*	
4.	**Introduction** – Authors try to optimize condition of making dried indigenous culture for fermenting corn flour. However, authors elaborate more on characteristic of corn flour and RSM method rather than focused on the intended topic. Authors must provide previous research reports related to the optimization of drying process of starter culture. – Problem statement is not well described, the author claimed that the use of starter culture required special expertise and thus suggested the use of dried starter culture. However it is a common practice in selling a starter culture for home or industrial used	

5.	**Research design/Methodology** – How did the author ensure that the culture viability is adequately recorded by the hemocytometer method since it can't differentiate between life and died cell. – *Penicillium citrinum*, *Aspergillus niger, Acremonium strictum,* and *Candida famata* were used as starter culture. The authors should describe the reason behind that decision? Did the author considered the interaction among those microorganisms in a mix-culture system or just mixed those four culture because of their amylolitic properties? – Furthermore, does the use of A. niger considered as safe? – Is the CFU/mL is an appropriate unit for hemocytometer observation? – Did the author count molds in the form of their spore?	
6.	**Data Analysis** – Discussion seems more focus on the minimal requirement of culture number for fermentation process, the authors must discuss on how the drying process affecting the culture viability – The author should observe the number of starter culture before drying process to see whether drying process or competition among the cultures affects the viability – Microorganisms in a dried starter culture is considered in a dormant (preserve) stage and it is expected to grow easily during reactivation when being applied in a fermentation process, thus it is not suitable to compare the Aw result with the required aw for growth as discussed in manuscript.	

	– At page 8, author implied that yeast play an important role in regulating pH value of the system, while its only contribute a quarter number from all the four microorganisms in starter culture. How did the author sure that the yeast over compete the growth of other culture. Moreover, the author didn't differentiate the count among the culture. – Many scientific names within the manuscript are not written in italic form	
7.	**Conclusion** – Conclusion should not contain method and hypothetical statement as seen within the manuscript – Suggestion is also lack in the conclusion part of this manuscript	
8.	**References** References are written in inconsistent format. Some journal article references missing their page number. The author must follow strictly the journal's format for references	
9.	**English Proficiency** The manuscript is written in poor English. The text must be subjected to language correction, many grammatical errors were found within the manuscript. It must be proof read by professional English editor before the manuscript be further reviewed and accepted.	
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Overall Evaluation
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2. Originality of Topic		√			
3. Manuscript Format		√			
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5. Data Analysis		√			
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(REVIEWER'S SECTION) REVIEWER'S COMMENTS/SUGGESTIONS		(AUTHOR'S SECTION) AUTHOR'S ACTION/RESPONSE *NOTE FOR AUTHOR: Please state your response to the reviewer's comments/suggestion below
1.	**Title** *It should reflect the article* The title is worth mentioning what factors are optimized	
2.	**Abstract** *Background, Aim, Methodology and Conclusion* The background is too long, while the results are less clear and the implications of the results do not yet exist	
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OPTIMIZATION OF TEMPERATURE AND DRYING TIME OF INDIGENOUS COCKTAIL YEAST MOLD CULTURE USING RESPONSE SURFACE METHODOLOGY (RSM)

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 h and the drying temperature was between 40 - 50 ^{o}C. The total of 16 formulas of 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: pH (AC) = -0.058A - 1.56 x 10^{-003}B + 7.13, where A = drying temperature (^{o}C), B = drying time (h). The AC optimization was achieved at a combination of drying temperatures and time of 50 ^{o}C for 48 hours. Desirability values were 0.729. The optimum formula for AC has a viability of total yeast mold of 7.39 x 10^{6} CFU / g, moisture content of 5.62%, a_w 0.303, and pH 4.18.

Keywords: cocktail yeast mold, dried culture, drying temperature and time, optimization, RSM

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 et al. (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 consist of indigenous yeast and mold. 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. 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 process and formulation optimization (Myers *et al*. 2009). In making dried cocktail starter 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 are the viability of microorganisms (total mold and yeast), moisture content, aw, 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. 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 *Penicillium citrinum*, *Aspergillus niger, Acremonium strictum,* and *Candida famata*, namely AC. The microorganisms used were previously isolated and identified from a spontaneous fermentation of corn grits (Rahmawati *et al.* 2013).

2.2 Culture Preparation and Enumeration

One 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 scrapping, 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 (Modified Rahmawati *et al.* 2013 and Rahmawati *et al.* 2017)

AC-indigenous cocktail yeast mold culture consists of indigenous amylolytic 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 10^6 CFU / mL per microorganism, then piped as much as 10% of the amount of water used. After that, all stir until homogeneous and put $\pm$ 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% alkolhol. 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 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 (^{o}C). The drying time is between 24 - 48 h and the drying temperature is 40 - 50 ^{o}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.

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 scale of interest 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.

3. RESULTS AND DISCUSSION

3.1 Optimization of Drying Process Parameter and Verification of the Model

Tables 2 showed that the model can describe the relationship of drying process parameters (drying temperature and time) to viability, moisture content, water activity, and pH value, 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. The model also has good that show in the precision adequacy values (Adeq. Precision> 4).

3.2 Yeast Mold Viability

The AC indigenous cocktail yeast mold culture has a viability value ranged from log 4.2041 - log 8.1139 cfu/g. Figure 1 showed that the viability of yeast molds tends to decrease with higher temperature 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 ^{o}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 h, has yeast mold availability as log 7,66 CFU/g. Beside that, the results are in line with Oliveira et al. (2002) where the minimum number of microorganisms for the starter is 10^6 CFU/mL. Yuliana and Rizal (2006) stated that the ideal conditions for using a starter culture as an inoculum is equal to $2.1x10^7$ CFU/g. As a compare, the CC indigenous cocktail yeast mold culture has a viability 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 (Fig. 1). The viability response model (AC) has an F value of 4.12 with a p value (Prob> F) was 0.0272. It mean's 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 culutre consist 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 may be there were competition for nutrients by microorganisms varies.

3.3 Water Content

The moisture content of AC-indigenous cocktail yeast mold culture ranges from 4.37 - 7.33%. Figure 2 showed that the percentage of moisture content tends to decrease with the higher temperature and longer drying time. Rasulu *et al.* (2012) stated that the water content value is influenced by drying process, because the drying process facilitate 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 (Fig. 2). This model response has F value 9.31 with p value (Prob>F) was 0.0031 that is significant by α = 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). Significant lack of fit values illustrate model not suitability with responses.

3.4 Water Activity

The a_w value for AC-indigenous cocktail yeast mold culture ranges from 0.262 - 0.464. Figure 3 showed that the a_w value tends to decrease with the higher temperature and longer drying time. This result 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). Product that have a lower a_w value will have a longer shelf life because microorganisms can only live in certain aw conditions. In general, yeast molds can live at certain minimum aw values. Aspergillus lives at a minimum of aw 0.78, Penicillium 0.88, and yeast can generally live at aw 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. Base on 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 (Fig. 3). This model showed that the response is 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:

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

a_W = water activity A = drying temperature (^oC) B = drying time (h)

This model has F value 4.06 with p value (Prob>F) is smaler than 0.05 (0.0427). It showed that the model is significant with α = 0.05, which means it can desribe that the aw response quite well. The ANOVA results also showed that the drying temperature and time had a significant effect on aw response with insignificant lack of fit values which were greater than 0.05 (0.3158). The value of lack of fit the value of lack of fit is greater than 0,05, which indicates that it is not significant which describes the model mismatch with the response (Keshani *et al*. 2010). The equation shows that the effect of temperature and drying time is inversely proportional to water activity. That is, the drying temperature and time higher can cause the water activity lower. While the interaction between drying temperature and time is inversely proportional or decreases the value of water activity which is indicated by negative values of constants.

3.5 The pH Value

The pH value of AC-indigenous cocktail yeast mold culture ranges from 4.12 - 5.05. Figure 4 showed that the pH value tends to decrease with the higher temperature and 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 is 4.37, 3.43, and 3.93 respectively in 96 hours 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 capable of fermenting nutrients contained in food will 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 reduce the pH value.

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 <0.0500 (Figure 4). The RSM equation or model for optimization of pH for AC-indigenous cocktail yeast mold culture is as follows:

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

A = drying temperature (^oC); B = drying time (h)

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 insignificant lack of fit values which were greater than 0.05 (0.2709). This 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*. Decreasing the pH value of CC yeast due to the activity of amylolytic molds which hydrolyzes amylose to sugar which will further disnite into organic acid and yeast that produces 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 lipase (Bussamara *et al*. 2010); Candida famata produces glucoamylase (Mohammed 2007) and lipase and protease enzymes (Wojtatowicz *et a*l. 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 3 showed that the actual value obtained from the four responses on the AC line is still in the range of 95% Confidence Intervals Low and 95% Confidential Interval High. Confidence Interval (CI) is a range that shows the lowest and highest values of the predicted range of the average results of actual measurements at the 95% significance level. 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 ^{o}C for 48 hours.

Conclusion

AC-indigenous cocktail yeast mold culutre 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, aw, and pH responses were measured to optimize 16 formulas. The result of optimization and verification is obtained by the model:

pH (AC) = -0.058A - 1.56 x 10^{-003}B + 7.13, where A = drying temperature (^{o}C), B = drying time (h). The AC optimization was achieved at a combination of drying temperatures of 50 ^{o}C for 48 hours. Desirability values are 0.729. The optimum formula for AC has a viability of 7.39 x 10^{6} CFU / g, moisture content of 5.62%, aw 0.303, and pH 4.18.

Acknowledgement

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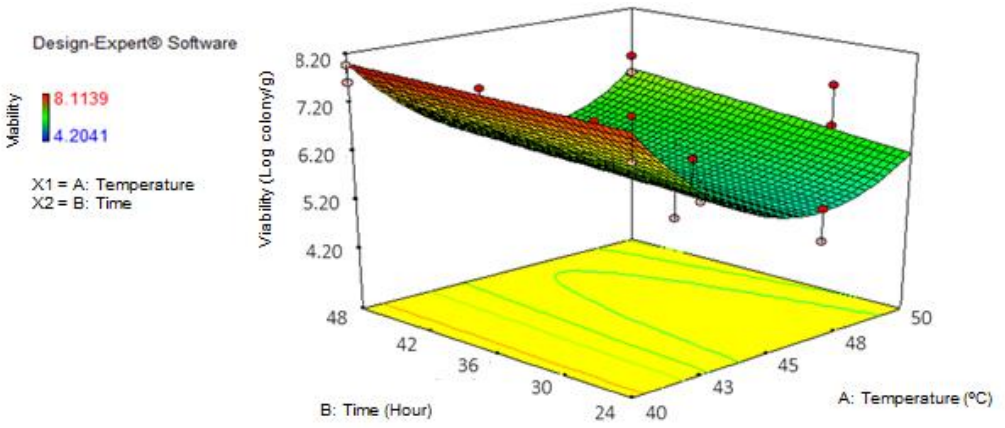

Figure 1. A 3D Graphical combination between Temperature and Drying Time to the viability of AC starter.

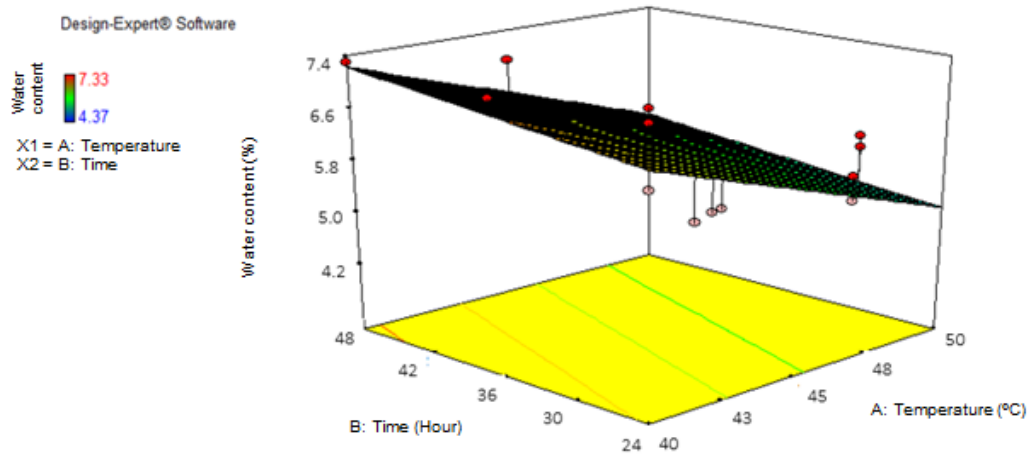

Figure 2. A 3D Grapihical combination between Temperature and Drying Time to the Moisture Content of AC starter

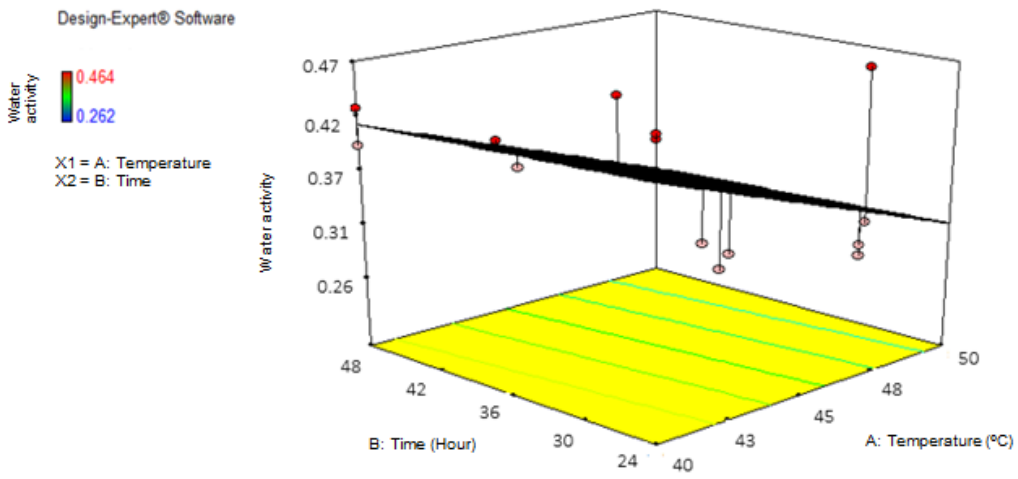

Figure 3. A3D Grapihical combination between Temperature and Drying Time to the Aw (water activity) of AC starter

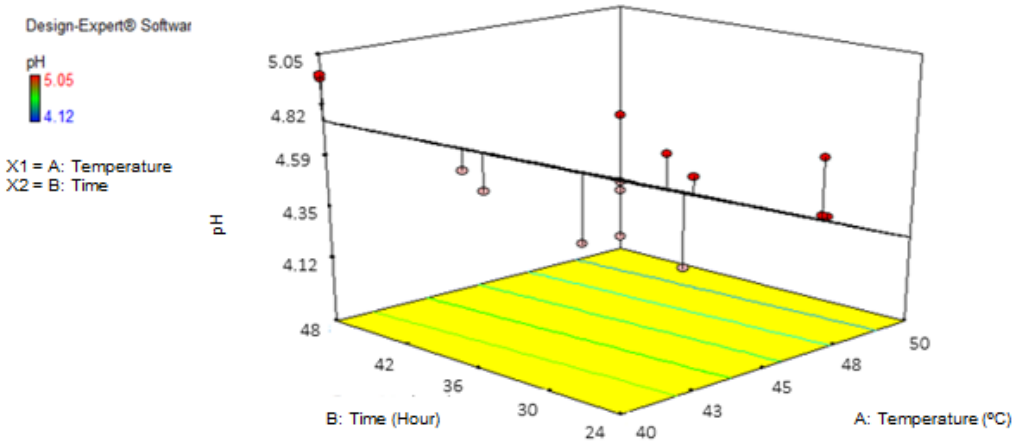

Figure 4. A 3D Grapihical combination between Temperature and Drying Time to the pH value of AC starter

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

Table 2. The math model of drying proses parameter as a response of the AC indigenous coctail 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 (%)	Linier	0.0031	0.0375	0.5255	0.4423	7.680
Water activity (a_w)	Linier	0.0427	0.3158	0.2897	0.1237	4.622
Degree of acidity (pH)	Linier	0.0032	0.2709	0.5234	0.3883	6.721

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

Table 3. The viability, water content, aw 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}

*Samples means with same superscripts in the same column are not significantly at α = 0.05

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

Responce	Actual	Prediction	*SE mean*	95% CI *low*	95% CI *high*
Viability (log koloni/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

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Manuscript FR-2019-247

Rahmawati Farasara <rahmafarasara@gmail.com> Mon, Sep 30, 2019 at 12:36 AM
To: Food Research <foodresearch.my@outlook.com>

Dear Prof. Son Radu, PhD.
Chief Editor
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I hereby submit the manuscript entitled "Optimization of Temperature and Drying Time of Indigenous Cocktail Yeast Mold Culture Using Response Surface Methodology (RSM)". There is a little bit change to the title. I added the words "temperature and drying time" to clarify the research parameters.
I have tried to accommodate reviewer comments. Would you like to inform me,
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Thanks & Regards,
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Before we can proceed with the article production, I would like to clarify a few points that I have commented in the manuscript. Please refer to the attachment. Please address the issues raised in the comments.

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