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Article

Application of the RSM-CCD Method in Making Mozzarella Cheese Using Pineapple Juice and Coagulation Time

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Abstract: Mozzarella cheese is a type of fresh cheese which is characterized by being elastic, fibrous and soft. This research was carried out using the RSM-CCD (Response Surface Methodology Central Composite Design) method which aims to determine the optimum conditions for coagulation time and pineapple juice concentration in making mozzarella cheese with a combination of 13 treatments obtained from *the expert-13 design software*. The research method uses two factors, namely coagulation time with a coagulation time limit of 25-35 minutes and pineapple juice concentration with a limit of 2.5-3.5%. The responses used are *curd weight* , water content, and elongation using a quadratic model. The research results showed that the optimal treatment combination was a coagulation time of 25 minutes with a pineapple juice concentration of 2.5%, the resulting desirability value was 57.4%. From the solution results, an estimate of the maximum response was obtained with an average value of *curd weight* of 248 grams, water content of 41.55%, and elongation of 17.95 inches. The confirmation results obtained an average of *curd weight data* of 244.5 grams, water content of 38.97%, and elongation of 17 inches. The average confirmation test data for each response showed that the results were in accordance with the predicted value with a confidence interval ratio of 95%.

Keywords: mozzarella cheese; RSM-CCD; coagulation; pineapple juice

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1. Introduction

Cheese is a product made from milk which is obtained through the process of coagulating the milk and separating the whey from the milk. One of the cheeses that is widely consumed is mozzarella cheese [1]. Mozzarella cheese is a type of soft cheese that is not ripened. The characteristics of mozzarella cheese are elastic, fibrous and soft. Mozzarella cheese is included in the "pasta-filata" cheese group, namely cheese that is heated and pliable during the manufacturing process. This cheese is classified as a type of fresh cheese which is usually used as a topping in making pizza [2]. Coagulation is the process of clumping milk casein protein which produces curd and whey as the final product. The curd formed is then processed into cheese. Coagulation can be carried out using acid, enzymes or with the help of a starter in the form of lactic acid bacteria [3]. In making mozzarella cheese, the enzyme commonly used is the rennet enzyme which contains proteolytic enzymes (proteases) [4]. The rennet enzyme obtained from the stomach of calves is increasingly expensive because it is difficult to obtain. Therefore, a

more affordable alternative protease enzyme is needed as a substitute for the rennet enzyme [5].

One of the protease enzymes that is commonly used as an alternative to rennet enzymes in the food industry is bromelain and papain [6]. The bromelain enzyme is a protease enzyme that is able to hydrolyze protein peptide bonds into smaller molecules, namely amino acids, making them easier for the body to digest. Pineapple is a fruit that has a high content of amino acids and bromelain. The specific activity of the bromelain enzyme in pineapple is 5-10 U/mg protein [7]. Pineapple is a horticultural commodity that grows widely in tropical and subtropical areas and is widely cultivated in Indonesia. Ripe pineapple fruit is generally bright yellow and has a fresh taste [8]. In 0.1 ml of pineapple stem juice there is a bromelain content of 0.095 mg [9]. Research related to cheese production using natural pineapple extract is still very limited, and can produce cheese that is soft, melts easily (high meltability), stretches easily (good stretchability) and forms fibers when stretched. so it is suitable as a pizza topping or spreadable cheese[10].

One of the main factors in the cheese coagulation process is the coagulation time and enzyme concentration used. The time taken for coagulation is very important from the addition of rennet to the formation of curd [11]. The time used in coagulation is also influenced by the enzyme concentration used. The difference in rennet concentration used causes the weight of the curd produced to be different. The coagulation temperature must be maintained to influence the quality of the cheese produced [12]. Optimization in making mozzarella cheese by using pineapple juice and coagulation time can be done using the Response Surface Methodology method. The RSM method with Central Composite Design (CCD) can help determine the number of experiments required which are then evaluated for response and variable optimization. It has three different points, namely the factorial point, axial point and central point [13], [14]. This research aims to determine the optimal results of making mozzarella cheese using pineapple juice using two factors, pineapple juice concentration and coagulation time.

2. Materials and Methods

2.1 Materials

The ingredients used are fresh milk from the SMK PP Wiyata Bakti Dau dairy farm, fresh pineapple with the characteristics of a slightly oval shape and yellowish green skin, citric acid, table salt (NaCl) and trisodium citrate (emulsifier).

2.2 Methods

This study used the Response Surface Methodology with a Central Composite Design (RSM-CCD) via Design Expert-13 software. The optimization stages included research design, making mozzarella cheese from pineapple juice, response model analysis, optimization, and verification. The study was conducted from November 2023 to February 2024. Mozzarella cheese was produced and physically tested at CV. Brawijaya Dairy Industry, Malang Jl. Sidomakmur No.26, Sengkaling, Mulyoagung, Kec. Dau, Malang Regency, East Java 65151. Moisture content testing was done at Jember State Polytechnic Analysis Laboratory.

2.2.1. Research Design

The factors used are coagulation time (A) in the range of 25-35 minutes and pineapple juice concentration (B) in the range of 2.5-3.5% with the responses used curd weight (Y1), water content (Y2) and elongation (Y3). Based on the factors and responses, 13 experiments were obtained from the design expert application which can be seen in Table 1.

Table 1. RSM-CCD Experimental Design Results

Std	Run	Coagulation Time (Minute)	Pineapple Juice Concentration (%)	Curd Weight (Grams)	Water Content (%)	Elongation (Inches)
11	1	30	3			
6	2	37.0711	3			
7	3	30	2.29289			
13	4	30	3			
1	5	25	2.5			
2	6	35	2.5			
5	7	22.9289	3			
12	8	30	3			
10	9	30	3			
9	10	30	3			
4	11	35	3.5			
3	12	25	3.5			
8	13	30	3.70711			

Source: Prosesed data, 2023

2.2.2. Making mozzarella cheese from pineapple juice

Making mozzarella cheese from pineapple juice begins by making pineapple juice by mashing the pineapple and then taking the pineapple juice. Next, the milk is heated to a temperature of 35°C and mixed with a solution of citric acid and pineapple juice, then stir until lumps form. Curd is cut, dried, and salt and sodium citrate are added. The processof cooking curd is done by steaming or cooking the curd in a pan placed on top of a pan filled with water at a temperature of 75-85°C. After that, the mozzarella cheese was storedfor ± 24 hours in the freezer before testing.

2.2.3. Response model analysis

The response model analysis was carried out using ANOVA to determine the optimal conditions in the research used. This research uses Design Expert-13 software with an RSM-CCD design. The results of the response values obtained are entered into the software so that the optimum point formula prediction output is obtained.

2.2.4. Optimization

Optimization is aimed at obtaining the perfect response from the best independent variables. Optimization is carried out to reduce costs and time used. Response Surface Methodology will perform optimization based on input of response measurement data and variable data. The desirability value is an optimization objective function value with a range of 0 to 1 which can indicate the program's ability to fulfill desires based on the specified criteria [15].

2.2.5. Verification

The verification process ensures the accuracy of predicted results by comparing actual values with previously obtained predictions. Two types of intervals are used: a 95%

confidence interval for expected average results and a 95% prediction interval for expected results under the same conditions. The verification results are then used to select the best predicted combination and proceed with further processing. [16].

2.3 Observation Parameter

Mozzarella cheese testing parameters that will be tested include curd weight, water content and elongation.

a. Curd Weight

The curd obtained from the coagulation process is weighed on a digital scale to determine the weight of the curd produced in each sample. Record the curd weight results obtained and enter them into the expert-13 design software for analysis.

b. Water Content (AOAC, 2012)

The first step was to dry the aluminum cup in an oven for 1 hour, then cool it in a desiccator for 30 minutes and weigh it. A cheese sample of 3 grams was weighed in a cup, then the cup was placed in the oven at 105°C for 20 hours. After that, the cup was cooled in a desiccator for 1 hour and weighed to obtain a constant dry sample weight. The percentage of water content is calculated using the following formula:

$$\text{Water Content } (\%) = \frac{(Initial\ Weight - Final\ Weight)}{Initial\ Weight} \text{ x } 100\%$$

c. Elongation

The mozzarella cheese sample was cut into 3x3 cm pieces and placed on a small heat-proof plate. Then put it in the microwave at medium temperature for 1.5 minutes. After that, spread the melted mozzarella cheese with a fork and measure it using a ruler or meter in inches.

3. Results and Discussion

The results of the analysis of observation parameters in making pineapple juice mozzarella cheese can be seen in Table 2. The results of the ANOVA on the response of curd weight, water content and elongation can be seen in Table 3.

Table 2. Response Analysis Results

Run	Coagulation Time (Minute)	Pineapple Juice (%)	Curd Weight (Grams)	Water Content (%)	Elongation (Inches)
1	30	3	218.3	37.1143	12.333
2	37.0711	3	196.1	38.5271	16.833
3	30	2.29289	256	42.2143	12
4	30	3	217.1	40.114	13.833
5	25	2.5	246.4	39.8749	15.5
6	35	2.5	224.5	40.3285	18.5
7	22.9289	3	212.7	40.0056	27
8	30	3	210.4	40.5382	8.667
9	30	3	210.9	39.7101	14.267
10	30	3	216.5	37.2809	8.967
11	35	3.5	206	45.6548	16.667
12	25	3.5	188.6	39.0347	15.667
13	30	3.70711	210.3	37.3199	8.667

Source: Prosesed data, 2023

Tabel 3. Response ANOVA Results

Parameter	Curd Weight (Grams)	Water Content (%)	Elongation (Inches)
Model	<0,0001	0,6770	0,0407
Lack of Fit	0,2888	0,1034	0,2465
R^2	0,9675	0,3142	0,7559
Adj. R^2	0,9442	-0,1757	0,5815
Predicted R^2	0,8458	-2,9422	-0,2057
Adeq.precision	21,7297	2,5649	6,6771
Information	*Significant*	*Not significant*	*Significant*

Source: Prosesed data, 2023

note: results are significant if model p<0.05; lack of fit is not significant p<0.05; predicted R^2 and Adjusted R^2 have a difference <0.2; Adeq.precision >4

3.1 Curd weight

The test results for making mozzarella cheese from pineapple juice showed that the best results were in run 3 with a coagulation time of 30 minutes and a pineapple juice concentration of 2.29% giving the largest curd weight (256 grams). The data were then used to develop a quadratic model that predicted 94.42% of the curd weight response. The ANOVA results show that the model is significant (p-value < 0.05) and does not have a lack of fit (P> 0.05). The Predicted R^2 and Adjusted R^2 values also comply with the requirements [13].

The final equation with the actual factors of the quadratic model of the curd weight response (Y_1) which is influenced by coagulation time (A) and pineapple juice concentration (B) is as follows:

$$Y_1 = 800.6 + 1.238\,A - 360.8\,B + 3.93\,AB - 0.23\,A2 + 34.62\,B2$$

Information :
Y_1= Curd Weight; A= Coagulation time; B = Concentration of pineapple juice

The results of the contour plot graph and 3D graph of the curd weight response can be seen in Figures 1 and 2.

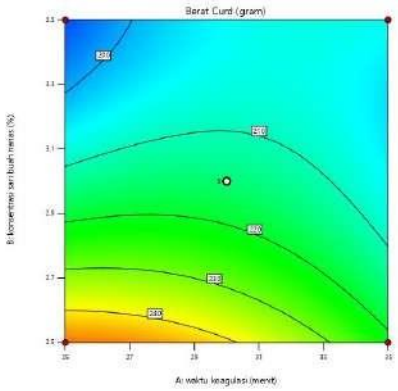

Figure 1. Surface Contour Plot Graph of Curd Weight Response

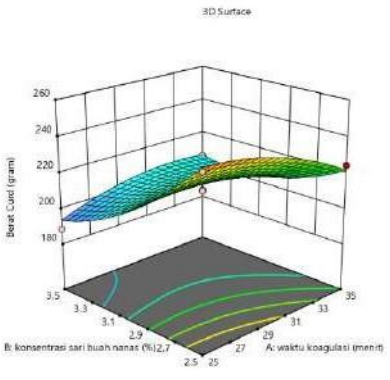

Figure 2. 3D Graph of Curd Weight Response

The contour plot is a two-dimensional depiction of the response presented using a prediction model for concentration values [17]. The contour plot graphic results of the curd weight response in Figure 1 show gradated blue, green, yellow and orange colors. The bluish colored area shows the lowest water content response result and the highest curd weight response result is in the slightly orange colored area. The results of the 3D graph of the curd weight response in Figure 2 show that the lower the concentration of pineapple juice used in making mozzarella cheese, the more curd will be produced. The weight of the curd obtained ranged from 188.6 grams - 256 grams.

3.2 Watercontent

The test results for making mozzarella cheese from pineapple juice showed the highest water content value was 45.65% and the lowest was 37.1143%. Water content response data was used to obtain optimal coagulation time variables and pineapple juice concentration. However, the ANOVA results show that the model is not significant (p-value 0.6770) and the F value is 0.6414. The R^2 value of 0.3142 and Adjusted R^2 -0.1757 also shows that the factors studied have no real effect on the water content response. The Adeq precision value of 2.56 is also not in accordance with the requirements [13].

The actual equation of the quadratic model of the water content response (Y_2) which is influenced by coagulation time (A) and pineapple juice concentration (B) is as follows:

$$Y_2 = 143.6 - 3.12A - 39.13B + 0.61AB + 0.02A2 + 3.34B2$$

Information :
Y_2= Water content; A= Coagulation time; B = Concentration of pineapple juice

The results of the contour plot graph and 3D graph of the water content response can be seen in figures 3 and 4.

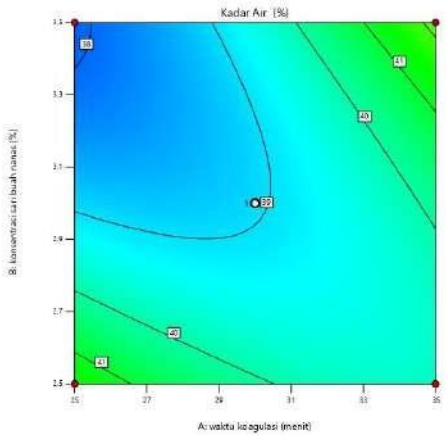

Figure 3. Surface Contour Plot Graph of Water Content Response

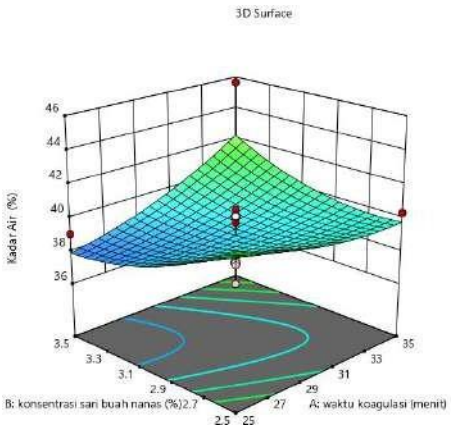

Figure 4. 3D Graph of Water Content Response

The results of the contour plot graph of the water content response can be seen in Figure 3 showing gradated green and blue colors. The light blue color in the middle of thecontour plot graph shows the lowest water content response, namely 37.11%, and the greencolor at the top right of the contour graph shows the highest water content response,

namely 45.65%. Figure 4 shows a 3D graph of the water content response. The water content response value obtained was in the range of 37.11% - 45.65%.

3.3 Elongation

The test results for making pineapple juice mozzarella cheese showed that the highest elongation value for mozzarella cheese was 27 inches and the lowest was 8.667 inches. Heated mozzarella cheese can melt completely at 232°C and has elongation characteristics of ≥ 3 inches. So the results obtained from the elongation response are very good because they are above the USDA standards (2005) [12].The ANOVA results show that the optimization model for coagulation time and pineapple juice concentration is significant (p-value 0.0407) and has a probability of error of 4.07%. The R^2 value of 0.7559 and Adjusted R^2 0.5815 shows that the factors studied have a real effect on the elongation response of 77.59%. The Adeq Precision value of 21.7297 also meets the requirements, namely greater than 4 [13].

The actual equation of the quadratic model of the elongation response (Y_3) which is influenced by coagulation time (A) and pineapple juice concentration (B) is as follows:

$$Y_3 = 176.8-12.29A+17.01B-0.20AB+0.21A2-2.1B2$$

Information :

Y_3= Elongation; A = Coagulation time; B = Concentration of pineapple juice

The results of the contour plot graph and 3D graph of the elongation response can be seen in figures 5 and 6.

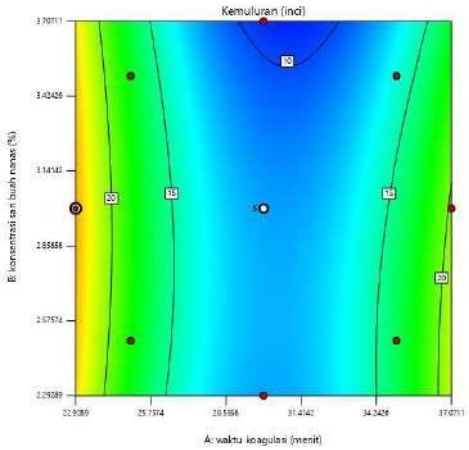

Figure 5. Surface contour graph plot of elongation response

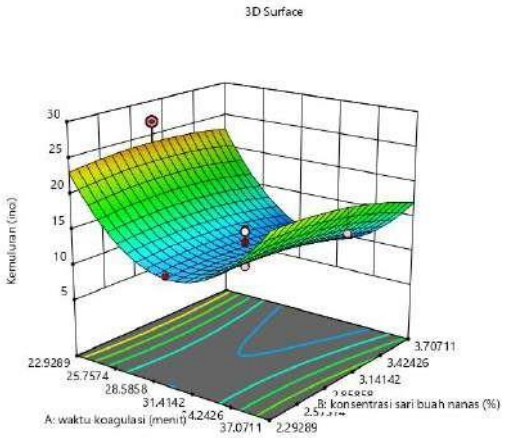

Figure 6. 3D graph of elongation response

The results of the contour plot graph of the elongation response can be seen in Figure 4.3 showing the gradated colors of blue, green, yellow and orange. The orange color in the graph shows the highest elongation response, namely 27 inches. Meanwhile, the dark blue color in the graph shows the lowest elongation response, namely 8.667 inches. The results of the 3D graph of the elongation response in Figure 4.6 show that the orange color is located at a coagulation time of 22.9 minutes and a pineapple juice concentration of 3% has an elongation result of 27 inches. Meanwhile, the lowest elongation value was dark blue at a coagulation time of 30 minutes and a pineapple juice concentration of 3.7%.

3.4 Optimization in Making Mozzarella Cheese with Pineapple Juice

Optimization process stage, optimum treatment conditions will be recommended based on the response surface method. The optimal factors and response points are determined based on the desired variable values and criteria. The criteria considered are in range, in target, maximize, and minimize. The desired importance value is in the range 1 (+) to 5 (+++++). The aim of optimization is to determine the best factor values that produce optimum response values [16]. The desired optimization criteria for each factor and the responses are presented in Table 4.

Table 4. Optimization Criteria

Name	*Goal*	*Lower Limit*	*Upper Limit*	*Lower Weight*	*Upper Weight*	*Importance*
Waktu Koagulasi	*is in range*	25	35	1	1	3
Konsentrasi Sari Buah Nanas	*is in range*	2,5	3,5	1	1	3
Berat *Curd*	*maximize*	188,6	256	1	1	4
Kadar Air	*minimize*	37,1143	45,6548	1	1	3
Kemuluran	*maximize*	8,667	27	1	1	5

Source: Prosesed data, 2024

The criteria used in this research were to determine the optimal coagulation time point and pineapple juice concentration in making mozzarella cheese. The in range criterion is used to determine the coagulation time and concentration of pineapple juice, with the aim of obtaining results that comply with the predetermined limits. The maximalize criterion is used to determine the curd weight and elongation of mozzarella cheese, with the aim of producing maximum mozzarella cheese in accordance with USDA (2005) [12]. Meanwhile, minimum criteria are used to determine the moisture content of mozzarella cheese, with the aim of producing mozzarella cheese with low moisture content.

Table 5. Selected Predicted Values

Coagulation Time (Minute)	**Pineapple Juice Concentration (%)**	**Curd Weight (Grams)**	**Water Content (%)**	**Elongation (Inches)**	**Desirability**	
25,00	2,50	248	41,55	17,95	0,574	*Seleected*

Source: Prosesed data, 2024

The optimal point of coagulation time and pineapple juice concentration that produces the best response according to the criteria and level of importance is the combination of a coagulation time of 25 minutes with a pineapple juice concentration of 2.5%. This combination was chosen because its desirability value is close to 1, namely 0.574 [15].

3.5 Validasi Data

The results of the optimization carried out showed that the selected solution was a coagulation time of 25 minutes and a pineapple juice concentration of 2.5% with the predicted average response data being a curd weight of 248.5 grams; water content 41.58%; and elongation of 17.95 inches. Then the results were confirmed by making mozzarella cheese with 3 repetitions of testing to check the research results. Confirmation results can be seen in Table 6.

Table 6. Confirmation results data

Treatment	Curd Weight (Grams)	Water Content (%)	Elongation (Inches)
U 1	244,5	39,09	16
U2	244,5	39,16	15
U3	244,5	38,66	20
Average	244,5	38,97	17

Source: Prosesed data, 2024

Note: U1, U2, U3 were obtained from the best prediction results which were confirmed by carrying out 3 repetitions of the test with the same sample.

The experimental results were analyzed using the predicted values with a 95% confidence level in the Design Expert program [11]. This confidence level represents the probability of the predicted range value. The results were compared to confirm the optimization outcomes. Comparison of optimization results with confirmation results can be seen in Table 7.

Table 7. Comparison of Confirmation Data and Prediction Results from Design Expert-13

Response	Data Average	Lowest Prediction	Prediction	Highest Prediction
Berat Curd (g)	244,5	235,60	248,51	261,42
Kadar Air (%)	38,97	34,10	41,59	49,08
Kemuluran (inchi)	17	8,28	17,94	27,61

Source: Prosesed data, 2024

Note : Confidence level = 95%

The results of comparing confirmation data and prediction results show that the difference in average data does not exceed or is not below the predicted value. Based on the comparative data that has been obtained, it can be seen that the confirmation results are not significantly different from both the test results and the prediction results. Therefore, the results of the response surface methodology design show valid results. So it can be said that this combination of treatments can be used to obtain good coagulation results and produce good quality pineapple juice mozzarella cheese.

4. Conclution

The use of Response Surface Methodology (RSM) optimized coagulation time and pineapple juice concentration to make mozzarella cheese, resulting in a quadratic model for curd weight, water content, and elongation. The optimal process combination, with a 25-minute coagulation time and 2.5% pineapple juice concentration, yielded a desirability value of 0.574. The predicted maximum response showed average values of 248.51 grams for curd weight, 41.59% for water content, and 17.94 inches for elongation. The confirmation and prediction values showed no significant difference at a 95% confidence

level, indicating that the RSM design can produce high-quality pineapple juice mozzarella cheese with good coagulation results.

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