

Optimization of Formulation and Operational Conditions of Vacuum Drying for Making Commercial Acacia Honey Powder: Study of the Chemical Quality of Honey

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Abstract: Acacia liquid honey can be processed into powdered honey using vacuum drying. The aim of this research is to obtain optimal conditions for powdered honey formulation consisting of the percentage of liquid honey and operational conditions, namely drying temperature. To obtain this, chemical tests are carried out, namely reducing sugar, diastase enzyme, and IC50. This research uses the Design Expert 12 application with the RSM (Response Surface Method) method, namely CCD (Central Composite Design) with 2 factors and 3 responses. The results of processing powdered honey obtained optimal results at a liquid honey percentage of 70% compared to maltodextrin of 30% and a drying temperature of 55°C. Chemical testing obtained optimal results for reducing sugar of 68%, diastase enzyme of 3.35, and IC50 of 18.88 mg/ml.

Keywords: optimization, acacia powdered honey, chemistry

I. Introduction

Making powdered honey consists of several ingredients, namely liquid acacia honey and fillers. This is supported by the [2] which shows that Indonesia produces 189,780 liters. The abundance of liquid honey provides an opportunity to process it into a food product, namely acacia powdered honey, so that it can increase the selling value of honey. The commonly used filler material is maltodextrin. Maltodextrin can help hydrate a structural molecule so that it can help dry a material. Maltodextrin is a derivative from the degradation of amyloosan and amylopectin chains, thus producing a derivative, namely dextrin.

The process of making powdered honey needs to be taken into account in the formulation of each ingredient. The formulation consists of comparing the percentage of liquid honey with the percentage of maltodextrin. Apart from this, to obtain the optimal powdered honey formulation based on several testing factors, namely reducing sugars, diastase enzymes, and antioxidant activity, namely IC50 (Inhibition Concentration 50). These three things can be benchmarks or standards for the chemical quality of powdered honey products. After the formulation process for making powdered honey is complete, continue with the drying process using vacuum drying with optimal drying temperature.

Therefore, it is necessary to optimize using 2 factors, namely the percentage of liquid acacia honey and the drying temperature. So, it can be seen that the formulation for making honey and operational conditions are vacuum drying temperatures. By using 3 responses, namely reducing sugar, diastase enzyme, and antioxidant activity (IC50). These optimal results will become the standard for making powdered honey and can be widely commercialized.

II. Materials and Methods

This research was conducted at the Lastrindo Engineering CV Science and Technology Laboratory. Lastrindo. The raw materials used are Riau acacia honey, maltodextrin, distilled water. The tools used are vacuum drying, baking pans, blenders, jars, beakers, plastic containers, spatulas. This research uses the Design Expert 12 application with the RSM (Response Surface Method) method, namely CCD (Central Composite Design). This research used 2 factors, namely the percentage of acacia honey and vacuum drying temperature with 3 responses, namely reducing sugar, diastase enzyme, and IC50 (Inhibition Concentration 50). The honey percentage for the lower limit is 60% and the upper limit is 70%, while the drying temperature uses a lower limit of 55°C and an upper limit of 65°C.

Making Honey Powder

Making powdered honey starts from making a dough by mixing the percentage of maltodextrin with distilled water using a ratio of 10:6. Then stirred with a mixer and mixed with a percentage of liquid honey. The total weight of the dough is 50 g. Mixing the maltodextrin mixture with liquid honey is carried out in a water bath to a temperature of 65-70°C. Next, drying was carried out using vacuum drying for approximately 4 hours at a temperature according to the Experimental Design Expert 12 table and at a pressure of -0.92 MPa. After drying, powdering was carried out using a blender and 2% of the weight of the dry mixture was added with tricalcium phosphate.

Reducing Sugars

Reducing sugars were tested using DNSA reagent. This testing process involves weighing 1.5 grams of NaOH and dissolving it to 20 ml and adding 1 g of DNSA while homogenizing using heating for 5-7 minutes. Potassium sodium tartrate is needed as much as

30 g and dissolved in distilled water. The finished DNSA is mixed with potassium while heating. Next, a standard curve was created using a glucose concentration of 0.2; 0.4; 0.6; 0.8; 1; 1.2 ; 1.4 and added 1 ml of reagent, and heated. Then it was measured using spectrophotometry at a wavelength of 540 nm. Sample preparation requires 0.1 gram diluted in 100 ml and 1 ml of sample plus 1 ml of heated reagent. After cooling, 8 ml of distilled water was added and the absorbance of the sample was measured with a wavelength of 540 nm (SNI 8664:2018).

Diastase Enzyme

Diastase enzyme testing is carried out by calculating the DN (Diastase Number) value which shows the total amount of starch that has undergone a 1 hour hydrolysis process at 40°C per 100 g. The stock solution used is iodine, then acetate buffer with pH 5.3, NaCl solution. A starch solution is needed as a parameter for hydrolysis by the diastase enzyme, 1 g of soluble amylum is needed and added with 44 ml of distilled water. After that, heating was carried out and 100 ml of distilled water was added and measured using spectrophotometry with an absorbance limit of 0.760 ± 0.02 with a wavelength of 660 nm so that it could be seen how many ml of distilled water was needed. It takes 5 g of sample to be added with 15 ml of distilled water, 2.5 ml of buffer solution, and 1.5 ml of NaCl solution. Then the absorbance was measured and recorded every 10 minutes to see changes in the absorption value. The regression equation is obtained from the absorbance and time graph, to get the time needed to get an absorbance of 0.235. The following is the DN (Diastase Number) formula (SNI 8664:2018).

$$DN = \frac{300}{t}$$

Information:

DN: Diastase enzyme activity

T : time used to reach the absorbance value (A)

IC50 (Inhibition Concentration 50)

The IC50 (Inhibition Concentration 50) test is used to determine the concentration required to capture 50 free radicals. This test uses the DPPH method. Weighed 0.01 g of DPPH powder and dissolved it in 100 ml methanol to make it 100 ppm and 20 ml was taken to dilute it with methanol to make it 40 ppm [1]. Each sample was extracted by maceration by weighing 4 g and distilled water was added with a total of 20 ml methanol. Maceration was carried out for 3 hours, centrifuged, and filtered using Whatman filter paper no. 42. The resulting filtrate was then made to concentrations of 20, 25, 30, and 35 mg/ml [3]. Next, measurements were carried out using a wavelength of 517 nm using spectrophotometry. The following is the formula for %inhibition. This will be used to determine the IC50 concentration, seen from the graph of the relationship between sample absorbance and %inhibition and to obtain the linear regression formula $50 = ax + b$.

$$\% \text{ inhibition} = \frac{\text{Absorbansi Blanko} - \text{Absorbansi Sampel}}{\text{Absorbansi Blanko}} \times 100\%$$

III. Results

Experimental design

This experiment used 2 factors, namely honey percentage and vacuum drying temperature, seen from 3 responses, namely reducing sugar, diastase enzyme, and IC50. In this case, the results obtained are the required and optimal percentage of honey. You need to pay attention to the drying temperature so that the bioactive content in powdered honey is not damaged. Using the Design Expert 12 application, by entering the upper limit and lower limit values for each factor.

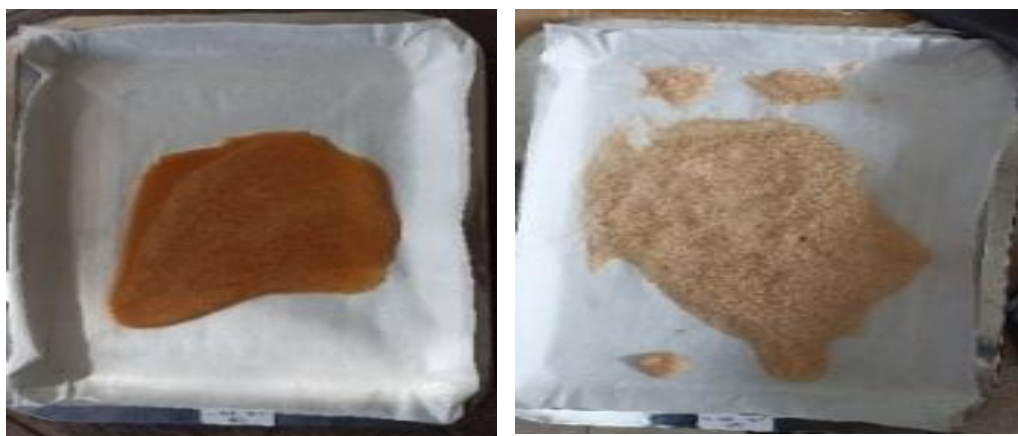


Fig.1 (a) Before drying (b) After drying

Table 1 Tried Centered Composite Points

Factor	$-\alpha$	-1	0	1	α
Liquid Honey Percentage (%)	58	60	65	70	72
Temperature (oC)	53	55	60	65	67

Tabel 2 Experimental Design

Code Variables		Original Variables		Response		
X1	X2	Liquid Honey Percentage (%)	Temperature (oC)	Reducing Sugars	Diastase Enzyme (DN)	IC50 (mg/mL)
-1,000	-1,000	60	55	66	3.33	31.33
1,000	-1,000	70	55	73	3.57	21.15
-1,000	1,000	60	65	70	3.09	16.06
1,000	1,000	70	65	76	3.27	39.77
-1,414	0,000	58	60	67	3.13	13.59
1,414	0,000	72	60	74	3.18	16.75
0,000	-1,414	65	53	65	3.31	38.43
0,000	1,414	65	67	72	3.19	23.94
0,000	0,000	65	60	69	3.2	45.52
0,000	0,000	65	60	68	3.19	35.15
0,000	0,000	65	60	69	3.21	31.22

Based on the research results, the IC50 value of powdered honey showed very strong antioxidant activity (below 50 mg/mL), with the three lowest values of 13.59 mg/mL, 16.60 mg/mL, and 16.75 mg/mL, respectively, at a combination of 59–72% honey percentage and a temperature of 60–65 °C. Increasing the percentage of liquid honey tends to increase antioxidant activity, although DPPH stability can be affected by storage conditions and room temperature [14]. These results are in line with [11] who stated that the higher the honey content, the greater the antioxidant activity. In addition, the highest reducing sugar content (74%) was obtained at 72% liquid honey and a temperature of 60 °C due to the increase in sugar concentration during heating [4]. The highest diastase (DN) enzyme activity of 3.57 was achieved at a temperature of 55 °C and 70% honey, indicating that although the drying process can reduce enzyme activity, the value is still considered good in the range of 0.0–7.2 as reported by [12].

ANOVA of Reducing Sugars, Diastase Enzymes and IC50

In the 11 experimental results, the data will be processed using the design expert application 12 so that the results are in the form of ANOVA to obtain the relationship between the results of each factor and the response.

Table 3 ANOVA of Reducing Sugar Response

Source	Sum of Squares	df	Mean Squares	F-value	P-value	
Model	101.25	2	50.62	20.60	0.0007	Significant
A-Percentage of Honey	65.55	1	65.55	26.67	0.0009	
B-Temperature	35.70	1	35.70	14.53	0.0052	
Residual	19.66	8	2.46			
Lact of Fit	18.99	6	3.17	9.50	0.0983	Not significant
Pure Error	0.6667	2	0.3333			
Total Cast	120.91	10				

Tabel 4 ANOVA Diastase Enzyme Response

Source	Sum of Squares	df	Mean Squares	F-value	P-value	
Model	0.0931	2	0.0465	4.94	0.0401	Significant
A-Percentage of Honey	0.0301	1	0.0301	3.20	0.1117	
B-Temperature	0.0630	1	0.0630	6.68	0.0323	
Residual	0.0754	8	0.0094			
Lact of Fit	0.0752	6	0.0125	125.26	0.0079	Significant
Pure Error	0.0002	2	0.0001			
Total Cast	0.1684	10				

Tabel 5 ANOVA Ic50 Response

Source	Sum of Squares	df	Mean Squares	F-value	P-value	
Model	943.94	5	188.79	4.27	0.0684	Not significant
A-Percentage of Honey	40.50	1	40.50	0.9166	0.3823	
B-Temperature	36.73	1	36.73	0.8314	0.4037	
AB	287.13	1	287.13	6.50	0.0513	
A2	574.73	1	574.73	13.01	0.0154	
B2	24.45	1	24.45	0.5535	0.4904	
Residual	220.90	5	44.18			
Lact of Fit	111.74	3	37.25	0.6824	0.6402	Not significant
Pure Error	109.16	2	54.58			
Total Cast	1164.84	10				

Table 3 is an ANOVA table of reducing sugars showing that the linear model is marked as significant at a P value <0.05. So, from this the percentage of honey and drying temperature very significantly influence the value of reducing sugar. According to [10] the 95% confidence level of the model will be considered significant if the P value <0.05. Next, the linear model equation for reducing sugar response is obtained as follows

$$Y_2 = 7.34817 + 0.572487X_1 + 0.422487X_2$$

Table 4 is an ANOVA table of the diastase enzyme which shows the linear model. Model B has a P value <0.05 so model B has a significant effect on the diastase enzyme response with a value of 0.0323. So, in this case the drying temperature affects the value of the diastase enzyme, when the drying temperature is higher, it can damage the diastase enzyme. From the linear model, the following equation is obtained. This quantitative value, when it is more positive, has a greater influencing value.

$$Y_3 = 3.50988 + 0.01268X_1 + 0.017743X_2$$

Table 5 is an ANOVA table of IC50 (Inhibition Concentration 50) showing the quadratic model. The value of the quadratic model A has a value of 0.0154 and a value of less than 0.05. In this case, it means that the factor of honey percentage has a significant effect on IC50, whereas factor B, namely drying temperature, has no significant effect on the response. The following is the quadratic equation formula

$$Y_1 = -649.09697 + 32.57531X_1 - 12.46905X_2 + 0.338900X_1X_2 - 0.403533X_1^2 - 0.083233X_2^2$$

Graphic Models

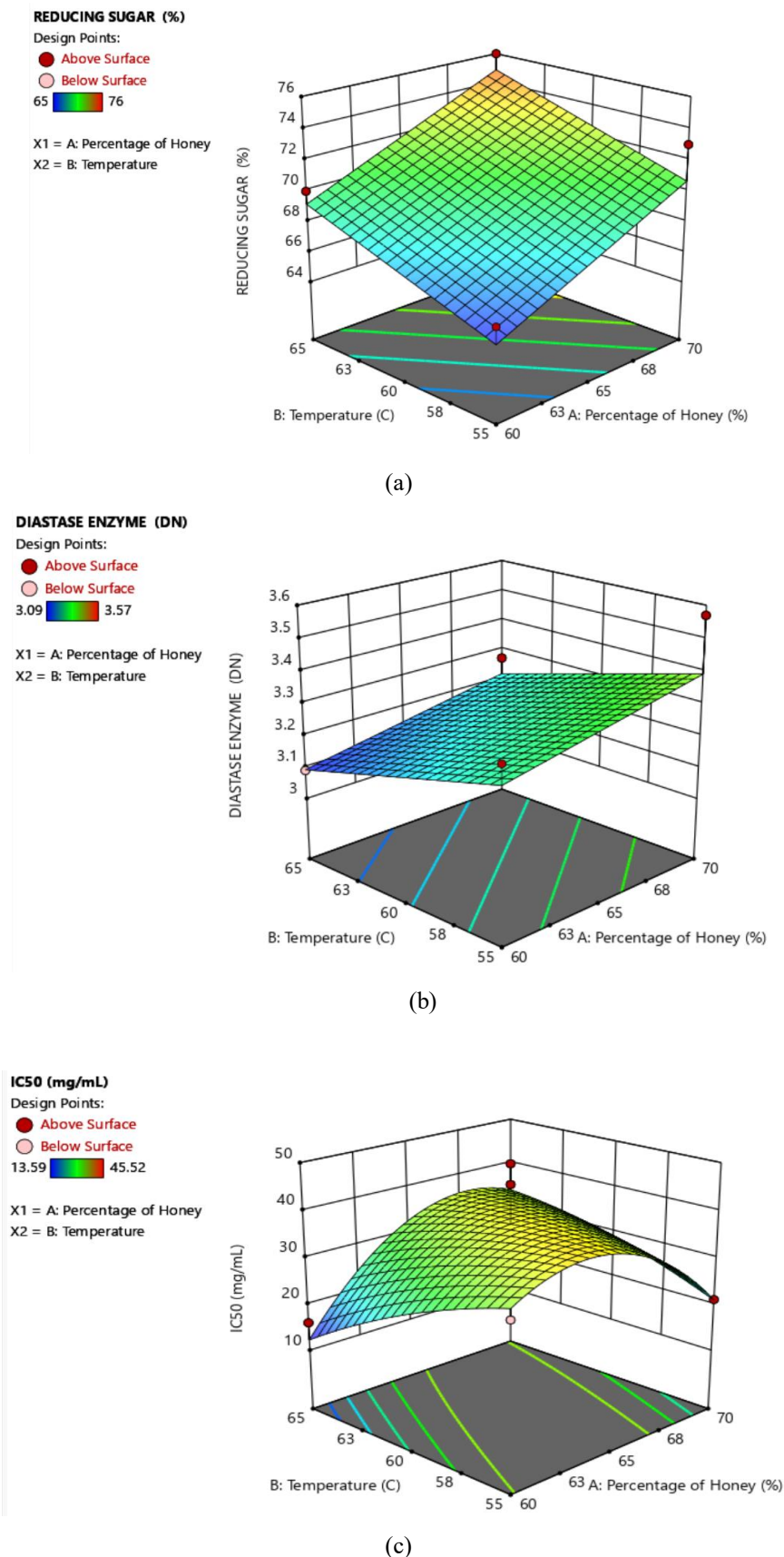


Fig 2 (a) 3D Model of Honey Percentage and Drying Temperature to Reducing Sugars (b) 3D Model of Honey Percentage and Drying Temperature to Diastase Enzymes (c) 3D Model of Honey Percentage and Drying Temperature IC50

The graph above shows a 3D image between the percentage of liquid honey and the drying temperature on the response. The graph shows the optimal point of the curve, namely at the midpoint or center. For reducing sugar, there is a value range of 65-76, the further to the right the red color, the higher the value. According to [9], the reducing sugar content with powdered honey is highest at a ratio of 70:30 with reducing sugar 69.28%. According to [13] reducing sugar is obtained in the inversion or hydrolysis process of sucrose into glucose and fructose. Furthermore, in the 3D graph the diastase enzyme range consists of a value of 3.09-3.57, the further to the right or the color red, the higher the value. Optimal results are marked by a red dot located right in the middle of the green color. According to [7], the diastase enzyme has a level of susceptibility to temperature, when the temperature is higher it will reduce the diastase enzyme. Furthermore, the IC50 (Inhibition Concentration) is shown in the image above that the value range starts from 13.59-45.52. In the image it is marked with a red dot located in the middle of the yellow color. The effect of honey percentage is more significant than drying temperature. According to [5], heating tends not to damage honey's secondary metabolites, because heating does not reduce antioxidant activity. The smaller the IC50 value, the higher the antioxidant activity and the antioxidants in powdered honey are good.

Optimal Validation

This optimal value validation is used to verify whether the optimal value suggested by Design Expert 12 is valid with the test results on a lab scale. The data obtained from the test results will then be processed by the application with the response optimization limits in Table 3.6 below. Next, the optimum solution results will be obtained in Table 3.7, namely that desirability has a value of 0.629. The desirability value has a value range of 0-1, when it gets closer to 1 it indicates that the prediction results from the application are getting better and more accurate. In this case, the factors namely the percentage of liquid honey and drying temperature have optimum values of 70% and 55°C respectively.

Table 6 Response Optimization Limits

<i>Name</i>	<i>Goals</i>	<i>Lower Limits</i>	<i>Upper Weight</i>
A:Percentage of Honey	Is in range	60	70
B:Temperature	Is in range	55	65
IC50	<i>minimize</i>	13.59	45.52
Reducing Sugars	<i>Maximize</i>	65	76
Diastase Enzyme	<i>Maximize</i>	3.09	3.57

Table 7 Selected Optimum Solution

<i>Numbers</i>	Honey Percentage	Temperature	IC50	Reducing Sugars	Diastase Enzyme	<i>Desirability</i>	
1	70,000	55,000	21,048	70,659	3,393	0.629	<i>Selected</i>

Table 8 Verification of Optimum Conditions for Model Prediction Results

Parameter	Lowest Prediction	Prediction	Highest Prediction	Verification Results	Difference	Accuracy
IC50 (mg/ml)	4.32139	21.0476	37.7738	18.88	2.1676	89.70%
Reducing Sugar (%)	67.6906	70.6591	73.6276	68	2.6591	96.23%
Diastase Enzyme (DN)	3,209	3.39278	3.57656	3.35	0.04278	98.73%

Verification of optimum conditions was carried out by carrying out laboratory scale testing, obtaining verification results at IC50 18.88 mg/ml, reducing sugar 68%, and diastase enzyme 3.35. Each has an accuracy value of 89.70%, 96.23%, and 98.73%. The verification results fall into the range of 95% PI Low and 95% PI High so that a high desirability value is obtained. So when the accuracy results fall into the range of 0.70-0.90 and >0.90 then they fall into high reliability and perfect reliability [8].

Comparison of Optimal Results with Commercial Powdered Honey

This research will obtain optimal results from data verification.

Based on the verification results, a 70% honey percentage and a drying temperature of 55°C were used. The verified data were compared with control results, namely 51 raw liquid acacia honey samples before processing and commercial powdered honey available on the market.

Tabel 9 Comparison of Optimum Treatment and Control

Parameter	Optimum Results (70% and 55oC)	Raw Acacia Liquid Honey	Commercial Powdered Honey on the Market
IC50 (mg/ml)	18.88	10.86	67.62
Reducing Sugar (%)	68	57	28
Diastase Enzyme	3.35	7.16	1.5

The comparison in Table 3.9 above shows the comparison of optimum results at 70% 55°C with raw acacia liquid honey and commercial powdered honey on the market. In the IC50 value, it can be seen that the results show that the optimum results have a value of 18.88 mg/ml compared to raw acacia honey which is 10.86 mg/ml, which is classified as a very strong IC50 because the value is less than 50. Meanwhile, for commercial powdered honey on the market, the IC50 value is 67.62, so the activity is still higher. antioxidants in powdered honey 70% and 55°C. According to [6], the greater the percentage of liquid honey used, the greater the IC50 value. Furthermore, for reducing sugar, the optimum value is 68%, liquid honey is 57%, so reducing sugar will increase when drying. Compared to commercial powdered honey on the market, the reducing sugar is very low, namely 28%. The optimum result for the diastase enzyme is 3.35 and raw acacia liquid honey has a value of 7.16, so the diastase enzyme tends to be damaged or decreased when drying the liquid honey. Meanwhile, acacia powdered honey has a diastase enzyme value which tends to be very low, only having a DN value of 1.5.

IV. Conclusion

Optimization results with 2 factors, namely the percentage of liquid honey and drying temperature and 3 responses, namely reducing sugar, diastase enzyme, and IC50. This research obtained optimal results at a honey percentage of 70% and a vacuum drying temperature of 55°C. The optimal response at IC50 is 18.88 mg/ml, reducing sugar is 68%, and the diastase enzyme has a DN value of 3.35. So, from this research it can be seen that the best formulation is to obtain powdered honey with optimal chemical quality and is not damaged even though processing is carried out in the form of vacuum drying.

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