

# Optimization of simple sugar extraction of nagara bean (*Vigna unguiculata* ssp. *Cylindrica*) on concentration and proportion of ethanol

*by Susi .*

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## Optimization of simple sugar extraction of nagara bean (*Vigna unguiculata ssp. Cylindrica*) on concentration and proportion of ethanol

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**Abstract.** Nagara bean, the native bean of South Kalimantan, is not only contains carbohydrates and protein, but also contains oligosaccharide fractions such as raffinose and stachiose that can cause flatulence. Raffinosa Family Oligosaccharide (RFO) is an undigested carbohydrate that is naturally found in beans. The presence of the oligosaccharide fraction can be identified through reducing sugar and total sugar content in nagara bean. The yield of this fraction depends on the type of material and extraction method, such as the type of solvent, solvent concentration, contact time and extraction temperature. This study was aimed to optimize the extraction of simple sugar fractions on nagara bean at several concentrations of ethanol with various ratio of nagara bean flour to ethanol using the Response Surface Methods (RSM). Optimization was carried out at the center point ethanol concentration of 50% and the ratio of nagara bean flour and ethanol of 1: 10. The optimization results of the simple sugar fraction extraction process in nagara bean flour were obtained at a concentration of 30% ethanol and a ratio of ethanol to nagara bean flour of 10: 1 at extraction conditions at 50° C and extraction time of 30 minutes.

### 1. Introduction

Raffinose family oligosaccharides (RFO) is one of the non-digestible carbohydrates that is naturally found in bean. Nagara bean is endemic cowpea to the swamps of Kalimantan Selatan, it contains high protein and quite high carbohydrates of 60% approximately. Besides containing starch, carbohydrates also have a RFO fraction in it. The absence of a galactosidase which able to hydrolyze the glycosidic bonds causes RFO undigested [1], and in the large intestine it will be fermented by bifidobacteria which produce gases such as carbon dioxide, hydrogen, methane and short chain fatty acids that play a role in prebiotic activity [2].

The existence of simple sugars such as glucose and fructose (reducing sugar) and sucrose (non-reducing sugar) contribute to the taste and flavor of the beans and also play a role in the process of browning reactions, such as the Maillard reaction. Li et al. [3] stated that the sucrose content reached 71% of the total dissolved sugar in soybeans. Nassourou et al. [4] explained that the total sugar in *Vigna Unguiculata L Walp* ranges from 11.12-40.79 mg.g<sup>-1</sup> and the reducing sugar ranges from 4.98-



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13.61 mg.g<sup>-1</sup>. RFO identification can also be done through extraction of the low molecular carbohydrate fraction. Generally, hydrolysis of RFO by galactosidase produces galactose and sucrose. While raffinose is a trisaccharide with galactose, fructose and glucose in it. The studies have shown that sucrose is one of the sugars in the dissolved sugar.

Several solvents can be used to extract Low weight Molecular Carbohydrate (LWMC) from beans flour including water ethanol mixture solvent. Previous studies mentioned the solubility of sugars (glucose, fructose, lactose and sucrose) in the methanol-water and ethanol-water mixtures [5–7]. Water-alcohol and alcohol-alcohol mixture solvents can be used for isolation by precipitation of certain sugar in a mixture of sugar. Xiao-li et al. [8] showed that the extraction conditions of the oligosaccharide fraction from chickpeas were obtained at ethanol concentration of 50% with ethanol and flour ratio of 10: 1 at extraction conditions 50°C for 30 minutes, the sugar analysis using HPLC obtained the highest glucose oligosaccharides content of 8.68% and sucrose content of 2.36%. It is necessary to obtain the right extraction conditions and optimum sugar yield. This study was aimed to optimize the extraction of simple sugar fractions in nagara bean at several ethanol concentration with various ratio of ethanol to nagara bean flour using the Response Surface Methodology (RSM).

## 2. Materials and methods

Material used in this study were nagara bean flour from Nagara Hulu Sungai Selatan South Kalimantan, ethanol, H<sub>2</sub>SO<sub>4</sub>, Dinitro salisilic acid (DNS), Na<sub>2</sub>SO<sub>3</sub>, NaOH, Na- K Tartrate, Phenol, standard of glucose, fructose, sucrose and maltose.

### 2.1. Fermentation process

The fermentation process used ratio of nagara bean : soaked water = 1: 4. Nagara beans were fermented for 48 hours, then were cleaned from the skin, washed, and dried at a temperature of 60 °C for 48 hours, then they were powdered and sieved 80 mesh.

### 2.2. Extraction method

As much as 1 gram sample was extracted 3 times using ethanol in concentration range of 30-70% with ethanol-water to flour ratio from 8 : 1 up to 12: 1 at waterbath of 50 °C for 30 minutes. At the end of each extraction, all samples were centrifuged at 3,500 rpm for 10 minutes. The supernatant of the 3 extractions were collected and concentrated using a rotary evaporator. The extract was reconstituted using sterile distilled water.

### 2.3. Design experimental of respon surface methodology

The extraction method uses a Response Surface Methodology design with a center point ethanol concentration of 50% and a center point of ethanol to nagara bean flour ratio of 10: 1.

**Table 1.** Design of response surface methodology

Std	Run	Factor 1		Factor 2	
		X <sub>1</sub> : concentration of ethanol (%)		X <sub>2</sub> : ratio ethanol to flour	
3	1	30		12	
1	2	30		8	
6	3	78.28427125		10	
8	4	50		12.82842712	
5	5	21.71572875		10	
13	6	50		10	
2	7	70		8	
9	8	50		10	
7	9	50		7.171572875	
11	10	50		10	
4	11	70		12	
10	12	50		10	
12	13	50		10	

#### 2.4. Parameter of analysis

Chemical characteristics of nagara bean flour were analyzed on moisture content, ash content, fat content, protein content, crude fiber content, carbohydrate content by difference, and calories (by calculation), reducing sugar (DNS method), glucose, fructose and sucrose, total sugar (Phenol-sulfuric method), and degree of polymerization (by calculation).

#### 2.5. Data analysis

The collected data for optimization was analyzed using Response Surface Methodology by Design Expert 11.

### 3. Results and discussions

#### 3.1. Proximate composition of nagara bean flour

Based on Table 2, the native nagara bean flour and nagara bean flour which were fermented spontaneously for 48 hours did not show significant different in proximate content. Decreasing of ash content (mineral content) in fermented nagara beans is presumably due to losses in dissolved solids. Research by Arora and Das [9] explained that 22 types of *Vigna Unguiculata L* Walp have starch content ranging from 50.66 to 67%, mineral content from 3.12 to 4.60%, and protein content from 17.94-27.56%.

**Table 2.** Proximate composition of nagara bean flour

Parameter	Nagara bean flour	
	native	Spontaneous fermented
Water content (%)	6.40 ± 0.16	6.64 ± 0.95
Ash content (%)	2.90 ± 0.12	1.28 ± 0.08
Fat (%)	1.34 ± 0.07	1.76 ± 0.02
Crude protein (%)	20.11 ± 0.95	19.71 ± 0.39
Crude fibre (%)	1.23 ± 0.04	1.35 ± 0.07
Carbohydrates (by difference)	69.26 ± 1.30	70.63 ± 1.41
Calorie (by calculation)	369.54 ± 0.78	377.12 ± 4.26

#### 3.2. Simple sugar fraction in nagara bean flour

Simple sugars that were analyzed are the fraction of monosaccharides (glucose and fructose), and the fraction of the disaccharide (sucrose and maltose). Monosaccharides can be divided into two parts, namely aldose which has an aldehyde group, and ketose which has a ketone group. The monosaccharides including galactose, glucose and fructose are all reducing sugars.

Disaccharides are carbohydrates formed from two monosaccharide molecules that bind through the -OH group by releasing a water molecule. Examples of disaccharides are sucrose, lactose, and maltose. Sucrose is a non-reducing disaccharide because it does not have a free aldehyde group.

**Table 3.** Simple sugar fraction in nagara bean flour

Parameter	Unit	Ranges
1 Glucose	mg.g <sup>-1</sup>	36.24 – 48.27
2 Fructose	mg.g <sup>-1</sup>	4.10 – 5.18
3 Sucrose	mg.g <sup>-1</sup>	5.43 – 7.01
4 Maltose	mg.g <sup>-1</sup>	52.66 – 66.43
5 Reducing sugar	mg.g <sup>-1</sup>	27.82 – 38.37
6 Total sugar	mg.g <sup>-1</sup>	38.81 – 51.76
7 Degree of polimerization		1.23 – 1.77

Table 3 showed the type of sugar that is quite dominant in nagara bean is glucose, while fructose and sucrose are lower. Measured reducing sugar is more dominantly influenced by glucose content.

The maximum measured total sugar content was 51.76 mg.g<sup>-1</sup>. It is assumed that the oligosaccharide content in nagara bean is quite small.

### 3.3. Optimization with response surface methodology

3.3.1. *Glucose, fructose and sucrose*. The content of glucose, fructose and sucrose extraction from nagara bean flour were varied according various concentration of ethanol and the ratio of ethanol: nagara bean flour. Glucose content ranged from 36.24 to 48.27 mg.g<sup>-1</sup>, fructose of 4.10 - 5.18 mg.g<sup>-1</sup> and sucrose of 5.43 - 7.01 mg.g<sup>-1</sup>. The results of the fit summary indicate that the appropriate model for glucose, fructose and sucrose is quadratic as presented in Table 4.

**Table 4.** Fit Summary of glucose, fructose and sucrose

Source	Sequential p-value	Lack of Fit p-value	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>	
<i>Glucose</i>					
Linear	0.5366	0.1849	-0.0595	-0.5673	
2FI	0.5159	0.1579	-0.1203	-1.2319	
<b>Quadratic</b>	<b>0.1560</b>	<b>0.2138</b>	<b>0.1529</b>	<b>-1.5213</b>	<b>Suggested</b>
Cubic	0.0794	0.9235	0.5695	0.6905	Aliased
<i>Fructose</i>					
Linear	0.5278	0.1913	-0.0560	-0.5617	
2FI	0.5032	0.1645	-0.1132	-1.2175	
<b>Quadratic</b>	<b>0.1613</b>	<b>0.2193</b>	<b>0.1502</b>	<b>-1.5152</b>	<b>Suggested</b>
Cubic	0.0824	0.9137	0.5618	0.6769	Aliased
<i>Sucrose</i>					
Linear	0.5318	0.1877	-0.0576	-0.5642	
2FI	0.5086	0.1609	-0.1163	-1.2236	
<b>Quadratic</b>	<b>0.1581</b>	<b>0.2165</b>	<b>0.1526</b>	<b>-1.5151</b>	<b>Suggested</b>
Cubic	0.0808	0.9186	0.5662	0.6843	Aliased

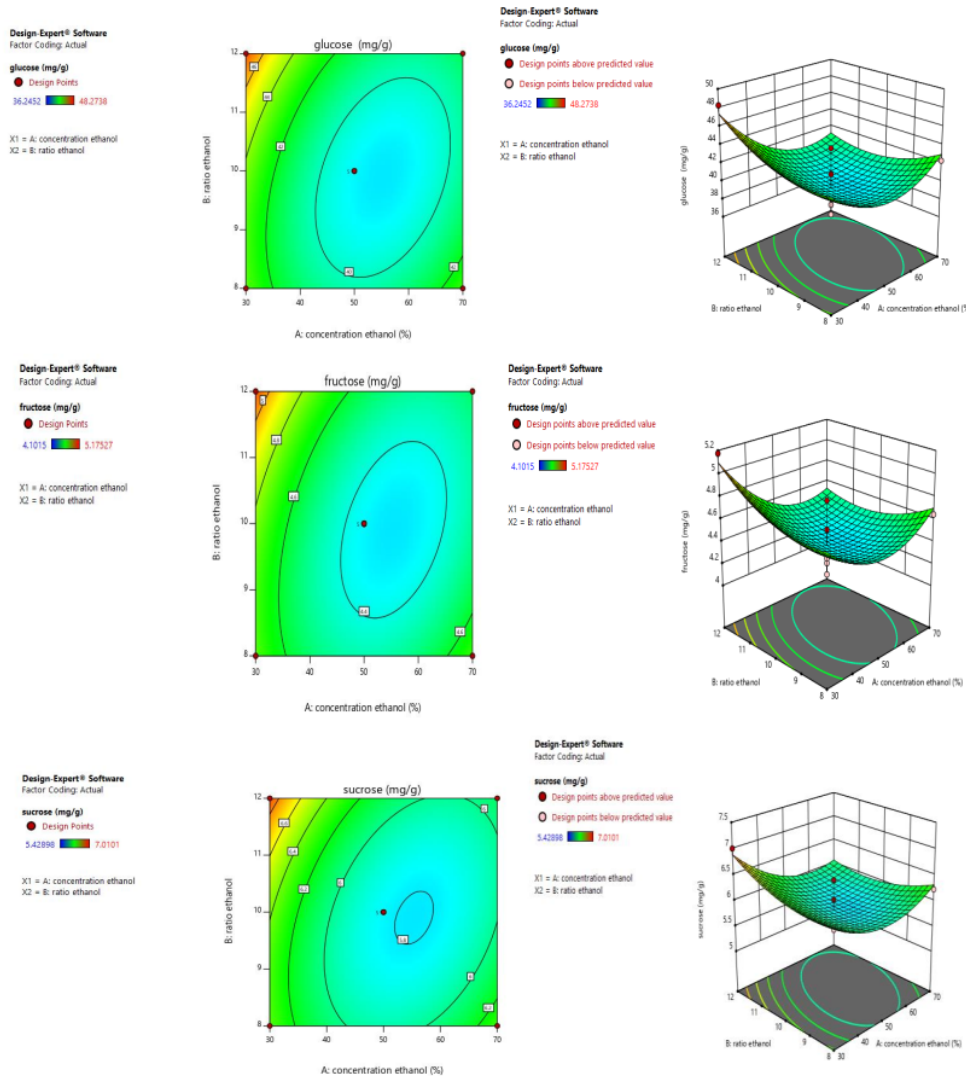
The results of ANOVA analysis showed that the lack of fit values of glucose, fructose and sucrose parameters were all greater than 0.05, glucose p-value is 0.2138, fructose p-value is 0.2139, and sucrose p-value is 0.2165. It means that there is no discrepancy in the model, the error does not significantly affect the model. The quadratic model formed in glucose ( $Y_g$ ), fructose ( $Y_f$ ) and sucrose ( $Y_s$ ) is shown in the following equation

$$\begin{aligned}
 Y_g &= 39.23 - 1.62X_1 + 0.56X_2 - 1.46X_1X_2 + 2.92X_1^2 + 1.56X_2^2 \dots\dots\dots 1) \\
 Y_f &= 4.37 - 0.1471X_1 + 0.0493X_2 - 0.1346X_1X_2 + 0.2591X_1^2 + 0.1385X_2^2 \dots\dots\dots 2) \\
 Y_s &= 5.82 - 0.2149X_1 + 0.0727X_2 - 0.1955X_1X_2 + 0.3829X_1^2 + 0.2049X_2^2 \dots\dots\dots 3)
 \end{aligned}$$

The quadratic equation of glucose, fructose and sucrose showed that ethanol concentration, interaction of ethanol concentration, and ratio of ethanol: flour give a negative correlation which means if the ethanol increases, the glucose concentration decreases, but the single factor ethanol to flour ratio gives a positive correlation, which means that the extracted glucose level increases. Glucose levels at optimum conditions were obtained at 48.166 mg.g<sup>-1</sup>, fructose at 6.89 mg.g<sup>-1</sup> and sucrose at 47.35 mg.g<sup>-1</sup> (Figure 1).

According to Alves et al. [10], the solubility of glucose in water increases with increases temperature, but in the ethanol-water mixture solvent up to 80% of ethanol concentration, the solubility of glucose decreases. The solubility of glucose in the ethanol - water mixture increases with increasing proportion of water [11]. With the addition of ethanol the solubility of saccharides will

decrease [1]. Thus, monosaccharides or oligosaccharides are retained in the supernatant after the addition of ethanol. In the industry this method used for separating saccharides. Cold temperature storage treatment will reduce the solubility of saccharides. Therefore, the solubility of the 3 types of sugar in a mixture of ethanol and water at low temperatures will help optimize the production process by Gong et al. [12].



**Figure 1.** Contour and response surface quadratic model of glucose, fructose and sucrose

Alavi et al. [13] stated that the solubility of fructose in the water-methanol solvent mixture is better than the solubility in water-ethanol at the same temperature and at the same percentage of water. It is because the hydrogen bonds between fructose and methanol are stronger than the hydrogen bonds

of fructose and ethanol. Increasing the percentage of water causes the solubility of fructose to be greater in the mixture.

3.3.2. *Reducing sugar*. Reducing sugar is a group of sugars (carbohydrates) that can reduce electron-accepting compounds, for example glucose and fructose. The end of the reducing sugar is the end which contains the free aldehyde or ketone group. All monosaccharides (glucose, fructose, galactose) and disaccharides (lactose, maltose) except sucrose and starch are reducing sugars. The model formed for reducing sugar parameters based on the fit summary test (Table 5) is 2F1, which is between linear to quadratic. The lack of fit value showed the p-value  $0.1335 > 0.05$ , it means that the lack of fit is not significant, and this showed there is no model mismatch.

**Table 5.** Fit Summary of reducing sugar

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	138.13	1	138.13			
Linear vs Mean	0.3162	2	0.1581	1.76	0.2213	
<b>2FI vs Linear</b>	<b>0.1811</b>	<b>1</b>	<b>0.1811</b>	<b>2.27</b>	<b>0.1659</b>	<b>Suggested</b>
Quadratic vs 2FI	0.1353	2	0.0676	0.8140	0.4810	
Cubic vs Quadratic	0.1156	2	0.0578	0.6202	0.5746	Aliased
Residual	0.4661	5	0.0932			
Total	139.34	13	10.72			

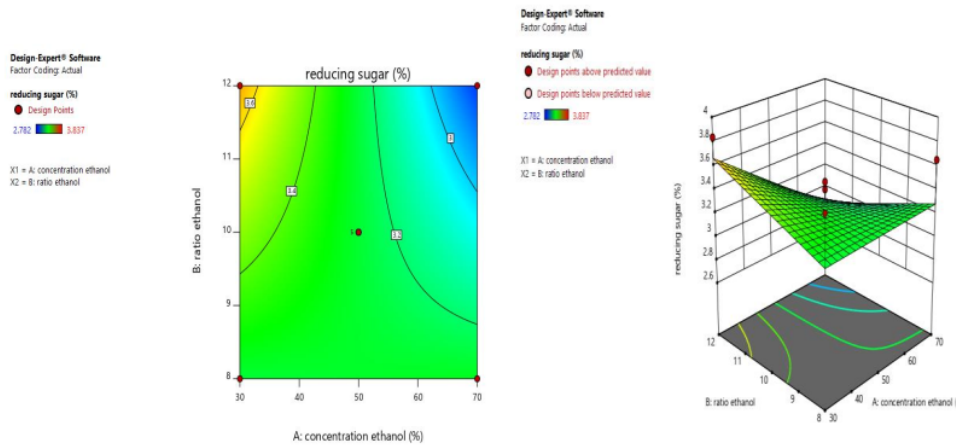
In the fit statistical test, the negative predicted  $R^2$  value reflects the average value to be a good predictor of the response model, while the adequate precision above 4 ( $5.2552 > 4$ ) shows that the model is representative. The reducing sugar model that is formed is:

$$Y_{rs} = +3.26 - 0.1986X_1 - 0.0084X_2 - 0.2127X_1X_2 \dots \dots \dots 4)$$

The  $Y_{rs}$  equation showed the ethanol concentration factor ( $X_1$ ), the ethanol to flour ratio ( $X_2$ ) and the interaction between the ethanol concentration and the ethanol ratio ( $X_1X_2$ ) have negative effect to reducing sugar content. Increasing the concentration of ethanol and the ratio of ethanol to flour will decrease reducing sugar levels.

Previous research by Alves [12] compared the solubility of glucose in water and ethanol-water mixture, the results showed that the solubility of glucose in water increased linearly with increasing temperature, while the solubility in water ethanol decreased following the increasing of ethanol concentration.





**Figure 2.** Contour and 3D respon surface 2FI model of reducing sugar

Figure 2 showed the 2FI contour of reducing sugar parameters where this model is between the linear and quadratic lines where the optimum conditions are obtained at a level of 36.63 mg.g<sup>-1</sup>

3.3.3. *Total sugar.* Total sugar content includes the sugar content of monosaccharides, disaccharides and the oligosaccharide fraction. Measurement of the oligosaccharide fraction, both raffinose and stachiose, can be described by measuring the total sugar using the phenol-sulfuric acid method (phenol method). The result of the fit summary test showed that the appropriate model for the total sugar parameter is quadratic (Table 6).

**Table 6.** Fit Summary of total sugar

Source	Sequential p-value	Lack of Fit p-value	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>	
Linear	0.5276	0.1959	-0.0559	-0.5624	
2FI	0.4952	0.1691	-0.1109	-1.2148	
<b>Quadratic</b>	<b>0.1672</b>	<b>0.2216</b>	<b>0.1432</b>	<b>-1.5302</b>	<b>Suggested</b>
Cubic	0.0836	0.9098	0.5555	0.6687	Aliased

ANOVA test results showed that the lack of fit is not significant (p-value 0.226), this indicates that the model represents the total sugar response. And the quadratic equation for total sugar that can be obtained is:

$$Y_{ts} = 3.93 - 0.1286X_1 + 0.0429X_2 - 0.1197X_1X_2 + 0.2246X_1^2 + 0.1196X_2^2 \dots\dots\dots 5)$$

The quadratic model equation (Y<sub>ts</sub>) showed the increasing the concentration of ethanol and its interaction with the ratio of ethanol to flour has a negative effect (-) or will reduce the measured total sugar, but the single factor of the ratio of ethanol to flour and the square of ethanol concentration and square of the ratio of ethanol to flour has an positive effect. Contour and 3D respon surface quadratic model of total sugar represented in Figure 3.

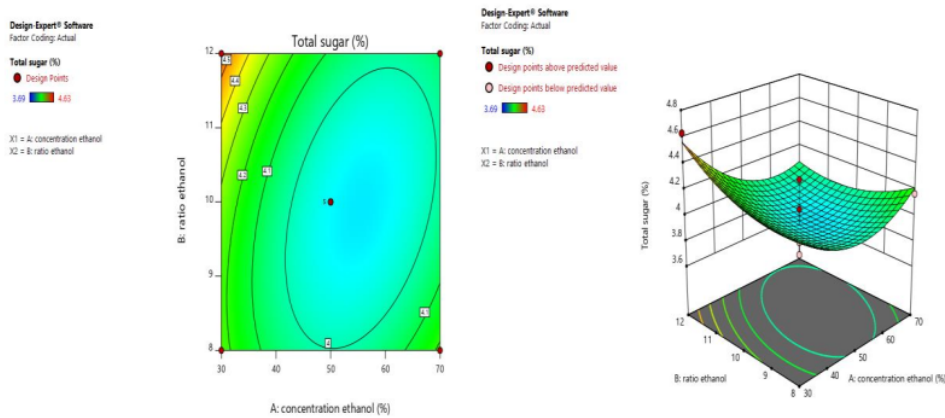


Figure 3. Contour and 3D respon surface quadratic model of total sugar

3.4. Solution of optimization

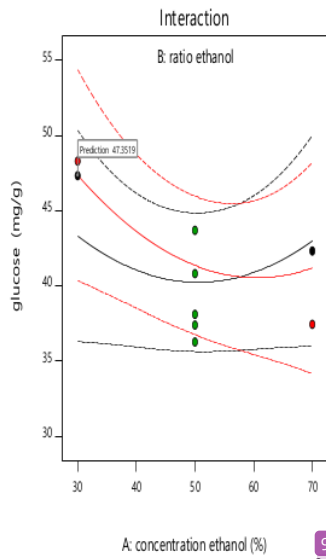
In optimization process, the maximum total sugar content of the extraction process results are obtained both in reducing sugar and total sugar. It is used to direct the optimum point of process conditions, especially the ethanol concentration and ethanol ratio (Table 7).

Table 7. Constraints of optimization

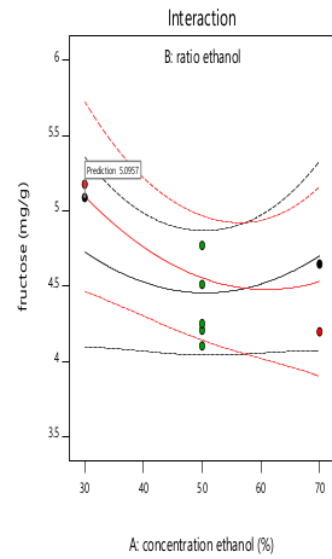
Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
X <sub>1</sub> :concentration ethanol	is in range	30	70	1	1	3
X <sub>2</sub> :ratio ethanol	is in range	8	12	1	1	3
reducing sugar	maximize	2.782	3.837	1	1	3
Total sugar	maximize	3.69	4.63	1	1	3
glucose	maximize	36.2452	48.2738	1	1	3
sucrose	maximize	5.42898	7.0101	1	1	3
fructose	maximize	4.1015	5.17527	1	1	3

Figure 4 showed the interaction between ethanol concentration and ethanol ratio in each parameter to determine the optimum point at the process condition for each parameter. This test resulted in an alternative solution with a desirability of 0.90 in the process conditions of 30% ethanol concentration and a ratio of ethanol to nagara bean flour 12: 1 with a reducing sugar value of 3.66% (36.63 mg .g<sup>-1</sup>), total sugar of 4.56% (50.58 mg .g<sup>-1</sup>), glucose of 47.35 mg .g<sup>-1</sup>, 5.09 mg .g<sup>-1</sup>, and sucrose 6.89 mg .g<sup>-1</sup>. The optimum condition for the extraction of sugar fractions at an extraction temperature of 50°C for 30 minutes with low ethanol concentration but requires a sufficiently large ratio of the amount of ethanol to nagara bean flour, namely 12 parts of ethanol. This suggests that the solvent-material ratio has a positive effect on the extraction yield [14]. In accordance with the principle of mass transfer, that the driving force of mass transfer is the concentration gradient of solids and solvents, a large gradient can be obtained through a high ratio of solvents and solids used [15,16].

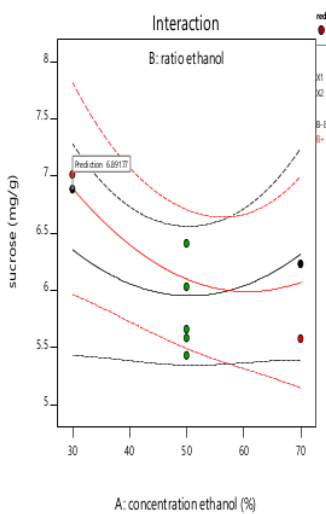
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glucose (mg/g)  
● Design Points  
— 95% CI Bands  
X1 = A: concentration ethanol  
X2 = B: ratio ethanol  
B=8  
B=12



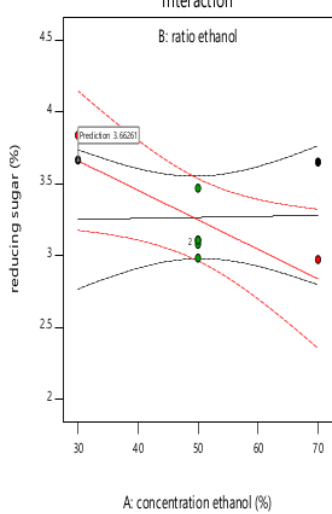
**9**  
Design-Expert® Software  
Factor Coding: Actual  
fructose (mg/g)  
● Design Points  
— 95% CI Bands  
X1 = A: concentration ethanol  
X2 = B: ratio ethanol  
B=8  
B=12

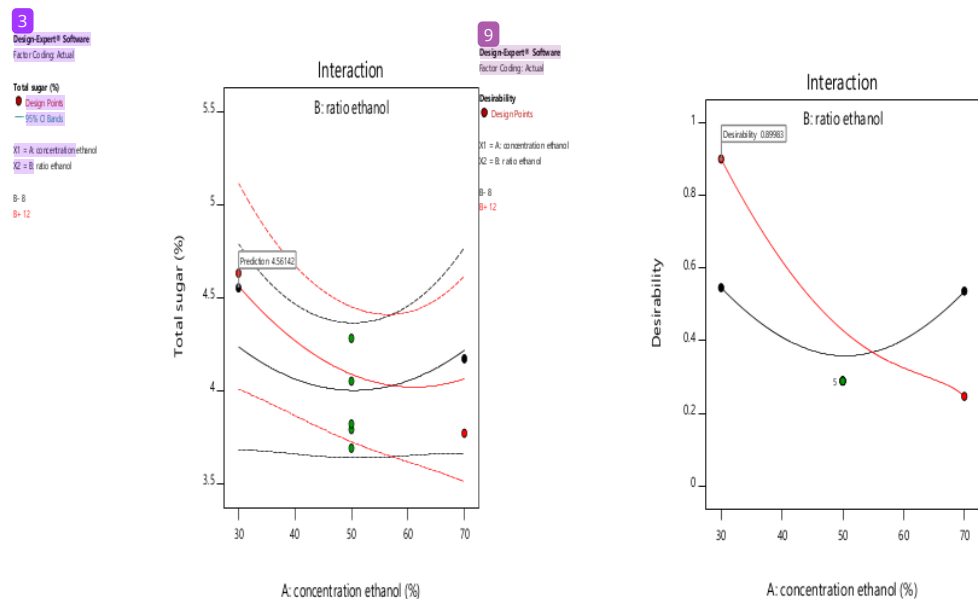


**3**  
Design-Expert® Software  
Factor Coding: Actual  
sucrose (mg/g)  
● Design Points  
— 95% CI Bands  
X1 = A: concentration ethanol  
X2 = B: ratio ethanol  
B=8  
B=12



**9**  
Design-Expert® Software  
Factor Coding: Actual  
reducing sugar (%)  
● Design Points  
— 95% CI Bands  
X1 = A: concentration ethanol  
X2 = B: ratio ethanol  
B=8  
B=12





**Figure 4.** Interaction concentration ethanol and ratio ethanol to flour

#### 4. Conclusion

The optimization results of the simple sugar fraction extraction process in nagara bean flour were obtained at concentration of 13 anol 30% and a ratio of ethanol to nagara bean flour of 10: 1 at extraction conditions at 50°C and extraction time of 30 minutes, with a reducing sugar value of 3.66% (36.63 mg.g<sup>-1</sup>), total sugar of 4.56% (50.58 mg.g<sup>-1</sup>), glucose of 47.35 mg.g<sup>-1</sup>, 5.09 mg.g<sup>-1</sup> and sucrose 6.89 mg.g<sup>-1</sup>.

#### Acknowledgment

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