OPTIMIZATION OF BIOETHANOL PRODUCTION FROM WASTE MANGO USING RESPONSE SURFACE METHODOLOGY

War War Thin[1], Khin Swe Oo[2], Soe Soe Than[3]

Abstract

In the present study, waste fruit of mango (*Mangifera indica* L.); Yin Kwe`, was used as raw material for the production of bioethanol. Baker's yeast (*Saccharomyces cerevisiae*) was used for fermentation of waste mango. Response Surface methodology (RSM) based on Box-Behnken Design (BBD) was applied to optimize the strength of ethanol during bioethanol production. The process variables for the maximum strength of ethanol were 0.73 % (w/w) amount of yeast, 76.08 % (w/v) of substrate concentration and pH of 4.6, respectively. Specific gravity (sp.gr) and Gas Chromatography (GC) methods were used to measure and identify the strength of ethanol. The observed strength of ethanol 25.05±1 % (v/v) was found to be very close to the predicted value 24.11 % (v/v). The coefficient of determination, R^2 value was 0.9867 that indicates the goodness of fit for regression model. The insignificance lack of fit (p=0.118) also proved that the model fitted well to the experimental data.

Keywords: waste fruit, *Saccharomyces cerevisiae*, optimization, RSM, BBD

Introduction

The excessive consumption of non-renewable energy has greatly resulted environmental deterioration and public health problems (Kahia *et al*., 2016). This in turn has resulted in the need to find a source of renewable energy. Ethanol is an alcoholic compound that has considered a renewable bio-energy source; it is clear-colorless liquid and eco-friendly potential fuel to power automotive engines (Hossain, 2015). The fermentation of sugar and starch containing crops or byproducts from industries based on such crops could be produced approximately 80% of world supply of alcohol. However, the use of food sources such as corn, sugarcane, wheat and sugar beet as raw material has been continuously debated. Therefore, other low-cost and abundant raw materials such as rice, sugarcane baggase, agricultural and kitchen residues have been investigated as alternative substrates (Uncu and Cekmecelioglu, 2011). The cheapest and easily available source of sugary material such as waste fruits was considered for the production of bioethanol. Among the fruit crops, mango is at the fifth rank of the most significant foodstuffs after rice, corn and milk. According to a report published by Reddy and Reddy in 2005, mango contains a high concentration of sugar 16-18 % (w/v) and acids with organoleptic properties and also contains antioxidants. Sucrose, glucose and fructose are the principal sugars in ripe mango with small amount of cellulose, hemicellulose and pectin. These pulpy fruits are more prone to spoilage due to their nature and this spoilage occurs at the time of harvesting, storage, marketing and processing resulting as wastes. The production of bioethanol from these food processing wastes could be an alternative and attractive disposal of the polluting residues. In the present study, the whole waste mangoes (pulp and peel) was used as the basic raw materials for the production of bioethanol by *Saccharomyces cerevisiae*.

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Materials and Methods

Materials

The waste mangoes were collected from Mawbi Township, Yangon Region. Hydrochloric acid, sodium hydroxide (Analar grade, BDH, England), and *Saccharomyces cerevisiae* (baker's yeast) were purchased from Super Shell (Chemical Store), 27th street, Pabedan Township, Yangon Region.

Preparation of Bioethanol

For the preparation of bioethanol from waste mango, the process flow diagram was shown in Figure (1). Firstly, waste fruits were thoroughly washed with water and seeds were removed. Flesh and peels of waste fruits were sliced into small pieces and pulped using a household blender. And then, pulps were sterilized in an autoclave at 121ºC for 15 min. The fermentation of waste pulp was carried out according to Box-Behnken Design (BBD) by *Saccharomyces cerevisiae* under anaerobic condition. Different ethanol fermentation conditions such as amount of yeast, substrate concentration and pH were used as process variables for experimental design. The initial pH of substrate was adjusted by applying (1 M) hydrochloric acid and (1 M) sodium hydroxide solution. The fermentation period and temperature were limited to 4 days and room temperature of 32ºC. Ethanol was then separated from the fermented broth by simple distillation at 78 ± 1ºC. The distillate was further purified by fractional distillation at 78 ± 1ºC using fractionating column for about 3 hours.

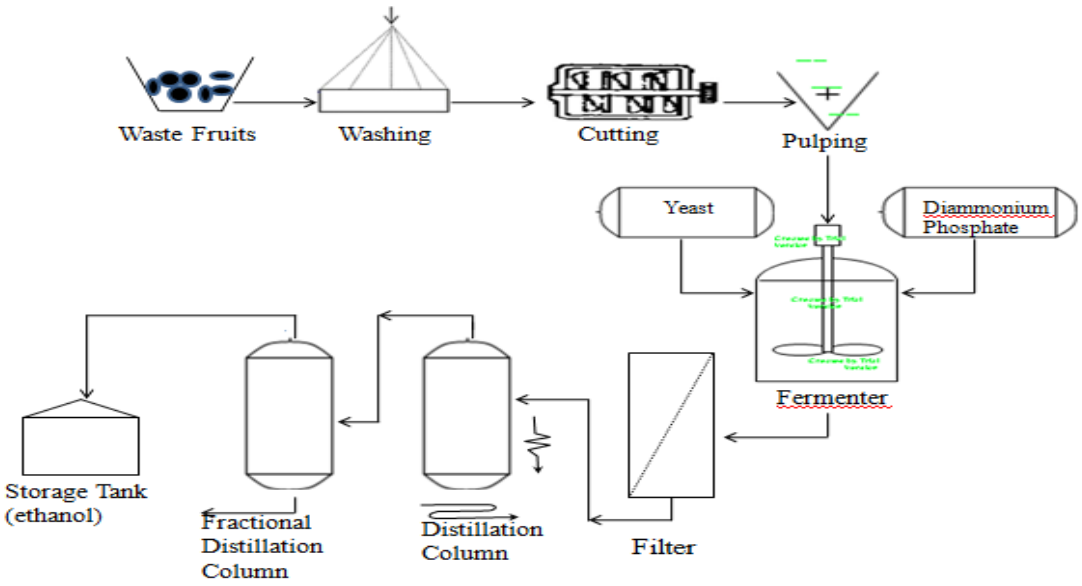


Figure 1 Process Flow Diagram for the Preparation of Bioethanol from fermentation of Waste Mango

Experimental Design

The response surface methodology (RSM) based on Box-Behnken Design was applied to estimate the number of runs and optimum conditions for three independent variables (amount of yeast, substrate concentration and pH) that effecting fermentation process. Table (1) shows the process parameters and levels for fermentation of waste mango.

Table 1 Process Parameters and Levels for Fermentation of Waste Mango

Sr. No.	Parameters	Coded	Levels	
			Low	High
1	Amount of Yeast %(w/w)	X_1	0.4	1.2
2	Substrate Concentration % (w/v)	X_2	50	100
3	pH	X_3	3.5	5.5

Determination of Ethanol Strength

Determination of Strength of Ethanol by Gas Chromatography (GC)

Ethanol strength was determined by gas chromatography by using GC 2010 SHIMADZU equipped with flame ionization detector (FID) at the laboratory of Amtt Co., Ltd. The sample was taken and gathered into a syringe and then injected into an injector port on the device. The temperature of the injector port must be in excess of the boiling point for the sample to obtain accurate readings. This allowed ethanol to convert into gas, which was then pushed into the filters by nitrogen carrier gas. As the gases were passed through the filters, the compound were identified by electronic detector and the alcohol content was then determined.

Analysis of Physicochemical Properties of Bioethanol

Determination of Specific Gravity (sp.gr)

The strength of ethanol was measured by specific gravity. The prepared ethanol sample 100 ml was weighed and filled into a 500 g round bottom flask. 50 ml of distilled water was added into it. The liquid was distilled until approximately 100 ml of solution was obtained. The ethanol sample was then cooled to 15ºC and the specific gravity of the ethanol was measured at 20ºC using a specific gravity bottle. A clean and previously weighed specific gravity bottle was used for this purpose. The specific gravity of the distilled water was also measured using a specific gravity bottle. After the density of the ethanol was determined from the ratio of the weight of liquid held in specific gravity bottle and the weight of water held in specific gravity bottle, ethanol content by volume from specific gravity at 20ºC was read from the table that tabulates the ethanol by volume at 15.56ºC from apparent specific gravity at 20ºC (Lees, 1975).

Determination of pH

The pH of the prepared sample was determined by using a digital pH meter (pH 300, HANNA, China). The glass electrode assembly was first calibrated by using buffer solutions of pH 4 and pH 7 and the electrode was adjusted to those values. After that, pH of the sample

Determination of Total Acidity

10 ml of bioethanol was put in a conical flask with a pipette and two drops of phenolphthalein was then added. It was titrated with 0.1 N sodium hydroxide solution from a burette until the end point was reached. The above procedure was carried out in triplicate. The acidity was calculated as follows:

$$\text{Total acidity as acetic acid } (\%) = \frac{\text{Titre} \times \text{Normality of NaOH} \times 0.006005}{\text{Volume of sample taken}} \times 100$$

1 cm^3 of 0.1 N of NaOH = 0.006005 g of acetic acid

Determination of Refractive Index

The refractive index of the bioethanol was measured by refractometer (Shibuya Optical Co., Ltd, Tokyo, Japan) at the Food Industries Development Supporting Laboratory (FIDSL), UMFCCI Tower, Lanmadaw Township, Yangon Region.

Results and Discussion

The experimental design and statistical analysis for fermentation of waste mango were performed according to the Box-Behnken Design of RSM using MINITAB Software (Version 18.1). (15) experimental runs were conducted according to Box-Behnken Design as tabulated in Table (2). All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors. Significance of each coefficient was determined by ANOVA using the resulting experimental data.

Table 2 The Observed values for Fermentation of Waste Mango

Run No.	Amount of Yeast % (w/w)	Substrate Concentration % (w/v)	pH	Strength of Ethanol % (v/v) Measured by Sp.gr
1	0.8	50	5.5	21.98
2	0.8	100	5.5	25.05
3	1.2	100	4.5	26.72
4	1.2	75	3.5	21.89
5	0.8	75	4.5	24.05
6	0.4	75	5.5	20.12
7	0.8	75	4.5	24.05
8	0.8	100	3.5	23.39
9	0.4	75	3.5	27.79
10	1.2	75	5.5	28.35
11	1.2	50	4.5	23.12
12	0.4	50	4.5	27.21
13	0.8	75	4.5	24.42
14	0.4	100	4.5	21.76
15	0.8	50	3.5	26.35

Based on the ANOVA results in Table (3), the statistically significance of coefficient were determined with a confidence interval greater than 95% ($p<0.05$). Hafid *et al*., (2011) stated that the smaller the p-value, the higher the significance of each variable because p-value represents the significance of variables. The results of ANOVA table revealed that the model was highly reliable with significant linear and interaction effects ($p<0.05$). Puligundla Pradeep *et al*., (2012) reported that the insignificance of the model terms ($p>0.05$) implies the factors have a more influence on the production of alcohol and changes in those variables will significantly affect the process. Moreover, the fitting of the experimental data to the regression model were checked by the coefficient of determination, R^2. Since the coefficient of determination, R^2 value was 0.9867, it is indicated that at least 98% of the total variation could

be explained by the model (Grahovac *et al.*, 2012) and revealed the good agreement between experimental and predicted values. Haaland (1989) and Chauha, *et al*., (2004) have explained on the acceptance of any model with $R^2 > 0.75$. The insignificance lack of fit (p=0.118) also proved that the model fitted well to the experimental data.

Table 3 Analysis of Variance (ANOVA) for Fermentation of Waste Mango

Source	Sum of Squares (SS)	Degree of Freedom (DF)	Mean Square (MS)	F-value	P-value
Model	84.2957	9	9.3662	41.35	0.000
Linear	3.6924	3	1.2308	5.43	0.050
Square	0.7139	3	0.2380	1.05	0.447
2-Way Interaction	79.8894	3	26.6298	117.58	0.000
Error	1.1324	5	0.2265		
Lack-of-fit	1.0412	3	0.3471	7.61	0.118
Pure Error	0.0913	2	0.0456		
Total	85.4281	14			
R-Squared	0.9867				
Adjusted R-Squared	0.9629				

To determine the model satisfies the assumptions of the analysis of variance (ANOVA), the normal plot with residue was analyzed. In the normal probability plot of the raw data, the analysis of variance shows more effective (straightforward) relationship with the residuals. The quadratic polynomial model satisfies the assumptions analysis of variance (ANOVA) i.e. the error distribution is approximately normal (Figure 2).

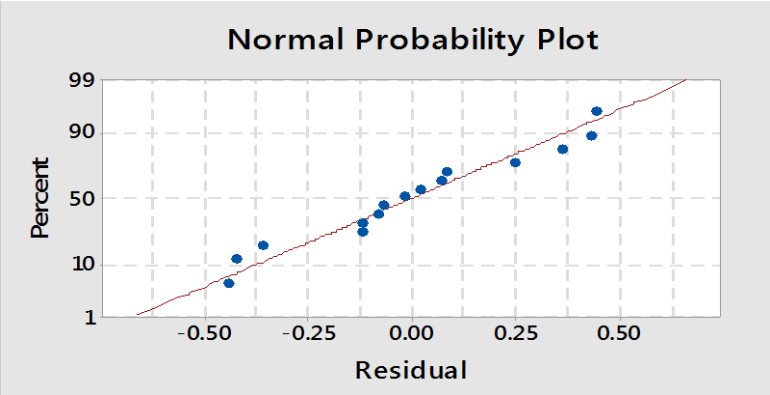
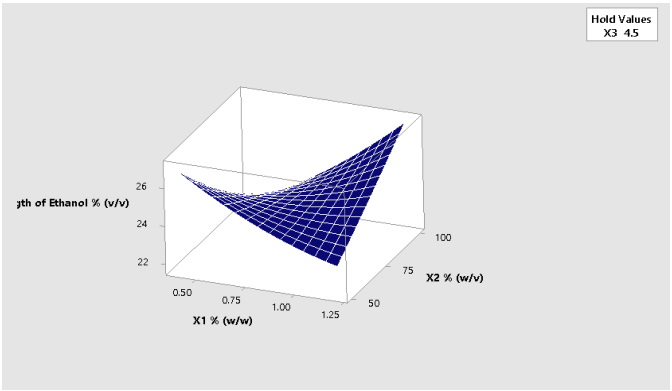


Figure 2 Normal Plot of Residual for Waste Mango

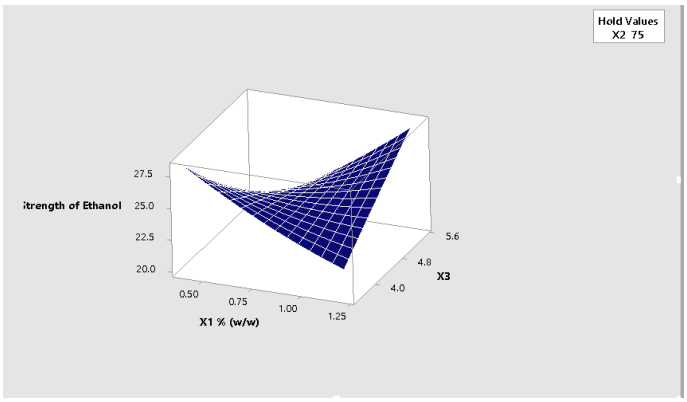
Using the results of experiments, the regression model of strength of ethanol for waste mango is given in equation (1).

$$\text{Strength of ethanol \% (v/v)} = 24.173 + 0.411X_1 - 0.229X_2 - 0.490X_3 + 0.426X_1^2 + 0.081X_2^2 - 0.062X_3^2 + 2.285\ X_1\ X_2 + 3.533\ X_1X_3 + 1.508X_2X_3 \quad \text{.....Eq. (1)}$$

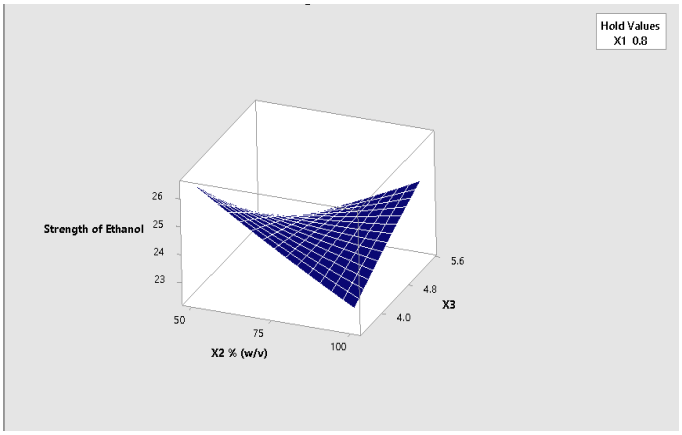
Where X_1, X_2 and X_3 are the coded values of the process variables; amount of yeast, substrate concentration and pH. The sign and magnitude of the coefficients indicate the effect of the variable on the response. The interactive terms of all variables in equation (1) indicated that positive effect on the strength of ethanol. Positive sign of the coefficient means increase in response when the level of the variable is increased while negative sign indicated decrease in the response (Montgomery, 2004). Similarly, the quadratic terms X_1^2 and X_2^2 have positive effect but X_3^2 has negative effect on the response. By using response surface 3D plots, the interaction between two variable factors and their optimum levels could be easily understood. Figure 3 (a, b and c) shows the maximum positive contribution of amount of yeast, substrate concentration and pH on the strength of ethanol during fermentation. Figures (a and b) revealed that the strength of ethanol decreased with increasing amount of yeast, independent of substrate concentration and pH. This may be due to high amount of yeast can adversely affect ethanol production because high increase of yeast level decreases the viability of yeast population and causes inadequate development of ethanol production (Powchinda *et al*., 1999). By increasing the level of pH from 4.0 to 5.6 in Figures (b and c) the strength of ethanol increased gradually. According to the results, the response surface suggests that pH and amount of yeast was a dominance interaction factor on the strength of yeast during bioethanol production from waste mango.



(a)



(b)



(c)

Figure 3 Response Surface Plots for fermentation of Waste Mango (a) Substrate Concentration and Amount of Yeast (b) pH and Amount of Yeast (c) pH and Substrate Concentration

The optimum values of the selected process variables and strength of ethanol were calculated from equation (1) by using MATLAB software. The observed experimental values and values predicted by the equations of the model are presented in Table (4).When compared the strength of ethanol, the predicted value 24.11 % (v/v) was closely agreed with the experimental value of 25.05 ± 1 % (v/v). Similar results were reported by Karuppaiya *et al*., (2009). Under optimum conditions, the variables substrate composition of 62 %(v/v), pH 5.5, incubation temperature 32ºC and fermentation time 37 hrs were utilized to obtain the maximum ethanol concentration of (12.64 g/l) from waste cashew apple juice by *Zymomonas mobilis.* Sasikumar *et al*., (2010) also reported that the maximum response for ethanol production was achieved under the optimum conditions at temperature 32ºC, pH 5.6 and fermentation time 110 hrs. The strength of ethanol identified by gas chromatography (GC) was shown in Table (5) and Figure (4). According to the GC analysis, 86.27% (v/v) strength of ethanol was obtained from second distillate. Some physicochemical properties of bioethanol such as specific gravity, pH, total acidity, refractive index and physical appearance were shown in Table (5). The results revealed that the properties of bioethanol from waste mango met some of the properties of standard bioethanol except the strength of ethanol (global biofuels, 2014).

Table 4 Optimum Process Conditions for Fermentation of Waste Mango

Sr. No.	Parameters	Values	Strength of Ethanol % (v/v) Analyzed by Sp.gr	
			Predicted Value	Experimental Value
1	Amount of Yeast % (w/w)	0.73	24.11	25.05±1
2	Substrate Concentration % (w/v)	76.08		
3	pH	4.6		

Table 5 Physicochemical Properties of Bioethanol after Fractional Distillation

Sr. No.	**Properties**	**Ethanol** (from waste mango)	**Literature Value*** **(**Anhydrous Ethanol**)**
1	Ethanol strength (% v/v)	86.27	99.3 (min.)
2	Specific gravity	0.8304	0.789
3	pH	7.0	6.5-9.0
4	Total acidity %(w/v)	0.0072	0.007
5	Refractive index	1.363	1.36
6	Physical Appearance	Clear and Colourless	Clear and Colourless `

*__Source of data:__ global biofuel, 2014

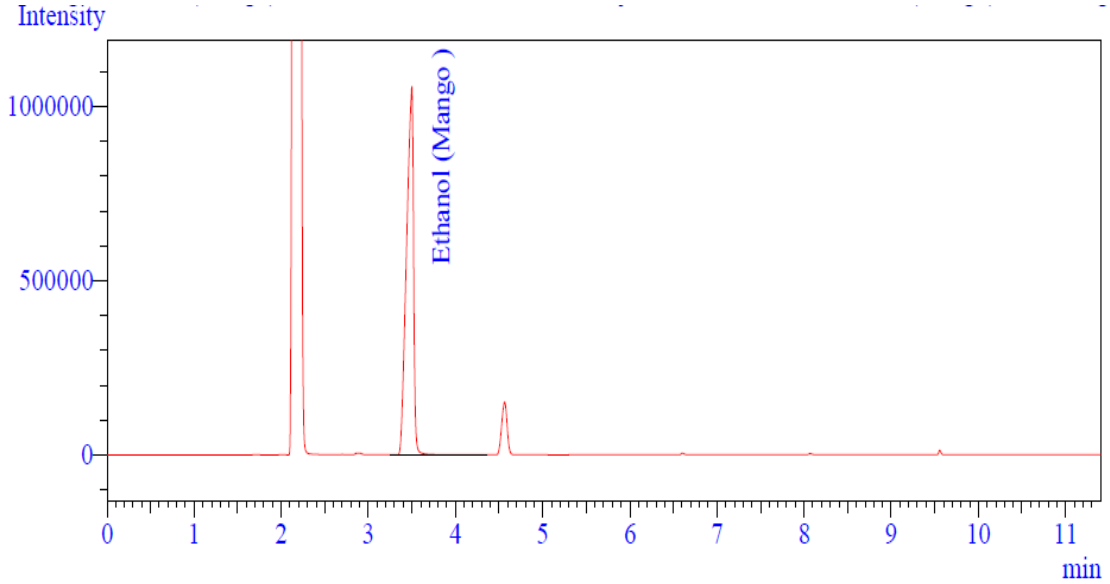


Figure 4 Gas Chromatogram of Bioethanol obtained by Fermentation of Waste Mango

Conclusion

The present study was employed RSM based BBD for the optimization of the strength of ethanol from waste mango using *Saccharomyces cerevisiae*. The results illustrated that the maximum strength of ethanol 25.05±1 % (v/v) was obtained at 0.73 % (w/w) amount of yeast, 76.08 % (w/v) of substrate concentration and pH of 4.6 at a fixed temperature and fermentation time. The slight discrepancies between the experimental and predicted strength of ethanol proved that the RSM was an accurate and applicable tool to optimize the ethanol production from waste mango. Besides, the high reducing sugar content 303 ± 30 (mg/g) of waste mango with small amount of cellulose, hemicelluloses and pectin showed the potential of waste mango as a good feedstock for bioethanol production, also it can be used as an alternative fuel to reduce the load on conventional fossil fuel resources.

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