Optimization of Ethanol Production from Fresh Jackfruit Seeds Using Response Surface Methodology

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Abstract: Fresh jackfruit seeds consist of about 36% carbohydrates, molecules which can be easily converted to ethanol. Ethanol fermentation conditions of jackfruit seeds using microorganisms from rice cake starter were optimized by the response surface methodology (RSM). The central composite design (CCD) was used to investigate optimum parameter levels in ethanol production (an anaerobic process), viz. temperature, pH and time. The optimum fermentation conditions were a temperature of 32.2°C, pH of 5.2 and time of 124.5 h. In this case, the highest amount of ethanol obtained was 11.5%v.

Keywords: Ethanol, Jackfruit seed, Response surface methodology (RSM), Rice cake starter

1. Introduction

Several feedstock materials can be used for ethanol production, the simplest being basic sugar, which can be fed directly into the fermentation tank, and digested by yeast. Jackfruit, *Artocarpus heterophyllus Lam*., is a popular fruit crop that is widely grown in Thailand, Malaysia, the Philippines and other tropical areas. Its seeds constitute around 10.0-15.0% of the whole fruit weight, and have a high carbohydrate and protein content [1-2]. The nutritive values per 100 g of the edible portion of fresh seeds are 38.4 g carbohydrate, 6.6 g crude protein, 0.4 g fat, 1.5 g fiber, 1.3-1.5 g ash and 51.6-57.8 g moisture [3]. The fresh seeds cannot be kept for a long time, so can be turned into seed flour as an alternative material which can be used for food, drink or ethanol fuel production. Ethanol can be used as an additive to gasoline fuel and is a promising alternative energy source [4]. The major fermentation products from cereals in the Asia-Pacific region are acids [5] and alcohols. Alcohol fermentation is more important than an acid fermentation in terms of the relative amounts of cereals used for fermentation and the varieties of products produced. Alcohol fermentation is carried out under mild acidic conditions, which prevents the growth of spoilage and pathogenic microorganisms during the initial stage of fermentation.

Loog-Pang Kao Mhark rice cake starter, is a microorganism source (yeast). It is isolated and characterized by its morphological, physiological, genetic and fermentation properties. It is a traditional starter culture of alcoholic production for the food and drink industries. Important microorganisms in rice cake starter are *Amylomyces sp., Aspergillus sp., Rhizopus sp., Mucor sp.* and *Absidia sp.* [6]. The microflora of marcha (a traditional amylolytic starter commonly used in Himalayan areas to produce sweet-sour alcoholic drinks) consists of filamentous molds such as *Mucor circinelloides* and *Rhizopus chinensis*, yeasts such as *Saccharomycopsis fibuligera* and *Pichia anomala*, and bacteria such as *Pediococcus pentosaceus* [7]. The majority of starter cultures are natural isolates of the desirable microorganisms found naturally in the substrates [8]. The use of starters also significantly influences the physical, chemical, biochemical, and sensory properties of cheeses [9].

Many statistical experimental design methods have been employed in bioprocess optimization. Among these, response surface methodology (RSM) uses statistical models, and therefore practitioners need to be aware that even the best statistical model is an approximation of reality. RSM can identify the effects of individual variables in efficiently seeking the optimum conditions of a multivariable system. A variety of products can be obtained from starchy or cellulosic biomass via hydrolysis [10-11]. In terms of volume, alcohol is one of the largest of these. Ethanol production can also be effectively promoted by optimizing the conditions of simultaneous saccharification and fermentation (SSF) using RSM [12]. RSM was an accurate tool for optimizing ethanol production from kitchen garbage. By utilizing statistical methodology, the optimum conditions for ethanol production from kitchen garbage was determined to be obtained at 33.05 g/L ethanol product, with a time of 67.60 h, pH of 4.18 and temperature of 35°C [13]. The application of experimental design using RSM for the fermentation process can result in impressive product yields, reduce process variability, and lower overall production costs [14]. RSM has been also applied to the optimization of nutrient concentration in a culture medium for enzymatic production [15]. The statistical designs have been used to reduce the number of experiments of anaerobic submerged fermentation processes [16]. RSM using the Box-Wilson experimental design method (Central Composite Design: CCD) can be used to develop a mathematical correlation between the time, pH and temperature of fermentation and the yield of ethanol [17].

In this work, we identified the optimum conditions for ethanol production from jackfruit seeds, and the corresponding correlations among these factors.

2. Experimental

2.1. Materials

2.1.1 Substrate

Fresh jackfruit seeds (*Artocarpus heterophyllus Lam.*) was purchased from a local market in Songkhla province, Thailand. The composition of the jackfruit seeds is shown in Table 1.

Table 1. Components of fresh jackfruit seeds.

Component	Fresh jackfruit seeds
Protein	5.48%
Crude fat	0.21%
Moisture	56.51%
Ash	1.42%
Crude fiber	1.27%
Total carbohydrate	36.38%
Energy	169.33 kcal
Total sugar	0.60%
Reduced sugar	133.2 µg/ml

2.1.2 Microorganism

Loog-pang Kao Mhark (Rice cake starter) were purchased from a local market. The starter culture media was stored at < 10°C in a refrigerator until used.

2.2 Studies of the physical pretreatment and thermal hydrolysis of the jackfruit seeds

Fresh seeds were cleaned and their white arils were peeled off along with other impurities. The seeds were sliced into thin chips, which were then crushed using a grinder and mashed through an 18-mesh number metal screen. A total of 30 g mashed seed together with 30 ml clean water was put into each of 250 ml Erlenmeyer flasks. The flasks were then immersed in an oil-bath at temperatures of 70, 75, 80, 85 and 90°C for heating times of 5, 10, 15 and 20 min. with a constant shaking rate of 100 rpm.

2.3 Studies of ethanol fermentation

The pretreated jackfruit seeds were mixed with rice cake starter in 250 ml air-locked Erlenmeyer flasks. Then nitrogen gas was added to the flasks for the anaerobic process, following inoculation with 3%wt active rice cake starter (from previous studies). The flasks were put in an oil-bath under a controlled temperature with a constant shaking rate of 100 rpm. The medium used was prepared with 0.1 M citrate-phosphate buffer with different pH values of 3.3 to 6.0. The broth was sampled at certain time intervals during analysis. After the fermentation process, a distillery process was carried out to purify the ethanol product. A block flow diagram of ethanol production is shown in Fig. 1.

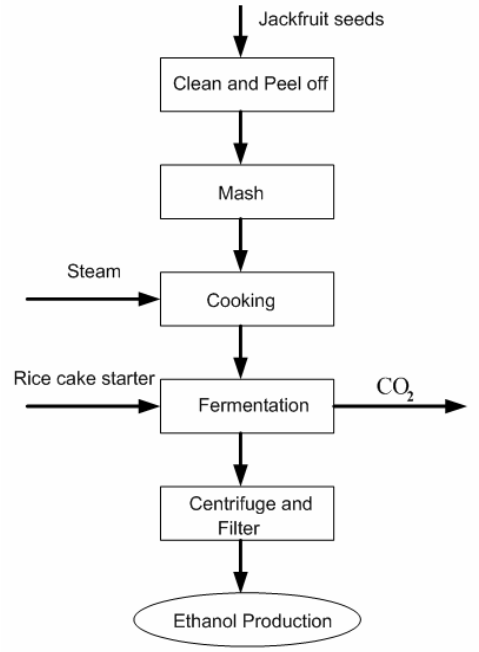

Figure 1. Block flow diagram of ethanol production.

2.4 Analytical methods

Ethanol was quantified by gas liquid chromatography (GC 6890 flame ionization detector, Hewlett Packard, USA). The column was a HP-FFAP (length 2.5 m, 0.32 mm ID.). The column oven was operated isothermally at 150°C, the detector and injection ports were kept at 250°C. Nitrogen was used as a carrier gas at a flow rate of 20 ml/min; the combustion gas was a mixture of hydrogen and air. Methanol was used as the internal standard and the injection volume was 30 μL.

Representative samples of jackfruit seeds were analyzed in triplicate for moisture, ash, protein, lipid and fiber content using standard methods of the Association of Official Analytical Chemists [18].

Reducing sugars were estimated by the dinitrosalicylic acid (DNS) method [19] using a double beam UV-Vis spectrophotometer (model HP 8453) with UV-Visible ChemStation software. To prepare a standard curve of glucose, 1 g of glucose was dissolved in a small quantity of distilled water, and its volume was raised up to 100 ml. This stock solution (10.0 g/L) was used to make eight appropriate glucose dilutions, with 0.25 to 2.00 ml of DNS reagent added into each tube. The tubes were placed in a water bath at 90°C for 30 min. Next, they were cooled at a room temperature and diluted to 20.0 ml with distilled water. A blank was run in parallel, replacing 2.0 ml of the sample dilution with distilled water. The % transmittance was measured at 575 nm on a spectrophotometer. A standard curve was plotted, taking the transmittance at the ordinate and sugar concentration at the abscissa. Total sugar was measured by the Lane-Eynon method [20]. Lane-Eynon is a titration method, so mixed Fehling's solution was titrated with the sample using methylene blue as an indicator.

2.5 Experimental design and optimization

Three 2^3-factorial central composite designs (2^3-FCCD) were carried out in order to identify optimum parameter levels for ethanol fermentation. Time (X_1, h), pH (X_2), and temperature (X_3, °C) were chosen as the independent variables as shown in Tables 2 and 3. Ethanol concentration in the product (Y, %v) was used as a dependent output variable. For statistical calculations the variable X_i was coded as x_i according to Eq. (1):

$$x_i = \frac{\left(X_i - \overline{x_i}\right)}{\Delta x_j}, \qquad i = 1, 2, 3, \ldots, k \tag{1}$$

where x_i is a dimensionless value of the independent variable, X_i is the real value of the independent variable, $\overline{x_i}$ is the real value of the independent variable at the center point, and Δx_j is the step change.

To optimize the parameters, 17 experiments were performed according to Table 2. Among these, six replications were at center points ($n_0 = 6$), while the distance of the axial points was ± 1.68, calculated from Eq. (2):

$$\alpha = (2^n)^{1/4} \tag{2}$$

where α is the distance of the axial points and n is the number of independent variables. The coefficient of the polynomial model was calculated using Eq. (3):

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_{11} X_1^2 + b_{22} X_2^2 + b_{33} X_3^2 + b_{12} X_1 X_2 + b_{23} X_2 X_3 + b_{13} X_1 X_3 \tag{3}$$

where: Y is the predicted response; X_1, X_2, X_3 are the independent variables; b_0 is the offset term; b_1, b_2, b_3 are the linear effects; b_{11}, b_{22}, b_{33} are the square effects; and b_{12}, b_{23}, b_{13} are the cross effects of the interaction terms.

Table 2. Variables of the experimental design for fermentation process.

Run No.	X_1	X_2	X_3
1	0	0	0
2	-1	-1	1
3	1	-1	1
4	1	1	1
5	0	0	0
6	0	0	0
7	-1.68	0	0
8	1	-1	-1
9	0	1.68	0
10	0	-1.68	0
11	0	0	-1.68
12	-1	1	1
13	-1	-1	1-
14	1.68	0	0
15	1	1	-1
16	-1	1	-1
17	0	0	1.68

Table 3. The central composite design matrix employed for the 3 independent variables (Actual values are given in Table 2).

Variables	Coded levels				
	-1.682	**-1**	**0**	**1**	**1.682**
Time (h)	79.63	96	120	144	160.37
pH	3.32	4	5	6	6.68
Temperature (°C)	26.59	30	35	40	43.41

3. Results and Discussion

3.1. Components of jackfruit seeds

Analysis of the components of fresh jackfruit seeds is shown in Table 1. The major components of the seeds are 36.4% carbohydrate that can be hydrolyzed to fermentable sugars before transforming into ethanol, and 56.5% moisture that provide good growth of microorganisms and save water material used in the fermentation process. These figures show that jackfruit seeds are an appropriate feedstock for ethanol production.

3.2. Effect of temperature on thermal hydrolysis

Fig. 2 shows the effect of boiling temperature on ethanol content. The ethanol content was increased with rising temperature. The optimum boiling temperature for starch can be obtained at the gelatinization point. The gelatinizing temperature is an important property in starch characterization. Gelatinization range has been reported to be dependent on differences in the degree of heterogeneity of crystallites within the starch granules. The optimum gelatinization was achieved at 85°C. It could provide 9.8%v ethanol product. The ethanol profile implied that the starch molecule imbibes water and the starch granules, small molecules of amylase, could be leached out into the aqueous medium [21]. The gelatinization of jackfruit seed starch gave two endotherm peaks. The first peak center was around 65°C which was the gelatinizing temperature and the second peak center was at about 80°C, the melting temperature of the amylose-liquid compound [22]. These were in agreement with previous studies with the first peak in the range 63.1°C to 73.3°C and the second peak in the range 79.6°C to 90.2°C [23].

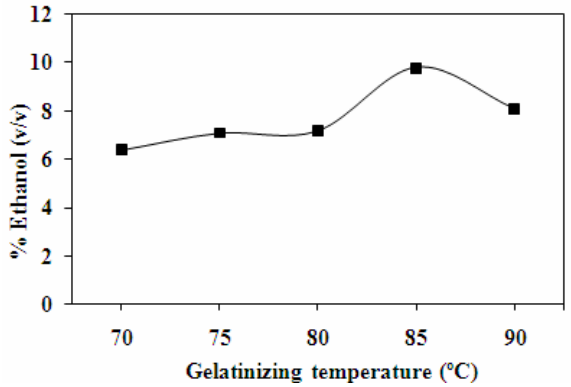

Figure 2. Effect of boiling temperature in the pretreatment step before ethanol fermentation at temperature of 30°C with Loog-pang 3% wt, pH of 5.0 for 180 h and a shaking rate of 60 rpm.

3.3. Response surface analysis for the optimization of three factors

The important factors which affect ethanol fermentation, were the temperature, initial pH, and time. The trials and results for 2^3- FCCD are shown in Table 4. Based on these results the model can be utilized to generate response surfaces and contour curves that indicated the effects of the factors on the ethanol content. The respond surfaces were fitted with Eq. (4):

$$Y = -56.02 + 0.01178\,X_1 + 10.17\,X_2 + 2.453\,X_3 - 0.000246\,X_1^2 - 0.884\,X_2^2 - 0.03536\,X_3^2 + 0.00625\,X_1X_2 - 0.05\,X_2X_3 + 0.000625\,X_1X_3 \quad (4)$$

The variable coefficient indicates the priority of the variables. Eq. (4) shows that each variable has very little interaction (X_1X_2, X_2X_3 and X_1X_3) and relationships are linear rather than curved (coefficients of X_1, X_2 and X_3 are more than that of X_1^2, X_2^2 and X_3^2). In addition, the linear effect of X_2 (pH) is the most important factor. The higher the pH (in the range of 4.4 to 5.9) the higher is conversion rate.

The predicted and experimental values of ethanol production are given in Table 4. To test the fit of the CCD model, the regression equation and determination coefficient (R^2) were calculated. The value $R^2 = 0.968$ implied that it was a quite good fit and that 96.8% of the variation could be explained by the model. As illustrated in Tables 5 and 6, a P value of ≤ 0.005 for any factor in an analysis of variance (ANOVA) indicates a significant effect of the corresponding factors. The predicted optimum levels of temperature, initial pH and time of fermentation were obtained by applying regression analysis to Eq. (4). However, the factor analysis results implied that time was less significant (P>0.05) [24-25]. As a result, the model, Eq. (4), might be modified into Eq. (5). Fig. 3 shows a satisfactory correlation between predicted and experimental values.

$$Y = -46.48 + 8.747\,X_2 + 2.160\,X_3 - 0.842\,X_2^2 - 0.03369\,X_3^2 \quad (5)$$

Table 4. Experimental and predicted contents by RSM for ethanol fermentation.

Run No.	X_1	X_2	X_3	%Ethanol	
				Experimental	Predicted
1	120	5	35	10.7	10.7
2	96	4	40	8.0	7.7
3	144	4	40	8.0	7.8
4	144	6	40	8.4	8.3
5	120	5	35	10.8	10.7
6	120	5	35	10.8	10.7
7	79.63	5	35	9.6	10.1
8	144	4	30	9.2	9.2
9	120	6.68	35	8.5	8.8
10	120	3.32	35	7.4	7.7
11	120	5	26.59	9.7	9.9
12	96	6	40	7.9	7.5
13	96	4	30	9.6	9.3
14	160.37	5	35	10.5	10.6
15	144	6	30	10.7	10.6
16	96	6	30	10.4	10.2
17	120	5	43.41	6.2	6.6

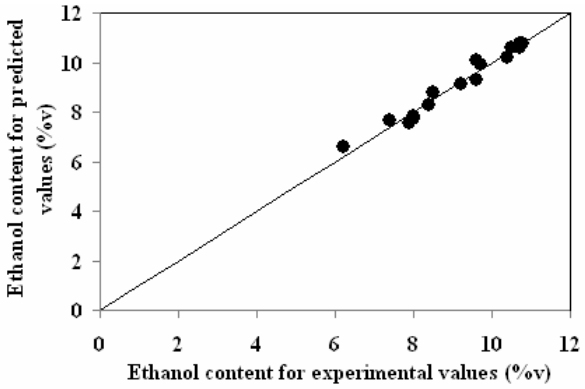

Figure 3. Parity plot showing the distribution of experimental vs. predicted values of ethanol content.

Table 5. Coefficients, *t*-statistics and significance probability of the model for Eq. (4).

Term	Coefficient	Value	Standard Error	*t*-value	*P*-value
Constant	b0	-56.02	11.06	-5.06	0.0010
Time	b1	0.012	0.06	0.17	0.86
pH	b2	10.17	1.61	6.30	0.004
Temperature	b3	2.45	0.36	6.65	0.002
Time × Time	b4	-0.00025	0.00019	-1.25	0.25
pH × pH	b5	-0.88	0.11	-7.84	0.0001
Temperature × Temperature	b6	-0.035	0.0045	-7.84	0.0001
Time × pH	b7	0.0063	0.0056	1.12	0.20
pH × Time	b8	-0.05	0.027	-1.86	0.10
Time × Temperature	b9	0.00063	0.001	0.56	0.59

Table 6. ANOVA for the full quadratic model.

Source of variation	%Sum of squares (SS)	Degrees of freedom (DF)	Mean squares (MS)	*F*-value	Probe > *F*
Regression	97	9	3.36	23.49	0
Residual	3	7	0.14		
Totla	100	16			

3.4. Interactions among the factors

Fig. 4 shows the effects of pH and temperature on ethanol content. The ethanol content of fermented product increased with increasing temperature and pH in the range of 27 to 36°C and pH of 4.4-5.9. However, the conversion rates were reduced for a further increase in temperature and pH value.

The effects of fermentation time and pH on ethanol content are shown in Fig. 5. The fermentation yield increased with an increase in pH and time. In contrast a higher pH from 5.9 to 6.7 caused a reduction in the yield. To obtain an optimum ethanol content the fermentation process should be operated at a pH in the range of 4.4 to 5.9 for a time in the range of 106.5 to 160.4 h.

The interaction effects of time and temperature on ethanol content (Fig. 6) imply that the fermentation process should be carried out at a temperature in the range of 28.5 to 35.9°C for a time of 97.6 to 106.4 h to achieve a maximum content of ethanol.

The results of the influence and interaction of the factors using CCD indicated that the highest yield could be reached near the center point of the operating conditions as on the contour curves (Figs. 4-6). The optimum conditions were at 32.2°C, pH 5.2 for 124.5 h which could provide 11.1%v for predicted ethanol content and 11.5%v for experimental content. These showed that the model, Eq. (4), could be useful.

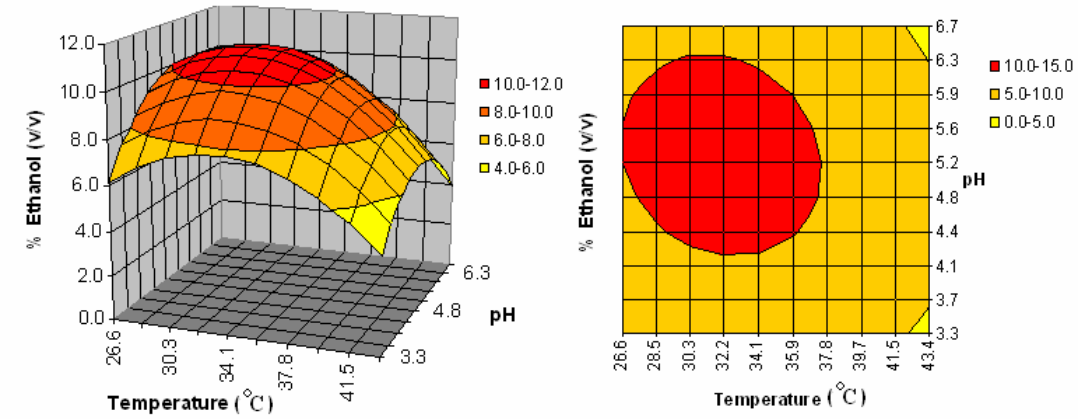

Figure 4. Response surface and contour plot of pH vs. temperature on ethanol content for a fermentation time of 120 h.

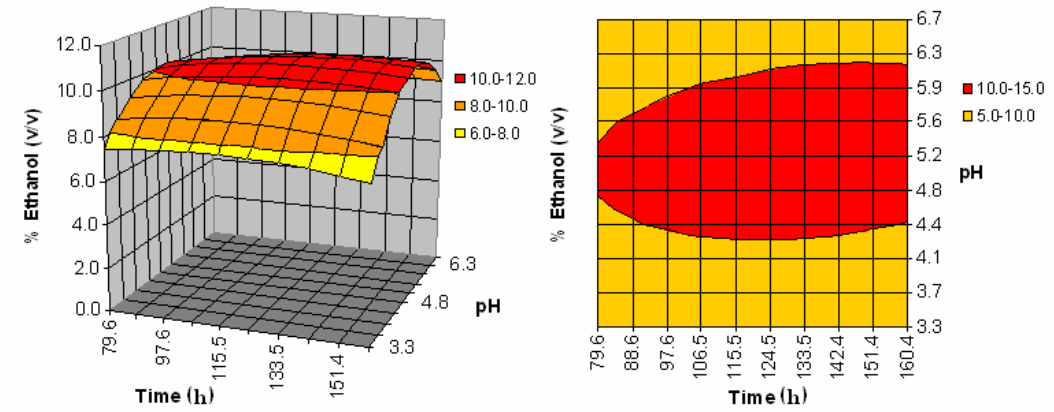

Figure 5. Response surface and contour plot of pH vs. time on ethanol content at a temperature of 35°C.

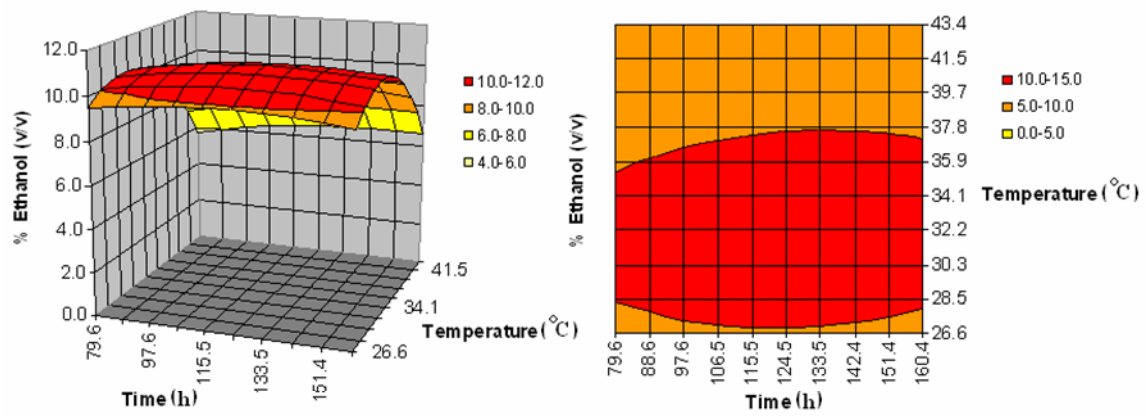

Figure 6. Response surface and contour plot of temperature vs. time on ethanol content with an initial pH of 5.

4. Conclusions

The boiling followed by fermentation using Loog-pang Kao Mhark for the ethanol production from fresh jackfruit seeds, a low cost biomass material, was evaluated. The heating pretreatment and hydrolysis could be explained by the gelatinization point. The range of temperatures, time and pH were established to optimize the fermentation condition by RSM which could save experiment times and cost.

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References

[1] Bobbio FO, El-Dash AA, Bobbio PA, Rodrigues LR, Isolation and characterization of the physicochemical properties of the starch of jackfruit seeds (*Artocarpus heterophyllus*), *Cereal Chem* 55 (1978) 505-511.

[2] Kumar S, Singh AB, Abidi AB, Upadhyay RG, Singh A, Proximate composition of jackfruit seeds, *J Food Sci Techno* 25 (1988) 308-309.

[3] Morton JF, Jackfruit, *Fruits of warm climates* (1987) Miami: Florida Flair Books, Available online: http://www.hort.purdue.edu/newcrop/morton/jackfruit_ars.html (May 21, 2010).

[4] Ananda SC, Saravanan CG, Study of Combustion Characteristics of an SI Engine Fuelled with Ethanol and Oxygenated Fuel Additives, *JSEE* 1 (2010) 85-91.

[5] Chooklin S, Kaewsichan L, Kaewsrichan J, Potential Use of *Lactobacillus casei* TISTR 1500 for the Bioconversion of Palmyra Sap to Lactic acid, JSEE 2 (2011) 83-87.

[6] Pichyangkura S, Kulprecha S, *Survey of mycelial molds in loogpang from various sources in Thailand* (1977) Symposium on Indigenous Fermented Foods, Bangkok, Thailand.

[7] Tamang JP, Sarkar PK, Microflora of marcha: an amylolytic fermentation starter, *Microbios* 81 (1996) 115-122.

[8] De Vuyst L, Vancanneyt M, Biodiversity and identification of sourdough lactic acid bacteria, *Food Microbiol* 24 (2007) 120-127.

[9] Hayaloglu AA, Guven M, Fox PF, McSweeney PLH, Influence of Starters on Chemical, Biochemical, and Sensory Changes in Turkish White-Brined Cheese During Ripening, *J Dairy Sci* 88 (2005) 3460-3474.

[10] Porrarath S, Optimizing enzymatic extraction of cereal milk using response surface methodology, *Songkla J Sci Techn* 33/4 (2011) 389-395.

[11] Leenakul W, Tippayawong N, Dilute Acid Pretreatment of Bamboo for Fermentable Sugar Production, *JSEE* 1 (2010) 117-120.

[12] Bandaru VVR, Somalanka SR, Mendu DR, Madicherla NR, Chityala A, Optimization of fermentation conditions for the production of ethanol from sago starch by co-immobilized amyloglucosidase and cells of *Zymomonas mobilis* using response surface methodology, *Enzyme Microb Tech* 38 (2006) 209-214.

[13] Wang Q, Ma H, Xu W, Gong L, Zhang W, Zou D, Ethanol production from kitchen garbage using response surface methodology, *Biochem Eng J* 39 (2008) 604-610.

[14] Rao KJ, Kim C-H, Rhee S-K, Statistical optimization of medium for the production of recombinant hirudin from *Saccharomyces cerevisiae* using response surface methodology, *Process Biochem* 35 (2000) 639-647.

[15] Kawaguti HY, Manrich E, Sato HH, Application of response surface methodology for glucosyltransferase production and conversion of sucrose into isomaltulose using free Erwinia sp. Cells, *Electron J Biotechn* 9 (2006) 482-493.

[16] Balusu R, Paduru RM, Seenayya G, Reddy G, Production of ethanol from cellulosic biomass by Clostridium thermocellum SS19 in submerged fermentation: screening of nutrients using Plackett-Burman design, *Appl Biochem Biotech* 113 (2004) 133-141.

[17] Box GEP, Wilson KB, On the experimental attainment of optimum conditions, *J Roy Statist Soc* Ser B Metho 13 (1951) 1-45.

[18] AOAC, *Official methods of analysis* (2000) Arlington, VA: AOAC International.

[19] Miller GL, Use of dinitrosalicyclic acid reagent for determination of reducing sugar, *Anal Chem* 31 (1959) 426-428.

[20] Lane JH, Eynon L, Determination of reducing sugars by means of Fehling's solution with methylene blue as internal indicator, *J Soc Chem Ind Trans* (1923) 32-36.

[21] Balagopalan C, Padmaja G, Nanda SK, Moorthy SN, *Cassava in food, feed and Industry* (1988) pp. 126-127, CRC Press Inc., Florida.

[22] Oates CG, Powell AD, Bioavailability of carbohydrate material stored in tropical fruit seeds, *Food Chem* 56 (1996) 405-414.

[23] Kategunya R, Sanguansri C, *Thermal properties and morphology of flour and starch extracted from jackfruit seeds (Artocarpus heterophyllus)* (2008) Congress on Science and Technology of Thailand.

[24] Wang Q, Ma H, Xu W, Gong L, Zhang W and Zou D, Ethanol production from kitchen garbage using response surface methodology, *Bioch Eng J* 39 (2008) 604-610.

[25] Bandaru VVR, Somalanka SR, Mendu DR, Madicherla NR and Chityala A, Optimization of fermentation conditions for the production of ethanol from sago starch by co-immobilized amyloglucosidase and cells of *Zymomonas mobilis* using response surface methodology, *Enzym and Microb Techn* 38 (2006) 209-214.

