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Optimization of enzymatic liquefaction of papaya (*Carica papaya* L.) and jackfruit (*Artocarpus heterophyllus* Lam.) pulp using response surface methodology

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Abstract

Enzymatic liquefaction of jackfruit and papaya pulp, using a commercial enzyme (pectinase) was investigated and evaluated by response surface methodology. The effect of enzyme concentration, incubation time and hydrolysis temperature were found to be significant in case of jackfruit whereas juice yield in case of papaya was influenced by enzyme concentration and incubation time. In the present study, polynomial equations were derived using multivariate analysis, for predicting the reduction in alcohol insoluble solids and increase in juice yield for jackfruit and papaya respectively. The models were verified experimentally against predicted response and were found to be suitable.

Key words: Enzymatic liquefaction, optimization, jackfruit, *Artocarpus heterophyllus* Lam, papaya, *Carica papaya*, response surface methodology.

Introduction

Use of enzymes as processing aids has been practiced in the fruit and vegetable processing industry to obtain low viscosity pulp, high yield of juices and solids, clarity and filterability of juices[1-3]. Recently, a mixture of cellulolytic and pectinolytic enzyme preparations has been increasingly employed in fruit and vegetable liquefaction due to their synergistic effects [4-6]. Few research papers on enzymatic liquefaction of banana[7-9] and mango[10, 11] have been published. World demand for fruit juices, including exotic or tropical juices is increasing. Value added products from underutilized fruits like papaya (*Carica papaya L.*) and jackfruit (*Artocarpus heterophyllus* Lam.) can be commercially exploited due to their high nutritive value. The fruit juices of papaya and jackfruit can also compete either as individual juice or as blends.

Although there are reports on enzymatic liquefaction of jackfruit and papaya[4], little information is available on their optimization with respect to operating parameters. In order to obtain juice from jackfruit and papaya pulp, pectinase enzyme is added which reduces the viscosity of the pulp and facilitates separation of the juice from the pulp. The enzymatic liquefaction is reported to be influenced by the enzyme concentration, incubation time and hydrolysis temperature[12]. The effect of enzyme concentration and incubation time and temperature has been investigated in the present study. A graphical optimization technique was used to minimize alcohol insoluble solids (AIS) and to increase juice yield of jackfruit and papaya respectively.

When many factors and interactions influence the desired response, response surface methodology (RSM) is an effective tool for optimizing the process. RSM is a statistical method that uses quantitative data from appropriate experimental designs to determine and simultaneously evaluate multivariate equations. Based on the preliminary studies, incubation time (X_1) and enzyme concentration (X_2) were found to be the two independent variables for papaya, and therefore a central composite rotatable design (CCRD) to fit a second order polynomial by a least square regression technique was adopted. Preliminary studies on jackfruit revealed that the interaction of enzyme concentration (X_1), incubation time (X_2) and temperature (X_3) had significant effect. Therefore, a shell design was adopted for its optimization. Shell design allows the selection of different levels (3, 5 and 7) of individual variables. The factor that has higher influences is studied at more number of levels and that with least effect at lower levels. An equation is used to describe the influence of variables on the response to determine the interrelationship among the variables and the response.

A new product has been developed in this work, namely jackfruit and papaya juice concentrates. The enzymatic liquefaction of jackfruit and papaya pulp is exploited for the reduction in the viscosity and AIS, in order to increase flux during microfiltration. The juice thus obtained can be concentrated by reverse osmosis without any phase change, retaining the important constituents intact, and later the juice is concentrated to obtain jackfruit and papaya juice concentrate. The enzymatic liquefaction step is the most critical one, which will influence the yield and clarity of the juice and the presence of AIS.

The objective of the present work is to 1) study the effect of enzyme concentration, incubation time and hydrolysis temperature on i) reduction in AIS during liquefaction of jackfruit ii) increase in juice yield in case of papaya and 2) optimize the enzymatic liquefaction of papaya and jackfruit pulp.

Materials and methods

Materials: Jackfruit and papaya pulp were prepared using a pulper (Bsen and Barry, India) having a sieve size of 0.80 mm. The pulp was pasteurized at the temperature of 80±1°C for 20 minutes, cooled to room temperature and stored at –18°C. The pulp was thawed and used for the experiments. The chemical and physical properties of the jackfruit and papaya pulp are given in Table 1. Pectinase enzyme was procured from Biocon, India. The polygalacturonase activity of the enzyme was estimated by the method of Collmer et al.[13], and was found to be 1590 U ml^{-1}.

Experimental procedure

Papaya liquefaction: Papaya pulp was homogenized and 250 g was taken for each experiment. The samples were kept in a temperature controlled water bath (29±1°C) with intermittent stirring and subjected to treatments as given in Table 2. Later the samples were kept in boiling water bath for 10 minutes to inactivate the enzyme.

Jackfruit liquefaction: Jackfruit pulp was homogenized and 250 g was taken for each experiment. The samples were kept in a temperature controlled water bath (29±1°C, 37±1°C, 45±1°C) with intermittent stirring and subjected to the treatments (Table 3). Later the samples were kept in boiling water bath for 10 minutes to inactivate the enzyme.

Alcohol insoluble solids: Alcohol insoluble solids were determined by AOAC procedure[14]**.**

Experimental design:

Papaya: A central composite rotatable design with two variables was used to study the response pattern and to determine the optimum combination of the variables[15]. The variables optimized were enzyme concentration (0.1-0.85%) and time of reaction (20-180 min) each at five levels (Table 2).

The CCRD was arranged to allow for fitting an appropriate regression model using a multiple regression program (Table 2). The CCRD combines the vertices of the hypercubes whose co-ordinates are given by *2n* factorial design to provide for the estimation of the curvature of the model. Five replicates (treatments 9-13) at the center of the design were used to allow for the estimation of the pure error sum of squares. Experiments were randomized in order to maximize the effects of unexplained variability in the observed responses owing to extraneous factors.

Jackfruit: A shell design as described by Doehlert[16] was planned for the optimization of jackfruit liquefaction process with three variables to study the response pattern and to determine the optimum combination of the variables (Table 2). The variables optimized were enzyme concentration (0.1-0.85%), time of reaction (20-180 min) and temperature of incubation (29-45°C). A total of 17 experiments were carried out, out of which the ninth experimental domain was repeated five times in order to determine the residual variance value for the polynomial equations derived (Table 3).

Statistical analysis: A second order polynomial equation was used to fit the experimental data (Table 2 and 3). The model proposed for the responses Y_{AIS} and Y_{YIELD} for jackfruit and papaya respectively are:

$$Y_{AIS}= 37.73263-13.28017x_1-0.09465x_2-1.406192x_3+2.617284x_1^2 +0.011944x_1x_2+0.20810x_1x_3+0.0000563x_2^2+0.002003x_2x_3+0.01553 \quad (1)$$

$$Y_{YIELD}=45.1828+0.2212x_1+36.5733x_2-0.0003x_1^2-0.125x1x_2-6.5733x_2^2 \quad (2)$$

where Y_{AIS} predicted responses for AIS (%), x_1 is enzyme concentration, x_2 is time of incubation and x_3 is temperature of incubation for jackfruit. In case of papaya, Y_{YIELD} is the predicted response for yield (%), x_1 is the time of incubation and x_2 is the enzyme concentration.

Optimization of the fitted polynomials was done graphically. The optimum condition was verified by conducting experiments at that condition. Responses were monitored and results were compared with the model predictions.

The fitted polynomial equation was expressed as surface and contour plots using the Microsoft EXCEL program (Microsoft Inc. 2000) in order to visualize the relation between the response and experimental levels of each factor and to deduce the optimum conditions.

Other statistical analysis: Three statistical parameters were chosen to evaluate the comparative results [17].

a. Root mean square difference (RSMD); (Equation-3)

$$RSMD=\sqrt{\frac{\sum\left(k_{exp}-k_{prd}\right)^2}{N}}$$

b. Standard deviation of difference (S_D); (Equation-4)

$$SD=\sqrt{\frac{\sum\left(k_{exp}-k_{prd}\right)^2-\frac{\left[\sum\left(k_{exp}-k_{prd}\right)\right]^2}{N}}{N-1}}$$

c. The average percent error; (Equation-5)

$$E=\frac{100}{N}\sum\frac{\left|k_{exp}-k_{prd}\right|}{k_{exp}}$$

where k_{exp} and k_{prd} are experimental and predicted values respectively, and N denotes the number of data points.

Results and Discussion

Softening of fruit flesh occurred as indicated by the rapid decrease in the amount of AIS and increase in juice yield, in both fruits investigated. The action of the enzyme leads to degradation and solubilisation of otherwise insoluble materials (pectin, hemicelluloses and some cellulosic materials) from the fruit pulp cell wall, resulting in increased juice yield [18]. The fruit processing industry takes advantage not only of the ability of enzyme preparations to facilitate juice release, but also of the increase in the juice yield.

Diagnostic checking of the models: Response was measured when the enzyme treated pulp was centrifuged at 10,000 rpm that is % of AIS (Y_{AIS}) in case of jackfruit and % of juice yield (Y_{Yield}) in case of papaya. The response under different combinations as defined in the design (Tables 2 and 3) was analyzed using the ANOVA appropriate to the experimental design. The ANOVA for the data obtained for jackfruit and papaya are presented in Tables 4 and 5. It is evident from the data presented that the second order terms were found to be significant and the lack of fit was not significant. The lack of fit measures the failure of the model to

Table 1. Physico-chemical properties of jackfruit and papaya.

Properties	Fruit *	
	Jackfruit	Papaya
TSS °Brix	28.86 ± 0.03	10.21 ± 0.04
Acidity (as % anhydrous citric acid)	0.23 ± 0.02	0.12 ± 0.01
pH	5.20 ± 0.03	4.88 ± 0.01
Total carotenoids (mg/100g)	0.31 ± 0.01	1.89 ± 0.01
Total sugars (%)	26.0 ± 0.2	9.2 ± 0.1

* Values are the mean of triplicate determinations (n ± SE).

Table 2. Treatment schedule for 2-factor CCRD for papaya and the response in terms of yield.

Run	Coded parameter		Actual parameter		
	X_1	X_2	x_1	x_2	(Y_{Yield})
1	-1	-1	180	0.3	77
2	-1	1	180	0.7	81
3	1	-1	240	0.3	81
4	1	1	240	0.7	82
5	0	-1.414	210	0.1	81
6	0	1.414	210	0.9	83
7	-1.414	0	150	0.5	79
8	1.414	0	270	0.5	83
9	0	0	210	0.5	83
10	0	0	210	0.5	83
11	0	0	210	0.5	83
12	0	0	210	0.5	83
13	0	0	210	0.5	83

X_1 and X_2, represent the coded variables for incubation time and pectinase concentration; x_1, x_2, represent the actual variables for incubation time (h), and pectinase concentration (g/100g pulp), Y_{Yield} represents response in terms of % juice yield.

Table 3. Treatment schedule for 3-factor shell design for jackfruit and the response in terms of alcohol insoluble solids.

Run	Coded parameter			Actual parameter			
	X_1	X_2	X_3	x_1	x_2	x_3	(Y_{AIS})
1	0	0	0	0.55	100	37	4.45
2	0	1	0	0.55	180	37	3.9
3	0.866	0.5	0	1.0	140	37	5.62
4	0.289	0.5	0.816	0.7	140	45	3.97
5	0	-1	0	0.55	20	37	5.47
6	-0.866	-0.5	0	0.1	60	37	6.29
7	-0.289	-0.5	-0.816	0.4	60	29	4.78
8	-0.866	0.5	0	0.1	140	37	3.77
9	-0.289	0.5	-0.816	0.4	140	29	3.62
10	0.577	0	-0.816	0.85	100	29	3.73
11	0.866	-0.5	0	1.0	60	37	5.29
12	0.289	-0.5	0.816	0.7	60	45	5.54
13	-0.577	0	0.816	0.25	100	45	3.79
14	0	0	0	0.55	100	37	4.26
15	0	0	0	0.55	100	37	3.72
16	0	0	0	0.55	100	37	3.73
17	0	0	0	0.55	100	37	3.75

X_1, X_2, X_3 represent the coded variables for pectinase concentration, incubation time and temperature; x_1, x_2, x_3 represent the actual variables for pectinase concentration (g/100g pulp), incubation time (h), and temperature (°C); Y_1 represents response in terms of % AIS.

represent data in the experimental domain at points, which are not included in the regression. The high values of the coefficient of determination (R^2) (0.94 in case of jackfruit and 0.93 for papaya) also suggest that the model is a good fit. The R^2 is the proportion of variability in the response values explained or accounted for by the model[19].

Response surface plotting: The effect of the conditions of the enzymatic liquefaction of papaya (enzyme concentration and incubation time) on the response (% yield) is reported (Table 2) by the coefficients of the second-order polynomials. The response surfaces based on these coefficients are shown in Fig.1. The effect of the conditions of the enzymatic liquefaction of jackfruit (enzyme concentration, incubation time) on the response (AIS) is reported (Table 3) by the coefficients of the second-order polynomials. The figure indicates the responses at room temperature. The trend was similar for other two temperatures also. The response surfaces based on these coefficients are shown in Fig.2.

Effect of enzyme concentration and incubation time on % juice yield of papaya: The juice yield of papaya was found to be a function of the linear and quadratic effects of enzyme concentration. The linear effect ($P \leq 0.01$) is positive, whereas the quadratic effect ($P \leq 0.01$) is negative, which results in a curvilinear increase in yield for all the incubation times. The yield is linearly related ($P \leq 0.05$) to the incubation time, and the quadratic term is

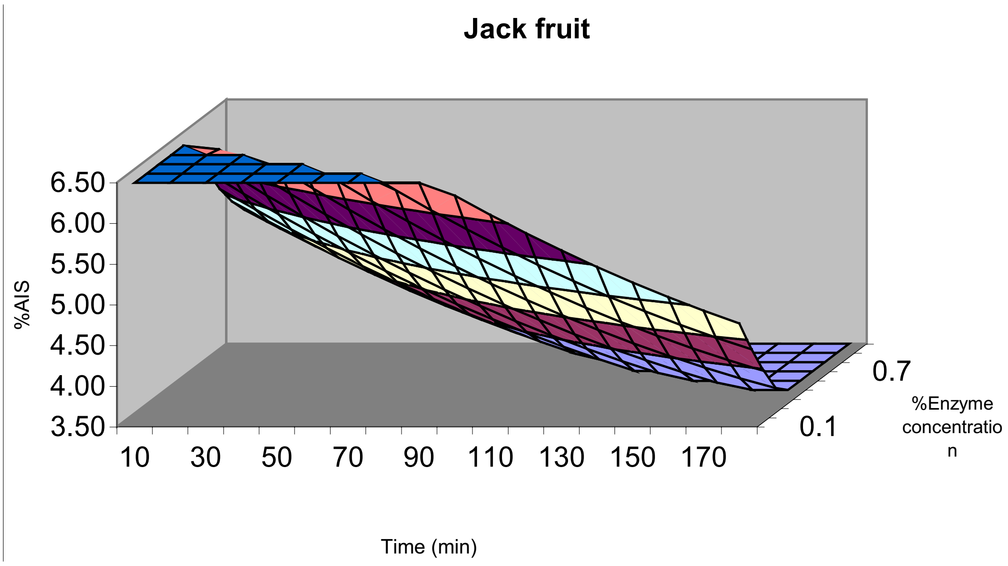

Figure 2. Response surface graph showing the effect of enzyme concentration and incubation time on % AIS of jackfruit at 29±1°C.

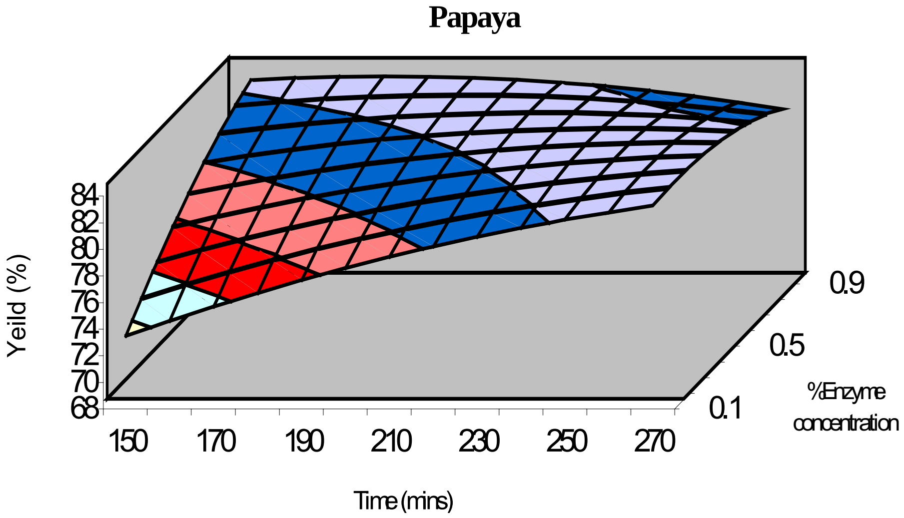

Figure 1. Response surface graph showing the effect of enzyme concentration and incubation time on % juice yield of papaya.

not found to be significant, which results in a linear increase in yield with the incubation time for all the enzyme concentrations ($P \leq 0.01$). It is interesting to note that the interaction is not significant ($P \leq 0.01$). The yield increases with enzyme concentration and incubation time up to a certain level and then remains constant (Fig.1). Based on optimized conditions, the process for the extraction of papaya juice was scaled up at pilot plant level[20].

Effect of enzyme concentration and incubation time on the % reduction of AIS of jackfruit: The AIS is the measure of the remaining crude pectin present in the jackfruit pulp. It depends on the enzyme concentration as well as the incubation time. The linear effects are negative and quadratic terms are positive for both the variables of enzyme concentration and incubation time; the interaction term is not significant. The AIS decreases with enzyme concentration and incubation time up to a certain level and then remains constant (Fig. 2).

Optimization: The graphical optimization technique was adopted to determine the workable optimum conditions for the enzymatic liquefaction of jackfruit and papaya pulp. This technique reduces the amount of time and effort required for the investigation of multifactor, multiresponse systems. It also provides comprehensive and informative insight into the system, leading to the process optimization. The specifications necessary for the response was set and these also served as constraints on optimization. An acceptable compromise was made based on the following criteria: in case of jackfruit: $Y_{AIS} \leq 3.91\%$, whereas in case of papaya $Y_{YIELD} \geq 75\%$. These constraints serve as a basis for further economically feasible option for commercialization of the process.

Verification of results: The suitability of the model equation for predicting the optimum response values was tested using the recommended optimum conditions. The set of conditions were used to predict the value of the responses using model equations. The predicted experimental values were found to be in excellent

Table 4. Analysis of variance (ANOVA) for the fitted second-order polynomial model and lack of fit for AIS as per shell design.

	Degree of freedom	Sum of squares	Mean square	F-Value	Significance F
Regression	9	10.60	1.18	5.84	0.015
Residual	7	1.41	0.20		
Total	16	12.01			

Table 5. Analysis of variance (ANOVA) for the fitted second-order polynomial model and lack of fit for % yield as per CCRD.

	Degree of freedom	Sum of squares	Mean Square	F-Value	Significance F
Regression	5	25.55	5.11	1.61	0.27
Residual	7	22.22	3.17		
Total	12	47.77			

Table 6. Actual versus predicted values for the increase in %yield of papaya juice.

Sl No.	Time (min)	Concentration (%)	Predicted value (%)	Experimental value (%)*
1	230	0.65	83	82.5±0.5
2	160	0.5	80	79.3±0.58
3	160	0.3	77	77.5±0.87
4	150	0.2	75	74.3±0.58
5	170	0.85	82	81.8±0.29

*Values are the mean of triplicate determinations (n ± SE).

Table 7. Experimental versus predicted values for the %AIS in jackfruit pulp.

Sl No	Time (min)	Concentration (%)	Predicted value (%)	Experimental value (%)*
1	170	0.6	3.91	3.88±0.01
2	165	0.7	3.86	3.83±0.01
3	155	0.8	3.82	3.78±0.06
4	90	0.75	3.66	3.64±0.02
5	80	0.7	3.73	3.73±0.00

* Values are the mean of triplicate determinations (n ±.SE).

Table 8. Statistical analysis of the comparison of experimental values*.

Fruit	RSMD	S_D	E (%)
Papaya	0.551	0.679	0.76
Jackfruit	0.018	0.021	0.74

*Values are the mean of triplicate determinations.

agreement with the experimental ones (Tables 6 and 7). The values of statistical analysis are given in Table 8, which defines the degree of fit of the model.

Enzymatic liquefaction of jackfruit and papaya pulp was found to be significantly influenced by the enzyme concentration and incubation time. The model developed for AIS could predict values very close to the experimental ones. Based on the set constraints, the optimum condition for enzymatic liquefaction of jackfruit and papaya have been determined.

Conclusions

The effect of the enzyme concentration, incubation time and incubation temperature seems to be significant in case of liquefaction of jackfruit and papaya using response surface methodology. The different conditions (time, temperature and enzyme concentration) for enzyme treatment revealed that, all these variables markedly affect the parameter under study. However, enzyme liquefaction does not have any significant effect on the nutritive properties of the juice. To explore whether the predictive model from RSM could be applied to scale up the process, the study was carried out at pilot plant level. The model equation developed for yield and AIS could predict values close to the experimental ones. The response surface methodology provides insight into the interaction and identifies the optimum combination of variables. The methodology developed in this research can also be extended to other fruits as well.

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