Optimizing Conditions for Enzymatic Extraction of Juice from Jackfruit (*Koozha)* Using Response Surface Methodology

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Authors' contributions

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ABSTRACT

The central composite design of Response surface methodology, statistical tool was used in this study for analyzing the effects of enzymatic treatment conditions namely; incubation time, incubation temperature and enzyme concentration on the percent yield. The jackfruit *koozha* pulp was treated with the pectinase enzyme at different incubation time (1- 5 hours), incubation temperature (30-45°C) and at various level of enzyme concentration (0.1-0.8 per cent). The effect of these independent variables on percent yield of juice was evaluated. Based on response surface and contour plots, the optimum condition for jackfruit *koozha* juices obtained were: incubation time

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2.75 hours, incubation temperature 42.3°C and the concentration of enzyme 0.5 percent with desired yield of 82 percentages. Enzymatic extraction, as a pretreatment condition in *koozha* type results in higher yield recovery. Statistical analysis showed that percent yield of juice was significantly correlated with the incubation time, incubation temperature and level of enzyme. The most significant variable enhancing the juice yield is enzyme concentration.

1. INTRODUCTION

Artocarpus heterophyllus is a fruit of the mulberry family Moraceae and is commonly known as Jack fruit (Eng.), Kathal and Panas (Hindi), Kanthal (Beng.), Palaa (Tamil), Phanas (Gujarati and Marathi) and Chakka (Malayalam). It is native to the Western Ghats of India and Malaysia [1]. They are widely grown in Bangladesh, Burma, Sri-lanka, Malaysia and Indonesia, Philippines, Brazil and other tropical countries [2].

The Jackfruit tree is well suited to tropical lowlands, and its fruit is the largest tree-borne fruit, with an average weight of 35 kg and average of 90 cm length, and 50 cm in diameter [3]. Jackfruit is the national fruit of Bangladesh and Indonesia. It is one of the important underutilized fruits which is known for its therapeutic and nutritive value, with excellent flavor and attractive color. It is often called as the "poor man's fruit" because it is cheaply available in large quantities, during the season and becomes a staple food in many regions [4].

Fresh juices are valued these days owing to their nutritional properties and are the best alternatives to the traditional caff/eine containing beverages such as coffee, tea, or carbonated soft drinks. Despite its vast potential, the fresh juice is used either in pure form or mixed with other juices to improve palatability [5]. In food processing industries three methods of juice extraction were employed *viz,* cold, hot, and enzymatic methods [6]. The enzymatic hydrolysis of pulp improved the juice yield and quality, in terms of viscosity, clarity. The enzymatic method was industrially applicable to improve the yield and quality of the juice [7]. The enzymes, mainly pectinases, assist in pectin hydrolysis, which cause a reduction in pulp viscosity and a significant increase in juice yield [8].

In the past, optimizing the various parameters during food processing has been performed through investigating the effect of one-parameter changes on a response while keeping all other parameters at a constant level [9]. The main disadvantages of this type of optimization are the interactive effects among the parameters were not considered and there is a lack of complete effect of the factors on the response [10]. In order to resolve this problem, optimization studies have been carried out by using multivariate statistical methods. Response surface methodology (RSM) is the most popular multivariate statistic technique, which has been used in the optimization of food processes [11]. RSM is a collection of statistical and mathematical methods, established on the fit of a polynomial model to the data that must depict the behavior of a data set, with the purpose of making statistical predictions. The approach is useful for optimizing, designing, developing, and improving processes where responses are affected by several variables [12].

The present investigation is aimed at studying the effect of the enzyme (pectinase) (at different time durations, temperature and enzyme concentration) on Jackfruit *koozha* type and optimization of the process condition by response surface methodology.

2. MATERIALS AND METHODS

2.1 Selection of Fruits and Enzyme

Fully matured Jackfruits of *koozha* type was purchased from the Instructional farm, College of Agriculture, Vellayani, KAU. The fruits were selected based on external maturity indices like distance between spines, colour of spines (greenish- yellow) and the number of days elapsed from fruit set (180) days. Fruits were cut in to equal halves and the parts like bulbs, rind and perigones were separated.

Commercial pectinase from *Aspergillus niger* (TCI 9032-75-1) with activity > 5 units/mg protein (Lowry) procured from Globe Scientific Pvt Ltd, Trivandrum was used for enzymatic treatment of Jackfruit pulp.

2.2 Juice Extraction

Koozha type Jackfruit were peeled, fruit bulbs were deseeded and blended into fine pulp in a food blender for 2-3 min until a homogenous fruit pulp was obtained. The juice was filtered using a cheese cloth. The obtained juice is yellow in colour with the pH 4.9 and the TSS of 13° Brix.

2.3 Enzyme Treatment

For each experiment, 100 ml of *koozha* juice was subjected to different enzymatic treatment conditions as shown in Table 1 using RSM. The independent variables were the incubation time, X_1 (1- 5 hours), incubation temperature, X_2 (30–45°C), and concentration of enzyme used, X_3 (0.1– 0.8 v/v %). The range of the variables for enzymatic treatment conditions were based on the preliminary experiments conducted at the Dept. of community science of College of Agriculture, Vellayani. The temperature of the enzymatic treatment was adjusted to the desired level using a constant temperature water bath [13]. The pH of the juice was kept at its natural pH value and was excluded from the RSM experimental design as the pH is considered optimal for exo-pectinase [14]. At the end of treatment condition, the enzyme in the sample was inactivated by heating the suspension to 90^0C for 5 to 10 min in a water bath. The treated juices were then centrifuged at 3000rpm for 10 min and the supernatant was collected. After that, the juice was filtered through a Whattman number filter paper. The filtrate collected was used for further analysis [15].

2.4 Experimental Design

A Design of Experiment (DoE) is a structured, organized method for determining the relationship between a number of factors affecting a process and the output of that process. In this study, Central Composite face centered (CCF) design from design of experiment was employed to determine the optimal conditions for the critical factors. This design is a kind of central composite design (CCD), in which the axial points are placed on the face centers of the cube; therefore, each factor has only three levels instead of five in CCD. With such cubical design, one or two center runs are sufficient to produce a reasonable stability of prediction variance [16]. The Design-Expert 6.0 (Stat-Ease Inc., Minneapolis, MN, USA) was used to determine

the analysis of variance (ANOVA) and the coefficient of determination (R^2 and adjusted R^2) to evaluate the quality of fitness of the model. The independent variables involve the time of enzyme treatment (X_1), temperature of treatment (X_2), and used enzyme concentration (X_3) while the dependent variable include yield of juice [17]. Each independent variable had three levels which were -1, 0 and +1; these three variables were responsible for the mechanism of enzyme activity in the juice [18]. A total 20 combination were carried out in a random order according to a CCD configuration for the three chosen variables. The experimental design matrix was coded- (x) form and actual form (X) of variables are shown in Table 1. The dependent variable (y) was the yield of juice. The dependent variables were expressed individually as a function of the independent variables, known as response function. The variance for each factor assessed was partitioned into linear, quadratic and interactive components and were represented using the second order polynomial function as follows:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1{}^2 + b_{22}x_2{}^2 + b_{33}x^2 \quad (1)$$

The coefficients of the polynomial were represented by b_0 (constant), b_1, b_2, b_3 (linear effects); b_{12}, b_{13}, b_{23} (interaction effects) and b_{11}, b_{22}, b_{33} (quadratic effects). The significance of all terms in the polynomial functions were assessed statistically using F-value at probability (p) of 0.001, 0.01 or 0.05. The regression coefficients were then used to generate contour maps from the regression models. The three-dimensional (3D) plots were generated by keeping one variable constant at the center point and varying the other variables within the experimental range using design software [19].

3. RESULTS AND DISCUSSION

3.1 Statistical Analysis

The variations in juice yield due to enzymatic hydrolysis are shown in Table 1. It is clear that the yield of juice increased significantly with the experiment conditions like incubation time, temperature and the concentration of enzymes. The response surface model developed for the variables were adequate, possessing significant fit and satisfactory R^2 values. The R^2 values for all these response variables were 0.94, indicating that the regression model explained the reaction well.

3.2 Response Surface Analysis of Juice Yield

Response surface model for the juice yield as a function of enzymatic extraction process variables was developed by using multiple regression technique. A complete second order model (Eq. 1) was tested for its adequacy to describe the response surface. The analysis of variance (Table 2) shows that the model with F value of 78.31 was highly significant (p<0.0001). There is only a 0.01% chance that a Model F value of this large could occur due to noise. Values of Prob>F less than 0.05 indicate model terms are significant. In this case, x_1, x_2, x_3, x_1^2, x_2^2, x_3^2, x_1x_2, x_1x_3 are significant model terms. Values greater than 0.10 indicate the model terms are not significant. The lack-of-fit F value of 0.57 implies that the lack-of-fit is significant. This means that some of the quadratic terms are not significant [20].

The Pred R^2=0.8829 is in reasonable agreement with the Adj R^2=0.93422. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. In the present case, the ratio of 37.80 indicates an adequate signal to use the model for prediction purposes [21]. Considering these criteria the response model for juice recovery was:

$$"y = 77.95 + 1.10x1 + 1.50x2 + 2.90x3 - 0.86x12 - 0.86x22 - 1.86x32 + 1.88x1x2 + 0.88x1x3 - 0.12x2x3 \qquad (2)$$

Where, y is the juice yield (%); x_1, x_2, and x_3 are coded variables

Effect of time and temperature on percent yield at a constant enzyme concentration is shown in Fig. 1 the juice yield increased with the increase in both time and temperature. The results are in agreement with the findings of [22], reported that the maximum juice yield from bael fruit is obtained by pectin lytic enzyme treatment of pulp at high temperature and time.

Fig. 2, presents the interaction effect of incubation temperature (X_1) and pectinase concentration (X_3) to juice yield. It is clear, that at higher temperature and enzyme concentration, the juice yield followed a linear behavior which reflects that with increase in juice yield due to the fact that pectinases degrade pectic substances leading to increase in juice yield [23]. Kaur et al. [24] studied the juice yield at interaction of treatment temperature and enzyme concentration. It is clear that at higher temperature and enzyme concentration, the juice yield followed a nonlinear behavior, suggesting maximal juice yield within the levels of the parameters studied.

Effect of enzyme concentration and incubation time on percent yield of jackfruit juice at constant temperature is shown in Fig. 3. As the enzyme and time concentration increases, the percent yield also increases. These above figures indicate the 3D surface for the effect of the independent variables on per cent yield. The total amount of juice increased because of degradation of pectin, which leads to lowered water holding capacity and consequently free water is related to the system that reduces the viscosity, thus facilitating higher percent yield [25].

3.3 Optimization of Processing Variables

The final optimizing process of juice extraction from *koozha* jackfruit with concerning enzyme treatment with incubation time, incubation
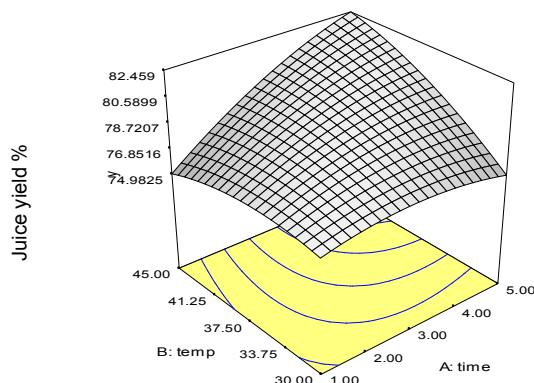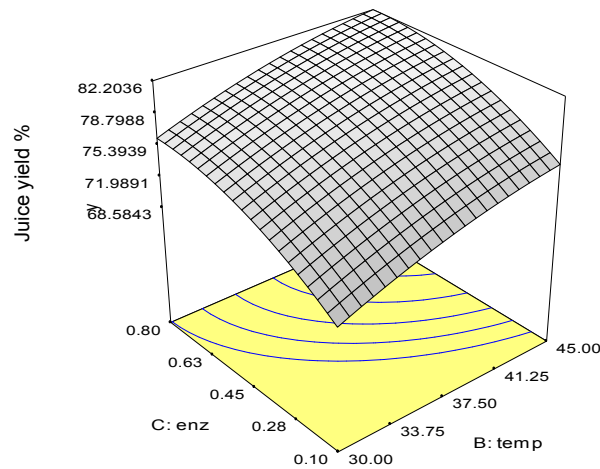

Fig. 1. Response surface of juice yield as a function of incubation time and temperature at the optimum enzyme concentration (0.5 per cent)

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Table 1. The central Composite Face Centered (CCF) experimental design employed for enzymatic hydrolysis pretreatment of jackfruit *koozha* pulp

Experiment no.	Coded variables			Uncoded/Independent variables			Dependent variable
	x_1	x_2	x_3	Time X_1 (h)	Temperature X_2 (^{0}C)	Enzyme X_3 (v/v %)	Juice yield (%)
1	0	0	0	3	37.5	0.45	78
2	0	1	0	3	45	0.45	79
3	0	0	0	3	37.5	0.45	78
4	-1	1	1	1	45	0.8	75
5	-1	-1	-1	1	30	0.1	72
6	0	0	0	3	37.5	0.45	78
7	0	0	-1	3	37.5	0.1	73
8	0	0	1	3	37.5	0.8	79
9	-1	0	0	1	37.5	0.45	75
10	1	1	1	5	45	0.8	82
11	0	0	0	3	37.5	0.45	78
12	0	0	0	3	37.5	0.45	78
13	1	1	-1	5	45	0.1	75
14	-1	1	-1	1	45	0.1	71
15	0	-1	0	3	30	0.45	75
16	0	0	0	3	37.5	0.45	78
17	1	0	0	5	37.5	0.45	79
18	1	-1	1	5	30	0.8	76
19	-1	-1	1	1	30	0.8	76
20	1	-1	-1	5	30	0.1	68

Table 2. Analysis of variance (partial sum of squares) for response surface quadratic model

Source	Sum of squares	DF	Mean square	F value	Prob> F	
Model	201.6886	9	22.40985	78.31877	< 0.0001	**Significant**
x_1	12.1	1	12.1	42.28753	< 0.0001	
x_2	22.5	1	22.5	78.63384	< 0.0001	
x_3	84.1	1	84.1	293.9158	< 0.0001	
x_1^2	2.051136	1	2.051136	7.168388	0.0232	
x_2^2	2.051136	1	2.051136	7.168388	0.0232	
x_3^2	9.551136	1	9.551136	33.37967	0.0002	
$x_1 x_2$	28.125	1	28.125	98.2923	< 0.0001	
$x_1 x_3$	6.125	1	6.125	21.40588	0.0009	
$x_2 x_3$	0.125	1	0.125	0.436855	0.5236	
Residual	2.861364	10	0.286136			
Lack of Fit	2.861364	5	0.572273			
Pure Error	0.43	5	0.08			
Cor Total	204.55	19				
R-Squared	0.94601					
Adj R-Squared	0.93422					
Pred R-Squared	0.882947					

Fig. 2. Response surface of juice yield as a function of enzyme concentration and temperature at the optimum time (2.75 hours)
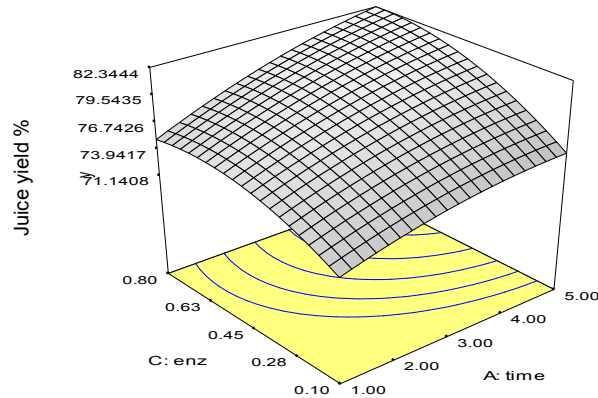
Fig. 3. Response surface of juice yield as a function of enzyme concentration and time at the optimum temperature (42.3)^0 C)

temperature and enzyme concentration as independent variables (time 1-5 hours, temperature 30-45°C and enzyme concentration 0.1-0.8 v/v %) has been performed by means of response surface method to investigate on maximum per cent juice yield. It was resulted that temperature (X_1) =42.3 °C; time (X_2) =2.75 hours and enzyme concentration (X_3) =0.5 v/v % indicate to obtain the maximum juice yield of *koozha* type.

To visualize the dependence of response (juice yield) with the independent variables X_1, X_2, and X_3, 3D plots were generated by keeping one variable fixed at the optimum point and varying the other two variables [8]. Using the contour plots or the response surface methodology, the optimum set of the operating variables are obtained graphically in

order to obtain the desired levels of the jackfruit juice.

4. CONCLUSION

The methodology of the experimental design was shown to be very useful for the evaluation of enzymatic extraction for *koozha* jackfruit juice. The study revealed that Jackfruit (*Koozha*) juice yield is a function of enzymatic treatment with specific conditions of concentration, time and temperature. Significant regression model describing the variation of juice yield with respect to the independent variables (enzyme concentration, temperature and incubation time) were established with the coefficient of determination, R^2> 0.8. Response surface methodology was successfully used for optimizing pretreatment condition for the

improvement of juice recovery of jackfruit. Enzyme concentration was the most significant ($p < 0.01$) variable affecting the juice yield. The recommended enzymatic treatment condition from the study was: enzyme concentration 0.5 v/v %; incubation time 2.75 h and incubation temperature 42.3°C.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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