# Jamun Juice Extraction Using Commercial Enzymes and Optimization of the Treatment with the Help of Physicochemical, Nutritional and Sensory Properties

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# Jamun Juice Extraction Using Commercial Enzymes and Optimization of the Treatment with the Help of Physicochemical, Nutritional and Sensory Properties

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***Abstract*—**Jamun (*Syzygium cuminii* L.) is one of the important indigenous minor fruit with high medicinal value. The jamun cultivation is unorganized and there is huge loss of this fruit every year. The perishable nature of the fruit makes its postharvest management further difficult. Due to the strong cell wall structure of pectin-protein bonds and hard seeds, extraction of juice becomes difficult. Enzymatic treatment has been commercially used for improvement of juice quality with high yield. The objective of the study was to optimize the best treatment method for juice extraction. Enzymes (Pectinase and Tannase) from different stains had been used and for each enzyme, best result obtained by using response surface methodology. Optimization had been done on the basis of physicochemical property, nutritional property, sensory quality and cost estimation. According to quality aspect, cost analysis and sensory evaluation, the optimizing enzymatic treatment was obtained by Pectinase from *Aspergillus aculeatus* strain. The optimum condition for the treatment was 44 $^{o}$C with 80 minute with a concentration of 0.05% (w/w). At these conditions, 75% of yield with turbidity of 32.21NTU, clarity of 74.39%T, polyphenol content of 115.31 mg GAE/g, protein content of 102.43 mg/g have been obtained with a significant difference in overall acceptability.

***Keywords*—**Jamun, enzymatic treatment, physicochemical property, sensory analysis, optimization.

## I. Introduction

AMONG several tropical fruits in India, Jamun (*Syzygium cuminii* L.) is known as wonder fruit for its medicinal value. The harvesting span of jamun fruit is very short (30 to 40 days) during monsoon months. The fruit is fleshy, dark purple coloured and ovoid in shape with a hard single seed inside. Compared to other popular fruits like sapota, papaya, banana and guava, jamun has higher level of antioxidant activity. The higher antioxidant activity in the fruit is attributed due to presence of antioxidant vitamins, tannin and anthocyanins. The fresh fruit is used for preparing juices, jam, squash and the seed has its own medicinal value mainly for diarrhea and diabetics. Due to the pectin-protein bond and fibrous texture, removal of juice is quite difficult from the fruit. Enzymatic treatment can be used to process the fruits into various products. This treatment mainly used in the many food industries.

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Fruit juices contain a high amount of pectin. Due to the presence of pectin, cloudiness creates a major problem in fruit juices industry. The cloudiness can be removed by enzymatic depectinization. Pre-treatment like enzyme clarification can be considered as one of the advanced processing method in beverage industry. Several studies have reported on depectinization using pectinases as enzymatic treatment for various fruit juices [1], [2]. The pectinase hydrolyses pectin and causes pectin-protein complexes. The juice obtained from the pectinase treatment will have a much lower amount of pectin with a lower viscosity, which will be beneficial for filtration processes. As a result, more concentrated, flavored, highly colored and clarified juice is obtained as a better final product.

In case of fruit juice depectinization and to increase the product yield, several enzymes have been used as a pre-treatment. Pectinase is one of the very common enzymes which have been used for decades to clarify the juices effectively [3]-[7]. The enzymatic treatment for hydrolysis of pectic substances are influenced by several factors such as incubation time, incubation temperature and enzyme concentration [6].

An enzyme acts differently with different strains. For example, pectinase obtained from *Aspergillus niger* will act on a biological system in a certain manner where pectinase obtained from *Aspergillus aculeatus* will work differently. Many studies reported that the strains acted differently with various fruits [8]-[10]. Tannase had been used to remove haze and cloudiness in fruit juice processing industry. Rout and Banerjee [11], for pomegranate juice, illustrated that tannase treatment brought about 25% debasement of tannin while Srivastava and Kar [12] reported that there was an exclusive loss of astringency in anola juice with an evacuation of 68.8% of tannin. Motoichi et al. [13] stated that the treatment of juice with tannase or chlorogenase increases the shelf life without any turbidity and precipitation. Fernández et al. [14] used several enzymes as well as tannase for the extraction of proanthocyanidins from País grape seeds and skins. Cashew apple juice was treated with 0.1 of Tannase enzyme for one hour at 30 $^{0}$C and passed through filtration resulted in lower astringency and better physical stability for longer days [15].

Box–Behnken design is a three-level factorial arrangement. All factor levels have to be adjusted only at three levels with equally spaced intervals between these levels [16], [17]. RSM has been used extensively for optimizing processes in the tropical fruit juice production [7], [9].

The objectives of the present study are to compare the changes in physical properties (turbidity, yield, clarity, and colour change), chemical properties (total polyphenol, protein concentration and TSS) and sensory properties of the juice treated with three different enzymes.

## II. Materials and Methods

### A. Sample Preparation

Fresh, mature and ripen Jamun (Ram *Jamun*) were obtained from the local area of Rourkela, Odisha, India (located at 84.54E longitude and 22.12N latitude). Jamun fruits are highly perishable. So, the samples were cleaned and washed manually and packed in perforated polythene bags and stored at -20 $^0$C for further use [18]. Before experiments, samples are taken out from deep freezer and thawed for 3 hrs.

Jamun fruits were stored in a deep freezer. Fruits were selected and washed properly to eliminate any microbial contaminations and to remove dust and foreign particles. Then, the fruits were kept at room temperature for 3 hours. Before juice extraction, seeds were removed manually from the fruits, to avoid the bitterness. Then, the thick pulp of the fruit was mixed in a mixer (Bajaj Mixer, India) for 5 min at its maximum speed. The used enzymes were Pectinase from *Aspergillus niger*, Pectinase from *Aspergillus aculeatus* and tannase from *Aspergillus ficcum*.

### B. Enzymatic Treatment

TABLE I
EXPERIMENTAL DESIGN INDICATING ACTUAL VALUES OF INDEPENDENT VARIABLES

| Exp. No | Real Variables | | |
|---|---|---|---|
| | $X_1$ (°C) | $X_2$(%) | $X_3$(min) |
| 1. | 30 | 0.01 | 80 |
| 2. | 50 | 0.01 | 80 |
| 3. | 30 | 0.10 | 80 |
| 4. | 50 | 0.10 | 80 |
| 5. | 30 | 0.06 | 40 |
| 6. | 50 | 0.06 | 40 |
| 7. | 30 | 0.06 | 120 |
| 8. | 50 | 0.06 | 120 |
| 9. | 40 | 0.01 | 40 |
| 10. | 40 | 0.10 | 40 |
| 11. | 40 | 0.01 | 120 |
| 12. | 40 | 0.10 | 120 |
| 13. | 40 | 0.06 | 80 |
| 14. | 40 | 0.06 | 80 |
| 15. | 40 | 0.06 | 80 |
| 16. | 40 | 0.06 | 80 |
| 17. | 40 | 0.06 | 80 |

After collecting the homogenized pulp from the mixer, 100 g of the homogenized pulp was treated with enzymatic conditions as per the experimental design shown in Table I. The scope of the variables for enzymatic treatment conditions depended on the preparatory trials (information not specified). The independent variables were incubation temperature X1, enzyme concentration, X2 and incubation time, X3. The mixture was kept in an incubated orbital shaker (REMI CIS24 PLUS) at 120 rpm with a vigorous mixing throughout the incubation period. So, at the end of the treatment, pectinase was inactivated by keeping the suspension at - 2 °C for 5 min [19]. The mixture was cooled and filtered through a muslin cloth to obtain the juice. The filtrate was collected to analyze various physical and chemical properties. Control samples, where considered where there was no enzymatic treatment.

ANOVA (analysis of variance) was used to validate the model. Keeping one variable constant at the center point and varying the other two variables within the experimental range, the three-dimensional plots were drawn.

### C. Analysis of Juice

Physical properties (turbidity, yield, clarity, and colour change) and chemical properties (protein concentration, polyphenol and TSS) were responses that were subjected to optimize. The viscosity of juice was dictated by a U-Tube slim viscometer (Zenith Glassware, Kolkata, India) at room temperature (28±10 °C). Digital Turbidity-meter was utilized to measure turbidity (Model 335, Deluxe Company, India) and the outcomes were accounted for as Nephelometric Turbidity Units (NTU) [7]. Yield was calculated according to [20]. Clarity of the concentrate was measured by transmittance (%T) at 660 nm utilizing the spectrophotometer (Model:AU 2701, Systronics India Ltd) [6]. Colour measurements were performed by Hunter colorimeter (Colorflex EZ). The change in colour was measured according to [21]. Protein concentration was determined with BSA as standard according to the dye binding method [22]. Polyphenol concentration was determined utilizing a spectrophotometer (Model:AU 2701, Systronics India Ltd) at 650 nm taking after the Folin-Ciocalteu method as portrayed by [23]. The outcomes are communicated as milligrams of Gallic acid equivalent per gram. The total soluble sugar (TSS) in the sample were determined with the help of an Abbe-type Refractometer, and the values were expressed as degree Brix ($^o$B).

## III. Results and Discussions

Fresh Jamun juice (without any treatment) has a viscosity of 1.21 mPaS, turbidity of 116 NTU and clarity of 0.165%abs with a yield of 65%. The colour value for the raw juice is L*(5.76), a*(2.65) and b*(-1.68). The untreated juice had TSS of 130B and enriched with protein (89.70 mg/g) and polyphenol content (78.08 mgGAE/g).

In case of Pectinase enzyme from *Aspergillus niger*, the optimum conditions for extractions were evaluated as incubation temperature of 33.5 $^o$C with 75 minute and the concentration of enzyme 0.09% (w/v) [24]. Overall desirability was 87% in this case (Fig. 1 (a)). For Pectinase enzyme from *Aspergillus aculeatus*, the optimized independent variables were incubation temperature of 44 $^o$C with 80 minute and the concentration of enzyme 0.05% (w/v) [25]. Overall desirability was 94% in this case (Fig. 1 (b)) For Tannase, the optimized enzyme concentration was 0.05% (w/v) with treatment time of 80 minutes at 40 °C [26]. Overall desirability was 62% in this case.

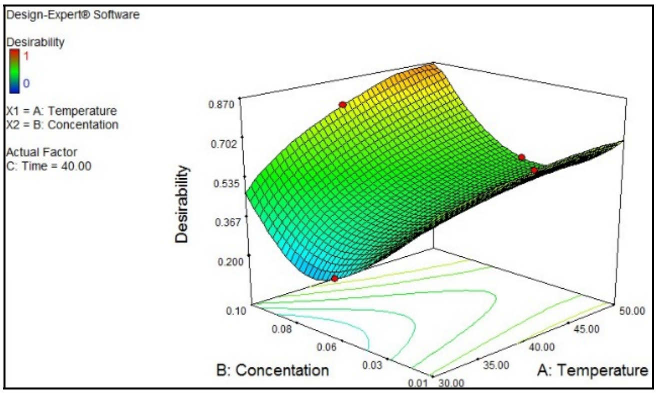


Fig. 1 (a) Overall desirability for Pectinase enzyme from *Aspergillus niger strain*

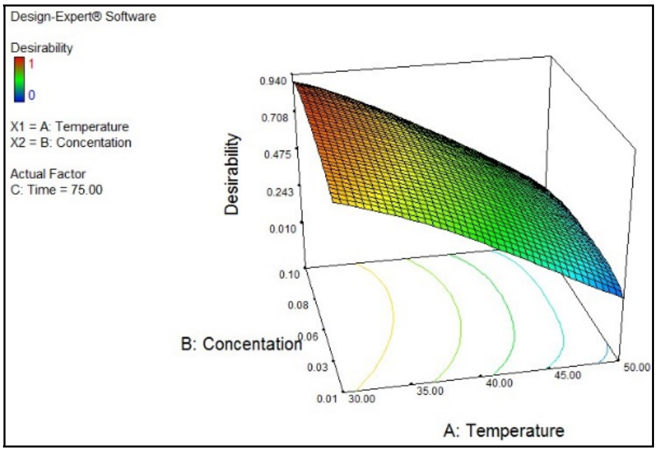


Fig. 1 (b) Overall desirability for Pectinase enzyme from *Aspergillus aculeatus* strain

### A. Effect on Physical Properties

From Fig. 2 (a), it can be observed that in case of raw juice, the yield was minimum whereas maximum yield was obtained by using Tannase enzyme up to 80%. But for the juice industry, turbidity and clarity plays the major role in the final product. In case of enzyme from Pectinase AA, turbidity is minimum (32.36 NTU) with higher clarity (30%T). There was a significant difference ($P<0.05$) with all values.

Color (Fig. 2 (b)) is divided into three different parameters where L* value represents the lightness and darkness of the juice, positive a* value represents the redness and negative b* value represents the blueness of the juice. The characteristics color of jamun juice can be represented by negative b* value. Due to the enzyme treatment, the lightness of the juice decrease. But jamun juice having a bluish – red color which can be visible in the juice treated with the Pectinase AA.

### B. Effect on Chemical Properties

Pectin forms gel structure which increases the viscosity at high temperature. But at temperatures between 35 and 45 $^{o}$C, the water holding capacity of pectin is reduced which results in lower viscosity. Viscosity was found minimum in case of juice treated with Pectinase AA. The pH of the control sample was 3.5 whereas Jamun juice treated with Pectinase AA possesses pH value of 2.8. Due to the natural acidic nature, enzyme treated juice was having better shelf-life than the control one. Total Soluble Solid or sugar present in the juice was another important nutritional parameter for the clarification process (Fig. 3).

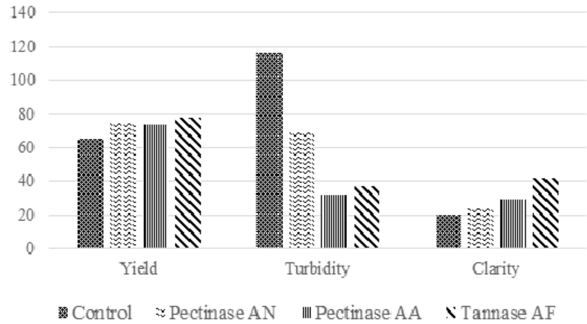


Fig. 2 (a) Comparison for Physical Parameters between 3 optimized treatment and raw juice

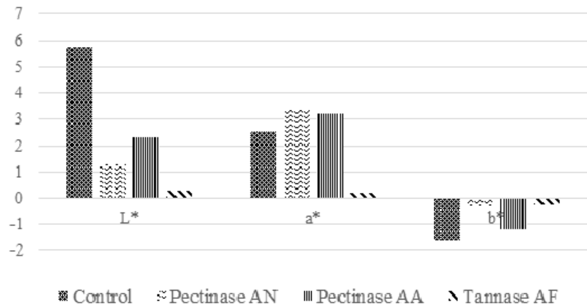


Fig. 2 (b) Comparison between 3 optimized treatment and raw juice (colour)

Jamun is a rich source of polyphenol content. But the cell wall of the fruit was made of protein and polyphenol content. Protein pectin bond breaks down and releases the protein into the juice. The highest polyphenol content of 115.31 mg GAE/g, protein content of 102.43 mg/g has been obtained with a significant difference treated with Pectinase AA juice.

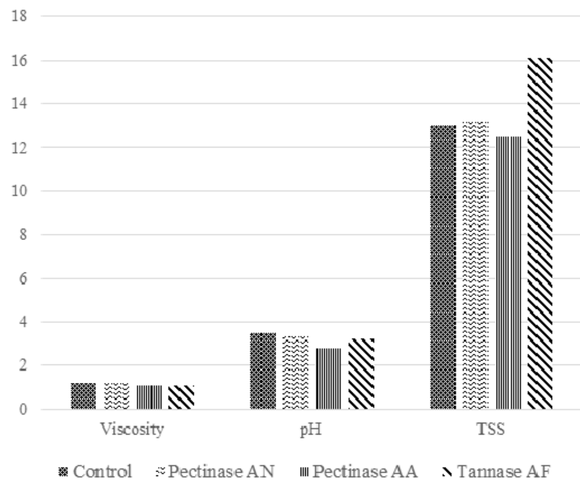


Fig. 3 Comparison between 3 optimized treatment and raw juice (chemical parameters)

### *C. Effect on Sensory Properties*

Nine point Hedonic scale was used for the sensory property. The highest overall score was obtained for the juice treated with Pectinase AA. Acceptance wise and odour wise it has a same point with juice treated with pectinase AN (Fig. 4).

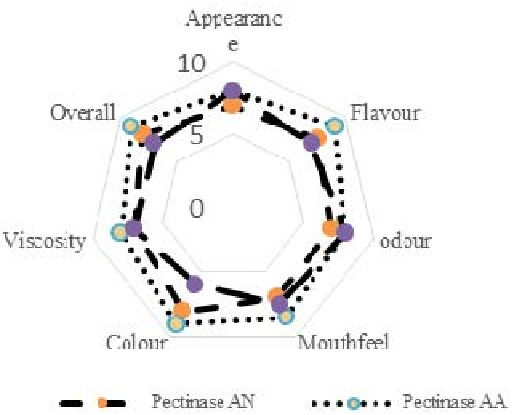


Fig. 4 Comparison between 3 optimized treatment (sensory properties)

## IV. Conclusion

Preservation of fresh produced samples over long time is energy consuming and expensive. After harvest, the nutritional and quality attributes of fresh juicy fruit such as Jamun get deteriorate as it comes in contact with surrounding. Low yield and low extraction efficiency of Jamun juice has been attributed to chemical structure of the fruit system. Presence of higher pectin and tannin content and storage linkage between protein and pectin molecules resulted in hindrance for extraction efficiency in case of jamun fruit juice. In case of perishable fruit system such as Jamun, there is a need for development of novel strategies for proper processing to get higher juice yield to achieve continuous supply. Meanwhile, the application of enzymes for enhancing the yield and extraction efficiency has been given wide range of commercial importance. Some properties are not best in case of the enzyme Pectinase AA but overall the maximum desirable characteristics was obtained by the enzyme. To produce per liter of juice pectinase AN costs Rs. 172/, pectinase AA Rs.88 and Tannase costs Rs. 32000. Finally, in terms of cost analysis also Pectinase AA has the lowest cost with high quality. So, this treatment can be successfully use in the industry as a pre-treatment method or for clarification method.

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