Research Article

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Crude pectinase parameter optimization for enhancing bael juice clarification

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ABSTRACT

This study investigates the optimization of crude pectinase concentration for clarifying bael juice, which contains fibrous pulp that limits juice recovery. The effects of different pectinase concentrations, incubation times, and temperatures on juice yield and turbidity were analyzed. The results showed that a 3% crude pectinase concentration, incubated for 60 minutes at 45°C, produced the highest juice yield (67.8%) and the lowest turbidity (568 NTU). The study underscores that the optimal conditions for maximizing juice yield while minimizing turbidity involve a careful balance of enzyme concentration and incubation parameters.

Introduction

Bael fruit juice, derived from the wood apple (*Aegle marmelos*), is packed with nutrients and offers several health benefits. Bael is a native Indian fruit from the Rutaceae family (Bhardwaj and Nandal, 2015). It originated in Northern India but is now found across the Indian subcontinent, as well as in countries such as Sri Lanka, Burma, Bangladesh, Thailand, and Indo-China (Brijesh *et al.,* 2009). Bael fruit holds significant medicinal and nutraceutical value and is often mentioned in ancient texts like the Vedas, Ramayana, and Buddhist and Jain literature. It is extensively used in Ayurvedic, Unani, and Siddha medicine systems for its healing properties.

Juice of bael fruit is highly valued for its exceptional nutritional, functional, and therapeutic qualities, making it an important part of many people's diets (Singh *et al.,* 2013). It is rich in vitamins (A, B, C, folate), minerals (iron, copper, potassium, iodine, zinc, selenium, sulfur, manganese, boron, molybdenum, magnesium), dietary fibre, antioxidants, amino acids, and bioactive compounds, all crucial for good nutrition and disease prevention. In addition to being regarded as a remedial plant, it has good nutritional value and is rich in carbohydrates, fibre, minerals and vitamins (Bhardwaj and Nandal, 2015).

Bael juice is an excellent source of fluids, which helps maintain hydration levels in the body. It is easily digestible, non-toxic, and has a cleansing effect on the blood and digestive tract. In short, bael fruit juice is a powerhouse of nutrition with numerous health benefits, contributing to taste and wellness. Bael fruit pulp is highly fibrous and complex, containing seeds (Singh *et al.,* 2014). The yellow-orange, flavorful pulp is rich in minerals like phosphorus, calcium, and vitamins such as vitamin C, thiamine, riboflavin, and niacin. Various products like wine, powder, toffee, marmalade, candy, slab, jelly, jam, and nectar can be made from bael fruit pulp for both therapeutic and food purposes (Bhattacherjee *et al.,* 2015).

However, the fibrous pulp of bael fruit poses a challenge for juice extraction. The complex texture and high pulp viscosity

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make it difficult to extract juice efficiently, leading to pulp losses. In the beverage industry, the enzymatic-mechanical extraction method is considered the best approach (Ghosh *et al.,* 2016). Enzymes like pectinase break down the cell walls and pectin substrates, releasing sap from the cells by enzymatic hydrolysis (Gurung *et al.,* 2013). Several researchers have reported the application of commercial enzymes to clarify different juices. Using crude enzyme (spore-free and produced from GRAS fermentation) is more economical to improve juice yield and clarity (Singh *et al.,* 2014). Therefore, the present study was undertaken to use crude enzyme from *A. niger* for the bael juice clarification. The present work optimized bael juice clarification using various partially purified pectinase enzyme concentrations at different incubation times and temperatures.

Material and Methods

The present investigation was conducted in the Centre of Food Science and Technology, CCS Haryana Agricultural University, Hisar during 2018-2019. Bael fruits were procured from the local market, Hisar. The chemicals used in the investigation were analytical reagents from CDH Chemicals, Sisco Research Lab and Himedia Ltd.

Juice extraction

Bael fruits were washed thoroughly in running water and broken by striking against a hard surface. The fruit pulp, along with its seeds and fibres, was scooped out with the help of a stainless steel spoon. The pulp was blended with distilled water in the ratio of 1:2 (w/v). The mixture of pulp and water was passed through a fruit pulper to obtain homogeneous pulp. The process flowchart for producing clarified juice from bael pulp is presented in Fig. 1.

Ripe bael
↓
Washing
↓
Breaking of fruits
↓
Scooping of bael pulp
↓
Blending with distilled water in the ratio of 1:2 (w/v)
↓
Passing through pulper
↓
Bael juice (100 ml) treated with crude pectinase at different conditions

Fig. 1. Flow sheet for the preparation of bael juice

Chemical analysis of extracted bael juice

Bael juice was analyzed for chemical constituents. Hulme and Narain's method (1931) estimated total and reducing sugars. Acidity was determined using the method of Ranganna (2014). Total carotenoids were analyzed using the Rodriguez-Amaya method (1999). Total phenols were estimated using the methods given by Amorium *et al.* (1997), and browning was estimated using the method of Ranganna (2014). The extracted bael fruit juice TSS in °Brix was determined using a hand refractometer (Erma Inc., Japan).

Effect of crude pectinase enzyme treatment

Erlenmeyer flasks (250 ml) with 100 ml of bael juice were inoculated with different concentrations of crude enzyme extract (1, 2, and 3% v/v) having pectinase activity of 8.12U/ml. The flasks were incubated at 35 °C, 40 °C and 45°C for 30, 45, and 60 minutes. The effect of the above enzyme treatments was observed on juice yield and turbidity.

Bael juice yield

The percentage and ratio of clarified juice to the bael pulp are defined as the clarified juice yield (%).
Clarified juice yield (%) = Weight of clarified juice/ Initial weight of bael juice × 100

Bael Juice turbidity

Turbidity (NTU) of juice was determined by Digital Turbidity Meter (Model: MT-134, India) (Sin *et al.,* 2006).

Statistical analysis

Each juice analysis was carried out in triplicates and converted to average values. The present investigation's data were analyzed according to a completely randomized design.

Results and Discussion

Fresh bael juice was analyzed for various chemical constituents (Table 1). Different studies have shown that *bael* fruit juice contains 28-39 per cent total soluble solids (TSS), 19-21 per cent carbohydrates, 11-17 per cent sugar, 1 per cent protein, 0.2 per cent fat and 7-21 mg 100 per g vitamin C (Singh *et al.,* 2014). The bael pulp is a base for various products like squash, jam, toffee, slab, powder, nectar, beverages and RTS. Bael fruit pulp of cultivar NB-5, CISH Bael-2 and Goma Yashi was used to make ready-to-serve drinks (Singh *et al.,* 2013). The physico-chemical studies revealed the little difference between fruit and fruit juice in bael's proximate composition and nutritional properties (Bhardwaj *et al.*, 2014).

Table 1. Chemical constituents of fresh bael juice (without enzymatic and incubation treatment)

S. No.	Parameter	Value±SD
1.	Total sugars (%)	37.08±1.02
2.	Reducing sugars (%)	25.42±1,04
3.	pH	5.2±0.12
4.	Acidity (%)	0.75±0.97
5.	Total carotenoids (mg/100 g)	1.24±2.14
6.	Total phenols (mg/100 g)	22.01±1.96
7.	Browning (440 nm)	0.65±1.01
8.	Total soluble solids (°B)	12±0.97
9.	Juice yield (%)	36±1.02
10.	Turbidity (NTU)	446±12.03

Effect of crude enzyme concentration on juice clarification

Minimum turbidity, 670 NTU and maximum juice yield (56.4%) were observed after 60 minutes of incubation with 3% crude pectinase concentration at 35°C (Fig. 2). The enzyme concentration was significant, and the incubation time was non-significant for turbidity, but for juice yield, both the pectinase concentration and incubation time were significant.

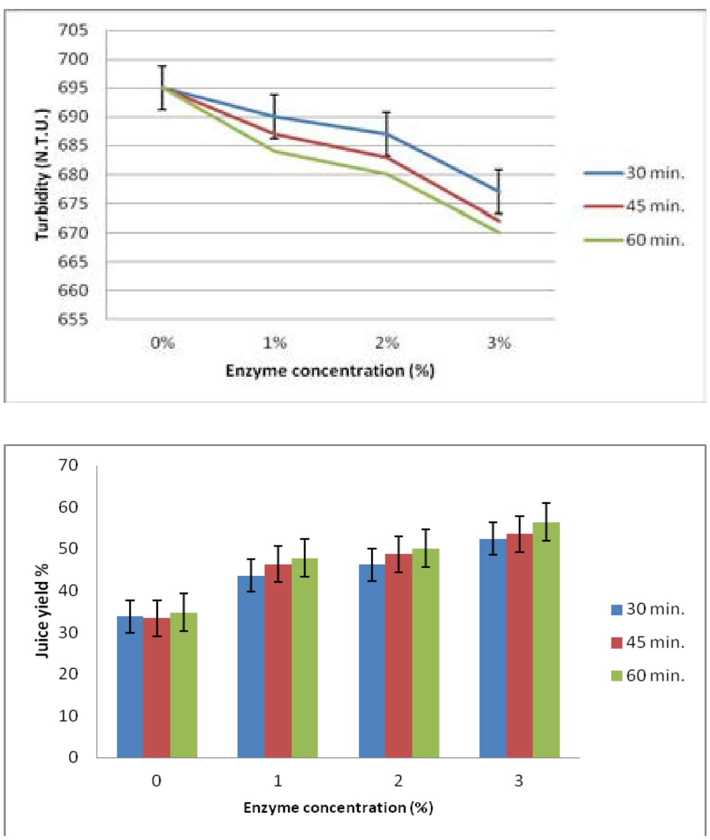


Fig. 2. Effect of enzyme concentration and incubation time on bael juice on turbidity (NTU) and yield (%) at 35°C

Minimum turbidity (638 NTU) and maximum juice yield (61.3%) were observed with 3% crude pectinase concentration after 60 minutes of incubation time at 40°C (Fig. 3). The enzyme concentration, incubation time, and temperature were significant factors in turbidity and juice yield. The variation in yield was due to the increase in the amounts of pectin and starch and the reduction of sugars in bael pulp.

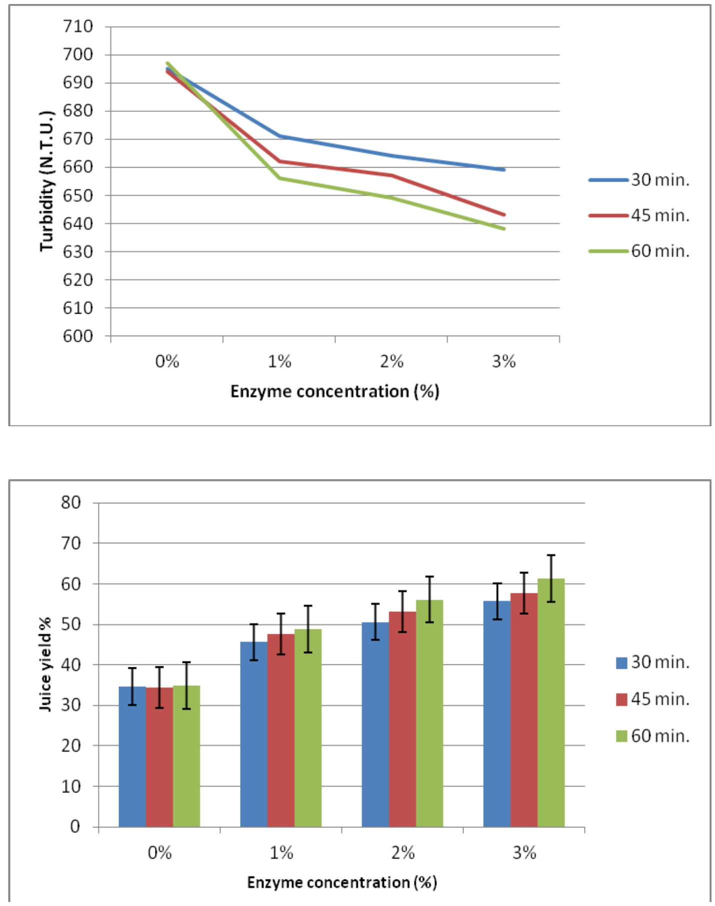
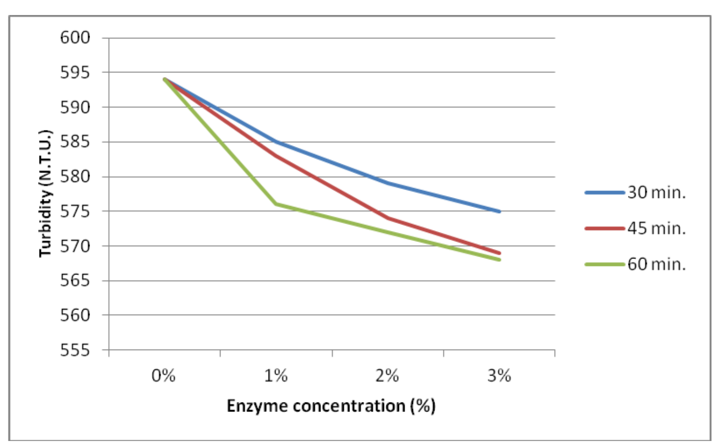


Fig. 3. Effect of enzyme concentration and incubation time on bael juice turbidity (NTU) and yield (%) at 40°C

After 60 minutes of incubation, 3% crude pectinase produced minimum turbidity (568 NTU), while maximum juice yield (67.8%), was observed after 30 minutes of incubation at 45°C (Fig. 4). The enzyme concentration, incubation time, and temperature were significant for turbidity and juice yield.



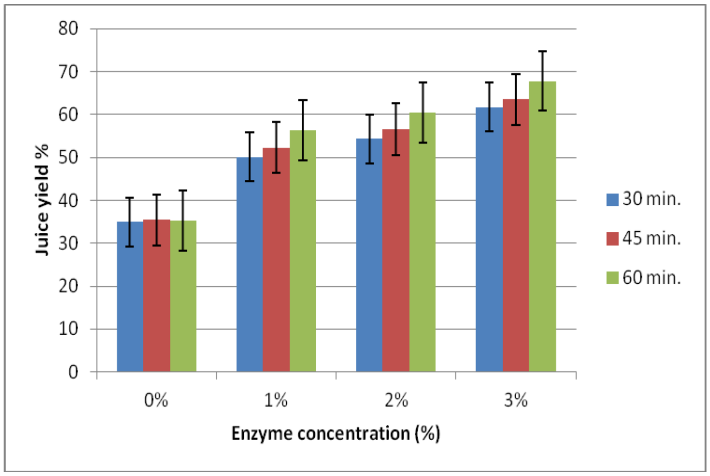


Fig. 4. Effect of enzyme concentration and incubation time on bael juice turbidity (NTU) and yield (%) at 45°C

This may be due to the higher tissue breakdown and the release of soluble solids. Abdullah *et al.* (2021) reported similar results in the study of pectinase-assisted extraction of cashew apple juice. Pectinase helps break down the pectin in bael fruit, reducing the cloudiness of the juice and resulting in a more transparent product. By breaking down the cell walls, pectinase allows for better juice extraction, increasing the yield. The enzyme helps reduce the juice's viscosity, making it smoother and palatable. Pectinase concentration, temperature, and time have shown a positive linear effect on juice yield.

The higher concentration of pectinase allows more cell wall breakdown, resulting in a higher release of sap from a cell. In addition, the fibres' solubility increases at the higher pectinase enzyme concentration, promoting a higher juice yield. Pectinase is a versatile and effective tool in the juice industry, providing a reliable method for juice clarification of different fruit types like apple, guava, jamun, pineapple, etc. This kind of trend for juice yield was obtained by Surajbhan *et al.* (2012) and Ghosh *et al.* (2016) for guava and Jamun juice. Crude enzyme with pectinase activity of 8.12U/ml was obtained by *Aspergillus niger* (MTCC 281) under solid state fermentation (Sadiq *et al.,* 2021).

The juice tends to be cloudy with sedimentation, which can be unappealing to consumers (Bhattacherjee *et al.,* 2015). Fresh bael juice has a relatively short shelf life, requiring proper preservation techniques to maintain its quality. Enzyme treatments (like pectinase) are often necessary to clarify the juice, which adds complexity to the extraction process. The juice and its nutrients can be sensitive to heat, requiring careful handling during pasteurization to retain its nutritional value.

The higher pectinase concentration would degrade the complex compounds, which may increase the juice yield. The viscosity and turbidity were minimal at higher enzyme concentrations and a high range of temperature and time. It is due to the breakdown of pectin and cellulose at higher pectinase concentrations, which gives a higher juice yield and dilutes existing juice. Thus, it results in lower viscosity and, consequently, the turbidity (Ghosh *et al.,* 2016). The pectinase concentration, temperature, and time have shown a negative linear effect on turbidity. Vivek *et al.* (2019) have reported similar turbidity behaviour in Sohiong fruit juice.

Conclusion

In conclusion, the clarification of bael fruit juice was optimized by adjusting key variables, including pectinase enzyme concentration, incubation temperature, and time. The highest juice yield (67.8%) and lowest turbidity (568 NTU) were achieved with 3% crude pectinase, incubated for 60 minutes at 45°C. This highlights the critical role of enzyme concentration and temperature in the clarification process of bael juice.

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Conflict of Interest

The authors have no conflict of interest.

Data Sharing

All relevant data are within the manuscript.

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