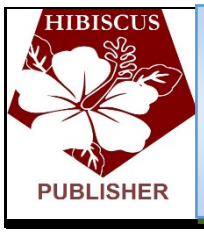
JOBIMB, 2024, Vol 12, No 1 SP1, 86-89
https://doi.org/10.54987/jobimb.v12iSP1.950



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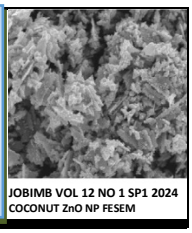

Effects of Operating Parameters on Enzymatic Clarification of Bambangan (*Mangifera pajang*) Juice Using Pectinase

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HISTORY

Received: 7th April 2024
Received in revised form: 5th July 2024
Accepted: 30th July 2024

KEYWORDS

Mangifera pajang
Bambangan juice
Clarification
Enzymatic treatment
Juice quality

ABSTRACT

Bambangan juice is known for its relatively high viscosity and cloudiness, often leading to sedimentation post-bottling. These attributes are attributed to the presence of polysaccharides like pectin and starch, which contribute to instability and degradation during storage. Clarification processes, typically involving enzymatic treatment, are essential to achieve a clear, bright juice with low viscosity. Hence, this research aims to identify the optimal operating conditions (enzyme concentration, temperature, and incubation time) for bambangan juice clarification using pectinase derived from *Aspergillus aculeatus*. Assessment of juice quality was based on yield and various physicochemical properties. The most effective parameters for bambangan juice clarification with pectinase were found to be an enzyme concentration of 0.10% v/v, a temperature of 40°C, and an incubation time of 30 minutes. These conditions substantially enhanced juice characteristics, including yield, clarity, and color (L*, a*, b*), with respective values of 87%, 0.5, and L* - 61.1; a* - 2.7; b* - 1.4.

INTRODUCTION

Bambangan (*Mangifera pajang*) is a native fruit originating from Borneo Island, specifically Sabah and Sarawak in Malaysia, Brunei, and Kalimantan in Indonesia. The fruit is ovoid in shape and possesses a sweet and sour taste with a strong aroma. Approximately 60-65% of the total weight of bambangan fruit consists of pulp [1], which is commonly eaten fresh, made into juice, or pickled by the local community. The cultivation of bambangan fruit for juice production has received more attention due to its high nutritional content. However, the bambangan juice available on the market is turbid, viscous, and cloudy. This is attributed to the presence of polysaccharides (such as pectin, cellulose, hemicelluloses, lignin, and starch), proteins, tannins, and metals [2], which resulted in hindrance for extraction efficiency. The turbidity causes instability in bambangan juice during storage, shortening its shelf life. Since the clear appearance of the juice is a determining factor for consumers, several efforts have been made to reduce its turbidity, viscosity, and cloudiness.

Enzyme application has emerged as a commercially significant tool for enhancing yield and extraction efficiency. Pectinase enzymes have been employed as pretreatment agents to depectinize fruit juice and enhance product yield. Almost all the commercial preparations of pectinases are derived from fungal species, primarily from the Aspergillus genus including *Aspergillus niger*, *Aspergillus aculeatus* and *Aspergillus carbonarius*. *Aspergillus aculeatus* produces dark brown to black conidia. It is closely related to *Aspergillus niger* which is only distinguished by the Aspergillus head containing phialides. *Aspergillus aculeatus* is more sensitive to temperature than *Aspergillus niger*.

Aspergillus aculeatus has a wide range of temperature to grow (10 and 42°C) as compared to *Aspergillus niger* (25 to 37°C) [3]. Therefore, *Aspergillus aculeatus* has become a prominent in industrial pectinolytic enzyme production. The commercial preparation Pectinex® Ultra SPL, obtained from this microorganism, was successfully employed to clarify and reduce the viscosity of juices from different fruits such as apple [4], guava [5] and red pitaya [6].

Several studies have reported that the clarification of fruit juices using pectinases has emerged as an effective strategy to reduce turbidity [7, 8]. Pectinases degrade pectin, reducing viscosity and promoting cluster formation, thus facilitating separation through centrifugation or filtration. This results in clearer juice with intensified flavor and color [9, 10]. Enzymatic treatment using pectinase enzyme from *Aspergillus aculeatus* is one effective way to reduce pectin content because of its ability to hydrolyze pectin polymer into smaller fragments through hydrolysis. Until now, no studies have reported on bambangan juice clarification using the enzymatic treatment method, specifically pectinase enzyme from *Aspergillus aculeatus*. Thus, this study aims to determine the effect of operating parameters on the clarification of bambangan juice using enzymatic treatment.

MATERIALS AND METHODS

Bambangan Juice Preparation

Ripe bambangan fruit was obtained from a local Kota Kinabalu, Sabah, Malaysia market. Upon arrival at the laboratory, the fruit was thoroughly washed and manually peeled to obtain the pulp. Next, the pulp was blended using a food processor for two to three minutes until a homogeneous fruit pulp was obtained.

Bambangan juice was prepared with a ratio of 1:1 (pulp to water, w/v), with extraction using hot water to facilitate the maceration process and increase juice production from the pulp [11]. Then, the pasteurization process was carried out at a temperature of 90°C for 60 seconds to destroy any pathogens that may be present in the raw juice. The quality of bambangan juice (clarity, color and pH) was conducted prior to the enzymatic treatment. The results obtained were used as a reference for comparison of treated bambangan juice.

Enzymatic Treatment on Bambangan Juice

Determination of the best enzymatic treatment conditions was used one factor at a time (OFAT) method. The optimal operating conditions are by varying one parameter while keeping the others at a constant level. Bambangan fruit juice was treated using pectinase enzymes from *Aspergillus aculeatus* which was commercially bought from Sigma-Aldrich (Catalogue no: P2611). Four parameters were studied, which include enzyme concentrations (0%, 0.05%, 0.10%, 0.20%, and 0.30% v/v), temperatures (30°C, 40°C, and 50°C), and times (30, 60, 90, and 120 min) to obtain optimal conditions for the juice clarification.

The selection of enzyme concentration range, temperature, and treatment time used in this study were based on previous studies [8, 9, 10] that used pectinase enzyme (*Aspergillus aculeatus*) to clarify various fruit juices. After the treatment, the enzymatic action was deactivated at 90°C for 5 min. The treated juice was centrifuged at 3000 x g for 10 min, and the supernatant was collected and filtered through Whatman filter paper no. 1 using vacuum suction at 25 mm Hg. The filtrate was then collected for juice yield measurement and further analysis of physicochemical properties. All analysis involved in this study were performed in duplicate.

Determination of Juice Yield

The volume of bambangan juice before and after enzyme treatment was measured using a measuring cylinder. The initial volume of bambangan juice was fixed at 200 mL. In order to determine the bambangan juice yield, the following formula was used [12]:

$$Juice\ yield\ (\%\ v/v) = \frac{Volume\ of\ clear\ fruit\ juice}{Volume\ of\ raw\ fruit\ juice} \times 100 \qquad \text{(Eqn. 1)}$$

Clarity Analysis

The clarity of bambangan juice was determined by measuring an absorbance at 660 nm using UV-Vis spectrophotometer (Lambda 35, Perkin Elmer Co., USA). USA). Distilled water is used as a reference [9]. The level of juice clarity is measured in percentage transmission (%T) using the following formula:

$$\%T = 100 \times 10^{A} \qquad \text{(Eqn. 2)}$$

Where the A is the optical absorption value at a wavelength of 660 nm.

Determination of Physicochemical Properties of Bambangan Juice

The color of bambangan fruit juice was measured using a Colorflex EZ Colorimeter (Hunter Lab, USA). Color values were measured using the system Comission Internationale de L'Eclairage (CIE) scale L*, a*, and b*. The pH value of bambangan juice was determined using a pH meter (Eutech Instrumental, Malaysia). The calibration process was performed using a pH standard buffer [14].

Statistical Analysis

SPSS version 25.0 was used in this study for statistical analysis. Means and standard deviations were determined for duplicate samples using one-way Analysis of Variance (ANOVA), and a confidence level of $p < 0.05$ was considered to indicate a significant difference. The three independent variables are enzyme concentration, temperature, and time, while the dependent variables measured are clarity, color, and pH. Tukey's test was also used to confirm whether there is a significant difference between each group analysis.

RESULTS AND DISCUSSION

Fig. 1 shows the effect of enzyme concentration, temperature, and incubation time on bambangan juice yield. The highest yield was obtained at an enzyme concentration of 0.10% v/v, a temperature of 40°C, and an incubation time of 30 minutes. Although the optimal enzyme concentration for the juice clarification is achieved at 0.10 to 0.20% v/v, there is no significant difference in juice yield. Therefore, 0.10% v/v is chosen to reduce production costs. Additionally, the optimal yield (86.75%) of bambangan juice is achieved at a temperature of 40°C. Beyond this temperature, the yield decreases, likely due to enzyme denaturation occurring at higher temperatures [15]. Furthermore, incubation time has minimal effect on bambangan juice yield.

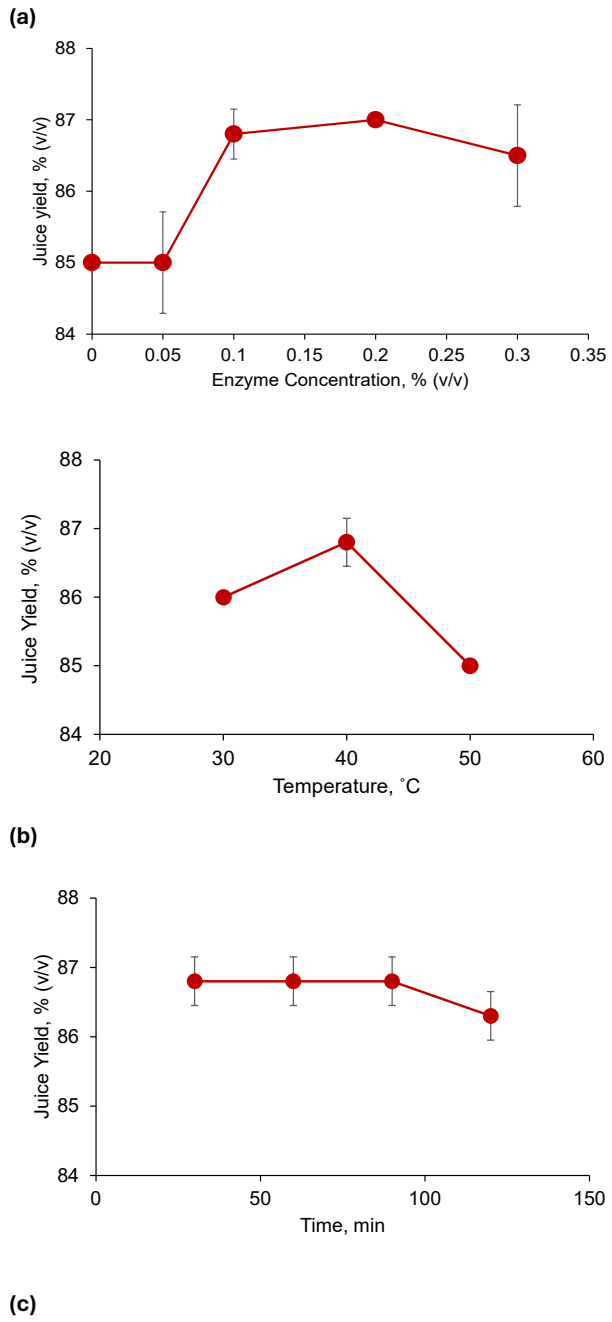

Fig. 1. Bambangan juice yield (% v/v) as a function of (a) enzyme concentration, (b) temperature, (c) incubation time.

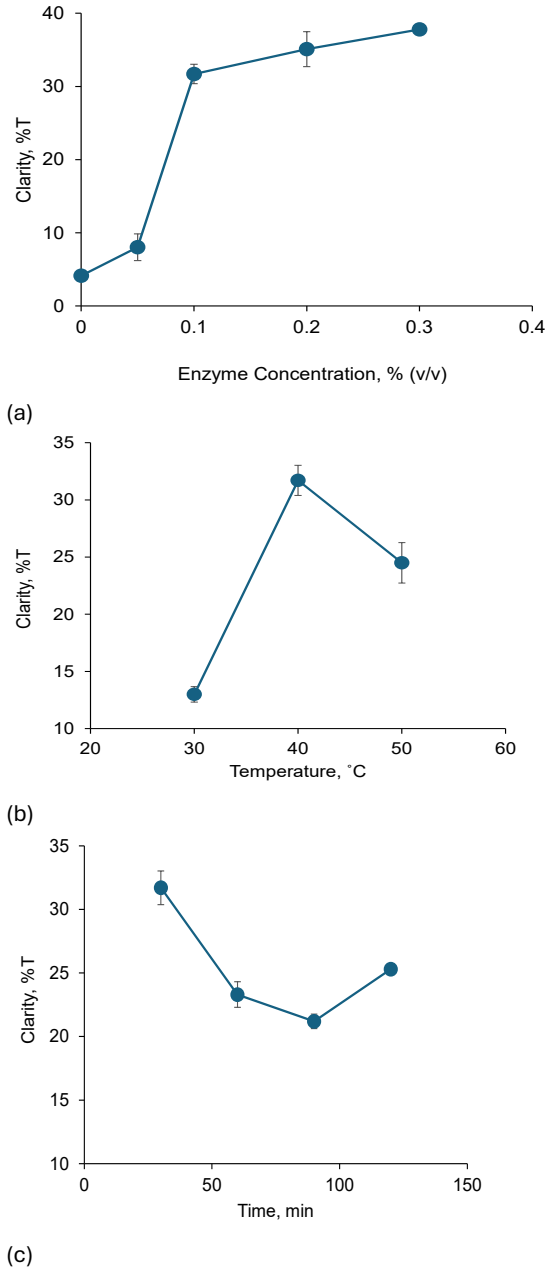

Fig. 2. Clarity of bambangan juice (% v/v) as a function of (a) enzyme concentration, (b) temperature, (c) incubation time.

The effect of enzymatic clarification operating parameters on the clarity of bambangan juice is illustrated in **Fig. 2**. A lower absorption value, as determined by UV-Vis spectrophotometer analysis, indicates higher juice clarity, with clarity levels measured in transmission percentage (%T). Prior to enzyme treatment, the clarity of bambangan juice was determined and found at value of 0.25. The clarity values of the juice increase significantly ($p<0.05$) with the rising enzyme concentration, likely due to the breakdown of pectin into monomers such as negatively charged galacturonic acid [16]. This study found that a temperature of 40°C yielded the lowest absorption value (0.50±0.02) and the highest clarity value (31.68±1.31). Additionally, an incubation time of 30 minutes produced the highest clarity.

Table 1 shows the effect of different enzyme concentrations, temperatures and incubation time in the color values (L*, a*, b*) of bambangan juice. Color plays an important role in the sensory attribute. A higher L* value indicates increased clarity in the juice produced. Significant differences were observed between the bambangan juice samples before and after enzyme treatment, with the most favorable color attributes demonstrated at an enzyme concentration of 0.10% v/v. The L* value (brightness) increased from 50.44 to 61.09, the a* value (redness) increased from 1.33 to 2.66, and the b* value (yellowish) decreased from 16.63 to 1.36. These findings suggest a clearer juice was obtained. At a different temperature, 40°C yielded the highest L* value of 61.09±1.00, indicating optimal temperature for achieving the highest juice clarity. The highest L* value was identified at 30 minutes regarding the optimal incubation time.

Table 1. Effect of different enzyme concentrations, temperature, and incubation time in the color values (L*, a*, b*) of bambangan juice.

Treatment	Color		
	L*	a*	b*
Different Enzyme Concentrations (% v/v)			
Untreated Bambangan Juice	50.44±0.64^{d}	1.33±0.02^{b,c}	16.63±0.83^{a}
0	54.47±0.51^{c}	1.05±0.66^{b,c}	9.81±0.10^{b}
0.05	57.00±0.83^{b}	1.70±0.94^{b}	5.98±1.87^{c}
0.1	61.09±1.00^{a}	2.66±0.17^{a}	1.36±0.06^{d}
0.2	61.23±0.05^{a}	2.77±0.04^{a}	1.03±0.01^{d}
0.3	61.42±0.02^{a}	2.85±0.06^{a}	0.17±0.11^{d}
Different temperature (°C)			
Untreated Bambangan Juice	50.44±0.64^{c}	1.33±0.02^{c}	16.63±0.83^{a}
30	57.91±0.68^{b}	1.85±0.57^{b}	7.11±1.03^{b}
40	61.09±1.00^{a}	2.66±0.32^{a}	1.36±0.06^{c}
50	59.56±0.06^{a,b}	2.09±0.01^{b}	3.65±0.17^{c}
Different Incubation Time (minutes)			
Untreated Bambangan Juice	50.44±0.64^{b}	1.33±0.02^{b}	16.63±0.83^{a}
30	61.09±1.00^{a}	2.66±0.09^{a}	1.36±0.06^{b}
60	60.47±0.10^{a}	2.72±0.54^{a}	2.23±0.08^{b}
90	60.42±0.21^{a}	2.48±0.08^{a}	3.56±0.05^{b}
120	60.21±0.08^{a}	2.74±0.12^{a}	2.12±0.88^{b}

*Data presented in mean ± standard deviation (n = 2). Values followed by different superscripts within each column indicate significant differences ($p < 0.05$).

Table 2 shows the effect of enzyme concentration, temperature, and incubation time on the pH of bambangan juice. There was a significant decrease ($p < 0.05$) in pH value as the enzyme concentration increased from 0% to 0.30% v/v compared to the pH value before enzyme treatment.

Table 2. Effect of enzyme concentration, temperature, and incubation time on the pH of bambangan juice.

Treatment		pH
Different Enzyme Concentration (% v/v)		
Untreated Juice	Bambangan	4.09±0.01^{a}
0		3.89±0.01^{b}
0.05		3.83±0.00^{b}
0.1		3.78±0.00^{c}
0.2		3.57±0.03^{e}
0.3		3.64±0.03^{d}
Different temperature (°C)		
Untreated Juice	Bambangan	4.09±0.01^{a}
30		3.79±0.04^{b}
40		3.78±0.00^{b}
50		3.81±0.02^{b}
Different Incubation Time (minutes)		
Untreated Juice	Bambangan	4.09±0.01^{a}
30		3.78±0.00^{b}
60		3.81±0.01^{b}
90		3.79±0.01^{b}
120		3.69±0.01^{c}

*Data presented in mean ± standard deviation (n = 2). Values followed by different superscripts within each column indicate significant differences ($p < 0.05$).

This increase in acidity is likely attributed to the formation of galacturonic acid resulting from the breakdown of pectin [17]. Although no significant difference was observed at different temperatures (30°C, 40°C, 50°C), the lowest pH value was recorded at 40°C, suggesting that this temperature may optimize the breakdown of pectin into galacturonic acid. Similarly, pH values decreased with varying incubation times from 30 to 40 to 50 minutes, yet no significant effect on pH values was noted.

CONCLUSION

In conclusion, the optimal operating parameters for enzymatic clarification to enhance the quality of bambangan juice, utilizing pectinase enzyme from *Aspergillus aculeatus*, were determined to be a 0.1% enzyme concentration, a temperature of 40°C, and an incubation time of 30 minutes. At these parameters, 86.75% bambangan juice yield was obtained with clarity of 31.68%, color of L*: 61.09, a*: 2.66, b*: 1.36, along with a pH of 3.78. These findings underscore the suitability of these optimal conditions for subsequent membrane-based clarification processes.

ACKNOWLEDGMENT

The authors acknowledge the financial support for this study by the Innovation Grant Scheme under Research University Grant Scheme (Universiti Malaysia Sabah) with grant number SGI0189.

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