Utilization of Cooking-Type 'Saba' Banana in the Development of Ready-to-Drink Juice with Improved Quality and Nutritional Properties

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Abstract: The 'Saba' banana cultivar is one of the most abundantly grown fruit crops in the Philippines. However, large postharvest losses were posed due to the rapid deterioration and challenges in marketing. This study was conducted to develop a ready-to-drink (RTD) beverage using the cooking-type banana cultivar [*Musa acuminata × balbisiana* Colla (ABB Group) 'Saba']. The pulp was subjected to treatment with α-amylase and pectinase enzyme concentrations ranging from 0.25% to 1.00% to facilitate juice extraction. The effect of α-amylase and pectinase enzyme combinations on juice yield, color and clarity was determined. The highest juice yield (69.83%) and clarity (72.56% by transmittance at 660 nm) were achieved using 1.00% α-amylase: 1.00% pectinase and 0.5% α-amylase: 1.00% pectinase enzyme treatments, respectively. The juice treated with 0.5% α-amylase: 1.00% pectinase was used in the formulation of the RTD beverage. Physico-chemical and sensory properties of the product were analyzed. The developed RTD 'Saba' juice with acceptable sensory characteristics had 11.45 cP viscosity, 0.33% titratable acidity, 5.38% protein, 1660 ppm potassium, 40 ppm sodium and 460 ppm calcium. Results showed that the cooking-type banana cultivar 'Saba' can be utilized in the development of the RTD beverage with enhanced sensory and nutritional quality.

Keywords: 'Saba' banana juice; ready-to-drink-beverage; enzyme-aided juice extraction

1. Introduction

Banana is known as "common man's fruit" and is considered as one of the most important global food crops (after rice, wheat and maize) grown in more than 100 countries worldwide [1]. Because bananas are fruiting all year round and contain sufficient nutrients for energy source, the crop is considered as a readily available food commodity, which may serve as a substitute for other seasonal food crops. Hence, it significantly contributes to food and income security of people engaged in its production and trade, particularly in developing countries like the Philippines. Also, it is a vital source of food especially in rural areas as a supplement or substitute to staple food such as rice and corn [2].

According to the Philippine Bureau of Agriculture Statistics, banana production was estimated at around 2 million metric tons [3]. However, a considerable amount of this fruit is wasted due to inadequate processing and preservation techniques [4], poor post-harvest handling, over ripening, poor storage, poor transportation, or overproduction at certain times of the year [5].

Food processing and preservation has always been a practical means in order to reduce fruit losses. Common products manufactured using bananas are wine and vinegar, puree, powder or flour, chips, jam and canned slices [6]. Bananas are also suitable for juice production and studies using different

approaches have been conducted in order to produce a commercial product out of banana juice. However, due to the high pectin content and starchy nature of bananas especially the cooking cultivar, problems on the juice yield, clarity and other sensory properties have been encountered. Hence, this study aimed to develop a ready-to-drink beverage from 'Saba' banana [*Musa acuminata × balbisiana* Colla (ABB Group) 'Saba'] with improved yield and quality through enzyme-aided processing of banana pulp.

2. Materials and Methods

2.1. Materials

Musa acuminata × balbisiana Colla (ABB Group) 'Saba' banana cultivar grown in Tiaong, Quezon, Philippines was purchased at the mature stage based on the standard peel color index [7]. Pectinase from *Aspergillus niger* and α-amylase derived from *Bacillus amyloliquifaciens,* both in crude form and each having an activity of 500 U/mL, were purchased from the Enzyme Laboratory at The National Institute of Molecular Microbiology and Biotechnology (BIOTECH), University of the Philippines Los Baños, College, Laguna, Philippines.

2.2. Banana Juice Extraction

Ten kg of ripened bananas were washed, peeled, weighed, cut into approximately $2.0 × 2.0 × 0.3$ cm and then soaked in 30 L of 0.1% citric acid and 0.05% NaCl solution for 30 min in order to prevent browning. The soaking solution was then discarded, the pulp was pureed, and the resulting mash was heated at 80 °C for 15 min and then cooled to 40 °C prior to enzyme treatment. The mash with an initial pH of 6.0 was treated with α-amylase in combination with pectinase at different concentrations ranging from 0.25% to 1.00% for 2 h to facilitate juice extraction. Mixing at 200 rpm for 10 min was done at the initial stage to ensure homogenization. The mixture was pressed and filtered using a Grade 90 cheese cloth to separate the juice from the pulp. Banana juice extract without enzyme pretreatment was also prepared in the same manner. The extracted juice was pasteurized in a bench scale stainless steel vat at 80 °C for 15 min. The juice was then packed into sterilized polyethylene bags and kept at −4° C until use.

2.3. Formulation of Ready-to-Drink 'Saba' Banana Beverage

In order to prepare the ready-to-drink beverage, the sugar content, expressed as total soluble solids (TSS), was adjusted to 10, 12 and 14 °Brix, respectively, by dilution with distilled water. The pearson square or box method was used to estimate the TSS and determine the necessary adjustment to obtain the target °Brix during the preparation of each formulation. The actual °Brix was measured using a hand-held refractometer (Atago, Master-20α). The pH of the beverage was also adjusted to 3.6 through acidification with malic acid for better stability.

2.4. Sensory Evaluation

The sensory characteristics of the ready-to-drink banana beverage namely color, aroma, sweetness, sourness, flavor, clarity, aftertaste as well as the general acceptability were evaluated by 20 laboratory panelists, which consisted of equal numbers of male and female, using quality scoring in a 15 cm line scale. The panelists were selected based on their knowledge and experience on conducting a discriminative sensory test and were re-oriented about the quality scoring test. Samples were then evaluated at room temperature (25 °C) under natural/white lighting condition in separate sensory evaluation booths and tests were conducted between 9:00 a.m. and 11:00 a.m. For each panelist, 25 mL of banana juice samples (chilled at 5 °C prior to sensory evaluation) placed in randomly coded 75 mL uniform glass containers were served. Panelists were instructed to sip water and rinse their mouths between the tasting of each juice samples provided [8].

2.5. Analysis of the Physico-Chemical Properties

2.5.1. Determination of Total Soluble Solids, pH, Total Titratable Acidity and Viscosity

The total soluble solids (TSS), pH and total titratable acidity (TTA) of the juice were measured using the AOAC (2012) method [9]. The viscosity of the sample was determined using the falling ball viscometer.

2.5.2. Color and Clarity

The color of the juice was measured by absorbance (A) at a wavelength of 420 nm while the clarity of the extract was measured by transmittance (%T) at 660 nm using a UV-Vis spectrophotometer (Shimadzu UVmini-1240) based on the method of Rai et al. (2006) [10].

2.5.3. Alcohol Insoluble Solids (AIS)

Alcohol insoluble solids are the measure of pectinaceous substances in banana [11]. Ten g of juice was weighed into a 250 mL beaker. One hundred fifty mL of 80% ethanol was added, stirred, brought to boil and then simmered gradually for 30 min. Whatman filter paper no. 2, which has been previously dried and weighed, was used for filtration. The content of the beaker was transferred to the filter paper and the residue was washed with 80% ethanol until the washing was clear and colorless. The filter paper and the alcohol-insoluble solids were dried for 2 h at 100 °C, cooled in a desiccator and weighed. AIS was calculated as a percentage (% w/w) using the following equation:

$$\%AIS = (\text{weight of residue/weight of sample taken}) \times 100.$$

2.5.4. Analysis of Total Sugar, Protein and Mineral (K, Na and Ca) Contents

The total sugar content was analyzed using the phenol-sulfuric acid method. The absorbance of the mixture was read at 480 nm and the sugar concentration of the sample was determined based on the glucose standard curve [12]. The total amount of protein in the sample was determined according to the dye binding method of Lowry, with bovine serum albumin as standard [13]. Calcium and sodium contents were determined using the atomic absorption spectrophotometric method (Perkin Elmer Aanalyst 200) while the potassium concentration was determined using the flame photometric method (Sherwood Scientific Model 410 Classic flame photometer) [9].

2.6. Statistical Analysis

The data gathered from the experiments were subjected to the Analysis of Variance (ANOVA) and analyzed using SAS General Linear Models (GLM) procedure with the SAS software 9.1.3. Duncan's New Multiple Range Test (DNMRT) was also performed to determine the significant difference among the treatments. The difference was defined at $p < 0.05$ level of significance.

3. Results

3.1. Effect of Enzymatic Treatment on the Characteristics of 'Saba' Banana Juice Extract

Table 1 shows the effect of varying amylase and pectinase enzyme concentrations ranging from 0.25% to 1% on the yield, color and clarity of the extracted 'Saba' banana juice.

Table 1. Juice yield, color and clarity of 'Saba' banana juice treated with different enzyme concentrations.

Enzyme Concentration (α-Amylase and Pectinase)	Juice Yield (%) [1]	Color (A) [1]	Clarity (%T) [1]
A1 (0.25% and 0.25%)	30.64 ± 1.93 [d]	1.10 ± 0.29 [ab]	35.41 ± 10.50 [c]
A2 (0.25% and 0.50%)	36.36 ± 2.32 [e]	0.94 ± 0.15 [ab]	38.21 ± 8.71 [c]
A3 (0.25% and 0.75%)	53.31 ± 7.51 [c]	0.86 ± 0.27 [bc]	44.03 ± 17.70 [bc]
A4 (0.25% and 1.00%)	55.06 ± 4.33 [bc]	0.94 ± 0.21 [ab]	45.78 ± 0.62 [bc]
B1 (0.50% and 0.25%)	59.59 ± 0.58 [bc]	0.87 ± 0.19 [bc]	45.86 ± 17.17 [bc]
B2 (0.50% and 0.50%)	61.19 ± 1.85 [bc]	0.96 ± 0.32 [ab]	42.22 ± 21.86 [bc]
B3 (0.50% and 0.75%)	60.52 ± 2.81 [bc]	0.76 ± 0.18 [bc]	56.49 ±18.66 [b]
B4 (0.50% and 1.00%)	62.21 ± 3.95 [ab]	0.63 ± 0.16 [c]	72.56 ± 15.98 [a]
C1 (0.75% and 0.25%)	61.25 ± 5.31 [bc]	0.85 ± 0.10 [bc]	52.29 ± 3.58 [bc]
C2 (0.75% and 0.50%)	61.99 ± 0.73 [ab]	0.82 ± 0.20 [bc]	57.65 ± 11.74 [bc]
C3 (0.75% and 0.75%)	63.04 ± 0.76 [ab]	0.64 ± 0.02 [c]	67.12 ± 3.31 [a]
C4 (0.75% and 1.00%)	65.47 ± 0.66 [ab]	0.78 ± 0.13 [bc]	52.32 ±13.92 [bc]
D1 (1.00% and 0.25%)	60.59 ± 6.13 [bc]	1.32 ± 0.05 [a]	36.86 ± 8.94 [c]
D2 (1.00% and 0.50%)	54.43 ± 2.57 [bc]	1.13 ± 0.43 [ab]	36.13 ± 10.81 [c]
D3 (1.00% and 0.75%)	62.90 ± 2.34 [ab]	0.81 ± 0.04 [bc]	57.51 ± 0.00 [bc]
D4 (1.00% and 1.00%)	69.83 ± 2.01 [a]	0.80 ± 0.05 [bc]	56.87 ± 1.14 [bc]

[1] Values are means ± standard deviation of triplicate measurements. Values followed by the same letter superscripts within a column are not significantly different from each other using DNMRT ($p < 0.05$).

Varying the enzyme concentration combinations increased the juice extraction yield from 30.64% to 69.83% juice. The enzyme combination 0.5% α-amylase and 1.0% pectinase resulted in a juice yield of 62.21% and appeared to be not significantly different with the samples treated with higher α-amylase enzyme concentration. The combination consisting of lower enzymes concentration, even if it was not significantly different from treatments involving higher amounts of enzymes, was chosen for further analysis and beverage formulation, considering the cost of enzyme as an important factor.

The juice yield, physico-chemical properties (color, clarity, total soluble solids, viscosity, pH, total titratable acidity, alcohol insoluble solids) and main composition (total sugar and protein) of the juice extracted with and without the aid of enzymes are presented on Table 2.

Table 2. Comparison of the physico-chemical properties of the 'Saba' banana juice extracted with and without enzyme pretreatment.

Parameters	Juice Without Enzyme Pretreatment [1]	Enzymatically Extracted Juice (0.5% α-Amylase and 1.0% Pectinase) [1]
Juice Yield, %	27.40 ± 3.54 [b]	62.21 ± 3.95 [a]
Color (A)	2.74 ± 0.05 [a]	0.63 ± 0.16 [b]
Clarity, %T	15.24 ± 3.46 [b]	72.56 ± 15.98 [a]
TSS, °Brix	14.07 ± 0.06 [b]	21.67 ± 0.60 [a]
Viscosity, cP	15.07 ± 0.09 [a]	11.66 ± 0.05 [b]
pH	4.57 ± 0.06 [a]	4.13 ± 0.06 [b]
TTA, %	0.21 ± 0.00 [b]	0.57 ± 0.01 [a]
AIS, %	5.37 ± 1.85 [a]	2.76 ± 0.08 [b]
Total Sugar, mg/ml	851.77 ± 19.41 [b]	928.77 ± 4.70 [a]
Protein content, mg/ml	7.39 ± 0.63 [b]	14.15 ± 0.59 [a]

[1] Values are means ± standard deviation of triplicate measurements. Means followed by the same letter within each row are not significantly different at $p < 0.05$ using DNMRT.

Significant differences between the banana juice extracted without enzyme pretreatment and the one which has undergone enzyme-assisted extraction were observed in the measured properties. Juice yield was increased from 27.40% to 62.21%. Improvement in the color and clarity of the juice was also pronounced. Moreover, it can be noted that the juice was easily pressed out from the enzyme treated pulp and filtered due to the decrease in viscosity from 15.07 cP of the untreated juice to

11.66 cP of the enzymatically treated one. Total soluble solids of the juice sample also increased from 14 °Brix to 21.67 °Brix. An increase in the total sugar of the sample was also observed, from 857.22 mg/mL to 928.77 mg/mL. A slight decrease in pH from 4.57 to 4.13 and increase in the total titratable acidity of the juice from 0.21% to 0.57% can also be noted. The presence of enzymes also doubled the protein concentration of the juice from 7.39 mg/mL to 14.15 mg/mL which is desirable for the nutritive properties of the juice.

3.2. Sensory Evaluation of Ready-to-Drink 'Saba' Banana Beverage

Sensory properties of the ready-to-drink (RTD) 'Saba' beverage with different dilutions and total soluble solids (10 °Brix, 12 °Brix and 14 °Brix) were assessed using quality scoring. The 20 panelists employed in the study were screened for sensory acuity and oriented regarding the conduct of a discriminative sensory test. They were asked to differentiate the intensities of the evaluated sensory attributes as well as to preliminarily assess the overall acceptability of the formulations.

Figure 1 presents the intensity of the sensory properties of formulated banana juice. The samples were significantly different in the attributes namely sweetness, flavor, sourness as well as in the general acceptability.
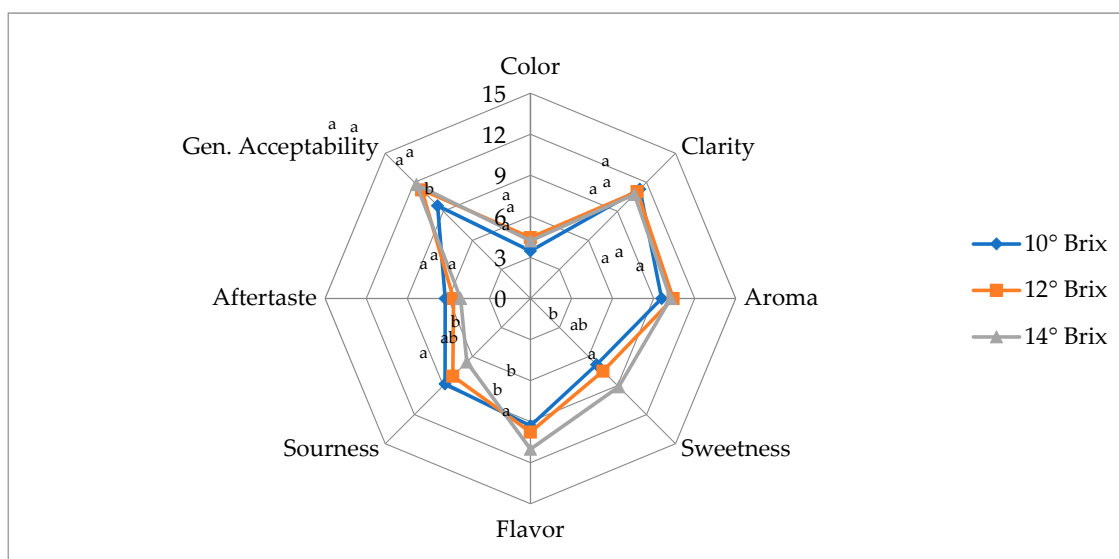

Figure 1. Plot comparing the intensities of the different sensory attributes of the formulated ready-to-drink 'Saba' banana juice evaluated using quality scoring.

The sample with TSS of 14 °Brix showed the highest intensity in terms of flavor and sweetness and lowest perceivable sourness. However, a balance in sourness, sweetness as well as a moderate intensity of flavor in the sample with TSS of 12 °Brix was perceived by the panelists. Moreover, the aforementioned beverage was assessed to have a high overall acceptability comparable with that of the sample with the highest TSS (14 °Brix).

The RTD beverage with TSS of 12 °Brix formulated using the enzymatically extracted juice was further analyzed for some of its nutritional components as shown in Table 3.

The quantified levels of minerals and other nutrients present in the developed RTD beverage can supply 6.98–9.25% protein and 5.75–6.13% calcium based on the recommended nutrient intakes for Filipino adults. Also, 8.30% and 0.80% of the electrolytes potassium and sodium requirement, respectively, in the diet of normal Filipino adults can be provided by every 100 mL of the beverage [14].

Table 3. Nutritional composition of Ready-to-drink 'Saba' beverage.

Nutrients	Amount, mg/100 mL [1]	RNI for Filipino Adults, mg [2]	Daily Value, %
Protein	538.00 ± 36.00	6200–7200	6.98–9.25
Potassium	166.00 ± 12.00	2000	8.30
Sodium	4.00 ± 0.86	500	0.80
Calcium	46.00 ± 2.00	750–800	5.75–6.13

[1] Values are mean ± standard deviation of triplicate measurements. [2] Source: Philippine Dietary Reference Intakes 2015.

4. Discussion

Enzyme application has been an effective method in order to improve the quality of banana juice. It involves the breakdown of the substances in the juice, which are closely related to the quality, appearance and flavor of the juice. The measured properties in this study namely juice yield, clarity, and viscosity were the primary parameters considered for the assessment of efficiency of enzyme-assisted processing of banana juice. Similar with the results of previous studies, the enzyme assisted extraction of the juice from the 'Saba' banana resulted in a significant improvement in these properties as compared with those of the juice extracted without the aid of enzymes. In the study conducted by Tapre and Jain (2014), a higher concentration of the enzyme pectinase also resulted in a higher juice yield [15]. Meanwhile, pectinase-aided extraction of banana juice in the study done by Sagu et al. primarily affected the alcohol insoluble solids content which consequently resulted also in the improvement of the clarity and viscosity of the product [16].

Pectinases in combination with other enzymes like amylases are used in order to increase the yield and decrease the cloudiness of the juice, increase the clarity, decrease viscosity, and increase the nutritional value of the extracted juice [17]. In the extraction of juice from the cooking-type 'Saba' banana pulp, the combined action of α-amylase and pectinase in hydrolyzing pectin and depolymerizing the middle lamellas of the treated banana mash resulted in the improved extraction process. Moreover, the breaking down of pectin and starch reduces the viscosity of the juice and thus increases the flow and shortens the press-time. The higher juice yield was also obtained from the enzyme treated samples. Solubilization of the cell wall components by pectinase facilitated the release of the juices inside the tissues. This is also in accordance with Rai et al. (2004) who had worked on the effect of pectinase for the pretreatment of mosambi juice [18].

A higher clarity and lighter color was also observed in the samples treated with higher levels of enzymes. A similar result was also observed by Lee et al. wherein the clarity of the banana juice was directly influenced by the concentration of the enzyme pectin [19]. The clarity of the juice can be affected by the presence of starch and homopolysaccharides, like araban (consisting of arabinose subunits), causing the post-clouding phenomenon, which cannot be easily removed during the post-extraction process with the aid of clarifying agents. Since pectinase hydrolyzes the pectin component of the substrate, this resulted in the ease of extraction and clarification process [20]. The presence of α–amylase also aided in the clarification by hydrolyzing the starches especially for the 'Saba' banana, which is high in the starch content.

Likewise, the viscosity of the 'Saba' banana juice was also affected by the pectinase treatment wherein increasing the amount of pectin caused a reduction in the viscosity. Tapre and Jain (2014) also concluded that the viscosity of pectin-rich juice is inversely influenced by the linear effects of the enzyme concentration [15]. Pectin is a binding agent which makes juice viscous. A decrease in the viscosity was observed upon the enzymatic treatment which is due to the degradation of pectin leading to a reduction of water holding capacity, releasing the free water to the system hence, reducing the viscosity of the juice [21].

An increase in the total soluble sugars was also observed in the enzymatically extracted juice. The presence of α–amylase facilitated the release of free glucose and maltose units from the starches present which contributed to the increased soluble sugars. Pectinase also caused an increase in the TSS

of banana juice as it aided in the release of free galacturonic acid residues from the pectin molecules during enzymatic extraction [19].

The enzyme concentration also plays an important factor [21]. The cost of enzyme is quite important thus, using the least amount with optimum effect is favored. The best enzyme combination was found to be 0.5% α-amylase: 1.0% pectinase, as this combination was not significantly different with the treatments with higher α-amylase enzyme concentration.

Sensory properties as well as the nutrient content of the beverage was significantly influenced as starch liquefaction and degradation of the protopectin and pectin also caused the release of components of the fruit that resulted in the improvement of the sensory quality and nutritional properties of the juice [17]. In the study by Joshi et al. (2011), enzyme-aided extraction resulted in the improvement of perceived color and clarity and the overall sensory quality of different juices [22]. Moreover, Sagu et al. (2014) showed in their study that the nutritional components of banana juice, specifically the protein content and concentrations of minerals such as calcium and potassium could be improved through enzyme-aided extraction under optimized conditions [16].

5. Conclusions

This study has elucidated that the cooking-type 'Saba' banana cultivar [*Musa acuminata × balbisiana* Colla (ABB Group) 'Saba'] has the potential to be utilized for banana beverage production. The use of the pectinase and α-amylase combination at 0.5% α-amylase: 1.0% pectinase in the enzyme-aided extraction resulted in greater efficiency and desirable characteristics for juice production in terms of ease of extraction, clarification and even on the sensory and nutritional properties of the extracted juice. A ready-to-drink 'Saba' banana beverage was formulated and the beverage with the TSS of 12 °Brix was found to have a balanced sensory characteristic and high overall acceptability as perceived by the panelists. Nevertheless, a separate acceptability testing, involving a larger pool of panelists with well-defined demographic characteristics, should be done in order to determine the best product formulation and its market potential among target consumers. The information obtained in the study can be helpful to prospective banana processors to profitably utilize the cooking-type 'Saba' bananas and produce a beverage with improved quality and nutritional property.

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