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Effect of Multienzymatic Treatment on Banana *(Musa sp.)* Fruit Juice: Optimization Studies on Processing Parameters to Produce Clarified Product

[1]Ravi Kumar, [2]Alak Kumar Singh, [3]Mahendra Kumar, [4]Shiv Kumar,
[1]Assistant Professor, Department of Food Technology, Institute of Engg. & Technology, Bundelkhand University, Jhansi, Uttar Pradesh, India-284128
[2]Professor, Department of Food Technology, School of Chemical Technology, Harcourt Butler Technical University, Kanpur, Uttar Pradesh, India-208002
[3]Project Engineer, Indian Institute of Technology, Kanpur, Uttar Pradesh, India-208016
[4]Professor, Institute of Food Technology, Bundelkhand University, Jhansi, Uttar Pradesh, India-284128

Abstract

This study was conducted with an objective to produce high-quality, high-yield clarified banana juice by using a multi-enzyme system. Various concentrations of the commercial enzymes pectinase, cellulase, and hemicellulase were used to treat banana pulp for varying incubation times (30–180 min) at 55 ^{0}C. For each enzyme, the effect of treatment conditions was examined on turbidity and yield of banana pulp, and the optimum process parameters were identified. For pectinase, cellulase, and hemicellulase, respectively, the optimum reaction conditions were 0.06% enzyme concentration with 60 min of incubation time, 0.40% enzyme concentration with 120 min of incubation time, and 1.20% enzyme concentration with 120 min of incubation time at 55 ^{0}C. The values of turbidity and yield obtained at these optimum conditions were 11 NTU and 74% for pectinase, 12 NTU and 80.9% for cellulase, and 62 NTU and 76.2% for hemicellulase. These results led to the simultaneous application of all three enzymes to banana juice under compromised processing conditions (Pectinase 0.06%, Cellulase 0.40%, and Hemicellulase 0.90% with 120 min incubation period at 55 ^{0}C). The simultaneous application of pectinase, cellulase, and hemicellulase on banana pulp with the aforementioned parameters provided clarified juice with turbidity of 7 NTU and yield of 79.2%.

Introduction

Banana is a well-known tropical fruit for its high nutritional value and excellent flavour and aroma. It is one of India's cheap and abundant fruits. The nation ranks first in production of bananas (26.4%) [1]. However, only 0.05% of the banana produced is exported; the remainder is consumed domestically as table fruit. Despite being the most popular fruit in international trade and ranks second after citrus fruits in terms of value, India, the world's largest producer of bananas, is hardly involved in it. Banana export volumes are poor for a number of reasons, including less-than-ideal post-harvest procedures, unsafe transportation methods, substandard storage facilities, and out-of-date handling procedures. Only 2% of produce gets processed leaving between 25–40% to be squandered due to improper handling [2,3]. Therefore, there is a lot of scope for producing high-value clarified banana juices to reduce this waste and increase foreign revenue through increased export of such valuable products.

The usual method of extracting fruit juices involves crushing and grinding. These procedures produce viscous, turbid, and cloudy juices. Because of the pulp particles and colloidal suspensions present, this occurs. This type of juice has a low yield and is exceedingly challenging to pasteurize and concentrate. Because bananas are excessively pulpy and pectinaceous, crushing and grinding do not yield juice from banana fruit [4]. After these processes with banana, a lumpy and sticky mass is produced. High viscosity is the most serious issue with processing banana pulp. Pectin and starch are the polysaccharides that contribute significantly to the viscosity and turbidity of banana juice [5]**.** Juice clarifying is a crucial step in the juice processing

business since it increases the product's acceptability [6]. Marketing juices with unfavorable cloud and muddy turbidity is not recommended [7]. Clarified juices are highly sought-after for use in the production of ready-to-serve beverages, jelly, cordials, nectars, nutritional carbonated beverages, concentrates, etc. [8]. Numerous items made from clarified juice are finding their way onto the market, including 100% canned fruit (with clear juice as syrup), clear juice blends, candies, fruit honey or fruit sugar, sparkling clear beverages (soft drinks, clear juice cocktails, cold teas with clear juice, alcoholic beverages), and translucent jelly products. This demonstrates that there are a variety of market prospects, including those for clarified juices made from fruits with a high pulp content in addition to the usual clear apple juice [9]. Large portions of the banana crop are unsuitable for the fresh market because they are too mature to ship [10,11]. Bananas have a distinctive, enticing flavor with a lot of sugar content in them. High-value clarified juices from these excess bananas could be turned into lucrative products from bananas that would have otherwise been discarded [11]. The process of clarifying involves breaking the semi-stable emulsion of colloidal plant polysaccharides that holds the insoluble cloud material in a freshly extracted juice. Juices treated with enzymes undergo pectin degradation and viscosity decrease, which makes it easier to separate them using filtering or centrifugation and increases juice clarity. Currently, the extraction and clarification of fruit juices is done using pectinases, cellulases, and hemicellulases, which are known as macerating enzymes. Pectinases hydrolyze the -1,4-glycosidic bonds of pectin molecules to generate polygalacturonic acid monomers [12]. Hemicellulases are a diverse group of enzymes that hydrolyze hemicelluloses, one of the most prevalent groups of polysaccharides found in nature [13]., while cellulases cleave β-1,4-D-glucan linkages of cellulose to yield oligosaccharides, cellobiose, and glucose [14,15]. Because polysaccharides are simultaneously degraded when combination of pectinolytic and cellulolytic enzymes are used for juice clarification, the yield and clarity are improved [16]. The influence of hydrolytic enzymes on juice extraction and clarity have been reported in papaya [17,18,19], guava [20,21,22,23], banana [24,25,26,27,5,28,29,30,10], mango [31,32], litchi [33,7], kiwifruit [34,35], pineapple [36,37], apple [38,39,40], asparagus [41], peach [42], carrot [43,44], pear [45,46], and plums [47].

Materials and Methods

Fruits: Fresh and mature bananas *(Musa sp.)* were purchased from the local market and used immediately or stored at 4 ^{0}C for not more than 2 days before being used.

Enzyme Source: Fruit juice was treated enzymatically using commercial enzymes from Biolaxi Carporation, Bhiwandi, India, including BL-Pectinase, BL-Cellulase, and BL-Hemicellulase. The food-grade enzyme preparation called BL-Pectinase is designed specifically for the extraction of pectic material and the breakdown of cell walls. 1200 PGU/g is the activity of BL-Pectinase. The recommended pH range for enzyme reactions is 3.5 to 6.0 (Optimum 3.8), and the recommended temperature range is 40 °C to 60 °C (Optimum 55 °C). The food-grade enzyme preparations BL-Cellulase and BL-Hemicellulase have activity of 100,000 CMCU/g and 100,000 HCU/g, respectively, and are used to extract and degrade cell walls. For BL-Cellulase, pH 4.5 to 6.0 (Optimum 4.8), temperature 40 ^{0}C to 60 ^{0}C (Optimum 55 ^{0}C), and BL-Hemicellulase, pH 4.5 to 6.5 (Optimum 4.5), and temperature 40 ^{0}C to 60 ^{0}C (Optimum 50 ^{0}C) respectively, are the recommended optimum enzyme reaction conditions. Table 1 is a list of the commercial enzymes with their enzyme activity.

Table 1: Commercial Enzymes used with their enzyme activities:

S.N.	Commercial Enzyme	Enzyme activity
1	BL-Pectinase	1200 PGU/g
2	BL-Cellulase	1,00,000 CMCU/g
3	BL-Hemicellulase	1,00,000 HCU/g

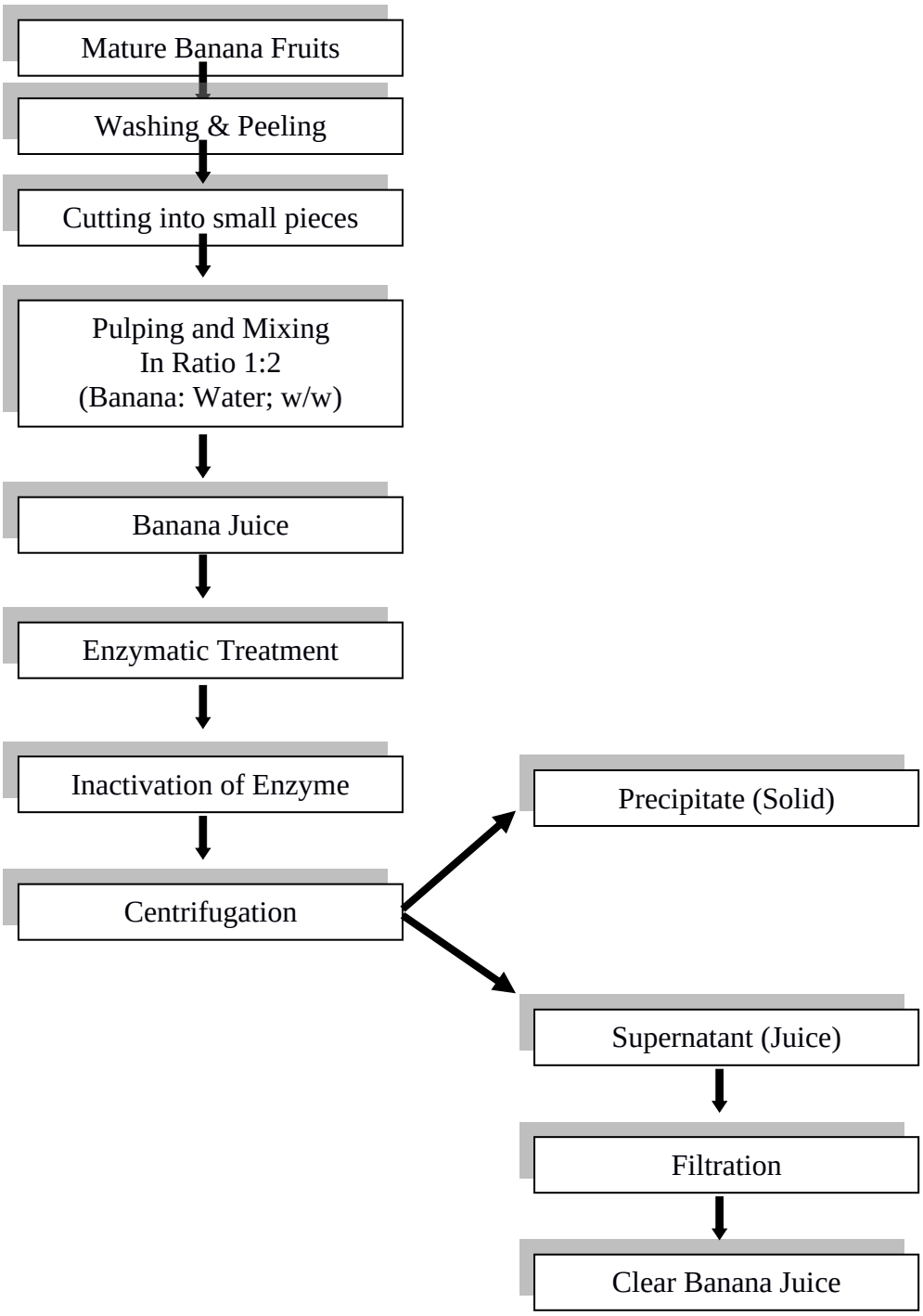


Fig 1: Steps for Banana Juice Extraction and Subsequent Clarification by Treatment with Enzymes

Juice Preparation: Mature bananas were washed, peeled and cut into small pieces. Based on previous works, a ratio of 1:2 (Banana: water; w/w) [25] was used in pulping using an electric blender. The juice obtained was subjected to different enzyme treatment conditions. The pH of the juice obtained was 5.1. Figure 1 shows the steps involved in extraction and clarification of banana juice by enzyme treatment.

Enzymatic Treatment and Optimization: The optimum conditions for enzyme dose and incubation time were determined by treating the juice with each enzyme separately. Fruit juice was

treated with various enzyme doses while maintaining the incubation time and temperature constant in each experiment for optimization of enzyme dose. Likewise, to optimize the incubation time the fruit juice was treated with different incubation times while maintaining the enzyme dose and incubation temperature constant in each experiment. Based on the optimum temperature recommendations for commercial enzymes, the reaction is carried out at incubation temperature of 55 ^{0}C. Fruit juice without the addition of an enzyme served as the control for experiments involving enzyme dose, and fruit juice with no enzyme addition and a retention period of 0 min served as the control for experiments involving incubation time. After the enzymatic treatment, the sample's enzyme was inactivated by heating the suspension in a water bath for five minutes at 90 ^{0}C before cooling it down to room temperature. The juices that had been treated were centrifuged for 20 minutes at 1100 RPM to separate the supernatant. At the adjusted optimum conditions based on the aforementioned trials, the influence of simultaneously adding all three enzymes on juice was investigated.

Turbidity Determination: The Insif India Digital Turbidity Meter (Labpro International, Ambala, Haryana) was used to measure turbidity. The results were reported as NTU (Nephelometric Turbidity Units).

Percentage Yield Determination: Percent yield was estimated as percentage of the clarified juice obtained based on the initial fruit pulp.

Results & Discussion

Juice was first treated individually with each enzyme to evaluate their effects and optimum process conditions. Juice was then treated simultaneously depending on the optimum conditions identified for each enzyme.

Optimization of enzyme reaction conditions for Pectinase

Effect of Enzyme Dose

For determination of optimum enzyme dose, the concentration of pectinase was varied starting from 0.01% (w/w), as shown in Table 2, at temperature of 55 ^{0}C for 60 min of incubation. The table shows the effect of pectinase concentration on juice clarity and yield. On increasing the enzyme concentration, the turbidity of juice decreased up to a minimum value of 11 NTU at the concentration value of 0.06% and thereafter it remained at almost constant value. On increasing the pectinase concentration, the decrease in juice turbidity was also reported by Karangwa et al. (2010) [48] for blended carrot-orange juice, Alam et al. (2014) [44] for carrot juice, Vinjamuri and Bhavikatti (2015) [49] for mixed fruit juices, Abdullah et al. (2007) [50] for carambola fruit juice, Umsza-Guez et al. (2011) [51] for caja-manga pulp, and Sin et al. (2006) [52] for sapodilla juice. Juice yield increased with the increasing concentration of enzyme with a maximum value of 74.2% and decreased slightly thereafter. At 0.06% enzyme concentration the turbidity was found minimum and % yield maximum. Both turbidity and yield was significantly affected by enzyme treatment as compared to control. This optimum concentration value was used in next experiment for optimisation of incubation time. These effects are graphically represented in Fig.2.

Table 2. Effect of pectinase concentration on banana juice clarity and yield

S. N.	Enzyme Conc. (%w/w)	Turbidity (NTU)	Yield (%)
1	0.00	190	50.6
2	0.01	131	70.6
3	0.02	60	72.0
4	0.03	26	73.4
5	0.04	19	73.8
6	0.05	15	74.0
7	0.06	11	74.2
8	0.07	11	74.0
9	0.08	12	73.7
10	0.09	11	73.7
11	0.10	12	73.6
12	0.12	11	73.5

Fig.2. Effect of pectinase concentration on banana juice clarity and yield

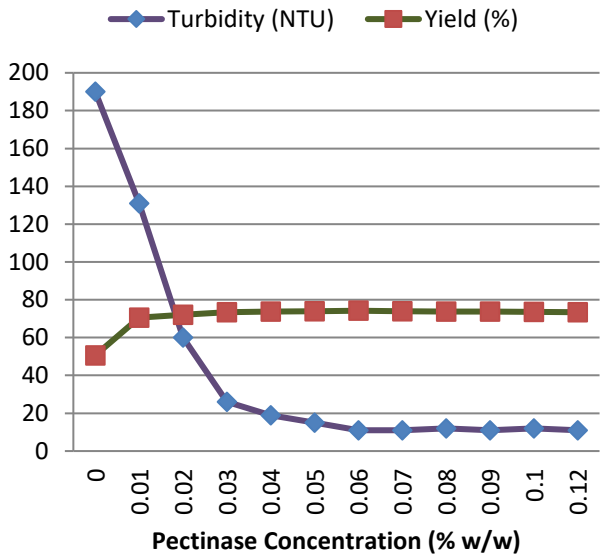
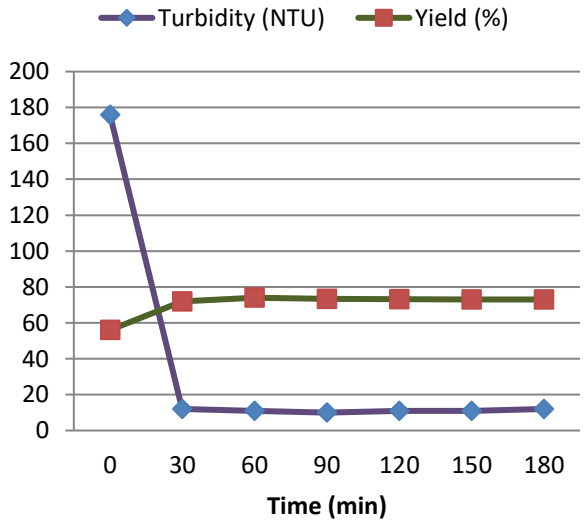


Table 3. Effect of incubation time on pectinase treatment on banana juice

S. N.	Enzyme Conc. (%w/w)	Time (min.)	Turbidity (NTU)	Yield (%)
1	0.00	00	176	56.0
2	0.06	30	12	72.0
3	0.06	60	11	74.0
4	0.06	90	10	73.4
5	0.06	120	11	73.2
6	0.06	150	11	73.0
7	0.06	180	12	73.1

Fig. 3. Effect of incubation time on pectinase treatment on banana juice



Effect of Incubation Time

The effect of varying incubation time (from 30 min to 180 min, as shown in Table 3) on the efficiency of pectinase was studied at a fixed enzyme dose of 0.06% (w/w) and at temperature of 55 ^{0}C. The table shows the effect of incubation time on juice clarity and yield. No significant change in turbidity was observed on increasing the incubation period. Maximum yield 74% was obtained for incubation period of 60 min. Turbidity was significantly reduced and yield was significantly increased on enzyme treatment as compared to control. Based on these results 60 min time may be taken as optimum. In Fig. 3, these effects are graphically represented.

Optimization of enzyme reaction conditions for Cellulase

Effect of Enzyme Dose

To determine the optimum enzyme dose for cellulase, the concentration of cellulase was varied starting from 0.10% (w/w), as shown in Table 4, at temperature of 55 ^{0}C for 60 min of incubation. The table shows the effect of cellulase concentration on juice clarity and yield. The turbidity of juice decreased on increasing the enzyme concentration up to a minimum value of 51 NTU at the concentration value of 0.40 % and thereafter it remained almost constant. Juice yield increased with the increasing concentration of enzyme with a maximum value 74.2% obtained with the enzyme concentration of 0.3% and decreased slightly thereafter. As compared to control, both turbidity and yield was significantly affected by enzyme treatment. Based on minimum turbidity value the compromised optimum enzyme concentration was taken as 0.40%. This optimum value was used in next experiment for optimizing incubation time. These effects are graphically represented in Fig.4.

Effect of Incubation Time

The effect of varying incubation time (from 30 min to 180 min, as shown in Table 5) on the efficiency of cellulase was studied at a fixed enzyme dose of 0.40% (w/w) and at temperature of 55 ^{0}C. Table 5 shows the effect of incubation time on juice clarity, yield and TSS. Minimum turbidity obtained was 12 NTU corresponding to 120 min of incubation time. Yield increased significantly with the increase of incubation time till 120 min, thereafter a slight increase was observed in it. As compared to control the turbidity after enzyme treatment was significantly decreased and yield was significantly increased. Based on these results the optimum incubation time was taken as 120 min. In Fig. 5, these effects are graphically represented.

Optimization of enzyme reaction conditions for Hemicellulase

Effect of Enzyme Dose

To determine the optimum enzyme dose for hemicellulase, the concentration of hemicellulase was varied starting from 0.10% (w/w), as shown in Table 6, at temperature of 55 ^{0}C for 60 min of incubation. The table shows the effect of hemicellulase concentration on juice clarity and juice yield. The turbidity of juice decreased slowly on increasing the enzyme concentration up to a minimum value of 122 NTU at the concentration value of 0.09 % and thereafter it remained almost constant. There was no significant change in Juice yield with the increasing concentration of enzyme. A slight decrease was observed in juice yield after the enzyme concentration of 1.1%. Based on minimum turbidity value the optimum enzyme concentration was taken as 0.09%. This value of enzyme concentration was used in next experiment for determining the optimum incubation time. Both turbidity and yield was significantly affected by enzyme treatment as compared to control. These effects are graphically represented in Fig.6.

Table 4. Effect of cellulase concentration on banana juice clarity and yield

S. N.	Enzyme Conc. (%w/w)	Turbidity (NTU)	Yield (%)
1	0.00	190	50.6
2	0.10	90	72.0
3	0.20	80	73.8
4	0.30	60	74.2
5	0.40	51	73.4
6	0.50	51	73.2
7	0.60	52	73.1
8	0.70	51	73.2
9	0.80	51	73.2

Fig.4 Effect of cellulase concentration on banana juice clarity and yield

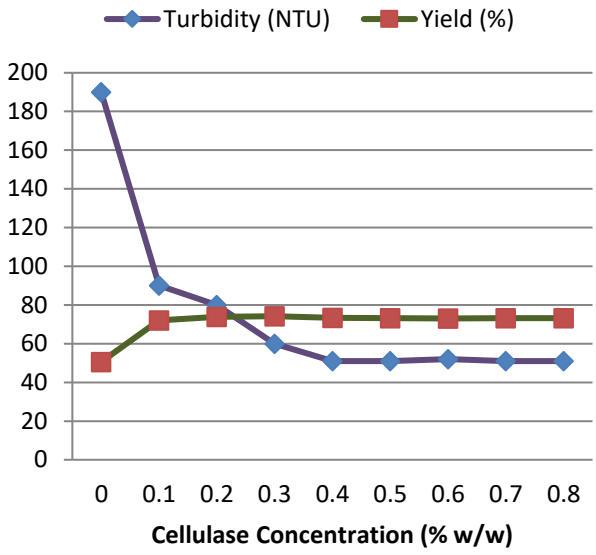
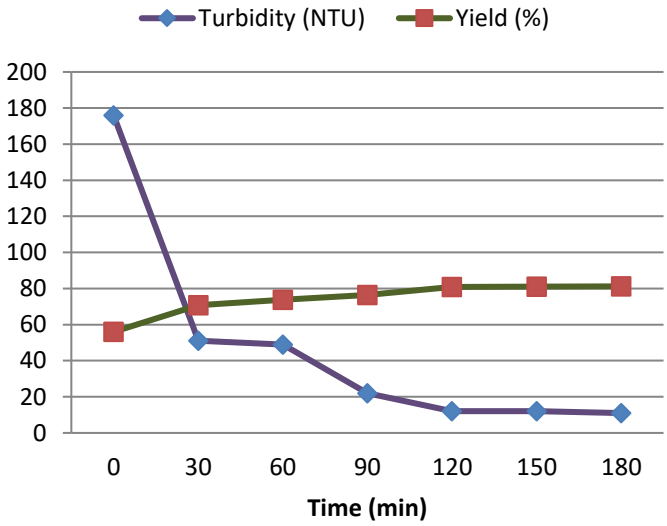


Table 5. Effect of treatment time on cellulase treatment on banana juice

S. N.	Enzyme Conc. (%w/w)	Time (min.)	Turbidity (NTU)	Yield (%)
1	0.00	00	176	56.0
2	0.40	30	51	70.7
3	0.40	60	49	73.8
4	0.40	90	22	76.4
5	0.40	120	12	80.9
6	0.40	150	12	81.0
7	0.40	180	11	81.2

Fig.5 Effect of treatment time on cellulase treatment on banana juice



Effect of Incubation Time

The effect of varying incubation time (from 30 min to 180 min, as shown in Table 7) on the efficiency of hemicellulase was studied at a fixed enzyme dose of 0.09% (w/w) and at temperature of 55 ^{0}C. The table shows the effect of incubation time on juice clarity and yield. Minimum turbidity obtained was 62 NTU corresponding to 120 min of incubation time; NTU value remained almost constant thereafter. There was a slight increase in yield up to incubation time of 120 min, thereafter it remained almost constant. Based on these results the optimum value for incubation time was taken as 120 min. Both turbidity and yield was significantly affected by incubation time as

compared to control. In Fig. 7, these effects are graphically represented.

Table 6. Effect of hemicellulase concentration on banana juice clarity and yield

S. N.	Enzyme Conc. (%w/w)	Turbidity (NTU)	Yield (%)
1	0.00	190	50.6
2	0.10	138	76.6
3	0.20	135	76.6
4	0.30	135	76.6
5	0.40	134	76.5
6	0.50	134	76.5
7	0.60	134	76.5
8	0.70	129	76.5
9	0.80	126	76.4
10	0.90	122	76.4
11	1.00	123	76.4
12	1.10	123	76.2
13	1.20	122	75.5
14	1.30	122	75.2
15	1.40	123	74.9

Fig.6. Effect of hemicellulase concentration on banana juice clarity and yield

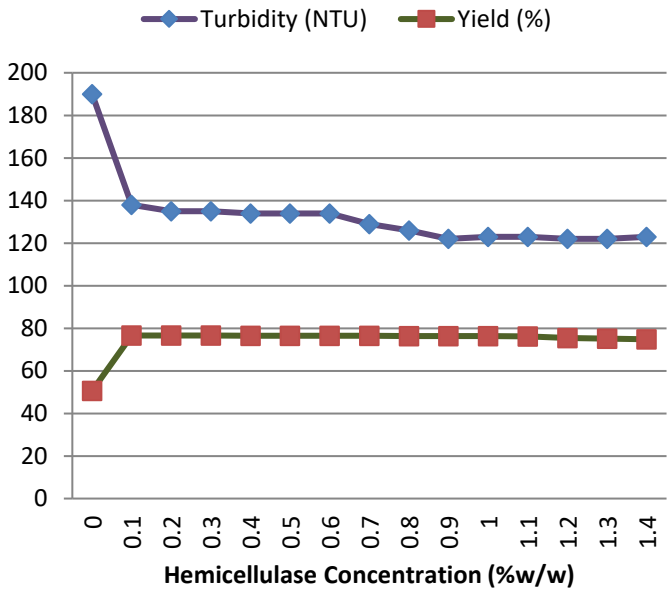
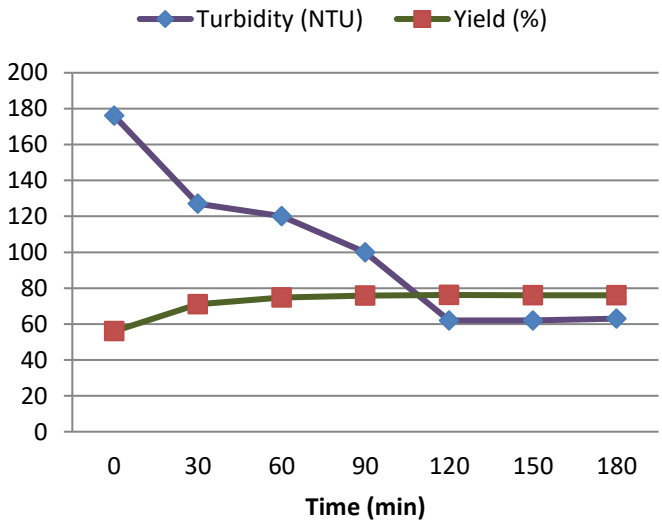


Table 7. Effect of treatment time on hemicellulase treatment on banana juice

S. N.	Enzyme Conc. (%w/w)	Time (min.)	Turbidity (NTU)	Yield (%)
1	0.00	00	176	56.0
2	0.90	30	127	71.0
3	0.90	60	120	74.8
4	0.90	90	100	75.8
5	0.90	120	62	76.2
6	0.90	150	62	76.0
7	0.90	180	63	76.0

Fig.7. Effect of treatment time on hemicellulase treatment on banana juice



Effect of simultaneous treatment of fruit juice with Pectinase, Cellulase and Hemicellulase

The optimum enzyme reaction conditions were obtained from the above experiments for the three enzymes separately. The optimum reaction conditions obtained for pectinase were 0.06% enzyme concentration and 60 min incubation time. The optimum reaction conditions obtained for cellulase and hemicellulase were 0.40% enzyme concentration with 120 min incubation time and 0.09% enzyme concentration with 120 min incubation time respectively. Based on the optimum treatment conditions obtained for individual enzymes, the compromised optimum conditions, for simultaneous treatment of enzymes, may be taken as Pectinase 0.06%, Cellulase 0.40%, Hemicellulase 0.09% with 120 min incubation time at a temperature of 55 ^{0}C. With these conditions, the effect of treating the fruit juice simultaneously with all the three enzymes is shown in Table 8. The fruit juice without addition of enzyme was taken as control. Simultaneous treatment of banana juice with pectinase, cellulase and hemicellulase produced clarified juice with turbidity 7 NTU and juice yield 79.2%. There was 96 % decrease in turbidity and 60.6% increase in yield compared to control enzyme-untreated juice.

The influence of multi-enzyme treatment on juice clarity and yield was also investigated by Shah and Nath (2007) [7] and they reported juice clarity and yield as a function of linear and quadratic effects of concentrations of the enzymes pectinase, cellulase, hemicellulase and incubation time for litchis. The effects of multi-enzyme (Pectinase and cellulase) treatment to clarify blended pineapple and mango pulp were reported by Jori et al. (2015) [53]. Koffi et al, (1991) [10] also showed the effect of different combinations of enzymes pectinase, cellulase and hemicellulase in improving filterability and reducing viscosity of green and ripe banana purees.

Table 8. Effect of treatment of fruit juice with compromised optimum treatment conditions of pectinase, cellulase and hemicellulase simultaneously.

	Control	**Test**
Enzyme Concentration (% w/w)		
Pectinase	0	0.06
Cellulase	0	0.40
Hemicellulase	0	0.90
Incubation Temperature (^{0}C)	55	55
Incubation Time (min)	120	120
Results		
Turbidity (NTU)	198	7
Yield (%)	49.3	79.2

Conclusion

The present study showed the effects of separate treatments of pectinase, cellulase and hemicellulase as well as the effect of simultaneous treatment with all three enzymes. The optimum reaction conditions obtained for pectinase, cellulase and hemicellulase for separate treatments, were 0.06% enzyme concentration with 60 min incubation time, 0.40% enzyme concentration with 120 min incubation time and 1.20% enzyme concentration with 120 min incubation time respectively at 55 ^{0}C. The turbidity and % yield improved significantly by simultaneous enzymatic treatment of banana pulp. Based on above study, the recommended

enzymatic clarification condition for simultaneous treatment of banana juice is 0.06%, 0.40% and 0.90% enzyme concentration for BL-Pectinase, BL-Cellulase and BL-Hemicellulase respectively, at incubation temperature of 55 ^{0}C and incubation period of 120 min. Under this condition, the juice was obtained with a very small value of turbidity 7 NTU and 79.2% of yield. Thus simultaneous enzyme treatment of banana pulp with enzyme is very effective in obtaining clarified juice with high yield. The commercialization of clarified juice production from the banana fruit by using enzymes may contribute significantly in increasing India's processed fruit and export volume.

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