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Effect of pectinolytic treatment on quality attributes of banana ready-to-serve drink during storage

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Abstract
The pectinaceous aspect of the fruits is challenging for juice extraction. Pectinases are known to be one of the most effective hydrolytic enzymes in fruit juices. In present research, crude pectinase enzyme produced under solid state fermentation by *Aspergillus Niger* (MTCC 281) having banana peel powder as substrate was used. Banana juice was treated with (1, 2 and 3% v/v) crude pectinase at different incubation time (30, 45 and 60 minutes) and temperature (35, 40 and 45 ºC). Treated samples were assayed for minimum turbidity and maximum juice yield. Treatment showing minimum turbidity was used for preparation of banana ready-to-serve drink. The samples were analysed fortnightly for quality attributes *viz.* physico-chemical, sensory and microbial parameters during 45 days storage period. The major variation in TSS, total sugars, pectin, acidity and turbidity found in banana RTS juice treated with pectinase. No microbial count found during 45 days of storage. The overall acceptability scores of RTS banana juice treated with pectinase were substantially higher than control over the storage period.

Keywords: Banana, pectinase, *aspergillus niger*, clarification, turbidity, shelf life

1. Introduction
Banana crop is leading in world agricultural trade and production. Average global banana production rose from 69 million tonnes in 2000-2002 to 116 million tonnes in 2017-19 with an approximate value of 31 billion USD (Voora *et al*., 2020) [13]. It is cultivated in over 130 countries, along the tropics and subtropics of Capricorn. It has originated from eastern Asia and then spread to all over the world through hybridization. Some of the varieties have been linked to India. Banana fruit is consumed by millions of people around the world as part of their daily diet and for nutrient enrichment in a variety of ways. Banana fruit is suitable for the processing of juice but its pectinous nature has problems in the process of juice extraction (Netshiheni *et al*., 2019) [8]. Enzymes are an integral component of modern fruit juice manufacturing and are highly suitable for optimizing processes. The enzymatic process is claimed to offer a number of advantages over mechanical-thermal combinations. Pectinases are a group of related heterogeneous enzymes, present mainly in plants that hydrolyze the pectic substances. Pectinases are commonly used to hydrolyze pectin and pectin-like colloids in fruit juices and to promote fruit juice clarity as well as preventing juice gelling during the cycle concentration level. The resulting juice has much lower pectin content and a lower viscosity, thereby shortening the corresponding clarification cycle (Karmakar and De, 2019) [6]. Many of the enzymes used in the food industry are of fungal origin since fungi are active producers of pectic enzymes and have GRAS (Generally Regarded as Safe) status. Acid pectinases produced by fungi are used for clarification of fruit juices particularly in food industries (Patidar *et al*., 2018) [9]. The acidic pectinases put down fruit juice's cloudiness by breaking down complex plant tissue polysaccharides into simpler molecules such as galacturonic acids.
Fruit processing, particularly bananas, generates significant quantities of by-products that often contain valuable compounds in their peels, making them valuable agro-industrial residues as substrates used to produce pectinase under solid-state fermentation (SSF). Effective agro-industrial residue utilization minimizes the additional economic burden of waste disposal in the production unit (Yusuf, 2019) [15]. Therefore, the present study explores the use of crude pectinase produced under solid state fermentation by *Aspergillus niger* (MTCC 281) having banana peel powder as substrate. Banana juice clarification was optimized by crude pectinase with different concentration (1, 2 and 3% v/v), incubation time (30, 45 and 60 minutes) and temperature (35, 40 and 45 ºC).

Treated samples were assayed for minimum turbidity and maximum juice yield. Banana juice treated at optimized conditions was used for banana RTS preparation. Quality attributes of banana ready-to-serve (RTS) juice were analyzed fortnightly for 45 days of storage period.

2. Material and methods

2.1 Material

The fungal culture *Aspergillus Niger* (MTCC 281) was obtained from Institute of Microbial Technology, Chandigarh, India. Banana (*Mussa spp.*) was procured from the local market of Hisar, India.

2.2 Crude pectinase

Crude enzyme with pectinase activity of 8.12U/ml was obtained by using banana peel powder as a sole source of carbon to grow *Aspergillus Niger* (MTCC 281) under solid state fermentation with 1:3 moisture levels, pH 5 and 3% of 10^8 spores/ml inoculum size at 40ºC with 120 hrs of incubation time. Pectinase activity was determined by measuring the release of reducing sugars during the enzyme substrate reaction using Miller's method (Miller, 1959) [7].

2.3 Optimization of physical factors for banana RTS juice yield and clarification

Ripe bananas were hand peeled and cut into pieces, mixed with 1:2 to distilled water and mixed with a commercial mixer and then TSS adjusted as 15ºBrix and acidity of 0.28%. Fifty milli litters of banana juice was subjected to different conditions *viz.* concentration of crude enzyme extract (1, 2 and 3% v/v) incubated at temperatures ranging from 35, 40 and 45 ºC with incubation time of 30, 45 and 60 minutes in order to optimize for maximum juice yield and minimum turbidity of the banana juice.

2.4 Biochemical analysis of banana RTS juice during storage

Banana juice obtained with maximum juice clarification after above mentioned treatments was used further for RTS preparation with and without (control) enzyme treatment. Banana RTS juice was filled in 200 ml glass bottles, pasteurized at 62±2ºC for 30 minutes and stored at refrigeration temperature (4 ºC). Samples were taken for chemical, microbial and sensorial analysis after every 15 days for 45 days of banana RTS juice storage (Figure 1).

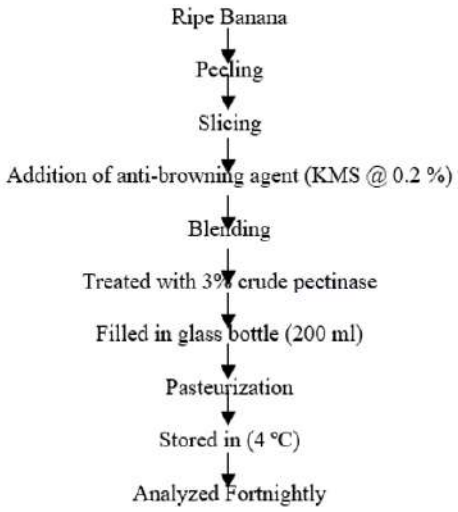

Fig 1: Flow sheet for the preparation of banana RTS

Pectin content of the sample was determined by Ranganna 2014 [10], titratable acidity, total sugars and ascorbic acid as suggested by A.O.A.C 2005 [1]. Total soluble solids were determined at ambient temperature by hand refract meter, Erma, Japan (0-32%). Microbial count was recorded on total plate count agar and potato dextrose agar by using serial dilution technique. The plates incubated at 28±2°C for 24-48 hours for total plate counts and at 28±2°C for 72 hours for mold counts. After the incubation period, the colonies were counted by using a colony counter and recorded in CFU/ml.

2.5 Sensory evaluation

The banana juice was subjected to sensory evaluation soon after preparation and after 15, 30 and 45 days of storage period by a panel of ten judges using 9-point hedonic scale as described by Ranganna 2014 [10]. The products were evaluated for colour and appearance, taste, aroma and flavor, body and texture, consistency and overall acceptability. The overall acceptability of juice was based on mean scores obtained from all the sensory parameters. The samples with mean scores of 6 and above out of 9 were considered acceptable.

2.6 Statistical analysis

The data in the present investigation were analyzed according to two-factorial completely randomized design. The critical difference value at 5 percent level was used for making comparisons among different treatments during storage period. For each individual experiment, two-way ANOVA was calculated using OP STAT software.

3.0 Results and Discussion

3.1 Juice extraction and clarification

3.1.1 Physico-chemical characteristics of fresh banana RTS

The data presented in (Table 1) shows that banana juice had 15% total soluble solids, 7.89% total sugar, 0.31% pectin, 7.64 mg ascorbic acid per 100 ml juice, 0.28% acidity and 4.6 pH.

Table 1: Physico-chemical characteristics of fresh banana RTS

Parameter	Banana juice Mean* ± S.D.
TSS brix (%)	15.00 ± 0.20
Total sugars (%)	7.89 ± 0.50
Pectin (%)	0.31 ± 0.02
Ascorbic Acid (mg/100ml)	7.64 ± 0.32
Acidity (%)	0.28±0.02
pH	4.6± 0.18

*The values are mean ± Standard Deviation of three replicates

3.1.2 Effect of physical factors on banana RTS clarification

Physical factors such as enzyme concentration, incubation time and temperature were optimized and observed for maximum juice yield and minimum turbidity of juice. Banana RTS (50 ml) was incubated at 35ºC, 40ºC and 45 °C with 1%, 2% and 3% concentration of crude pectinase for 30, 45 and 60 minutes juice yields and turbidity, respectively. Maximum juice yield 54.6% and minimum turbidity 568 N.T.U were observed after 60 minutes incubation with 3% crude pectinase concentration at 35ºC. Enzyme concentration was significant and the incubation

time was non-significant for turbidity whereas in juice yield the pectinase concentration and incubation time both were significant. Maximum juice yield was 59.7% observed with 3% crude pectinase after 60 minutes incubation time at 40 ºC. Minimum turbidity 539 N.T.U was obtained with 3% crude pectinase after 60 minutes incubation at 40 ºC**.** Enzyme concentration is more significant and the incubation time is less significant for turbidity but in juice yield the pectinase concentration and incubation time both are significant. Maximum juice yield was 52.30% observed with 3% crude pectinase after 30 minutes incubation time at 45 ºC. Minimum turbidity 579 N.T.U was obtained at 3% crude pectinase after 60 minutes incubation at 45 ºC. Among the set experiments, it observed that at 40 ºC the maximum 59.7% juice yield and minimum turbidity 539 N.T.U achieved at 3% crude pectinase concentration at 60 minutes incubation time (Figure 2).

Therefore, these conditions were kept as optimized for banana juice treatment for RTS preparation and its storage studies. Tadakittisarn *et al*. 2007 [12], found the ideal state for enzymatic extraction to be 0.15% of the pectinase enzyme incubated at 50°C for 120 min. Ultrasound paired with pre-treatments of both the enzymes (cellulase and pectinase) resulted in a gross yield of 89.40% compared to 47.30% in the control (Bora *et al*., 2017) [2]. Nevertheless, pectinase was more efficient in increasing juice yield compared with cellulase; and juice yield was not substantially improved by ultrasonic pretreatment alone. The juice's viscosity decreased with enzyme addition and ultrasound application. Cellulase treatment did not affect the consistency of the juice but it increased with pectinase treatment.

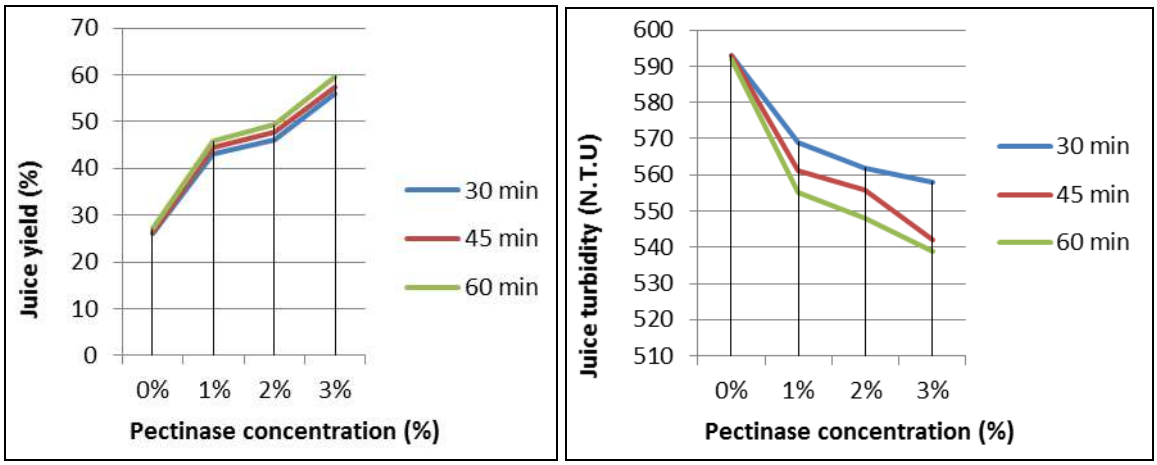

Fig 2: Effect of crude pectinase concentration on banana juice yield and turbidity at 40 ºC

3.2 Effect of storage on physico-chemical composition of banana RTS beverage

3.2.1 TSS and total sugars

The TSS of the banana RTS was adjusted at 15ºBrix during preparation. The amount of TSS significantly increased from 15ºBrix at 0 day (Table 2) to 15.6ºBrix at 15 days, 16.3ºBrix at 30 days and 16.40ºBrix at 45 days of storage.

There were significant differences in TSS observed among juice prepared with or without enzyme treatment. There was a significant increase in TSS of banana juice with increasing storage period. This increase could be due to dissolving of juice constituents and hydrolysis of polysaccharides (starch) into oligosaccharides and monosaccharide's during storage. The total sugars of ripe banana pulp increased with increasing storage period. Total sugar in banana RTS increased significantly (7.89 to 8.39%) in control and enzyme treated juice during 45 days storage. There was also a significant difference in total sugars of banana juice prepared with or without enzyme treatment. There was an increase in total and reducing sugars with an increase in storage period (Hussain *et al*., 2014) [3].

Table 2: Effect of enzyme treatment on physico-chemical composition of banana RTS during storage

Treatments	Period of storage (Days)				Mean
	0	15	30	45	
TSS (ºBrix)					
Controlled	15.0	15.2	15.3	15.5	15.0
3% Pectinase	15.0	15.6	16.3	16.4	15.8
Mean	15.0	15.4	15.8	15.9	
C.D. at 5%	Treatment (T)=0.40 Storage (S)= 0.57 T×S = N.S				
Total sugars (%)					
Control	7.89	7.91	7.94	8.10	7.96
3% Pectinase	7.89	8.46	8.52	8.67	8.38
Mean	7.89	8.18	8.23	8.38	
C.D. at 5%	Treatment (T)=0.13 Storage (S)= 0.19 T×S = 0.26				
Pectin content (%)					
Control	0.31	0.29	0.29	0.29	0.29	0.29
3% Pectinase	0.31	0.27	0.27	0.27	0.27	0.27
Mean	0.31	0.28	0.28	0.28	0.28	
C.D. at 5%	Treatment (T) = 0.014 Storage (S) = N.S T×S = N.S					

Ascorbic acid (mg/100 ml)					
Control	7.64	7.23	7.10	6.93	7.22
3% Pectinase	7.64	6.21	5.82	5.53	6.30
Mean	7.64	6.72	6.46	6.23	
C.D. at 5%	Treatment (T)=0.13 Storage (S)= 0.19 T×S = 0.26				
Acidity (%)					
Control	0.28	0.28	0.28	0.29	0.28
3% Pectinase	0.28	0.29	0.31	0.32	0.30
Mean	0.28	0.29	0.30	0.30	
C.D. at 5%	Treatment (T)=N.S. Storage (S)= 0.01 T×S = N.S				
pH					
Controlled	4.66	4.58	4.54	4.49	4.568
Pectinase	4.60	4.48	4.46	4.41	4.488
Mean	4.63	4.53	4.50	4.45	
C.D. at 5%	Treatment (T)=N.S Storage (S)= N.S T×S = N.S				

3.2.2 Pectin content

In the present study, the pectin content of fresh banana RTS was 0.31%. It was observed that pectin content significantly decreased during storage. There was a significant difference in treatments pectin contents 0.27% and 2.95% observed at 45 days storage among juice prepared with or without enzyme treatment (Table 2). But no significant differences were observed with storage time. Pectinase treatment in fact pertaining to the theory that the main clarification action of pectinases is to decrease the immediate turbidity by promoting the agglomeration of oppositely charged pectin–protein particles (Netshiheni *et al*., 2019) [8]. Siddiq *et al*. 2018 [11] found significantly ($p \leq 0.05$) higher juice yield (86.91–87.29 ml/100 g with Pectinex and Crystalzyme as compared to 79.45 ml/100 g from control), higher juice clarity, titratable acidity and lower extraction loss.

3.2.3 Ascorbic acid

The ascorbic acid of the banana RTS at 0-day of storage was 7.64 mg/100ml. There was a continuous decrease in ascorbic acid content of banana juice during storage. It was observed that the ascorbic acid progressively and significantly decreased from 7.64 mg/100 ml at 0 day, 6.21 mg/100 ml at 15 days, 5.82 mg/100 ml at 30 days and 5.53 mg/100 ml at 45 days during storage (Table 2). Significant differences in ascorbic acid were observed among RTS prepared with or without enzyme treatment. Similar decrease in ascorbic acid content during storage has also been reported in pulps of mango (Jangir *et al*., 2017) [4]. The decrease in ascorbic acid content might be due to the oxidation of ascorbic acid to dehydro ascorbic acid and then further degradation to 2, 3- diketo-gluconic acid with the passage of time by the action of oxidase enzyme.

3.2.4 Acidity and pH

The acidity of the banana RTS was adjusted at 0.28% initially. There was progressively increase in acidity of banana juice prepared with or without enzyme treatment during storage. It was observed that the acidity progressively and significantly increased from 0.280% at 0 day, 0.297% at 15 days, 0.31% at 30 days and 0.321% at 45 days during storage. Significant differences in acidity were observed among RTS prepared with or without enzyme treatment. The increase in acidity of banana juice might be due to the formation of acidic compounds by degradation or oxidation of reducing sugars, polyphenols and conversion of protein to amino acids by the breakdown of peptide bonds. Degradation of pectic substances, polysaccharides and uric acid could also be the reason to increase the acidity of the juice during storage. The increase in acidity during the storage may be attributed to the conversion of sugars to acids. The pH of the RTS at 0-day of storage was adjusted at 4.6. There was a decrease in pH of banana juice prepared with or without pectinase during storage. It was observed that the pH progressively and significantly decreased from 4.6 at 0 day, 4.48 at 15 days, 4.46 at 30 days and 4.41 at 45 days during storage. According to the statistical analysis there were non-significant differences observed among RTS prepared with or without enzyme treatment (Table 2). The results of the present study were in similarity with Patidar *et al*. 2018 [9]. Figure 3 depicts the visual changes occurred during storage of banana RTS due to increase or decrease of various up-listed physicochemical parameters.

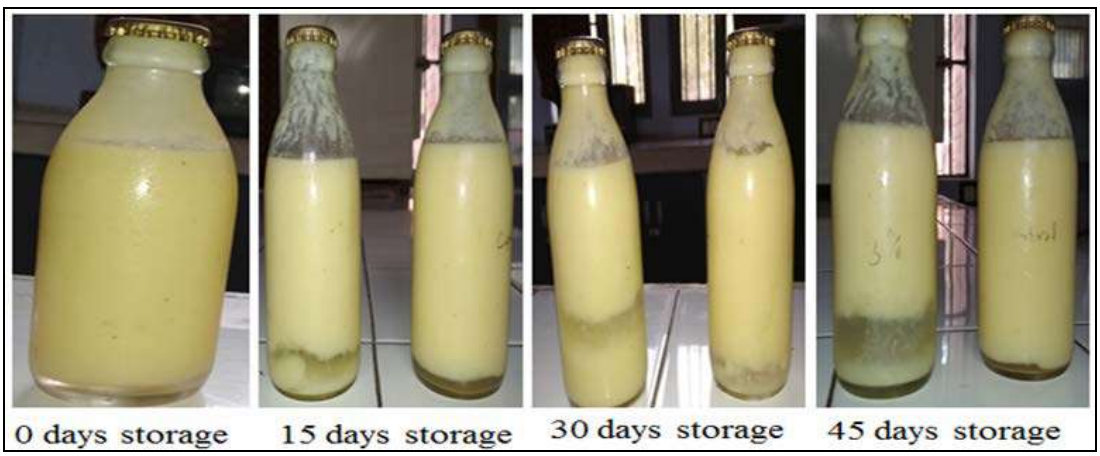

Fig 3: Visual appearance of banana RTS during storage

3.2.5 Microbial Quality
No total plate count detected up to 45 days of storage in banana RTS. It was observed that enzymatic treatment did not affect the microbial count.

3.3 Sensory quality of banana RTS during storage
A gradual decrease in sensory scores for all sensory attributes *viz*., colour and appearance, taste, aroma and flavor, consistency of both controlled and enzyme treated banana juice observed during storage (Figure 4). But pectinase treated banana juice body and texture score was stable and no significance decrease was in body and texture in controlled banana juice score. Slightly but significant decrease in overall acceptability was observed in both banana juice prepared with or without enzyme. This might be due to changes in quality of juice during storage at low temperature and without any preservatives. Similar results have been reported in ready to serve banana beverages during storage (Yadav *et al*., 2014) [14].

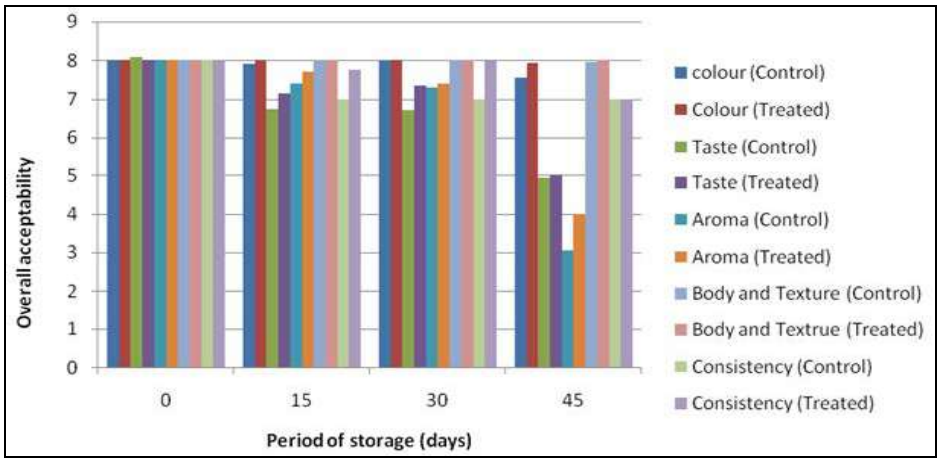

Fig 4: Effect of storage on overall acceptability of the banana RTS

4. Conclusion
Maximum juice yield (59.7%) and minimum turbidity (539 N.T.U.) was achieved with 3% crude pectinase concentration at 60 minutes incubation time at 40 ºC. During storage studies of banana RTS, significant differences were observed in TSS, total sugars, pectin, acidity and turbidity among control and pectinase treated banana RTS. The overall acceptability scores of enzyme treated juices throughout the storage period were significantly higher than control. No bacterial count was detected up to 45 days of storage. However, no significant differences were observed among banana RTS prepared with or without enzyme treatment. It has been concluded that banana peel could be potentially utilized as substrate for pectinase production under SSF and further it can be applied in banana juice clarification. This study has concluded that pectinase enzyme clarified juice can be potentially used for RTS and other beverages production.

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