Research Article

Effect of Enzymatic Treatment and Ripening Stages on Physico-Chemical Parameters of Clarified Banana Juices

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Abstract

Clarified banana juices of banana pulp of maturity stages 5, 6 and 7 were prepared by enzymatic clarification process using the commercial pectinolytic enzyme i.e. Pectinex Ultra SP-L and cellulase enzyme i.e. Celluclast 1.5L. The recommended optimized combination of a particular ripening stage was applied to the pulp of other ripening stages. The clarified juices thus obtained were used during subsequent investigation to study the effect of enzymatic treatment and ripening stages on various physico-chemical parameters viz. Total Soluble Solids (TSS), sugar/acid ratio, total sugar, reducing sugar, acidity, pectin content of clarified juices. Both these factors were found to be affected the physico-chemical properties of prepared juices but the effect of ripening stages was found be more pronounced. Enzymatic combinations with higher enzyme concentration yielded clarified juices with higher TSS (22.57^{0}Bx), total sugar (17.40%), reducing sugar (10.27%) and titratable acidity (0.49%); and lower sugar to acid ratio (45.86) and pectin content 0.11% as compared to treatments with lower concentration of enzyme. In advancements of stages of ripening (stage 5 to stage 7) significantly marked increased in TSS (19.98^{0}Bx to 23.94^{0}Bx), total sugar (13.95% to 19.12%), reducing sugar (7.03 to 11.83%) and titratable acidity (0.4025 to 0.52%); and decreased in sugar to acid ratio (49.72 to 46.10) and pectin content (0.4 to 0.175%) of prepared juices were observed.

Keywords: Banana pulp, ripening stages, enzymatic clarification, clarified juice, pectin, total soluble solids

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Introduction

Banana (*Musa* sp.) is a non-seasonal, climacteric fruit and shows marked physiological changes during ripening. In banana, ripening has been divided into seven stages. Commercial standard colour charts are available in which 7 stages of ripening were reproduced and translated to a numerical scale where Stage 1=all green, 2= green with trace of yellow, 3= more green than yellow, 4= more yellow than green, 5= yellow with trace of green, 6= full yellow, 7= full yellow with brown spots. In banana, ripening stages of fruit are associated with changes in texture, color and flavor leading to the best eating stage. Mostly, banana peel color changes from green to yellow while going through the 7 stages of ripening [1]. Texture softens at different rates for each ripening stage partly due to the hydrolysis of starch and pectin in banana pulp**.**

Generally, fruit juices are extracted by simple crushing and or grinding of fruits. However, in case of banana this process results in a sticky, lumpy mass with no juice. Bananas are usually too pulpy and pectinaceous to yield juice by simple pressing or centrifugation. Several studies have reported on depectinization of banana pulp using enzymatic treatment. Application of enzymes such as pectinase and amylase improved the clarification process for banana fruit juices [2,3].

Most of the researchers used fully ripened stage i.e. stage 7 for enzymatic clarification study of banana pulp. During ripening as there is drastic changes occur in the composition of pulp viz. starch, sugar content; enzyme requirement for clarification will also be vary at different ripening stages. So some researchers worked on enzymatic clarification of banana pulp with respect to their ripening or maturity stages and optimized the enzymatic combination for respective stages. Mostly yield and clarity of prepared clarified juices were used as a criterion for enzymatic optimization but researchers have not focused on other physico-chemical parameters of the prepared clarified juices. Furthermore there has been no systematic report on the effect of optimized enzymatic condition of a particular ripening stage on other stages and the effect of ripening stages on various physic-chemical parameters of clarified juices. Thus the present research study was undertaken. In the present investigation, the optimized combination of a particular ripening stage suggested by researchers [4] was applied to the pulp of other ripening stages and the clarified juices thus obtained were used during subsequent investigation to evaluate the effect of enzymatic treatment and ripening stages on various physico-chemical parameters of clarified juices.

Materials and Methods

Bananas (*Musa* sp.) of variety 'Robusta' were procured at the maturity stage 1 (all green) from selected banana orchards near Anand city (India). The procured material was sorted, tagged and kept for ripening as per the method suggested by Kulkarni *et al.* [5]. As the ripening progressed, fruit passes through various ripening stages. In the present investigation, advanced maturity stages of banana i.e. stage 5 (yellow with a trace of green), stage 6 (all yellow) and stage 7 (all yellow with a brown speckles); according to Standard Banana Colour Chart have been selected. Commercial pectinolytic enzyme i.e. Pectinex Ultra SP-L (with enzymatic activity 26000 PG U per ml, optimum pH 3.5 to 6 and temperature below 50^0C) and cellulase enzyme i.e. Celluclast 1.5L with cellulose activity 158 U per ml were obtained from Novozymes South Asia Pvt. Ltd., Bangalore

Banana pulp clarification process

Enzymatic clarification of banana pulp was done by using the commercial pectinolytic enzyme i.e. Pectinex Ultra SP-L and cellulase enzyme i.e. Celluclast 1.5L. Clarified banana juices of banana pulp of each selected maturity stage i.e. stage 5, 6 and 7 were prepared as per the process and optimized combinations suggested by [4]. The most desirable recommended enzymatic combination for ripening stage 5, 6 and 7 were denoted as A, B and C respectively and displayed in **Table 1**.

Table 1 Optimized enzymatic combination for clarification of banana pulp of different ripening stages*

Enzymatic Combination	**Ripening stage**	**Enzyme Concentration (%)**	**Temperature (^{0}C)**	***Incubation Time (min)***
A	5	0.15	35.0	*180*
B	6	0.12	40.0	*135*
C	*7*	*0.11*	*40.0*	*145*
*Source: Tapre and Jain (2013)				

The optimized combination of a particular ripening stage was applied to the pulp of other ripening stages and the clarified juices thus obtained were used during subsequent investigation to study the effect of enzymatic treatment and ripening stages on various physico-chemical parameters of clarified juices. Juice obtained without any enzymatic treatment treated as control.

Physico-Chemical Parameters of Clarified Banana Juices

TSS was measured as ^{0}Brix at 20^0C by using Hand Refractometer (measuring range 0-32^0Bx).

The sugar acid ratio also termed as Brix : Acid ratio was calculated by taking the ratio of total soluble solid and acidity of respective samples. Total sugar, reducing sugar and titratable acidity in terms of mallic acid was determined as per the method described by [6]. Pectin was determined as calcium pectate method [7].

Statistical Analysis

Analysis of variance (ANOVA) was used to detect treatment effect. Mean separation was performed by using least significance difference (LSD) at the $p< 0.05$ level.

Results and Discussion

In the present investigation, clarified banana juices of different maturity stages i.e. stage 5, 6 and 7 were prepared using the different enzymatic combinations. The optimized combination of a particular ripening stage suggested by previous researchers was applied to the pulp of other ripening stages and the clarified juices thus obtained were used during subsequent investigation to study the effect of enzymatic treatment and ripening stages on various physico-chemical parameters of clarified juices.

Effect enzymatic treatments and ripening stages on TSS and sugar/acid ratio

The total soluble solids content (TSS) shows high positive correlation with sugars content and is therefore generally accepted as an important quality trait of fruits. Clarified juices prepared by using different enzymatic combinations and control showed statistically significant differences for TSS as shown in **Table 2**. Clarified juice prepared with highest enzymatic combination 'A' showed the highest value of TSS (22.57^0Bx) followed by 'B' and 'C' combinations. The lowest TSS (21.0^0Bx) was found in untreated i.e. control juice sample. The high TSS content of enzyme-extracted juice may be explained by the degradation of cell wall of pectin, cellulose and hemicelluloses by enzymatic treatments, resulting in the release of some sugars and leading to a Brix increase [8]. Similar results were reported by [9] who showed that treatment of banana pulp with enzymes resulted in increased in TSS in the juice. In other fruits like apple, pears, apricots and carrot juice also same trend were reported by [10] and [11].

Table 2 Effect of enzymatic treatments on physico-chemical parameters clarified banana juices

Enzymatic Combination	TSS (^{0}Bx)	Sugar/Acid ratio	Total Sugars (%)	Reducing sugars (%)	Titratable acidity (%)	Pectin content (%)
A	22.57^a	45.86^c	17.40^a	10.27^a	0.49^a	0.11^c
B	21.83^b	48.34^b	17.00^b	9.53^b	0.45^b	0.15^c
C	21.47^b	48.25^b	16.67^b	9.17^c	0.45^b	0.21^b
Control	21.00^c	50.17^a	16.18^c	8.67^d	0.42^c	0.72^a
LSD (0.05)	0.3904	1.6424	0.3792	0.2564	0.01373	0.0512

Means followed by same alphabet in a column do not differ significantly ($p<0.05$)

It is the sugar to acid ratio which contributes towards giving many fruits their characteristics flavour and so as an indicator of commercial and organoleptic ripeness. From Table 2 it is evident that clarified juice without enzymatic treatment showed highest value of sugar to acid ratio (50.17) as compared other enzymatically prepared clarified banana juices. The lowest sugar to acid ratio (45.86) was found in clarified juice prepared with enzymatic combination 'A'. Sugar to acid ratio decreased by the addition of pectinases apparently due to more increased in titratable acidity than total soluble sugar of treated juice. This may be due to unesterified galacturonic acid units are released in this enzymatic breakdown [12].

From **Table 3** it is revealed that clarified juices prepared from different ripening stages showed significant differences for TSS. Marked increase in TSS of clarified banana juice of 7th stage of ripening (23.94^0Bx) was observed as compared to 5th stage (19.98^0Bx) and 6th stage (21.23^0Bx) juices. Increased in ripening stage, increased the TSS content of juice. This may be due to the conversion of starch into sugar during ripening [13].

Table 3 Effect of ripening stage on physico-chemical parameters clarified banana juices

Ripening Stage	TSS (^{0}Bx)	Sugar/Acid ratio	Total Sugars (%)	Reducing sugars (%)	Titratable acidity (%)	Pectin content (%)
5	19.98^c	49.72^a	13.95^c	7.03^c	0.4025_b	0.4^a
6	21.23^b	48.65^a	17.37^b	9.38^b	0.4375^b	0.3125^b
7	23.94^a	46.10^b	19.12^a	11.83^a	0.52^a	0.175^c
LSD (0.05)	0.3381	1.4223	0.3284	0.2221	0.01189	0.0443

Means followed by same alphabet in a column do not differ significantly ($p<0.05$)

The significant effect of ripening stages (stage 5 and 7; stage 6 and 7) was observed on sugar to acid ratio of prepared clarified juices (Table 3). As ripening progressed banana pulp yielded clarified juices with lower sugar to acid ratio. This may be due to sharp increase in acidity in course of banana fruit ripening as reported by [14].

From **Figure 1**(a), it is evident that irrespective of the different enzymatic treatments, banana pulp of maturity stage 7 yielded clarified juices with higher TSS as compared to other two stages of ripening. For all the ripening stages, clarified juices prepared by enzymatic combination 'A' content higher TSS as compared to other enzymatic combinations and control. This may be due to higher concentration of pectinase used in enzymatic combination 'A' responsible for greater degree of tissue breakdown and released more components that contributing to soluble solids [8].

From Figure 1(b) it can be observed that in every stage of ripening clarified juices prepared without enzyme treatment i.e. control showed comparatively higher sugar to acid ratio than enzymatically treated juices. This may be due to higher acidity in enzyme treated juices form due to release of unesterified galacturonic acids by enzymatic breakdown of pectin.

Effect enzymatic treatments and ripening stages on total and reducing sugars content

Sugars are the important carbohydrates in juices as they not only give body and mouth feel to the juice in addition to sweetness but also mask the astringency effects caused by tannins on its taste [15]. Of the total sugars present in banana pulp, sucrose, glucose and fructose are predominant and contribute heavily to the sweet taste of ripe bananas.

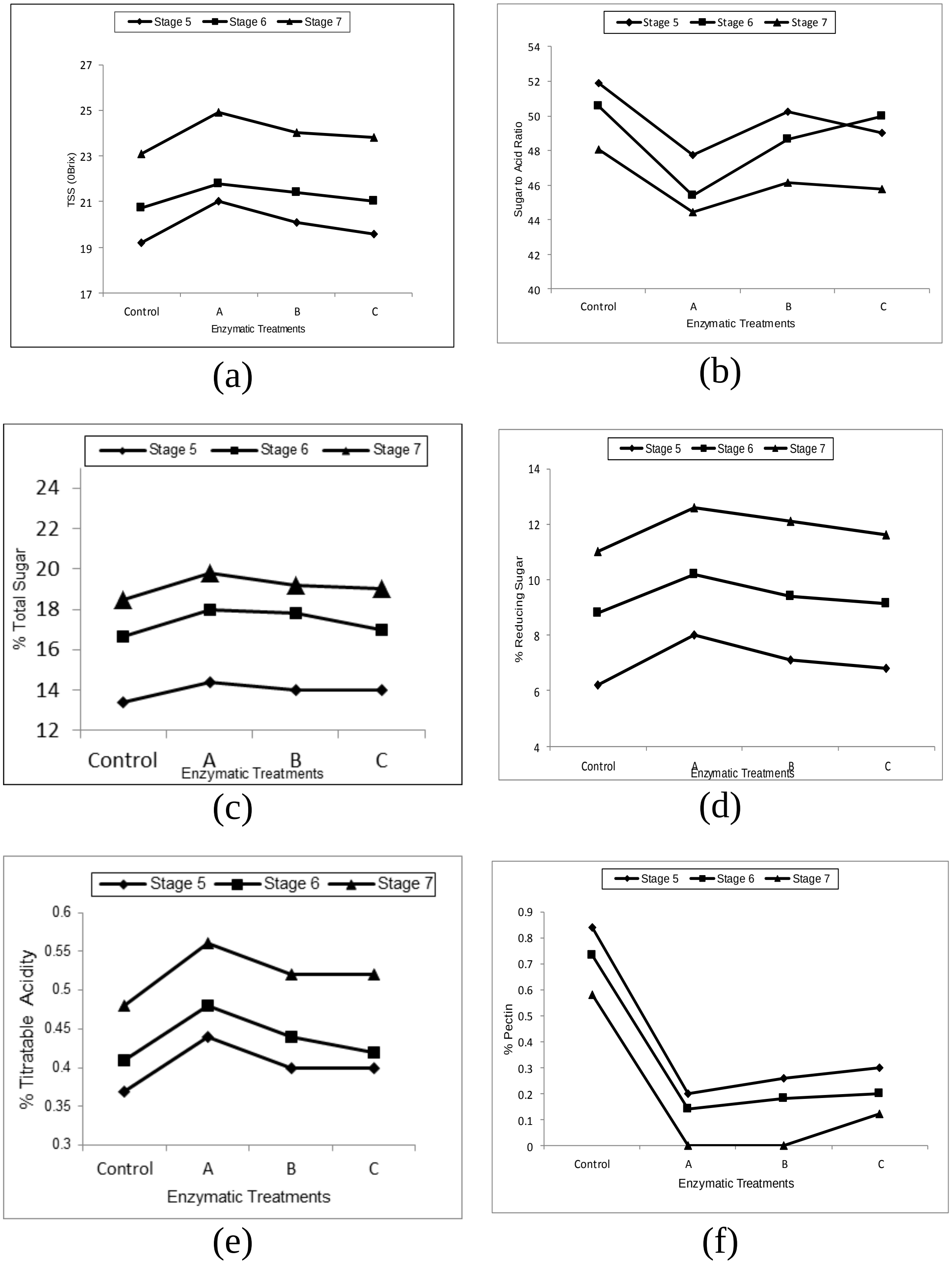

Figure 1 Changes in (a) Total Soluble Solids (b) Sugar to Acid Ratio (c) Total Sugar content (d) Reducing Sugar (e) Titratable Acidity (f) Pectin content of Clarified Banana Juices with Enzymatic Treatments and Stage of Ripening

Clarified juices prepared with different enzymatic combinations and without enzymatic treatment were studied for their total and reducing sugar content. In all the enzymatically prepared juices higher total sugar and reducing sugars content were recorded as compared to control (Table 2). Statistically significant differences were observed for both these parameters in all the enzymatic treated as well as untreated juice samples. Enzymatic combination ‘A’ yielded clarified juice with highest total sugar (17.40%) and reducing sugar content (10.27%) followed by enzymatic combination ‘B’ and ‘C’. The lowest total sugar (16.18%) and reducing sugar content (8.67%) were observed in untreated i.e. control juice sample.

Higher total sugar content in enzymatic clarified juices may be due the degradation of polysaccharides like pectin by enzymatic treatments, resulting in the release of simple sugars and higher reducing sugar content may be due to conversion of non-reducing sugars into reducing one during enzymatic treatment [8]. The results supports the finding of [16] who treated the banana must with pectinase and α-amylase enzymes for preparation of banana wine.

Similarly the effect of ripening stages on both these parameters was studied in prepared clarified juices. Clarified juices prepared by using the banana pulp of ripening stages 5, 6 and 7 showed statistically significant difference with respect to their total and reducing sugars content (Table 3). From the fully ripened stage of banana i.e. stage 7, clarified juice obtained showed the highest values of total and reducing sugar content i.e. 19.12% and 11.83% respectively followed by 6^{th} and 5^{th} stage. This may be due to the higher conversion of starch into sugar during ripening stage 7 [17]. Reducing sugars are initially present in small quantities and become abundant during ripening [18].

Combined effects of both i.e. enzymatic treatments and ripening stages on total and reducing sugar content are shown in Figure 1(c) and Figure 1(d) respectively. It is evident that the total and reducing sugar content in prepared clarified juices were mainly influenced by stages of ripening. Both these sugars content were found to be increased in juices prepared at later stages of ripening irrespective of the enzymatic treatments applied for juice clarification. Clarified juice at fully ripened stage i.e. stage 7 was observed to be with highest total and reducing sugar content followed by 6^{th} and 5^{th} stage of ripening

Effect enzymatic treatments and ripening stages on titratable acidity and pectin content

The principal acid in banana was investigated [9] and found to be malic acid. The average titratable acidity values of prepared clarified juices depicted in Table 2 shows that there was a significant change ($p<0.05$) in titratable acidity of the enzymatically treated juice samples as compared to untreated one. Clarified juice prepared with enzymatic combination 'A' showed the highest value of titratable acidity (0.49%). No significant difference was observed between enzymatic combination 'B' and 'C'. The lowest average acidity value of 0.42% was shown by control sample. It had been earlier reported [19] and [20] that pectinases de-esterify (pectinesterases) or depolymerize (polygalacturonases, polymethyl galacturonases, pectate lyses) specific pectic substances. In addition, during the enzymatic breakdown of pectin and hemicellulose, unesterified galacturonic acid units are released [12]**.** This may be the reason why there was increased in titratable acidity of enzyme treated juices than in untreated one. The results obtained in this study on titratable acidity agree with those of previous researcher [9] who had observed similar trend when treated the banana pulp with pectinase enzyme.

The turbidity and viscosity of banana pulp are caused mainly by the polysaccharides such as pectin and starch. Pectins make the clarification process harder because of their fibre-like molecular structure [3]. Pectin degradation is prerequisite for juice clarification. To evaluate the effectiveness of clarification treatment, determination of pectin content in prepared clarified juices is important. In the present investigation, significantly lower pectin content was observed in all the enzymatic treated juice samples as compared to control (Table 2). The maximum pectin content of 0.72% was observed in control and lowest of 0.11% in juice prepared with enzymatic combination 'A'. This may be due to the degradation of pectin in treated juice sample by the pectinase enzyme.

Ripening stages of banana were also found to be affecting significantly on titratable acidity and pectin content of clarified banana juices (Table 3). Data on titratable acidity and pectin content indicate that pectinase enzyme treatment for banana pulp was more effective in later stage of ripening. Clarified juice at maturity stages 7 shows the highest value of titratable acidity i.e. 0.52% and lowest level of pectin content i.e.0.175% followed by maturity stage 6 and 5. This may be due to increase in acid content [14] and decrease in pectin levels occurring during ripening of bananas [21].

It is evident from Figure 1 (e) and Figure 1 (f) that irrespective of enzymatic combinations, ripening stages affects significantly on titratable acidity and pectin content values of clarified banana juices. Later stages of ripening were observed to be more effective to produce juices with higher acidity and lower pectin content. For all the ripening stages viz. 5, 6 and 7; enzymatic combination 'A' was found to be more effective as compared to other enzymatic combinations and untreated sample.

Conclusion

In enzymatic clarification of banana pulp, the applied enzymatic combinations and ripening stages of banana affects the physico-chemical parameters of prepared clarified juices. In general, both these factors affects the physico-chemical properties of prepared juices but the effect of ripening stages is more pronounced. Enzymatic combinations with higher enzyme concentration yield clarified juices with higher TSS, total sugar, reducing sugar and titratable acidity; and lower sugar to acid ratio and pectin content as compared to treatments with lower concentration of enzyme. Advancement in the stage of ripening (from stage 5 to stage 7) significantly increases the TSS, total sugar, reducing sugar and acidity; and decreases the sugar to acid ratio and pectin content of clarified juices.

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