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Synergistic Effect of Multiple Enzymes on Apple Juice Clarification

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Abstract

Objectives: To clarify the apple juice enzymatically using different commercial enzymes like amylase, pectinase and cellulase at a standard concentration of 1U/ml both in isolation and in combination. To standardize the clarification conditions like temperature (40°C and 50°C) and different incubation time intervals (15 and 30 minutes). **Methods/ Statistical Analysis**: Apple juice was prepared from good cleaned fruits using distilled water by blending and extraction of juice. The extracted juice was filtered centrifuged and pasteurized before subjecting it to clarification. Different commercial enzymes like amylase, pectinase and cellulose were taken at a concentration of 1U/ml in sodium acetate buffer (pH 5) for clarification studies both in isolation and in combination. The clarification was measured in terms of viscosity reduction. The tested parameters for the present study were time of incubation (15-30 min), concentration of enzyme (1 U/ml) and temperature (40-50° C). **Findings:** The apple juice was **c**larified using pectinase at 1U/ml in 30 min with reduction of viscosity to 1500 cP units at 40° C. When amylase and cellulase are used separately for apple juice clarification under similar enzymatic conditions there was viscosity reduction to 500 cP units at 40°C for 30 min incubation time and viscosity reduction to 600 cPunits 50° C for 30 min incubation time respectively. Pectinases in combination with amylases showed viscosity reduction up to 510 cP units and with cellulases 609 cP units at 50° C for 30 min of incubation time. Clarification of apple juice was found to be taking 30 min and there was reduction of viscosity to 400 (cP) when I U/ml concentration of amylase, pectinase and cellulase was used at 50° C in combination. **Application/Improvements:** The results indicated that there was a significant viscosity reduction of apple juice when enzymes were used in combination for fruit juice clarification. This can be applied for clarification of other fruit juices The study is significant as perishable and seasonal Indian apples grown in abundance can be used for commercial production of clarified juices.

Keywords: Amylase, Apple Juice, Pectinase and Cellulase, Viscosity

1. Introduction

Apple (*Pyrus malus*), a fruit of the domesticated tree *Malus domestica* is one of the most widely cultivated tree fruits. India is one of the top five producers of apple fruit. The hilly States of Jammu and Kashmir, Himachal Pradesh and Uttar Pradesh of North India are the predominant apple growing states. An apple fruit composition consists of 10% carbohydrate, 4% vitamins and minerals and more than 80% water. Apple consists of mainly starch, pectin and considerable amount of cellulose. Presence of starch gives the thickness and viscosity which contribute to the turbidity of juice. Amylase is a starch degrading enzyme which breaks down starch into amylose and amylopectin. Pectin is present in the skin or rind of the apple and due to its presence the juice has thick and sticky consistency. Cellulose and hemicellulose are present as roughage (fiber) on which cellulase enzyme can act. Apple juice has antioxidant properties as it contains polyphenols and flavonoids which contribute to heart's health. It is the best

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defender against tumors and cancer, improves eye health, keeps away dandruff, reduces cholesterol and boosts immune system.

Enzymes like pectinases, amylases and cellulases reduce fruit juice viscosity and turbidity. When these enzymes are applied on fruit pulp, it degrades these carbohydrates and thereby reduces the viscosity of the fruit juice[1]. Enzymes addition results in the viscosity reduction of the fruit juice which inturn improves pressability of the pulp, yielding more juice without the necessity for pressing aids and hence is commercially cost effective. Use of enzymes like pectinases, amylases and cellulases improves both clarity and yield of fruit juices[2]. Pectinases have multiple applications in food industry like processing plant materials, de-pectinization of fruit juices, vegetable oils extraction and fruit maceration[3]. There are reports on use of pectinases for reduction of viscosity of pulp along with simultaneous increase in juice yield[4-6]. A simple method was reported for determining the enzymatic activity with commercial pectinases for clarification of apple juice[7]. Pectic enzymes of *Saccharomyces cerevisiae* were used for pine apple juice clarification[8]. Earlier surplus fresh fruits were processed into fruit juices locally for consumption, but now processing of fruit juices has been established as a major industry for diverse applications.

With increased demand for fruit juices, the production of fruit juices presently has increased considerably. This triggered more research to develop better methods for fruit processing. In present study different commercial enzymes like pectinases, amylases and cellulases were used in clarification of apple juices and parameters like time of incubation, temperature and concentration of enzyme were studied to obtain maximum clarified juice.

2. Materials and Methods

2.1 Preparation of Enzyme

Commercial enzymes pectinase, amylase and cellulase at a concentration of 1U/ml were prepared in sodium acetate buffer (pH 5). These enzymes were maintained under cold conditions.

2.2 Fruit Juice Preparation

Fresh ripened apples of different varieties were purchased from the fruit market and these were used for the juice preparation. The apples after thorough washing and cleaning were used with peel, and later chopped into small pieces which were blended in mixer without addition of water. The homogenous pulp obtained was used for the clarification studies. This homogenous fruit juice was diluted by 50% with addition of distilled water and pasteurized at 60° C for 1 hour. This treatment was followed by centrifugation and filtration. The filtrated juice which was about 80ml was made up to 100 ml. The pH of this fruit juice was 5, which is in accordance with general pH of most fruit juicesl[9].

2.3 Enzymatic Treatments

Twenty five milli litres of apple juice extracted was subjected to various enzymatic clarification treatment conditions. The different clarification conditions tested were time of incubation (15-30 min), concentration of enzyme (crude enzyme 1 U/ml) and temperature (40-50°C). Post treated apple juice was heated upto 90° C for a duration of five minutes for enzyme inactivation. Further this juice was subjected to centrifugation at rpm of 10,000 for time period of 15 min. This was then filtered using Whatman no 1 filter paper and this collected filtrate was used for further analysis.

2.4 Fruit Juice Viscosity Studies

The apple juice viscosity was measured using DV-II+ Pro Brook field Viscometer LV-6.3V at 30^{0}C. The viscosity is expressed in cP units (centi Poise).

3. Results

Fruit juices rich in pectin, starch and cellulose need enzymatic treatment for clarification. Use of multiple enzymes can significantly reduce viscosity resulting in clarified juice. Pectinases, amylases and cellulases in isolation and in combinations were tested for clarification effect on apple juice. The conditions of testing for juice clarification were variables like incubation time and temperature at constant enzyme concentration. Viscometric studies were carried out for the apple juice clarification. The juice was incubated at 40 and 50^0 C for clarification at different time intervals of 15 and 30 min at constant enzyme concentration 1 U/ml. Figure 1 shows the effect of temperature on clarification of apple juice at temperatures 40 and 50° C when pectinase enzyme alone is used at 1U/ml concentration. Clarification of apple juice was found

to be taking 30 min and there was reduction of viscosity to 1500 cP units at 40°C. Under similar enzymatic conditions when amylase and cellulase are used separately for apple juice clarification there was viscosity reduction to 500 cP units at 40° C for 30 min incubation time and viscosity reduction to 600 cPunits 50^0 C for 30 min incubation time respectively Figure 2, Figure 3. Pectinases in combination with amylases showed viscosity reduction up to 510 cP units whereas pectinases in combination with cellulases reduced viscosity to 609 cP units for 30 minutes of incubation time and at 50° C Figure 4 and Figure 5. Clarification of apple juice was found to be taking 30 min and there was reduction of viscosity to 400 (cP) when I U/ml concentration of amylase, pectinase and cellulase was used at 50° C Figure 6.

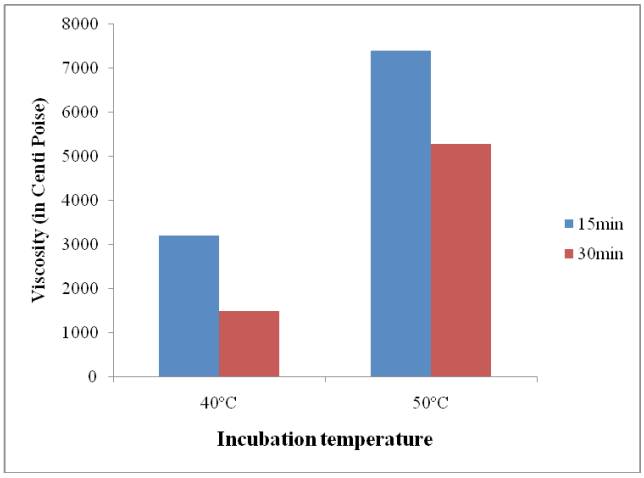

Figure 1. Effect of Incubation Time on Clarification of Apple Juice with Pectinases at 40^{0}C and 50^{0}C Temperature (by Viscometric Method).

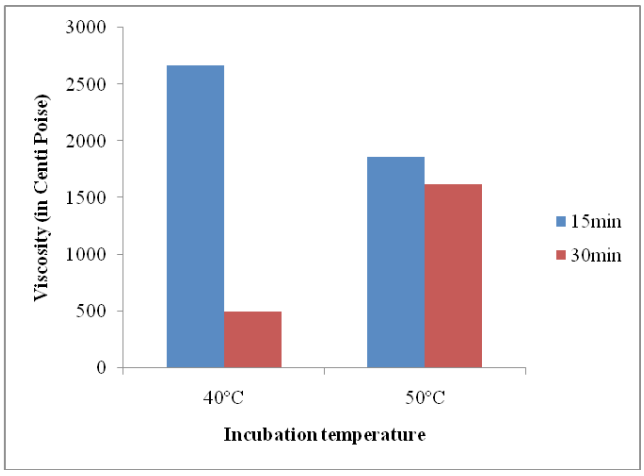

Figure 2. Effect of Incubation Time on Clarification of Apple Juice with Amylases at 40^{0}C and 50^{0}C Temperature (by Viscometric Method).

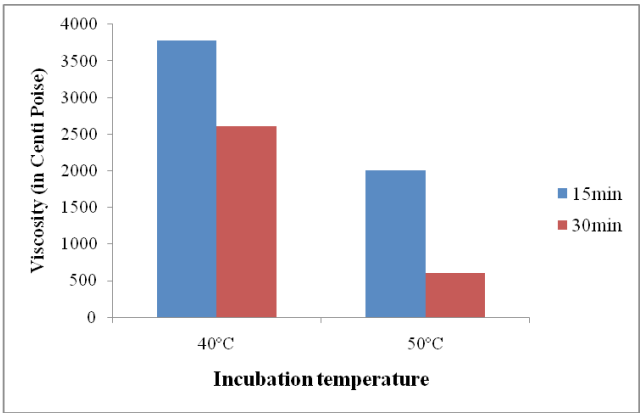

Figure 3. Effect Of Incubation Time on Clarification of Apple Juice with Cellulases at 40^{0}C and 50^{0}C Temperature (by Viscometric Method).

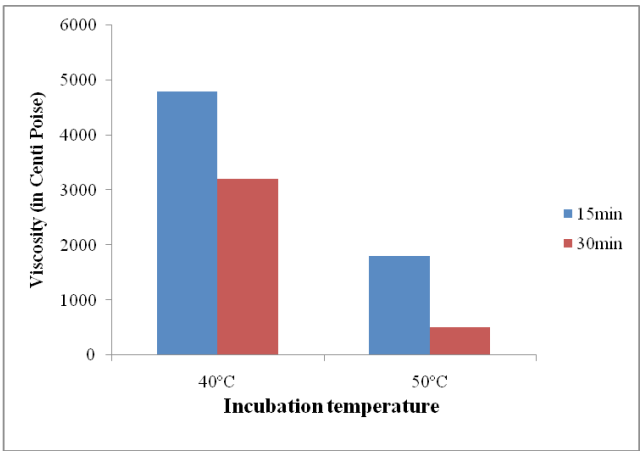

Figure 4. Effect of Incubation Time on Carification of Apple Juice with Pectinases and Amylases at 40^{0}C and 50^{0}C Temperature (by Viscometric Method).

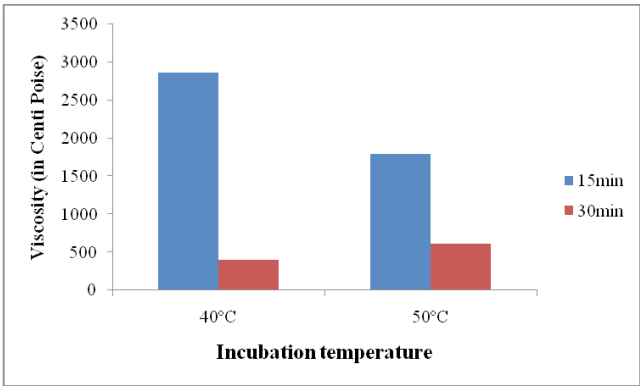

Figure 5. Effect of Incubation Time on Clarification of Apple Juice with Pectinases and Cellulases at 40^{0}C and 50^{0}C Temperature (by Viscometric Method).

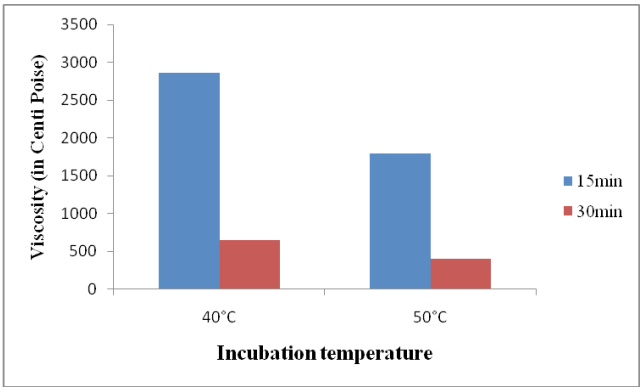

Figure 6. Effect of Incubation Time on Clarification of Apple Juice with Pectinases, Amylases and Cellulases at 40^{0}C and 50^{0}C Temperature (by Viscometric Method).

4. Discussion

The best quality apples are grown abundantly in India. Fruit mash when treated enzymatically results in 80% juice yield. The Pectic substances treatment by enzymatic hydrolysis depends on several variables such as enzyme type and concentration, hydrolysis time, temperature of incubation and pH[10-12]. The commercial enzymes pectinases, amylase and cellulase had been used separately as a single enzyme and as multiple enzymes for clarification of apple juice under different enzymatic conditions like incubation time, temperature at constant enzyme concentration. Mixture of hydrolytic enzymes like pectinases, amylases are reported to be used for clarifying fruit juices resulting in both in clarification and decrease of time of filteration by almost 50%[13]. Pectinolytic treatment of banana, grapes and apple fruit pulps showed significant increase in fruit juice volume[14]. Pectinases in combination with other multiple enzymes like cellulases, xylanases and arabinases have been not only used to increase the pressing efficiency of the fruits but also for juice extraction[15]. Apple and grape juices are produced and consumed as clarified juices[16]. Clarified fruit juices are the most preferred form of juices by all consumers and so clarification of fruit juices is a necessity and hence studies on clarification are significant. Clarification of apple juice under study as indicated by remarkable viscosity reduction was found to be good with enzyme concentration of 1 U/ml, time of incubation 30 min and at temperature of 50° C. Apple juice clarification using multiple enzymes showed good synergistic effect on clarification. Comparative study of viscosity reduction by various enzymatic treatments in isolation showed that amylase gave highest viscosity reduction followed by pectinase respectively. Based on our studies optimum enzymatic conditions recommended for apple juice clarification using pectinases, amylases and cellulases were 50° C, an incubation time of 30 minutes with 1U/ml enzyme concentration.

5. Conclusion

Application of multiple enzymes for fruit juice clarification is a novel approach in the fruit juice industry. Standardized enzymatic clarification conditions like incubation time, temperature and enzyme concentration influence the yield of highly clarified apple juice, as it is the most preferred juice by all the consumers. The maximum apple juice yield can be obtained at a temperature of 50° C with enzyme concentration 1 U/ml for 30 minutes of incubation time. Significant reduction in viscosity was observed when enzymes were used in combinations for fruit juice clarification. The study is significant as perishable and seasonal Indian apples grown in abundance can be used for commercial production of clarified juices.

6. Acknowledgment

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