

Amylase Based Clarification of Apple, Orange and Grape Juice

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Abstract: Amylases are starch-degrading enzymes produced by many bacteria, plants, fungi and animals. It has found tremendous application in industry. One of its applications is in fruit industry wherein amylases are used to clarify fruit juices and reduce its viscosity. In fruits, high amount of starch is present which resulted in increased viscosity of the prepared juices. This may also lead to settling of juice at bottom. Amylases act on starch component and degrade it. In the current study, amylase from *B. licheniformis* was used for the clarification of apple, orange and grape juices. Total suspended solids, viscosity and total acidity was found to decrease with increasing amylase concentration. The results revealed that after amylase treatment the color, texture and flavor of juices were also improved.

Keywords: Amylase; juice clarification; apple juice; orange juice; grape juice

I. INTRODUCTION

Amylases are ubiquitous and have the highest market share of enzymes[1]. In the food industry, starch is used for liquefaction and sacrifice, for making corn syrups, for antistalling in baking, and for reducing fog in beverages[3]. Amylases are used in juice resolution to increase clear juice production[4]. The enzyme is used to debit fruit juices and darken the juices. Enzymes degrade pectin from cell wall [5]. Apples, grapes and oranges are mainly processed for juice production. Ripe apples contain up to 15% carbohydrates and up to 1% juice after extraction, milling and pressing[6]. Starch can cause problems during juice processing, which complicates the processing process and contributes to the post-processing cloud [7]. Therefore, amylase can be used to improve the yield and clarity of the juice. The use of amylase in terms of low viscosity, turbulence and improved filtration improves the quality of the juice. Therefore, a proposed study was conducted to evaluate the utility of amylase in apple, grape, and orange juice processing.

II. MATERIALS AND METHODS

Fruit sample

Apple, grape and orange fresh fruit samples were collected from a local market in Mohali, Punjab, India and brought to the laboratory for further processing.

Production of amylase

Amylase was produced in solid state culture condition using paddy straw as substrate[8,9]. Substrate was washed 2–3 times with distilled water, treated with 1% NaOH for 30 min, and dried in an oven at 80°C overnight. Dried substrates were ground in the grinder and sieved through a mesh to obtain equal size particles. Fermentation was carried out by using 5g substrate consisting of 1.81% starch with 70% initial moisture content and 0.94% Tween-80 inoculated with 10% inoculum. Incubation was carried out at 37°C for 48h.

Extraction and assay of enzyme

Fermented solids were collected using 50 ml 0.1 M phosphate buffer and incubated in a rotary shaker (150 rpm) for 30 min at 30 ° C. Slurry is squeezed through a wet cheesecloth. The suspension was centrifuged at 10000 rpm for 15 minutes at 3700 C to separate small particles,

cells and spores. Clear supernatant was collected and further used for amylase assay. Amylase was assayed using supernatant containing crude enzyme by Dinitrosalicylic method [10] with 1% starch as substrate at pH 7.0, 50°C for 30 min and optical density was taken at 540nm, on UV- spectrophotometer. One unit of enzyme activity was defined as micromoles of glucose released per minute per one gram of substrate.

Enzyme purification

The crude enzyme was purified by fractionation by ammonium sulphate followed by dialysis and ion exchange chromatography [11].

Effect of varying concentrations of amylase on fruit juice yield and clarification

After washing, rinsing and masking, the fruit (apple, grape, and orange) was treated with amylase at various concentrations 0-1.25 IUml⁻¹ of juice. After 15 min, physico-chemical parameters i.e. and TSS, acidity and viscosity were noted.

Total soluble solids

Total soluble solids were determined by filter paper assay method. 100ml of sample was passed through pre-weighed Whatman filter paper. The filter is dried at 104 ± 1°C. After drying the filter is reweighed and the TSS was calculated as per following equation:

$$TSS(mg\ L^{-1}) = \frac{Weight(g)_{Final} - Weight(g)_{initial}}{sample\ volume\ (ml)} \times 10^6 \quad (i)$$

Acidity

The titratable acidity (TA) of apple and orange juice was determined by aliquots of 10 mL sample from standard 0.1 N NaOH solution to pink endpoint using 0.1% phenolphthalein as an indicator[12]. The values of titratable acidity are expressed as percent tartaric acid:

$$TA = \frac{volume\ of\ NaOH\ (ml)}{volume\ of\ juice\ taken\ (ml)} \times 0.75 \quad (ii)$$

Viscosity

A clean and dry Ostwald capillary viscometer was used to measure the viscosity. Double distilled water was used as reference. The time required for the juice sample to flow through the capillary section of the Ostwald viscometer was observed using a stopwatch and a sample at 20±2°C.

Sensory evaluation of juice

The sensory evaluation of apple and orange juice was based on color, taste, taste and overall acceptability on the hedonic scale. The tasting panel (7-9 members at a time) includes faculty members and PG students of the Department of Biotechnology, Landron, Chandigarh College of Technology. Attempts have been made to organize a single panel for sensory analysis for the entire period. Coded samples of juice were placed in 100 ml glass bakens. Panelists were given plain filtered water to take the air during the sensory evaluation.

Statistical Analysis

The data obtained are subjected to the analysis of the variance technique using a fully randomized design (RBD) for a laboratory experiment and a random block design for the sensory features developed by Gomez and Gomez [13].

III. Results and Discussions

Apple, orange and grape juices are amongst the top drunk juices in the world. Raw juices obtained after crushing, grinding and pressing are turbid and very viscous. This results in settling of juice at bottom during storage[14]. Therefore, for commercial production of juices, it must be clarified and non-viscous for long term storage and consumption. Polysaccharides including starch are mainly responsible for the turbidity of juices. Starch also resulted in slowing down the filtration rate, membrane fouling, gel formation in concentrated solutions and haziness[15]. Therefore, destarching is an essential step for juice clarification. Amylase is a hydrolytic enzyme that acts on starch. It eliminates starch embedded in protein thereby removing the haziness formation in juices. In the present study, clarification of apple, grape and orange juice was carried out with using purified bacterial amylase. The concentration of amylase was varied so as to obtain best concentration which can be utilized for juice extraction. The results of obtained were as follows:

Total suspended solids (TSS)

TSS is a main factor governing the viscosity of the juice. Reduction in TSS leads to reduction in viscosity which enhanced the membrane processes[16]. Treatment with amylase resulted in decrease of TSS in all the three juices tested. Increase in amylase concentration lead to increase in reduction of TSS (Fig. 1). This shows that with increase in enzyme concentration TSS decreases which is possible due to the degradation of various polysaccharides by amylase enzyme[17].

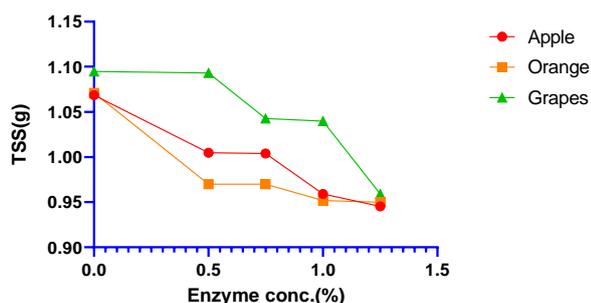


Fig. 1 Effect of varying concentrations of amylase on TSS of fruit juice

Acidity

The acidity of the treated juice decreases with the increase in concentration of amylase (Fig. 2). In the case of orange juice, the untreated juice (Control) shows the maximum acidity, i.e., 2.65ppm, while the treated juice with 1.25% enzyme concentration showed the minimum acidity i.e., 0.48ppm. In case of grape juice, same trend was observed i.e. the untreated juice (controlled) shows the maximum acidity of 4.65ppm, while the treated juice with 1.25% enzyme concentration showed the minimum acidity of 1.2ppm. These results were in conformation to the results where two varieties of grape juice gave acidity of 0.47-0.96ppm in one variety and 0.83-1.46ppm in other variety[18]. In case of apple juice, acidity of untreated juice was maximum i.e., 2.01ppm which decreases to 0.18ppm with 1.25% amylase. Jan et al., 2016 conducted a research on production of apple juice treated with amylase enzyme and measured its acidity, the two varieties of apple juice gave acidity to be 0.48ppm in one variety and 0.18ppm in another variety which are comparable to our findings [19].

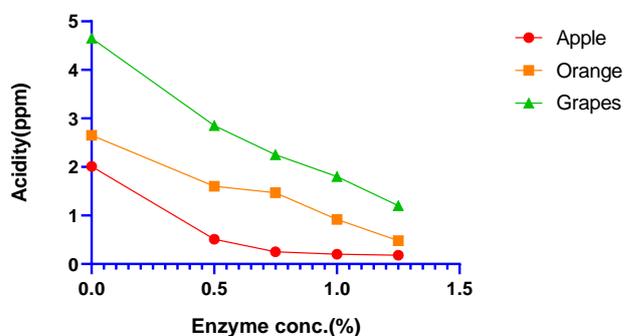


Fig. 2 Effect of varying concentrations of amylase on Acidity of fruit juice

Viscosity

In case of orange juice, the treated juice resulted in decrease of viscosity (Fig 3). The total viscosity decreases from 0.00253 g/cc in 0.5% enzyme to 0.00221 g/cc in juice treated with 1.25% enzyme, which is lower than the control, i.e., 0.00263 g/cc. According to Kareem et al. [20], the viscosity was reduced to 51% in treated orange juices. Utami et al. [21] concluded that the viscosity of amylase treated orange juice samples ranged from 1.1113 to 1.1767 cp. In case of grape juice, viscosity was calculated for amylase treated grape juice with maximum and minimum value at 0.5% and 1.25% to be 0.00094 g/cc and 0.00082 g/cc respectively which is comparable to the research done on white grape juice which concluded the decrease in viscosity by 25% in the process of clarification[22]. In case of apple juice, viscosity was calculated of amylase treated apple juice with maximum and minimum value at 0.5% and 1.25% to be 0.00087 g/cc and 0.00075 g/cc respectively. The starch content diminution is significant in the juice elucidation by amylase [22]. The loss in viscosity in the juices amplifies the efficiency of the juice concentration process and accelerates the filtration process [23]

High-viscosity fruit juices have been shown to cause problems in the purification process[24]. Soluble pectinaceous substances, hemicellulose, soluble polysaccharides and colloids lead to high viscosity. Enzymes degrade these substances by hydration. Ural et

al. [25] reported a reduction in the viscosity of fruit juices by enzymatic hydrolysis of pectin.

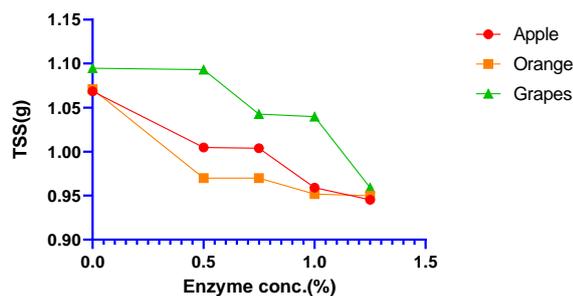


Fig. 3 Effect of varying concentrations of amylase on viscosity of fruit juice

Sensory evaluation of juice

Addition of amylases when preparing fruit juice increases yields, but also increases their taste, color and flavor, as well as their acceptability as shown in Fig. 4. Color is an important sensory attribute. A dark product usually does not make customers happy, because it represents a decline. The enzyme, amylase, reduces carbohydrates into smaller units and prevents post-bottling fog. Amylase derived from *Bacillus licheniformis*, to improve the yield and clarity of orange, apple and grape juice. Degradation polysaccharides are responsible for the elucidation of juices using amylase enzymatic treatment. These enzymatically named juices reduce viscosity, so the juice is more concentrated in terms of taste and color [26]. Srivastava and Tyagi[27] reported that juice yield and resolution are a function of enzymatic hydrolysis.

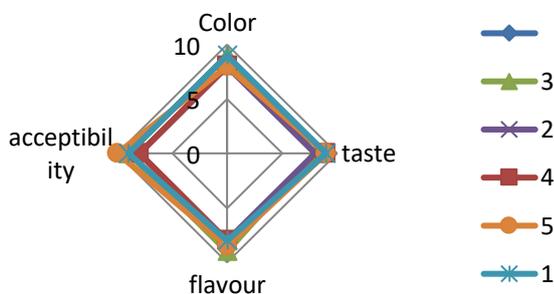


Fig. 4 Sensory evaluation of juice

IV. CONCLUSION

Enzymes are becoming a basic need for the beverage industries now-a-days. Application of amylase from *Bacillus licheniformis* enhanced the clarification, taste, color and flavour of orange, apple and grape juices along with reducing total suspended solid, viscosity and acidity. Therefore, enzymatic treatment is an effective way to reduce the cloudiness in the fruit juices. This further enhanced the overall acceptability of the juices. Thus, amylase from *Bacillus licheniformis* can be utilised in fruit juice manufacturing.

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