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Application of bacterial amylase in clarification of juices and bun making

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Abstract

Amylases have found applications in juice processing, starch processing, desizing of textiles, paper sizing, detergent additives, bread improvement, utilization of waste biomass for valuable products, treatment of waste water and other fermentation processes including malting barley and bakery industries. In the present investigations, amylase producing thermophilic bacterial strain M13 was isolated from spent mushroom compost. M13 isolate produced amylase activity of 61.35 IU after 72 h of incubation period at a pH of 9.0 and temperature of 45°C. It was identified as *Bacillus subtilis* M13 by using 16S rDNA sequence analysis and deposited in NCBI gene bank (KY962809). Further, the application of extracted amylase was evaluated for apple and kiwi juice yield and clarification as well as for the preparation of buns. An application of 1 and 0.75 per cent of amylase yielded 60 and 55 per cent apple and kiwi juice and enhanced their color, taste, flavour and overall acceptability. Whereas, 1.25 per cent of amylase resulted in the maximum leavening activity (2.15 ml/h), loaf volume (177.43 cm³) and crumb grain (0.145%) of dough. Sensory characteristics of dough viz., color, taste, flavour and overall acceptability were also enhanced at 1.25 per cent of amylase.

Keywords: amylase; apple; *Bacillus subtilis*; mushroom compost; starch

Introduction

Amylase is well known calcium containing enzyme which catalyses the hydrolysis of α -1, 4 glycosidic linkages in starch to yield products like glucose and maltose (Senthikumar *et al.*, 2012; Sundarram and Murthy, 2014) [20, 27]. It represents approximately 30 per cent of world enzyme production (Calik and Ozdamar, 2001; Rajagopalan and Krishnan, 2008) [6, 14]. It was the first enzyme to be commercially produced and Dr. J. Takamine established the first industrial production of alpha amylase from *Aspergillus oryzae* known as taka diastase, which was used as a digestive aid. Since then, amylases have been reported to have diverse applications in various industries such as in the food, bread making, paper industries, textiles, sweeteners, glucose and fructose syrups, fruit juices, detergents, fuel ethanol from starches, alcoholic beverages and spot remover in dry cleaning. Amylases also find their applications in the degradation of environmental pollutants and conversion of starch to desired products by microorganisms (Veille and Zeikus, 2001). Bacterial α -amylases are also being used in clinical, medicinal and analytical chemistry (Pandey *et al.*, 2000) [13]. β -amylase catalyzes the hydrolysis of the second α -1,4 glycosidic bonds, cleaving off two glucose units at a time. During the ripening of fruit, β -amylase breaks starch into maltose, resulting in the sweet flavour of ripe fruit. In food industry amylase is used for starch liquefaction and saccharification, manufacturing of corn syrups, antistaling in baking, enhance shelf life of breads and reduction of chill haze formation in beverages (Christopher and Kumbalwar, 2015; Singh *et al.*, 2016) [9, 23]. Amylases are utilized in the clarification of juices to maximize the production of clear juice (Sivaramakrishnan *et al.*, 2006) [24].

Apple and kiwi fruits are two important commercial fruit crops of Himachal Pradesh which are mainly processed for juice making. Apple juice contains considerable amounts of starch, particularly at the beginning of the harvest season. Unripe apples contain as much as 15 per cent starch and up to 1 per cent in the juice after extraction, milling and pressing (Carrin *et al.*, 2004) [7]. Starch creates problem during apple juice processing as it complicates filtration and causes post process cloudiness. The enzyme amylase degrades starch into smaller units and contributes to prevent post bottling haze formation. Thus, the amylase can be used to improve the yield and clarification of apple and kiwi juices. Amylase is also employed in baking industry to supplement the natural enzymes present in the grains during fermentation by yeast which increases the quality of baking products. Microorganisms are regarded as predominant source of amylase production due to optimum growth requirements, accessibility and they are efficient, eco-friendly and cost-effective as compared to other resources such as animals and

plants (Tanyildizi *et al.*, 2005) [28]. Selection of right organism plays a key role for high yield of desirable enzymes with suitable properties. The increasing demand for enzymes in various industries has led to interest in research on enzymes suitable for applications. Therefore, the proposed study was undertaken to evaluate the application of amylase in juice processing and bakery products.

Materias and Methods

Sample collection and isolation of amylase producing bacteria

Compost samples were collected from mushroom fields of Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan and homogenized to form bulk sample. These samples were brought to the Microbiology Laboratory for further isolation and characterization work in aseptic conditions. Isolation of amylase producing bacteria was done by the standard procedure of Subba Rao (1999) [26].

Amylase assay

Qualitative assay

Starch hydrolysis test was performed by Shaw *et al.* (1995) [21]. The starch agar plates were spot inoculated with the isolated strains and incubated at 45 °C for 72 h. The growth thus obtained was flooded with 2 ml of iodine solution. Bacterial colonies producing clear zones were selected and purified using streak plate technique on the starch medium and refrigerated at 4 °C for further studies.

Quantitative assay

Amylase activity was determined by measuring the amount of

$$\text{Total acid (\%)} = \frac{\text{Titre} \times \text{Normality} \times \text{volume made up} \times \text{equivalent weight of an acid}}{\text{Volume of sample taken for estimation} \times \text{aliquot taken for estimation} \times 1000} \times 100$$

The fruit after washing, peeling and pulping was treated with amylase at different concentrations viz., without amylase, 0.5% amylase, 0.75% amylase, 1.0% amylase and 1.25% amylase. With every 15 min of intervals, physico-chemical parameters i.e. juice yield, absorbance and acid content were noted. Sensory evaluation of apple and kiwi juice was conducted on the basis of color, flavor, taste and overall acceptability on hedonic scale according to the method of Amerine *et al.* (1965) [2]. Taste panel (7-9 members at a time) comprised of faculty members and PG students of Department of Microbiology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan. Efforts were made to keep the same panel for sensory analysis throughout the entire period. Coded samples of juice were served in 100 ml glass beakers. Plain filtered water was provided to the panelists for mouth rising in between the sensory evaluation.

Softening of dough for buns making

A refined flour of 1.5 kg was taken with 65% of hot water. Water was added in the small amount to mix the flour then 6% of sugar, 45 g of milk powder, 3% of yeast, 1% oil were added and mixed properly. The above mixture was treated with different concentrations of amylase viz., without amylase, 0.5% amylase, 0.75% amylase, 1.0% amylase and 1.25% amylase. Leavening activity was checked. The mixture was incubated for 15 min in hot air oven at 55 °C. After 15 min salt was added in small amount and was mixed properly. Further, the above mixture was kept in oven for fermentation for 1.5 h at 35 °C followed by proofing of dough for 1 h at 35 °C. Baking was achieved at 175-200 °C for 30 min.

hydrolyzed starch using the method of Xiao *et al.* (2006) [32]. 0.5 ml of enzyme solution was incubated with 0.2 per cent starch at 37 °C for 15 min. 3 ml of DNSA reagent was added to it and the mixture was heated on boiling water bath for 15 min. Absorbance of reaction mixture was read at 540 nm.

Morphological, biochemical and 16S rDNA sequence analysis of isolate M13

Morphological and biochemical characterization of isolate M13 was done by standard methods as given by Sherman and Cappuccino (2005) [22].

Clarification of apple and kiwi juice

Physico-chemical analysis

Various physico-chemical characteristics of apple and kiwi juice was analysed by following standard procedures (AOAC, 1984; Thiamiah, 1997; Ranganna, 1997) [3, 29, 17].

Total soluble solids

The total soluble solids (TSS) in fruit pulp was determined with the help of hand refractometer and expressed in degree brix.

Acidity

The titratable acidity of apple and kiwi juice was determined by titrating the aliquots of known quantity of sample against a standardized 0.1 N NaOH solution to a pink end point using 0.1 per cent phenolphthalein indicator (Ranganna, 1997) [17]. The values of titratable acidity were expressed as per cent anhydrous citric acid on fresh basis of a given sample.

Physical characteristics of bun

Loaf volume

The loaf volume expressed in cubic centimeters was determined by using seed displacement.

Leavening activity

The leavening activity was estimated in the prepared sample (wheat flour-20gm, water-15 ml, sugar-1.2 gm, salt-0.4 gm and baker's yeast-0.3 gm) in 100 ml volumetric cylinder (Caballero *et al.*, 1995) [5]. Maximum leavening rate (ml/h) at 30 °C was calculated.

$$\text{Leavening activity (ml/h)} = \frac{\text{Volume of sample reached at 120 mins at } 30^{\circ}\text{C}}{\text{Initial volume of the sample}}$$

Crumb grain

The crumb grain was collected during cooling and slicing of bread. A total weight of crumb grain was recorded and calculated:

$$\text{Percent crumb grain} = \frac{\text{Weight of crumb grain}}{\text{Weight of sample}} \times 100$$

Crumb colour

A known weight of bread sample was macerated with distilled water and then filtered. Absorbance was measured at 420 nm (Ranganna, 2009) [18].

Sensory evaluation

The samples of coded sliced bun were served in cleaned white plate to panelist at room temperature (25 °C) for sensory evaluation by hedonic scale (Larmond, 1997) [12]. Attributes evaluated in bun were colour, taste, flavor and overall acceptability.

Statistical analysis

The data obtained were subjected to analysis of variance technique using (CRD) Completely Randomized Design for laboratory experiment and (RBD) Randomized Block Design for sensory characteristics developed by Gomez and Gomez (1976) [17].

Results and Discussion

Isolation and screening of amylase producing bacteria

Three isolates viz., M13, M15 and M51 showed clear zones of starch hydrolysis with varying diameters from the pooled samples of mushroom. However, isolate M13 exhibited maximum zone (10.00 mm) with enzyme index of 37.00. In quantitative assay, isolate M13 produced amylase activity of 61.35 IU after 72 h of incubation period at a pH of 9.0 and temperature of 45 °C. Chauhan *et al.* (2011) [8] isolated *Bacillus* spp. A1 from mushroom compost giving maximum amylase activity (264.4 μg^{-1}) on deodar wood dust after 48 h of incubation period at a pH of 10.0 and temperature of 35 °C. Further, production and optimization of amylase from *Bacillus thuringiensis* J2 isolated from hot water spring has been carried out by Rana *et al.* (2017) [16].

Morphological, biochemical and 16S rDNA sequencing of isolate M13

On the basis of morphological and biochemical characteristics, the M13 isolate was identified as *Bacillus* sp. as per the criteria of Bergey's Manual of Systematic Bacteriology. Similar morphological and biochemical characteristics of *Bacillus* have been reported by Bhardwaj *et al.* (2017) [4]. It was identified as *Bacillus subtilis* M13 by 16S rDNA sequence analysis.

Effect of amylase on apple and kiwi juice yield and clarification

The TSS and acidity of raw pulp of apple was found to be 10.2 °B and 1.67 per cent, whereas, TSS and acidity of raw pulp of kiwi was recorded to be observed to be 14 °B and 1.74 per cent. It was revealed that the 1 per cent and 0.75 per cent of amylase has not only increased yield of apple and kiwi juice, but also improved their taste, colour, flavor and over all acceptability as depicted in Fig 1.

The enzyme amylase degrades the starch into smaller units and contributes to prevent post bottling haze formation. Thus the amylase produced by *Bacillus subtilis* M13 was subjected to improve the yield and clarification of apple and kiwi juice. The possible reason for juice yield and clarification of juices with amylase enzymatic treatment may be due to degradation polysaccharides. Enzymatically clarified juice results in

viscosity reduction and cluster formation, which facilitates the separation through centrifugation or filtration. As a result, the juice becomes more concentrated in respect to flavor and colour (Abdullah *et al.*, 2007) [1]. Srivastava and Tyagi (2013) [25] reported that the juice yield and clarification are the function of enzyme hydrolysis which assesses the variables of enzymatic treatment of fruit pulp particularly temperature, time, pH and amylase concentration. Further, Vinjamuri and Bhavikatti (2015) [31] also reported the optimal conditions for the enzymatic treatment of a mixture of apple, banana and sapodilla fruit juices in order to minimize the turbidity in the mixed juices. They showed that enzyme preparations containing a combination of pectinase and amylase in the ratio of 2:1 reduced the viscosity.

Effect of amylase on the softening of dough for buns making

The data revealed that the best leavening activity was observed to be 2.15 ml/h at 1.25 per cent amylase concentration [Fig 2 (a)]. The loaf volume was recorded to be 177.43 at the concentration of 1.25 per cent [Fig 2 (b)]. The similar results were obtained for the bun quality and sensory characteristics viz., taste, colour and overall acceptability of buns [Fig 2 (c) and (d)].

The prime function of amylase is to liquefy and hydrolyze starch. The action of amylase is the saccharification which provides fermentable sugars to yeast. Our results are in agreement with those of Sanz-Penella *et al.* (2014) [19] who reported that the use of α -amylase in bread making processes could provide technological advantages improving quality of breads without markedly changes in their glycaemic index. David *et al.* (2014) [10] also reported that small dose (10g/100kg) of β -amylase showed the improvement in quality of dough for bread making. They further suggested that amylase acts on the starch resulting in increase in the fermentable sugars and change the rheological characteristics of dough which improves the bread quality as well as its nutritive value.

Further results were supported by Rakita *et al.* (2015) [15] who evaluated the influence of different climatic conditions on the activity of alpha-amylase in wheat samples and bread quality parameters. They reported that decrease in Mixo lab parameter torque C3 and specific bread loaf volume, as well as increase in the breakdown torque (C3-C4) of samples harvested in 2013 were also observed, which could be attributed to rainy weather influencing increase in alpha-amylase activity.

Conclusion

The optimum reaction temperature, pH and incubation period were 45 °C, 9.0 and 72 h giving enzyme activity of 61.35 IU. Application of amylase from *Bacillus subtilis* M13 enhanced the clarification and yield of apple and kiwi juices alongwith improving the quality of bun. Thus, potential of amylase from *Bacillus subtilis* M13 inhabiting spent mushroom compost can be utilised in food industry.

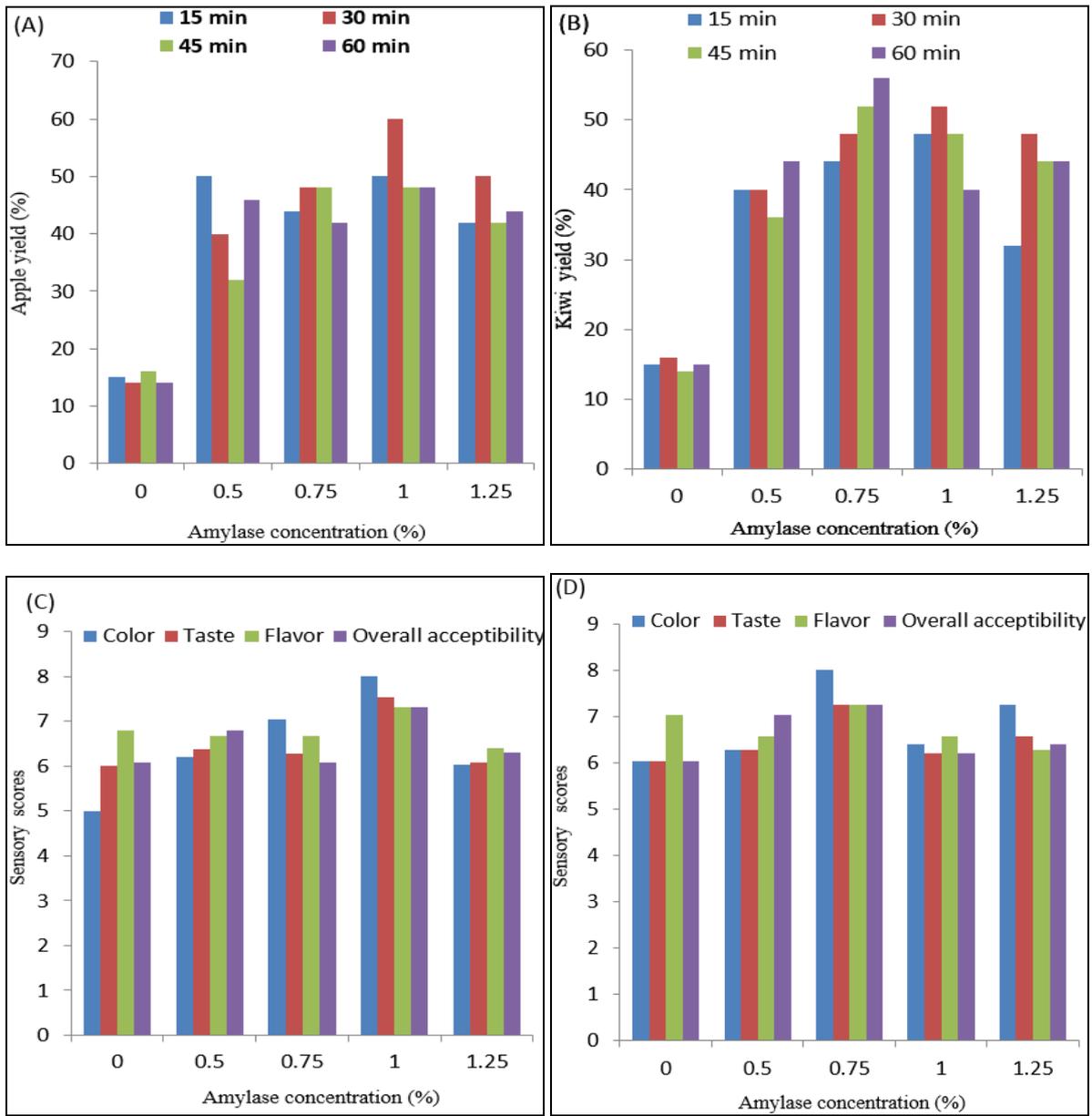
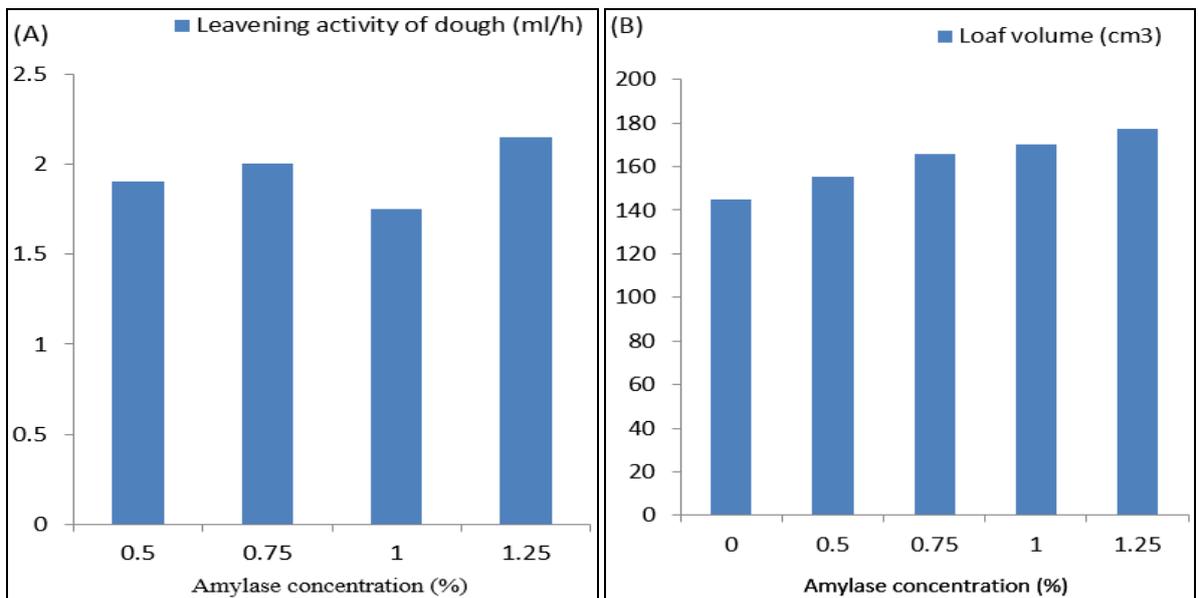


Fig 1: Effect of amylase concentration (%) on (A) Apple yield (%), (B) Kiwi yield (%), (C) Sensory characteristics of apple and (D) Sensory characteristics of kiwi



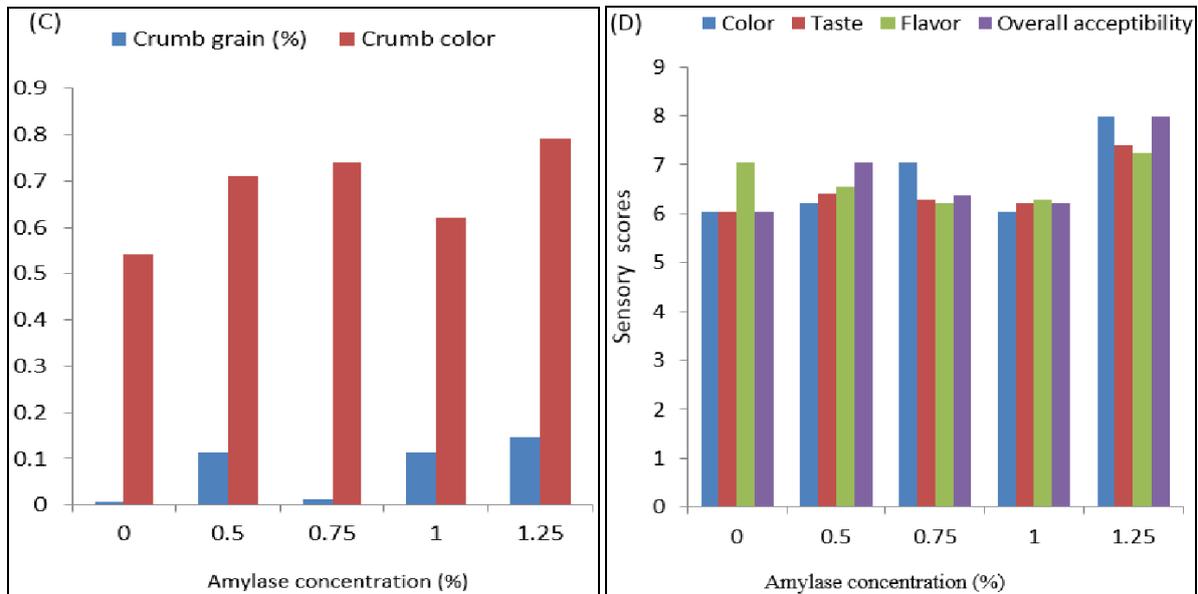


Fig 2: Effect of amylase concentration (%) on (A) Leavening activity of dough (ml/h), (B) Loaf volume (cm³), (C) Crumb grain (%) and crumb color, (D) Sensory characteristics of bread

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