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COMPARATIVE STUDY ON THE NOVEL AMYLASES PURIFIED FROM APPLE AND ORANGE SEEDS

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Abstract

Amylases are starch-digesting enzymes that are naturally present in seeds, fruits, tubers, roots, stem and pith of all plants. The present study is focused on the comparative studies on the α-amylase from apple and orange seeds to seek the better source. The enzyme extract was subjected to 40-60% salt precipitation. Further purification was carried out by traditional chromatographic procedures. The crude extracts obtained from apple and orange seeds presented the activities of 4.43 and 3.48 U mL^{-1} respectively. The purification through ion exchange and size exclusion chromatography resulted in an increase in specific activity for the apple source. The fold purification for the enzyme was 257.78 and 11.17 from apple and orange seeds, respectively. The study concluded that apple seeds are better source for purified α-amylase as compared to orange seeds. This work will also help to explore the natural sources like seeds for the isolation and purification of enzymes.

Introduction

Amylases are one of the most widely distributed groups of enzymes in nature. These are capable of attacking starch and breaking either every glycosidic bond to produce solely alpha-glucose or every alternate bond to produce maltose. Starch is one of the nature's major energy storage compounds found in seeds, fruits tubers, roots, stem and pith of all plants notably in corn, potatoes, wheat, rice and food stuff (Hanery & Furtado, 1999). During cereal seed germination, α-amylase in the aleurone layer plays an important role in hydrolyzing the endosperm starch into metabolizable sugars (Murtaza & Asghar, 2012). Thus provides energy for the growth of roots and shoots (Beck & Ziegler, 1989). α-Amylase is utilized to hydrolyze the glycogen, the reserved food material in the animals and starch in the plants during seed germination (Niaz *et al.,* 2010).

Amylases are among the most important enzymes and are of great significance in present-day biotechnology. They can be derived from several sources, such as plants, animals and microorganisms (Malik *et al.,* 2011). Plant derived amylases could be potentially useful in the pharmaceutical and fine chemical industries if enzymes with certain suitable properties could be prepared. With the advent of new frontiers in biotechnology, the spectrum of amylase application has widened too many fields. These include clinical, medicinal and analytical chemistry as well as their widespread application in starch saccharification and in the textile, food, brewing and distilling industries (Haq *et al*., 2012; Abdulla *et al*., 2013).

In starch processing and bread making amylases are the most extensively used hydrolytic enzymes. As a pharmaceutical aid for the treatment of digestive disorders, the enzyme was firstly used in 1984. In present scenario, amylases are applied in most of known industrial practices, such as food, detergent, textile and paper industry. In these sectors the demand for this versatile enzyme will always remain evergreen (Pandey, 2006). That is why α-amylase has a major world market share of the enzymes (Aehle & Misset, 1999).

Although amylases from different sources have been studied extensively including fruits and their pulp. A limited work is reported on the enzyme from the seeds of fruits and vegetables. The present study reports the comparative overview of the purification of α-amylase from apple and orange seeds, where no significant work has been done on seeds.

Materials and Methods

Preparation of crude extract: Apple and orange seeds were procured from the local market. Fifty gram of each was subjected to blend in 200mL distilled water. The crude extract was filtered by Whatman filter paper No. 1 and centrifuged for 15 min. at 10,000 rpm at 4ºC (Bernfeld, 1955).

Purification by salt extraction method: For salt extraction ammonium sulphate at 40 to 60% was used for the α-amylase. The crude enzyme was 38.8g mL^{-1} ammonium sulfate was added to achieve 40% saturation of the solution. After centrifugation the supernatant were added with 15.4g more salt for the 60% saturation level. The sediments after centrifugation at 4ºC were further used (Kanwal *et al.,* 2004; Iqbal *et al.,* 2011). The precipitated sediments of the enzymes were dialyzed for the removal of salt using phosphate buffer (pH 6.9) as the dialysis medium.

Purification by Chromatographic methods: DEAE cellulose column was prepared for the ion exchange chromatography. The column was activated and equilibrated. 0.5mL of the desalted enzyme sample was applied on the column. The elution was carried out using a range of buffers having different pH. A total of 50 fractions each having a volume of 2mL were collected. The samples were analyzed on spectrophotometer for the enzyme assay and proteins contents determination (Kanwal *et al*., 2004; Ammar *et al.,* 2002).

The purified fraction by DEAE-cellulose column chromatography was further subjected to sephadex-G-100 column. Thirty fractions (2mL) were collected and analyzed for the enzyme assay and protein contents (Ahmed *et al*., 2011; Zia *et al*., 2012).

Enzyme and protein assay: Activity for α-amylase is expressed in terms of ceralpha units that is the only procedure which employs a well-defined substrate i.e., end-blocked *p*-nitrophenyl maltoheptaoside. One unit of the enzyme is defined as the "amount of enzyme determining 1mL of soluble starch in 1 minute. The activity was determined by DNS reagent using 1% starch solution as a substrate for the enzyme. Protein contents were determined by using freshly prepared Biuret reagent which was stored at 4ºC (Gornall *et al*., 1949; Iftikhar *et al*., 2011).

Results and Discussion

It has been reported that the amylase activity decreases with ripening of fruits. It is affected by gibrellic acid synthesis that inhibits the α-amylase activity. Kanwal *et al*., (2004) reported that same process occurs in seeds during maturation. A notable work has been reported on juicy pulp extracted from different varieties of fruits but very little work has been performed on seeds of fresh fruits like apple and orange. Here, we report the study containing the comparison on α-amyalse from apple and orange seeds.

All the purification steps were investigated for the enzyme activity and specific activity. Crude enzyme preparation from apple and orange seeds attained 4.43 and 3.48 UmL^{-1} activity respectively. The specific activity for both preparations was 0.9 and 1.37 Umg^{-1} (Fig. 1). Kanwal *et al*., (2004) reported the 0.78 and 0.95 Umg^{-1} specific activity of the α-amylase in crude extract from orange and apple pulp respectively. While from the germinating beans, 0.75 Umg^{-1} of specific activity of amylase has been reported (Mar *et al*., 2003). From the shoots of pea the activity was observed to be 1.175 UmL^{-1} (Beers and Stanley, 1990).

The saturation of 40% with ammonium sulfate had specific activity of 1.56 Umg^{-1} in supernatant while 0.35 Umg^{-1} in sediments of apple seeds. This was subjected to 60% ammonium sulfate saturation and the resultant specific activity was 2.9 Umg^{-1} and 8.4 Umg^{-1} in supernatant and sediments of apple seeds, respectively. While for the enzyme from orange seeds, supernatant and sediments after 60 % saturation attained 0.339 and 3.22 Umg^{-1} specific activity. The desalted enzyme from apple seeds attained the specific activity of 13.87, while from orange seeds it was 6.8 Umg^{-1}. The results indicate that apple seeds have greater enzyme levels as compared to the orange seeds, while the pulp presented lesser specific activity as reported by Kanwal *et al*., (2004). The protein content during purification of enzyme decreased stepwise but the specific activity increased progressively. These findings are in agreement with the previous workers. They reported progressive loss of enzyme activity after partial purification (Niaz *et al.,* 2010).

The enzyme α-amylase was further purified by traditional chromatography using DEAE-cellulose and Sephadex G-100. After the elution from DEAE-cellulose column, the enzyme from apple seeds was 208 folds purified while it was 7.37 folds purified in case of orange seeds. Specific activity for the enzyme reached to 187.2 Umg^{-1} from apple seeds (Fig. 2). After treatment on Sephadex G-100 column, specific activity was obtained to be 232 and 15.3 Umg^{-1} from apple and orange seeds respectively (Fig. 3). The enzyme was 257.78 from apple while the notably less folds were purified from orange seeds. From germinating seeds of sunflower, 131.5 folds purification of the enzyme after chromatography have been reported (Elarbi *et al*., 2009). There is a notable difference between the fold purification of the enzyme from both (apple and orange); while the sunflower seeds also show a marked difference from the above mentioned sources. Nouadri *et al*., (2010) performed the enzyme purification using Sephadex G-100 as the separating medium and obtained 104.2 Umg^{-1} of specific activity, while the enzyme was 26 folds purified.

The specific activity of apple pulp was 38.95 Umg^{-1} and activity of purified enzyme was 5.025 UmL^{-1} (Kanwal *et al*., 2004). Chang *et al*., (1995) obtained less purified and stabilized enzyme as compared to fresh fruit seeds. Nirmala & Muralikarishna, (2003) reported an α-amylase from finger miller with 31 folds purification. Prakash & Jaiswal, (2010) also reported that soya-bean seeds were 20 folds purified with 85% recovery after affinity precipitation.

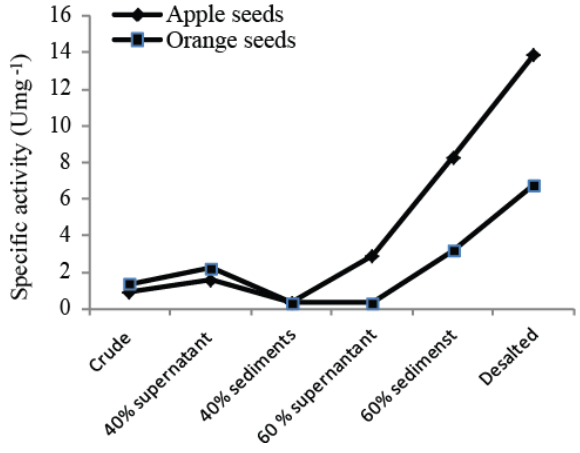

Fig. 1. A comparative analysis for the specific activity of α-amylase from apple and orange seeds after $(NH_4)_2SO_4$ treatment.

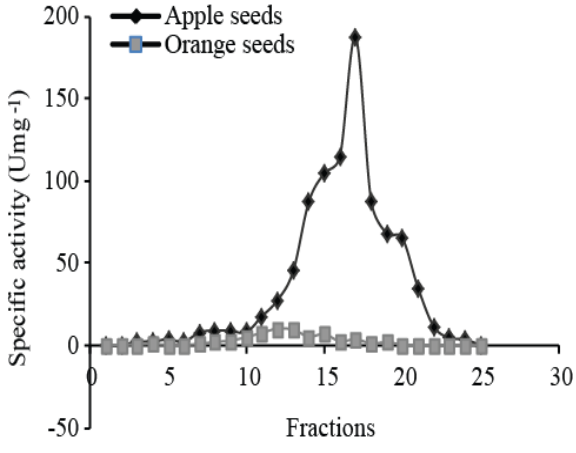

Fig. 2. Specific activity of α-amylase from apple and orange seeds after DEAE-cellulose column chromatography.

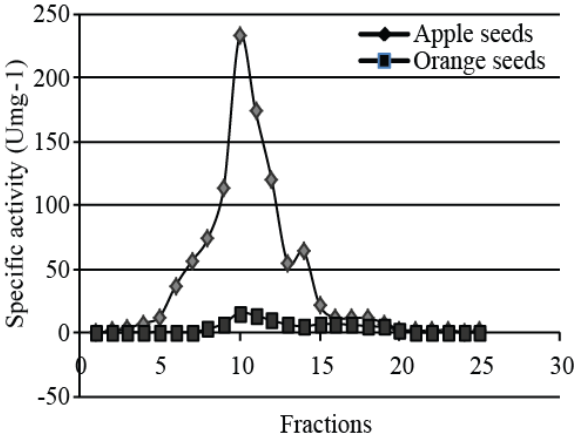

Fig. 3. Specific activity of α-amylase from apple and orange seeds after Sephadex G-100 column chromatography.

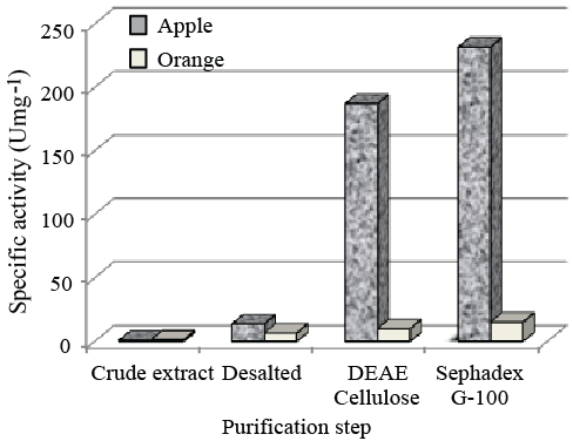

Fig. 4. A comparison of α-amylase from both sources at different purification steps.

Table 1. Summary for purification of α-amylase from apple seeds and orange seeds.

Purification steps	Activity U mL^{-1}		Protein (mg mL^{-1})	Specific activity (U mg^{-1})	Yield %	Purification fold
Crude	*AS*	4.43	4.91	0.9	100	1
	OS	3.48	2.5	1.37	100	1
Desalted	*AS*	2.22	0.16	13.87	50.1	15.41
	OS	2.04	0.3	6.8	58.6	4.96
DEAE-Cellulose	*AS*	2.06	0.01	187.2	46.5	208
	OS	1.21	0.12	10.1	34.7	7.37
Sephadex G-100	*AS*	2.32	0.01	232.0	52.3	257.78
	OS	1.53	0.1	15.3	43.9	11.17

Key: *AS*: Apple Seeds *OS*: Orange Seeds

Comparison between the apple and orange seeds for occurrence of α-amylase: Both the sources were used in same concentration and purified under similar conditions. Enzyme from apple and orange seeds obtained the activity of 4.43 and 3.48 UmL^{-1} in crude extracts, while the specific activity was 0.9 and 1.37 Umg^{-1} respectively. The protein contents of the analyte from orange seeds were less in crude extract as compared to apple seeds. Enzyme purification through salt treatment resulted in an increase in specific activity from 0.9 to 13.87 for apple seeds and 1.37 to 6.8 Umg^{-1}. Both the specific activities increased but for the analyte recovered from apple seeds were comparatively increased. After the chromatographic studies again apple seeds sample attained the higher specific activity and enzyme yield (Fig. 4 and Table. 1).

Conclusion

It is concluded from the above research article that apple seeds has the significant potential for starch processing than orange seeds. This forecasts the apple seeds to be a far better source of α-amylase as compared to other seeds and juice pulps. These could be more economical when used in place of juice or pulp of such edible fruits.

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