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Study of Some Enzymatic Activity from Two Varieties of Water Chestnuts (Trapa sp)

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ABSTRACT

The activities of some enzyme like amylase, cellulose, invertase lipase and protease were studied in locally available two varieties (green and red) of water chestnuts, an Asian aquatic fruits popular for its nutritive value and medicinal properties. All the tested enzyme activities were found slightly higher in green variety than in red variety. The amylase, cellulase, invertase, lipase and protease activities were 0.3532, 0.1922, 0.0587, 0.0234 and 0.0548 mg/ml/min, respectively in green variety and 0.2514, 0.1221, 0.0520, 0.0204 and 0.0515 mg/ml/min, respectively in red variety. From the enzyme activity assay it was found that water chestnuts might be used as a source of some enzymes such as amylase, cellulase, invertase, lipase, protease etc. These enzyme activity could be major factor for determine the nutritive and medicinal value of the water chestnuts.

Keywords: *Trapa fruit, amylase, invertase, Celulase , Lipase, protease, Enzyme activities.*

1. INTRODUCTION

Trapa bispinosa Roxb. is an annual aquatic fruit plant found in tropical, sub-tropical and temperate zone of the world. It's a starchy fruit and used as minor fruit in Bangladesh. It is the fruit plant of Trapaceae family comprising about 30 species that are distributed in tropical, subtropical and temperate zone (Daniel *et al*., 1983; Kumar *et al*., 1985; Kusum and Chandra, 1980; Mazumder, 1985; Srivastava and .Tandon, 1951). Trapa has two varieties, one of them green in color with green stem, swollen and fruit, another type is red in color with red stem, swollen and fruit. The plant has a folkloric reputation as a cure for various diseases. The acrid juice is used for diarrhoea and dysentery (Vhotracharcho, 1987) and fruit are used in aphrodisiac, astringent to the bowels, leprosy, inflammations, urinary discharges, fractures, sore throat, bronchitis, leucorrhoea, bad teeth and malaria (Kirtikar and Basu, 1987). It is also a drug of good reputation in Yunani and Ayurvedic medicine in Indian subcontinent, still the plant is being used by the rural people of the northern part of Bangladesh in the treatment of diarrhoea and dysentery.

Natural products play an important role in drug development programs of the pharmaceutical industry (Baker *et al*., 1995). There are hundreds of medicinal plants which have a long history of curative properties against various diseases. However, screening of plants for their activity is very essential and needs urgent attention in order to know the value of the higher plant. The screening of the plants for their biological activity is done on the basis of their chemotaxonomic investigation or ethno-botanical knowledge for a particular disease. Identification of a particular compound against a specific disease is a challenging issue. Importance of the plant lies in their biologically active principles.

Higher plants have the capacity to produce a large number of organic phyto-chemicals with complex structural diversity that is known as secondary metabolites. Some of these secondary metabolites are produced for plant's self defense (Evans *et al*., 1986).The secondary metabolites are produce by the action of enzymes, a biological catalysts, or chemicals that speed up the rate of reactions between substances without themselves being consumed in the reaction. Scientists believe there are thousands of different enzymes, many of which have yet to be discovered. It controls catalytically the vast majority of chemical reactions in living system. The striking characteristics of all enzymes are their immense catalytic power and high specificity.

Over the last 20 years, a large number of secondary metabolites from different plant species have been evaluated for their antimicrobial activity. The demand on plant-based therapeutics is increasing in both developing and developed countries due to their recognition as natural products, non-narcotic, readily biodegradable, has no adverse side-effects and availability at affordable prices. Another driving factor for the renewed interest in past 20 years has been the rapid rate of plant species

extinction. Infectious diseases account for high proportion of health problems in the developing countries (Sashi *et al*., 2003). Besides these as enzyme play an important role for the formation of secondary metabolites and degradation of starch, sucrose and ester of fatty acids.

Enzymes catalyze all aspects of cell metabolism, including the digestion of food, in which large nutrient molecules (including proteins, carbohydrates, and fats) are broken down into smaller molecules; the conservation and transformation of chemical energy; and the construction of cellular materials and components. Enzymes have many uses in addition to their natural functions in the body. Enzymes are used in the manufacture of antibiotics, beer, bread, cheese, meat tenderizers, vinegar, vitamins, and many other products.

Proteases, Amylase, Invertase, celluloase and lipase play a major role in carbohydrate metabolism and other secondary metabolism in several plant tissues (WHO, 1985 and NHMRC, 1881). Proteases are a group of enzymes that break the long chain like molecules of proteins into shorter fragments (peptides) and eventually into their components, amino acids. Proteolytic enzymes are present in bacteria and plants but are most abundant in animals. In plant during germination the rapid mobilization of storage protein in the cotyledons of seedling require the action of protease.

Amylase, an enzyme having physiological, commercial, and historical significance. Amylase is also an important industrial enzyme, is used in starch industry (Liquefection of starch for production of glucose, fructose and maltose), backed goods, brewing, paper, textiles, detergent and sugar industries (Crueger.W. and A.Gruger, 1990). Starch is the major component of most of the world's crop yield and the degradation of starch is essential in the germination of these plants (Yoshiki and Yamasaki, 2003). Starch degradation in seeds requires the action of α-amylase and β-amylase.

Invertase, which hydrolyzes sucrose into glucose and fructose, occurs in many plants and microorganisms. The expression and distribution of plant invertases has been especially well documented, because these are considered to play an important role in sugar metabolism (Kastle, J. H. and M. E. Clark, 1963; Krishan *et al*,1985*)*.

Lipases constitute an important group of enzymes since they are associated with fat metabolism as well as with fat digestion. Lipases are versatile enzyme that catalyze the hydrolysis of ester linkage, primarily in neutral lipid such as triglycerids and are widely distributed in various animals, plants and microogranisms (L. Sarda and P. Desnuelle, 1957) During germination the rapid mobilization of storage triacylglycerols (TAGs) in the cotyledons of seedling require the action of lipases (E. D. Wills, 1965).

Although water chestnut have pharmaceutical and nutritive value (Kirtikar and Basu 1994). But some limited work of enzyme activity have been done on water chestnut. Therefore in this present study, we focused on the study of some enzymatic activity from the two varieties (green and red) of water chestnut.

2. MATERIALS AND METHODS

2.1 Preparation of Crude Enzyme Extract

At first 10 g of water chestnuts were cut into small pieces and pasted in a mortar with pestle and then homogenized well with Pre-cold buffers of respective pH (for amylase: 0.1 M phosphate buffer pH 6.7and for invertase pH 7.0, for cellulase, citrate buffer, pH 5.0 and for protease 0.1M phosphate buffer, pH 7). The homogenate was filtered through a double layer of muslin cloth. After centrifugation at 6,000 rpm for 10 min the supernatant was used as crude enzyme extract.

2.2 Assay of Amylase Activity

Amylase activity was assayed following the method as described in laboratory Manual in Biochemistry (Jayaraman, 1981).

2.3 Assay of Cellulase Activity

Cellulase activity was assayed following the method as described in Biochemical Methods for Agricultural sciences (Sadasivam S and Manickam A, 1992)

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2.4 Assay of Invertase Activity

Invertase activity was assayed following the modified method as described in methods in physiological Plant Pathology (Mahadevan and Sridhar, 1982). Sucrose was used as substrate. The invertase activity was measured by estimating the release of glucose. The amount of glucose released was calculated from the standard curve (data not shown) prepared with glucose. The enzyme activity was measured as amount of glucose released per minute per ml of crude enzyme extract.

2.5 Procedure

Three sets of experiments (Blank, Control and Sample) were performed for the measurement of invertase activity. The following different solutions were taken in different test tubes.

The contents in the test tubes were mixed uniformly and the test tubes were incubated in a water bath at 37°C for 10 min. Then 0.5 ml of crude enzyme extract and 0.5 ml of distilled water were added to the sample and control tubes respectively, whereas 1 ml of distilled water was added to the blank test tube. Immediately after the

addition of crude enzyme extract and distilled water, 0.5 ml of 2 N NaOH was added to the control tube.

The rest of the test tubes were incubated at 37°C for 15 min and the reaction was then stopped by the addition of 0.5 ml of 2 N NaOH. Then 0.5 ml of DNS (Dinitrosalicylic acid) reagent was mixed to all the tubes. The tubes were heated in a boiling water bath for 5 min. After cooling at room temperature the absorbance was measured at 650 nm.

2.6 Measurement of Lipase Activity

Lipase activity was assayed essentially as described by Sugihara et. al., (1984) Olive oil was used as substrate. The Lipase activity was measured by estimating the release of free fatty acids.

2.7 Measurement of Protease Activity

The protease activity was measured following the method of Kunitz. The milk protein casein was used as substrate. The activity was measured by detecting the release of amino acid (Tyrosine). The amount of tyrosine released was calculated from standard curve (data not shown) constructed with tyrosine. The enzyme activity is measured as amount of tyrosine released per minute per ml of crude enzyme extract (Abser, N., and Funatzu, G 1984).

3. RESULTS AND DISCUSSION

3.1 Activity of Amylase in Water Chestnuts (Trapa Sp.)

Amylase, an enzyme having physiological, commercial, and historical significance. Amylase rapidly breaking down large molecules into smaller units. Payven, A. et al, 1883 were the first to become aware of enzymatic starch hydrolysis, they found that malt extract converted starch to sugar..

Starch are the stored form of energy, with the help of the enzyme amylase, must first break down the polymer into smaller soluble sugars, which are eventually converted to the individual basic glucose units. So, amylase activity measurement is essential for the determination of nutritive value of different varieties of water chest nuts. The amylase activity was measured by estimating the release of maltose. The amount of maltose released was calculated from the standard curve (data not shown) prepared with maltose. 1% starch solution was used as substrate. The enzyme activity is measured as amount of glucose release per minute per ml of crude enzyme extract.
The amylase activity of the two species of water chestnuts were shown in the table 1 and diagrammatically represent in Fig.1. The mean, SD values of the amylase activity on the it two varieties of water chest nuts were 0.3532 mg/ml/min, 0.02120 mg/ml/min and 0.2514mg/ml/min, 0.0219 respectively. In this analyses, it is indicated that both the mean and SD values of the amylase activity in the Green and Red varieties of water chestnut were significant ($p< 0.05$). Figure 1 represented that the amylase activity is higher in Green variety (0.3532 mg/ml/min) and lower in red variety (0.2514 mg/ml/min). The highest amylase activity on Green variety could be due to the highest content of starch in Green variety of water chest nut. Afsana et al mentioned that Green content highest content of Starch & sugar as compare to Red variety of water chest nut. The figure 1 also represent that the amylase activity was highest on the both varieties of water chest nut as compare to other enzyme activities. This highest activity of amylase on water chest nut is due to high contents starch in water chest nuts.

3.2 Activity of Cellulase in Water Chestnuts (Trapa Sp)

Cellulolytic enzymes are group of hydrolytic enzymes (cellulases) capable of hydrolyzing cellulose to glucose. Many plant pathogens are also known to produce either adaptively proteolytic, cellulolytic, and various polysaccharides (Wood. R.K.S, 1960). They are used to perform various functions including removing cell walls or crude fiber to release valuable components (flavors, enzymes, polysaccharides and other proteins) from plant cells to improve nutritional value of animal feeds or to prepare plant protoplast for genetic research (Mandels, M 1985).

The activity of cellulase was observed in both of green and red varieties of chestnuts, which shown in table 1 and Fig. 1. Table 1 indicated the mean, SD values of the cellulase activity on the Green and Red varieties of water chest nut \were 0.1922 mg/ml/min, 0.00286 mg/ml/min and 0.1221mg/ml/min, 0.0023mg/ml/min respectively. The p-values of this test were significant. In the present study a significantly higher cellulase activity was found in Green variety as compared to Red variety (shown in Fig. 1). The figure 1 also represents that the cellulase activity was significant as compare to other enzymes activities.

3.3 Activity of Invertase in Water Chestnuts

The enzyme occurs widely in plant, microbial and animal sources. Invertase plays an important role in the hydrolysis of sucrose to glucose and fructose in higher plants, especially in the storage organs. Sucrose in an early product of photosynthetic reaction and is the most abundant transportable free carbohydrate in the plant kingdom. Sucrose serves as an important carbohydrate in plants, especially in such storage organs as tuber, root and seed, sucrose is a readily degradation source of energy (Klysov, A.A. 1990).

Table 1 represent the mean and SD activity of invertase on Green and Red varieties of water chestnuts were 0.0587mg/ml/min, 0.0007mg/ml/min and 0.0520 mg/ml/min, 0.0008mg/ml/min respectively. The results suggesting that the invertase activity is significant ($p<0.05$) in both the Green and Red varieties of water chest nuts. Figure 1 indicates that the invertase activity is slightly higher in Green variety as compared to Red variety. This result implies that the activity of invertase is higher in Green variety as compare to Red due to high content of sucrose in Green variety (Omar M Sc. thesis 2010 unpublished data). The figure 1 also indicates that the invertase activity was moderate in both varieties of water chest nut as compare to other enzymes activities.

3.4 Activity of Lipase in Water Chestnut

Lipases or lipolytic enzymes catalyzes the hydrolysis of fats as well as esters of fatty acids with alcohols (Desnuelle, 1972., McCrae, 1983., Verger, 1984., Huang, 1984).

Lipases are versatile enzyme that catalyze the hydrolysis of ester linkage, primarily in neutral lipid such as triglycerids and are widely distributed in various animals, plants and microogranisms. In this present study were carried out to observe the lipase activity on the two varieties of water chestnut.

The results obtained in this analyses, which shown in table 1 indicates that the mean and SD values of lipase activities in Green and Red varieties of Water Chestnuts were found to be 0.0234 mg/ml/min, 0,0009 mg/ml/min and 0.0204 mg/ml/min, 0.0062 mg/ml/min respectively. The p- values of the lipase activity on the two varieties were apposing to significant. In our previous studied we observed that Green variety contents highest amount of total lipid as compare to Red variety. Therefore our present result indicated that lipase activity might be slightly higher in Green variety of water chest nut (shown in Fig.1). Figure 1 also represents the lowest activity of lipase on both varieties of water chest nut as compare to other enzymes like, amylase, cellulase, invertase and protease. Therefore this result reveal that the lowest contents of lipid in water chest nut as compare to carbohydrates, sucrose and protein contents (Alfsana et al 2011).

3.5 Activity of Protease in Water Chestnut

Proteins play an important role in the whole development of water chestnuts. Protein content rather plant size may be main factor influencing seedling development. Proteases hydrolyze peptide bonds in polypeptides into amino acid. The most well characterized proteases are associated with seed germination, and are employed to mobilize stored reserve to provide amino acids and amides for embryogenesis and/or early seedling development.

Table 1 indicate the mean and SD values of protease activities on Green and Red varieties of water chestnuts were 0.0548 mg/ml/min, 0.0014mg/ml/min and 0.0515mg/ml/min, 0.0005 mg/ml/min , respectively. Figure 1 represents that the protease activity on the Green variety of water chestnut is slightly higher as compare to Red variety of water chest nut. The graphical observation from figure 1 also represent that the moderate activities of protease were seen in both Green and Red varieties of water chest nuts as compare to amylase, cellulose, invertase and lipase. The highest activity of the protease on Green variety might be the highest amount of total water soluble protein content in the Green variety of water chest nut (Alfasane *et al*.2011).

The enzyme amylase, cellulase, invertase, lipase and protease activity were measured in the two varieties of water chest nut as evidenced that all the enzymes activity were higher in Green variety as compared to the Red variety. This finding indicated that enzyme activity is high due to highest amount of nutrient such starch, fibres, sucrose, total lipid and water soluble protein content in Green variety as compare to Red variety of water chest nut. Therefore, the enzyme activity is higher in Green variety of water chest nut. This finding could useful for choosing the high quality of water chest nut during its processing for food or medicinal use.

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FIGURE LEGENDS

Fig.1: The enzymes amylase, cellulase, invertase, lipase and protease activities on two varitieties of water chest nut. The Green bar indicates the Green variety of water chest nut and the Red bar indicates the Red variety of water chest nut. The longest bar indicates the highest amylase activity both in Green and Red varieties of water chest nut. The middle bar also indicates the significant and moderate activities of Cellulase, invertase and protease on the Green and Red varieties of water

chest nut. The lowest bar represents the less activity less activity of lipase on both the Green and Red varieties of water chest nut.

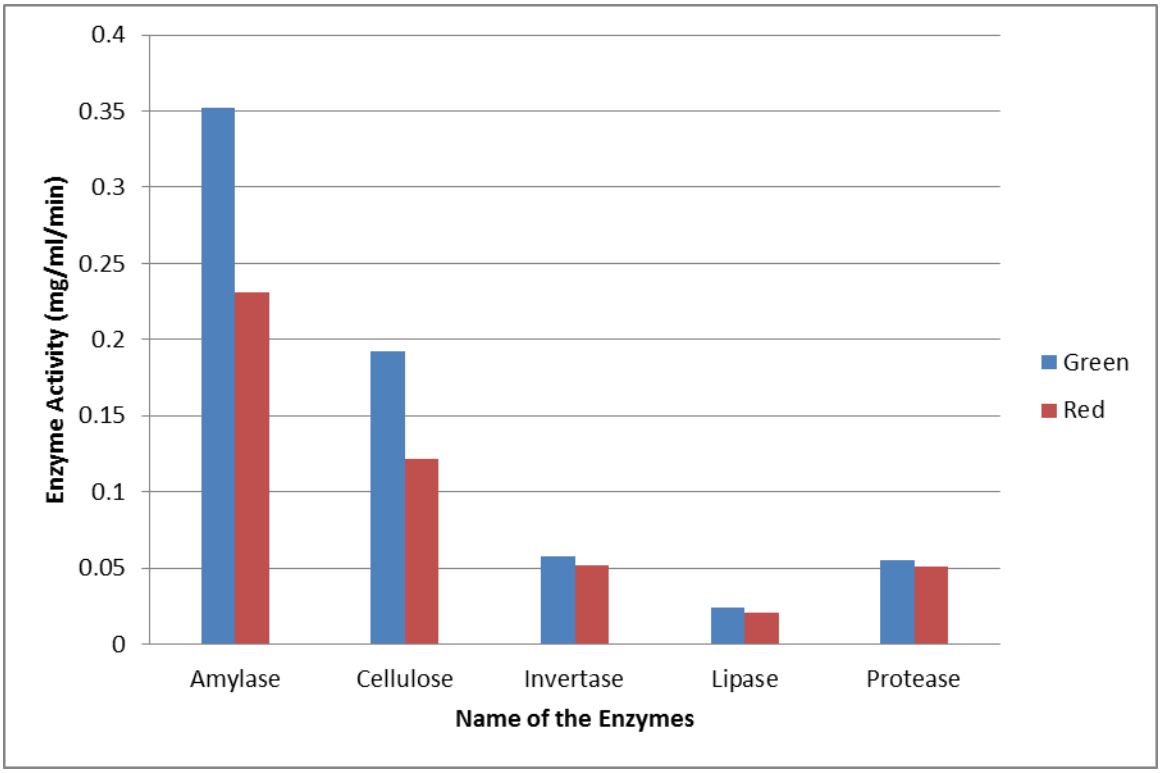

Figure1: Amin et al. 2013

Table-1: **Comparative means of different enzymes activities of green and red water chest nuts**

	Green (mg/ml/min)		Red (mg/ml/min)		p-value
	Mean	SD	Mean	SD	
Amylase*	0.3532	0.02120	0.2514	0.0219	0.000
Cellulase*	0.1922	0.00286	0.1221	0.0023	0.000
Invertase*	0.0587	0.0007	0.0520	0.0008	0.000
Lipase	0.0234	0.0009	0.0204	0.0062	0.082
Protease*	0.0548	0.0014	0.0515	0.0005	0.000

*Values are significant at $p<0.05$