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RESEARCH ARTICLE

Extraction, Purification and Characterization of Proteolytic Enzyme from Fig (*Ficus carica*) and Kachri (*Cucumis trigonus*)

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Abstract

Fig (*Ficus carica*) and Kachri (*Cucumis trigonus*) contain high concentration of proteolytic enzymes. In current research work, purification and characterization of papain (a proteolytic enzyme) from Kachri (*Cucumis trigonus*) and Fig (*Ficus carica*) was carried out. Kachri and Fig was crushed separately in a food blender by using 0.1M $(PO_4)^{-3}$ buffer of pH 8.00, distilled water and 70% ethanol. Liquid mixture obtained was centrifuged and supernatant used as enzyme source. Purification of papain was carried out by Ammonium Sulphate precipitation and dialysis followed by Gel filtration by Sephadex G-50. Then characterization of papain such as protein estimation, determination of proteolytic activity (International Unit) of enzyme and SDS-PAGE analysis were performed to determined molecular weight. Finally, the yield and proteolytic activity of papain was measured and compared with the commercial products. Crude preparation of enzyme has a wide specificity due to the presence of various proteinase and peptidase isozymes. These enzymes being present in natural fruits were free from any toxic effects and consequently can be used in food and pharmaceutical industries.

Key words: Kachri, Fig, Papain, SDS-PAGE, Sephadex G-50, Isozymes

Introduction

Plant proteases engaged for cheese production in different areas of the world include papain, bromelain, ficin, oryzasin, cucumisin, sodom apple protease and lettuce protease extracted from *Carica papaya, Ananas comosus, Ficus carica. Oriza sativa, Cucumis melo sp., Calotropis procera and Lactuca sativa* respectively. Due to immense improvements in enzyme biotechnology, stability and high activity of enzymes are maintained in various processes. (Uchikoba, 1996). Since the recent trends of developing technologies, proteases have been shown to have widespread applications in leather industry and in pharmaceutics for debrisment of wounds (Slim et al, 2009; Sjodahl et al, 2002). Method of crystallization of papain was established by Monti et al. (2000). The fruit of kachri a melon variety fruit is available throughout the drier upland tracts of India, West Pakistan, Afghanistan and Persia. Among the widely used plant proteolytic enzymes cucumin, which is obtained from kachri has been reported to have proteolytic activity and coarsely ground dried fruits of kachri are traditionally used as a food tenderizing agent (Hajjatullah and Baloch, 1970).

Crude papain has a wide specificity due to the presence of different peptidase isozymes and proteases. The enzyme activity is dependent on the plant source, conditions for growth, isolation and purification methods (Asakura et al, 1997). In the healthy fruit, enzyme found is always more active. Papain, the enzyme which is responsible for the clotting of milk, was found in papaya latex (*Carica papaya).* Latex is rich in proteolytic enzymes

commercially called papain. It is extracted from the latex of unripe papaya fruits, which are grown in subtropical areas of west and central Africa and Asia (Tanzania, Uganda, Zaire, Sri Lanka, Thailand and India). It is extensively used in industries as a meat tenderizer and in pharmaceutics, detergents, veterinary and food industries (Monti et al, 2000).

Papain activity towards the deprivation of Levetir acetam and Granisetron HCl drugs which are showing lethal effect on human cellular system. Papain enzyme breaks peptide bonds by deprotonation of Cys-25 by His-159. The sulfhydryl group on Cys-25 often forms covalent bonds with substrates. His-159 supports Cys-25, and while Arg-175 keeps histidine-159 in its stabilized imidazole form (Kim et al, 2004). Both histidine-159 and cysteine-25 involve in the actual catalytic mechanism. Cys-25 acts as a nucleophile and it attacks on the carbonyl carbon of a polypeptide. So amino terminal of the peptide become free, and forms a complex covalent (acyl-enzyme) intermediate (Khanna et al. 2007). Water de-acylate and releases the carboxyl terminal part of the peptide and it may be combined in papain enzyme that's why lethal effect of drug molecule may be reduced on living organ system (Hitesh et al, 2012).

Chymopapain is a polypeptide consists of 218 amino acids. It has significant structural similarity with papaya proteinase and papain, as well as conservation of the catalytic site of the disulfide bonding. Chymopapain like papaya proteinase has four extra residues at position 168 and 169, but differs from composition of its subset S2, in addition to having a second thiol group, Cys-117 (David et al, 1974).

Materials and Methods

Preparation of Crude Papain Extract

The *Ficus carica* and *Cucumis trigonus* fruits were washed with distilled water and air dried. 80g sample of each Kachri and Fig was taken and crushed separately in a food blender using 400mL of 0.1M $(PO_4)^{-3}$ buffer of pH 8.00, 400mL distilled water and 400mL 70% ethanol. The fluid obtained after each extraction was centrifuged for 30 min at 4^0C. The supernatant obtained (Crude Papain Extract) was used as enzyme source. Protein concentration of each crude extract was determined by using the Bradford's method.

Standard Curve for Protein Estimation

Standard curve for protein estimation was obtained by using different concentrations of casein as standard and concentrations were determined with the help of UV spectrophotometer at 280nm.

Crude Papain Assay for Determination of Proteolytic Activity

After extraction of crude papain enzyme, its proteolytic activity was determined through casein digestion in 0.1M PO_4^{-3}buffer of pH-8, 0.1M EDTA and 0.03M cystein by incubating at 37^0C for 30 minutes and trichloroacetic acid added to stop the reaction. Both sample and standard were centrifuged and pallets were discarded, 1mL of supernatant was diluted to 10mL by adding 0.01M $(PO_4)^{-3}$ buffer of pH 8 and absorbance was measured at 280nm spectrophotometrically. Supernatant was used for protein content determination as tyrosine produced in the supernatant after casein hydrolysis by papain. One unit of enzyme is defined as the amount which transforms one micromole of substrate into its product in one minute.

Purification of papain

Ammonium Sulphate Precipitation

Crude extract of papain was subjected up to 80% saturations of ammonium sulphate. All the saturations were allowed to stand overnight in refrigerator. After overnight the solutions were centrifuged for 30 minutes at 4^0C. The supernatant was then separated from the pallet and studied both supernatants and pallets separately for proteolytic activity assay of papain enzyme. Ammonium sulphate was removed by dialysis (Monti et al, 2000).

Purification and Molecular Weight Determination

Papain purification was done by gel filtration column chromatography through sephadex G-50 and equilibrated with a 0.01M sodium

phosphate buffer, 1mM EDTA at pH 8.00 (Azarkan et al, 2003). Flow rate applied was 0.5mL/min and each fraction was collected separately of Kachri (*Cucumis trigonus*) and Fig (*Ficus carica*). The fraction presenting casein hydrolysis was applied to SDS-PAGE for molecular weight determination by using protein ladder (Laemmli et al, 1970).

Results and Discussion

Standard Curve for Protein Estimation

Standard solutions of different concentrations were prepared and their absorbance was taken at 280nm by using spectrophotometer after proteolytic activity assay and used for estimation of protein in crude extracts.

Determination of Amount of Protein (mg/mL) and Enzyme Activity (IU/mg) in Crude Extract from Kachri

The amount of protein as 2.45, 2.25 and 1.60 mg/mL and enzyme activity of 3.38, 2.20 and 1.03 IU/mg with 70% ethanol, $(PO_4)^{-3}$ buffer and distilled water respectively. Maximum activity and protein was observed in sample with 70% ethanol, so sample with 70% ethanol used for further analysis. These results indicate the presence of protein in Kachri fruit and can be used as natural source of papain in food industry and pharmaceutical industry.

Determination of Amount of Protein (mg/mL) in Crude Extract from Fig (*Ficus carica)*

The amount of protein as 3.00, 2.85 and 2.55 mg/mL and enzyme activity (IU/mg) 5.54, 3.62 and 2.67 IU/mg with 70% ethanol, $(PO_4)^{-3}$ buffer and distilled water respectively. Maximum enzyme activity and protein was observed in sample with 70% ethanol, so sample with 70% ethanol used for further analysis. These results indicate the presence of high concentration papain in *Ficus carica* fruit.

Comparison of Enzyme Activity (IU/mg) of Supernatants Obtained from Kachri (*Cucumis trigonus*) and Fig (*Ficus carica*) with 70% Ethanol.

When compared the enzyme activity (IU/mg) of supernatants from Kachri (*Cucumis trigonus*) and Fig (*Ficus carica*) with ethanol (70%), obtained after ammonium sulphate precipitation, it was observed that maximum enzyme activity was obtained in Fig (*Ficus carica*).

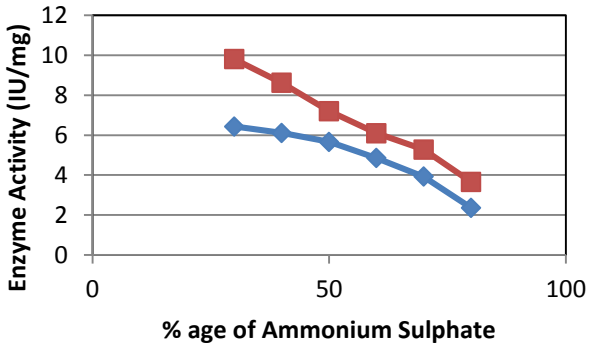

Figure 1: Comparison of enzyme activity (IU/mg) of supernatants obtained from Kachri (*Cucumis trigonus*) and Fig (*Ficus carica*)

Comparison of Enzyme Activity (IU/mg) of Pellets from Kachri (*Cucumis trigonus*) and Fig (*Ficus carica*) with Ethanol (70%)

When compared the enzyme activity (IU/mg) of pellets from Kachri (*Cucumis trigonus*) and Fig (*Ficus carica*) with ethanol (70 %), obtained after ammonium sulphate precipitation, it was observed that maximum enzyme activity was obtained in Fig (*Ficus carica*).

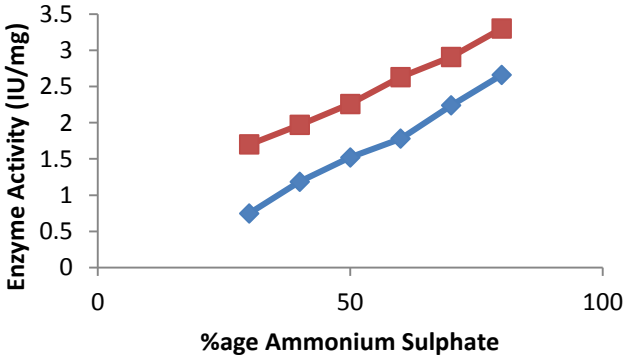

Figure 2: Comparison of enzyme activity (IU/mg) of pellets obtained from Kachri (*Cucumis trigonus*) and Fig (*Ficus carica*)

Gel Filtration through Sephadex G-50

Proteolytic activity of different fractions of Fig (*Ficus carica)* and Kachri (*Cucumis trigonus*) with 70% ethanol was determined by casein digestion. The maximum activity was observed in fraction 5 of Kachri (*Cucumis trigonus)* and fraction

7 of Fig (*Ficus carica).* The highest amount of protein (mg/mL) in Fig and Kachri samples was found 3.891 in fraction 7 of Fig and 3.560 in fraction 5 of Kachri sample respectively.

SDS-PAGE Analysis

For the characterization of papain enzyme on the basis of molecular size, SDS-PAGE analysis was carried out. The number of bands obtained in column 4 and 5 was found higher in case of crude extract of papain showing more impurity in crude sample of Fig and Kachri. Column 2 and 3 showed single band in each, because proteins other than papain were removed. It was noticed that all the five columns showed bands below than 29 KDa which is most probably the band of papain showing its molecular weight less than 29 KDa that is approximately 23 KDa as reported in the literature (Slim A, 2009).

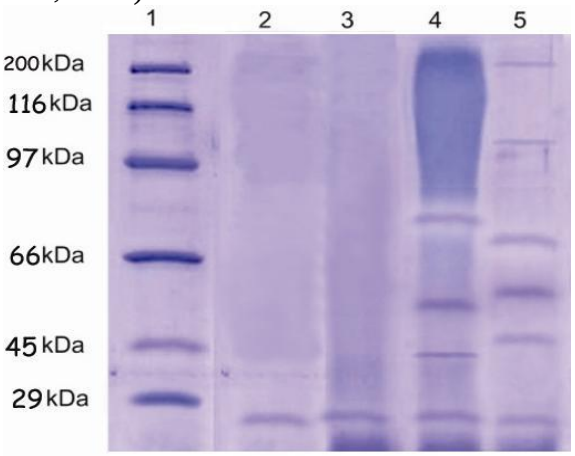

Figure 3: Different fractions of Gel filtration of papain
1= Molecular marker SDS6H2 Sigma
2= Fraction of column chromatography (Kachri sample)
3= Fraction of column chromatography (Fig sample)
4= Crude Fig sample
5= Crude Kachri sample

References

Asakura T, Watanabe H, Abe K, Arai S. 1997. Oryzasin as an aspartic proteinase occurring in rice seeds: purification, characterization and application to milk clotting. J. Agri. Food Chem 45:1070-1075.

Azarkan M, Moussaoui AE, Van Wuytswinkel D, Dehon G, Looze Y. 2003. Fractionation and purification of the enzymes stored in the latex of *Carica papaya*. J.Chrom B: 790: 229-38.

David E, Burke, Sidney D, Lewis Jules AS. 1974. A two-step procedure for purification of papain from Extract of papaya latex. Arch biochem and biophys 164:30-36.

Hitesh, Bhoi MN, Borad MA, Dalvadi AD, Dalsania KV. 2012. Extraction and application of papain enzyme on degradation of drug patel. Int. J. Biochem. 113-115

Hujjatullah S, Baloch AK. 1970. Crystallization of proteolytic enzymes. Food Science 35:276-278.

Khanna N, Panda PC. 2007. The effect of papain on tenderization and functional properties of spending hen meat cuts. Ind. J. Anim. Res. 41:55-58.

Kim J, Chang S, Yun S. 2004. Multiple layered patches for teeth whitening. EP Patent 1:385-401.

Laemmli S, Basilio CA, Trevisan HC, Contiero J. 1970. Purification of papain from fresh latex of *Carrica papaya*. Br. Ar. Bio. Tech 43:501-507.

Monti R, Basilio CA, Trevisan HC, Contiero J. 2000. Purification of papain from fresh latex of Carrica papaya. Br. Ar. Bio. Tech 43:501-507.

Sjodahl J, Emmer A, Vincent I, Roeraade J. 2002. Characterization of Proteinase from Antartic Krill (Euphausiasuperba). Protein Expression and Purification. 26:1.

Slim A, Hiroyuki O, Nathalie B, Benjamin F, Regine L, Michel P, Frantz S, Pierre V, Frederic C. 2009. Identification and biochemical characterization of a GDSL-motif carboxylester hydrolase from *Carica papaya* latex. Biochem Biophys. Elsevier: 1048–1056.

Uchikoba T, Kaneda M. 1996. Milk-clotting activity of cucumisin, a plant serine protease from melon fruit. Applied Biochemistry and Biotechnology 56:325-330.