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Mini Review

Efficacy of selected purification techniques for bromelain

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Abstract

Bromelain is one of the vegetal proteases found in pineapple plant. It has numerous applications in food and pharmaceuticals. This review discussed different bromelain purification techniques which will assist in determining the effect of processing conditions on the purification efficacy. There are four purification techniques to be discussed, namely; reverse micellar system, aqueous two phase extraction, cation exchange chromatography and ammonium sulphate precipitation. Of the four techniques, cation exchange chromatography had shown the best bromelain purification technique with purification fold of 10.0 followed by reverse micellar system containing CTAB/ isooctane/ hexanol/ butanol, ATPE containing PEG polymer, ammonium sulphate precipitation and ATPE containing PEO-PPO-PEO with purification fold of 5.2, 4.0, 2.81 and 1.25, respectively.

Introduction

Bromelain has numerous applications in the food industry as well as in pharmaceutical industry and hence, it is desirable to obtain bromelain in its highest purified form. However, the preparation of bromelain in pure form has always proved difficult (Devakate *et al*., 2009). Thus, an effective and economically viable technique needs to be developed for the purification of bromelain (Babu *et al*., 2008). The aim of this paper is to discuss the potential application of different purification techniques for bromelain.

Bromelain

Bromelain is the name of proteolytic enzyme found in pineapple plant [*Ananas comosus* (L.) *Merr.*] (Hale *et al*., 2005; Hebbar *et al*., 2008). It is present in the stem and fruit of the pineapple. The enzyme extracted from the stem is called stem bromelain and from fruit is called fruit bromelain (Babu *et al*., 2008). Stem bromelain (EC 3.4.22.32, formerly EC 3.4.22.4) is the most abundant proteinase within bromelain preparations derived from pineapple stems. Other proteinases that are present at lesser amounts include fruit bromelain (the major proteinase present in pineapple fruit; EC 3.4.22.33, formerly EC 3.4.22.4 and 3.4.22.5) and ananain (EC 3.4.22.31) (Hale *et al*., 2005). Bromelain is also present in pineapple wastes such as core, peel, crown and leaves in relatively smaller quantities as compared to those in the stem (Hebbar *et al*., 2008). The name 'bromelain' was first applied to the fruit enzyme. Later, the term 'bromelain' was introduced and originally applied to 'any protease from any plant member of the plant family Bromeliaceae' (Rowan *et al*., 1990). 'Bromeliaceae' is a plant family whose members usually produce large amounts of proteases with no apparent function in plant growth and development (Lopez *et al*., 2000). The bromelain contains a complex mixture of thiol proteases and non-protease components. Proteases constitute the major components of bromelain and include stem bromelain (80%), fruit bromelain (10%), and ananain (5%). Among non-protease components are phosphatases, glucosidases, peroxidases, cellulases, glycoproteins and carbohydrates (Chobotova *et al*., 2009). The N-terminal sequence of bromelain is AVPQSIDWRDYGAVTSVKNQNPCGACW (Lopez *et al*., 2000). Stem bromelain presents isoelectric point at 9.5 and fruit bromelain presents isoelectric point at 4.6 (Rabelo *et al*., 2004) Bromelain can be absorbed in human intestines without

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degradation and without losing its biological activity (Chobotova *et al*., 2009). Bromelain has earned universal acceptability as a phytotherapeutical drug because of its history of safe use and zero side effects (Bhattacharryya, 2008).

Application of bromelain

Bromelain is known for its clinical applications, particularly modulation of tumor growth, blood coagulation, third degree burns, improvement of antibiotic action and anti-inflammatory properties of therapeutic value (Fernández and Pomilio, 2003). Bhattacharya (2008) reported that bromelain is a natural-inflammation enzyme, whereby it has many uses such as reduce the swelling that causes joint pain in patients of arthritis, relieving the pain, numbness, tingling and loss of motor and sensory function in fingers. Oral exposure to bromelain is safe, with N10 g kg^{-1} required for lethality in 50% of rats or mice tested. Humans have been treated orally with up to 12 g day^{-1} without major side effects (Hale *et al*., 2006). Bromelain as well has a potential as an anti-cancer agent whereby it affects major pathways and regulators implicated in cancer (Chobotova *et al*., 2009). It is also used in food processing for meat tenderization (Rohrbach *et al*., 2003) for example in decreasing toughness of coarse dry sausage (Melendo *et al*., 1996). Bromelain, the plant thiol protease affects the structure of myosin and the actin filaments of myofibrillar proteins (Shin *et al*., 2008). Besides, bromelain had also been used for brewing to solubilize grain proteins and stabilize beer (Singh *et al*., 2003), for cookie baking to improve crispiness, for production of protein hydrolysates (Vallés *et al*., 2007) and as a dietary supplement (Babu *et al*., 2008).

Efficacy of bromelain purification using different purification techniques

Reverse micellar systems

Purification of bromelain from the core of pineapple plant by reverse micellar system of Cetyltrimethylammonium bromide (CTAB)/ isooctane/ hexanol/butanol resulted in purification fold of 5.2 with the following conditions: forward extraction: aqueous phase pH 8.0; CTAB concentration of 150mM; NaCl concentration of 0.1M, backward extraction: aqueous phase pH 4.2; KBr concentration of 0.5M. Reverse micelles are surfactant-stabilized water droplets dispersed in organic solvents. Surfactant, also termed as amphiphiles are organic compounds with lipophilic and hydrophilic portions. Its advantages are no loss of native function or activity, low interfacial tension, ease of scale up, and potential for continuous operation (Hebbar *et al*., 2008). This method is of particular interest since many enzymes have been found to remain active when solubilized in these systems (Savelli *et al*., 2000). In the reversed micellar extraction process, amino acid or proteins are transferred from an aqueous feed phase to a reverse micellar organic phase by forward extraction, and subsequently to an aqueous stripping phase by backward extraction (Nishiki *et al*., 2000). Reverse micellar extraction provide the system for selective solubilization of protein whereby the solubilization is controlled by electrostatic interaction energies between the charged protein and the micelle's inner layer which can be manipulated by system's ionic strength, pH and aqueous/ organic phase ratio (Soni and Madamwar, 2000).

Aqueous two phase extraction (ATPE)

Aqueous two phase extraction is an efficient method for separation and purification of mixture of proteins or enzymes. It can remove undesirable byproducts present in the system such as unidentified polysaccharides, pigments and interfering proteins that lower the activity of enzymes. Its advantages are ease of scale-up, low cost, scope for continuous operation and environment friendly (Babu *et al*., 2008). Besides, it is also biocompatible, easily processed and has high sensitivity in the recognition of ligand–protein interactions (Bassani *et al*., 2007). These systems are suitable for purification of biological molecules as the phases contain 70-90% water, thus reducing the denaturation of labile molecules (Rabelo *et al*., 2004). These high water content systems (70–90%, w/w) have low interfacial tension, and are safe, non-toxic, non-flammable, and relatively environmentally benign as extraction media (Li *et al*., 2002). In the ATPE, the selective partitioning of the desired enzymes or proteins to one phase and contaminant proteins to the other phase, not only purify the enzymes but also concentrate them into one of the phases (Madhusudhan *et al*., 2008). The partitioning behavior of an enzyme, or protein, in ATPE is influenced by factors including the polymer molecular weight, polymer/ salt concentrations, system pH and temperature, the size and the hydrophobic/ hydrophilic nature of the biomolecule among others (Gautam and Simon, 2006).

Purification of bromelain in fruit of pineapple plant using ATPE systems containing poly (ethylene oxide) PEO-poly (propylene oxide) PPO- poly (ethylene oxide) PEO block copolymers resulting in purification factor of 1.25 with the following conditions: copolymer with 10% ethylene oxide (EO) (mm) and molecular mass of 2000g/mol, copolymer

concentration of 5% (m/m) and temperature 5˚C above and the cloud point of 25˚C, pH 6.0 and salt concentration of 15mM (Rabelo *et al*., 2004). The block copolymers PEO–PPO–PEO is a class of synthetic polymers whose properties have recently drawn the attention for the formation of new ATPE whereby the copolymers formed by three different homogeneous chains: a block of polyethylene oxide (PEO), a block of polypropylene oxide (PPO), and a symmetrical block of PEO (de Oliveira *et al*., 2007).

Purification of bromelain in fruit of pineapple plant using ATPE systems with polyethylene glycol (PEG) polymer resulting in purification factor of 4.00 with the following conditions: PEG 1500 (18%) and potassium phosphate (14%) system at pH 7.0 (Babu *et al*., 2008). PEG potassium phosphate is the most frequently used polymer-salt system but this salt lead to high phosphate concentration in the effluent streams, and therefore they are of environmental concern (Porto *et al*., 2008).

Cation exchange chromatography

Cation exchange chromatography is the most common and well-accepted methods of chromatography for the separation of protein mixtures. They offer good dynamic capacity, high specificity, scalability, consistency, relatively low cost, simple buffers, high working flow rates and a large choice of resins materials (Ng *et al*., 2009). The principle of ion exchange chromatography is that a charged analyte is bound to the stationary phase by means of electrostatic attraction. Ions in the mobile phase selectively displace components of the analyte from adsorption to the stationary phase (Swadesh, 2001). In cation exchange chromatography, the stationary phase is usually composed of resins containing sulfonic acid groups or carboxylic acid groups of negative charges and, thus, cation metallic species are attracted to the stationary phase by electrostatic interactions (Ali *et al*., 2010). Salts, or electrolytes, provide ionic strength to the eluent, which weakens the ionic interactions between the proteins and the column and leads to elution of the protein (Arakawa *et al*., 2007). Raweeritha and Ratanabanangkoon, (2003) had used cation exchange chromatography for the fractionation of equine antivenom. They found that the recovery of antibody activity was higher (65%) as compared to that achieved by ammonium sulfate precipitation (50%). The bromelain in the fruit of pineapple plant was found as much as 10-fold pure using cation exchange chromatography technique (Devakate *et al*., 2009).

Ammonium sulphate precipitation

Ammonium sulphate precipitation has been widely used to precipitate proteins in a partially purified form (Saxena *et al*., 2007). Ammonium sulphate is the most commonly used salt as it is cheap and sufficiently soluble. Ammonium sulphate concentration is usually quoted as percent saturation, assuming that the extract will dissolve the same amount of ammonium sulphate as pure water (Harris, 1989). Brovko *et al*., (1998) had used ammonium sulphate to separate proteins from phenols in cereal leaf extract. Barros *et al*., (2001) found that precipitation with ammonium sulfate is an effective way to produce substantial amounts of active proteases from the flowers of *C. cardunculus*. The bromelain in fruit of pineapple plant was 2.81-fold pure at 40-60% saturation level using ammonium sulphate precipitation (Devakate *et al*., 2009).

Conclusions

It can be concluded that out of the four purification techniques, cation exchange chromatography had shown the best purification technique for bromelain purification with purification fold of 10.0. This shows that processing conditions greatly affected the purification efficacy.

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