Review Article

FRUIT ENZYMES AND THEIR APPLICATION: A REVIEW

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ABSTRACT

Fruit refers to the edible part of the plant consisting of seeds & its covering, which includes pulp fruits, dry fruits, grains, nuts, herbs etc. Enzymes are those proteins, which acts as catalyst within the living cells. Catalysts are substances which increases the rate of reaction without being a part of it or permanently altered themselves. In this paper, we have discussed about enzyme i.e. papain, ficain, bromelain, and lipoxygenase. During the discussion, we have detailed explained about enzymes with their origin, extraction process and their application in food and other industry.

KEYWORDS: Papain; Enzymes; Fruits; Catalyst.

INTRODUCTION

The term “fruits” refers to the edible part of the plant consisting of seeds & its covering, which includes pulp fruits, dry fruits, grains, nuts, herbs etc. Consumption of fruits is important & highly nutritious for human health as they are the source of certain essential nutrients as well as phytochemicals [1].

Enzymes are those proteins, which acts as catalyst within the living cells. Catalysts are substances which changes (increases/decrease as per requirement) the rate of reaction without taking part in it or permanently altered themselves. Similar to all proteins, the structure of enzyme consists of one or more long chains of interconnected amino acids. Each enzyme composed of a unique sequential structure, made up of amino acids, and forces it to fold into a characteristic shape. This sequence of amino acid in an enzymes is determined by specific genes present in the cells nucleus. On each enzymes, an active site is present where reagents meet and react. The active site on an enzyme can only accommodate certain types of reagents and only one type of reaction can be catalysed by a specific enzyme [2]. For example, during the manufacturing of haemoglobin, the oxygen carrying green pigment in red blood cells (RBCs) becomes functional by inserting a single atom of iron at it's centre. An enzyme named ferrochelatase brings the molecules together and catalyses them. This is the only reaction catalysed by ferrochelatase.

DOI: 10.5455/ijcbr.2018.42.18

eISSN: 2395-0471
pISSN: 2521-0394

Food enzymes: Enzymes plays an important role in digestion of food, as they consists of protein would help to perform various functions in body. Certain enzymes produces in the body, while some are provided by foods. The enzymes present naturally in plants are metabolised after ingestion and are considered safe. They are important for the quality of fresh fruits i.e. growth and ripening of fruits and also maintaining the same during the transportation & storage.

Most of the enzymes are important to maintain the quality and metabolism of fruit, but some can have an undesirable effect on color, flavor, taste etc of the fruits. Enzyme lipoxygenase can alter the development of flavor & odour of certain fruits. The other enzymes which affects the flavor and odour are lipase and peroxidase. The discoloration occurs in fruits and vegetables along with negative effect on their taste and nutrition is mainly due to phenol oxidase. The fruits and vegetables contains various substances which affects their physical and chemical appearance. The presence of pectic substances affects texture; activity of pectinase present in fruits ensures the fruit softening; presence of ascorbic acid affects vitamin availability [5].

Enzymes in fruits: Most common enzymes present in fruits is Protease. Protease speed up the breakdown of proteins into smaller units amino acids. Fruit enzymes that breakdown proteins have their own uses.

Some fruit enzymes with respect of their fruits sources are listed below [6]:

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Table1. Names of Enzyme and their Sources

Fruit Enzyme	Source
Papain	Papaya
Bromelain	Pineapple Fruit & Stem
Ficain	Fig
Lipoxygenase	Soyabean

All food items are rich in protein, carbohydrate, fat, minerals, and vitamins. Enzymes are those workhorses that puts all the necessary ingredients together and convert them into substances which the body can utilise. Certain enzymes are studied in detail in this chapter.

Papain: Papain is cysteine protease enzyme, which helps in protein digestion more extensively as compared to pancreatic protease enzymes. It mainly consists of single peptide chain with three sulphide bridges and a sulfhydryl group. Among the various application of papain, the most common use in food industry is enzymatic synthesis of amino acids, peptides and other molecules [6].

Origin: This protease enzyme is isolated from the papaya latex. The latex collected after cutting the unripen papaya is dried. The more greener the fruit, the more active the enzyme. It has a crucial application in various biological processes, food and medicines. This enzyme shows extensive proteolytic activity towards proteins, short chain peptides, amino acids esters and amide links [7].

Papain has a globular structure, with 212 amino acids stabilized by three disulphide bridges in its structure and a molecular weight of 23,406 DA (1 DA = 1g/mol). Papain is a type of cysteine protease that degrade protein and is stable and active under a wide range of temperature, concentration and pH. This enzyme is highly active even at high temperature, provide resistance to higher concentration of denaturing substances and also remain active from 3 to 9 pH range [8]. Papain as a crystalline suspension is stable at 5^0C for 6-12 months. Stabilizing agents are cysteine, EDTA and dimercaptopropanol.

Papain, structurally, is stabilized by three disulphide bridges and found to be folded around these bridges, which makes an active site along these side chains for the interactions with new molecules. This strong interaction with other molecules leads to the stability of enzyme. The 3D structure of papain mainly consist of two distinct complex molecules with a cleavage between them that carries an active site over which the catalysis takes place.

Extraction Process: The raw material required for preparing papain enzyme is the white milky latex. The latex is extracted after cutting the skin of unripe papaya and then collected in a container. Also the latex coagulated on the surface of fruit is scrapped and collected in a container. The latex is then passed through a number of sieves in order to separate dust, impurities and any other extraneous material and then mixed with potassium meta bisulphate. Now, the cleaned latex is collected and spreaded over the trays for drying under vacuum conditions at a temperature of 55^0C for 4-5 hrs. The entire manufacturing process can also be described as follows:-

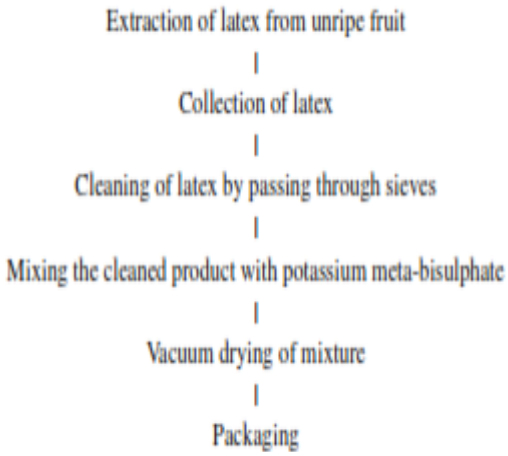

Fig 1. Extraction Process of Papain

The dried product is packed in the form of dried flakes in air tight containers and stored in cool, dry place. Dried flakes were preferred over powdered form as it would decrease the stability of papain. Papain is transported at a temperature of 20^0C. If properly stored and transported under required conditions, then its shelf life is 5-6 months [9].

Applications: There are several application of papain at different level as per the requirement. Some of them are as follows :

- It act as a debris-removing agent, with no negative effect as it acts at a specific tissue, which particularly lacks in a1- antitrypsin plasmatic antiprotease. a1-antitrypsin plasmatic antiprotease has the property to inhibit proteolysis in healthy tissue [10].
- Papain shows features similar to cysteine protease and also the folding pattern around active site that has its own use for drug manufacturing [11].
- Papain can be used as meat tenderizer. This enzyme can also be used extensively as a common ingredient in brewery [12].
- Papain act as a digestant which helps in proper digestion of protein in human body and thus, helps in preventing diseases like dyspepsia and digestive disorders and disturbances of the gastrointestinal tract [13].

Bromelain: It is a general name for a family of sulfhydryl proteolytic enzymes and is also the protease enzyme mainly obtained from the pineapple plant, mainly consists of mixture of enzymes that helps in protein digestion.

Origin: Pineapple is considered to be the main source from which bromelain is extracted. Bromelain is extracted from fruit as well as stem. The one extracted from fruit is known as fruit bromelain, while the one extracted from stem is called as stem bromelain. It consists of 212 amino acids, with a molecular weight of 33 kDA [14]. This enzyme helps in protein breakage & remain stable in pH ranges from 3 to 7 and temperature between 40^0C & 60^0C [15]. The bromelain showed a maximum activity at pH 7 and 50°C at the simple extraction and most proteolytic activity at pH 8 and 60°C [17].

Extraction Process: The complete manufacturing process in divided into 2 steps, first extraction process and second purification process. In the extraction process, pineapple is procured initially, cleaned in distilled water and then the peel is removed from the fruit. Now, the peel and fruit are to be cut into small pieces as per the requirement. Then, the fruit is grounded and a fine slurry is extracted using a mixture of ethanol, methanol & distilled water. Then, the slurry is heated up to a temperature of 55^0C and then centrifuges at 10000 rpm for 15 min and finally filtered, which is stored at a temperature of 4^0C.

In the second step of purification process, the filtered extract of enzyme is mixed with ammonium sulphate & left for about 30 min for proper mixing. Then, the mixture is transferred and centrifuged to get a supernatant, which is further passed through dialysis. During dialysis, the strips are heated at 80^0C for 10 min, followed by boiling with sodium bicarbonate for 2 min. Then, they are rinsed with distilled water to open holes of one side of membrane and leave the other side to prevent leakage. Finally, the supernatant is passed through membrane to get bromelain enzyme [16].

Application: Similar to papain, the main function bromelain is the break down and proper digestion of protein. Bromelain has various applications in food, pharmaceutical, cosmetics and other industries. Some of the applications of bromelain are as follows:

- It can be used for meat tenderization, grain protein solubilization, beer clarification, baking cookies etc.
- It has been studied and verifies that bromelain acts as enzymatic browing inhibitor in fresh apple juices [18].
- It helps to hydrolyse fish protein to generate fish protein hydrolysate.
- It also helps to treat acne, wrinkles, and dry skin [19].

Ficain: It is also known as Ficin, is a proteolytic enzyme extracted from fig, which belongs to a class of proteinases known as sulfhydral enzymes. It is extracted from the clarified latex of fig tree. It is a specific enzyme which can hydrolyze the chemical bond in natural protein, which helps in proper digestion of protein.

Ficin has good stability, and its structure and hydrolysis mechanism is much similar to that of papain and has a wide application in various sectors like food sector, healthcare etc. The process of separation and purification of raw ficin enzyme can be carried out in various ways like electrophoresis, chromatography, precipitation etc. Ficin has different applications in food, and health care industry.

Manufacturing Process: Initially, the raw extract of ficin is extracted from the latex of fig. Now, the extract is centrifuged for 30 min at 4^0C in order to separate gums and other impurities. A supernatant solution is prepared from the obtained extract by ultracentrifugation for 60 min at 4^0C. The obtained supernatant extract is mixed with 0.01M phosphate buffer (pH 7.5). Then, the prepared solution is transferred for cation exchange chromatography, where it is mixed with sodium chloride. This process helps in separating the enzyme from the remaining solution. [20]

Lipoxygenase: Lipoxygenase, also known as lipoxidase, is an enzyme widely found in plants, animals and fungi. This enzyme helps in catalyzing the reaction of oxidation of fatty acids containing cis, cis-diene units to get converted into hydroperoxidienoic compounds. Ames and King have recently identified the effect of pH on the ionic strength of the substrate medium. [21]

They are widely present in different sources, but are abundantly available in legumes (beans and peas) and potato tubers. The main polyunsaturated fatty acids in plant tissue, which are catalysed by lipoxygenase at different locations, are linoleic and linolenic acids.

Origin: Lipoxygenase is found in vegetative tissues and plays an important role in plant defence system, but it is yet not identified about their presence in vegetative tissue in large quantity. This enzyme in tissue provides hydroperoxides substrate that helps the plants in defend themselves. Rance *et. al.* claimed that lipoxygenase converts a strain of tobacco, which resists *phytophthora parasitica var nicotiana* to one that is susceptible [22]. Royo *et. al.* identified that lipoxygenase from potato leaves eliminates the production of jasmonate and/or proteinase inhibitor and thus help in reducing the susceptibility of insect attack [23].

Extraction Process: As lipoxygenase is widely available in plants, animals and fungi, thus, there are different ways to isolate and purify this enzyme from these respective sources. In this section, we will discuss the isolation and purification of lipoxygenase from wheat [24].

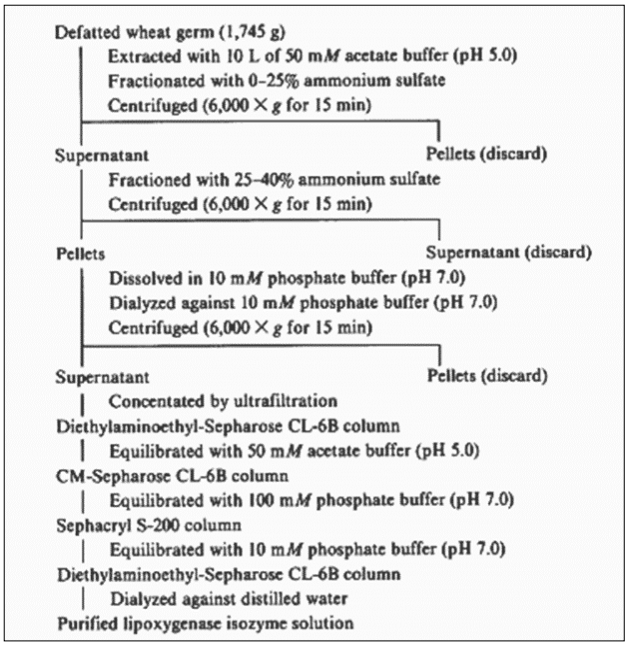

Fig 2. Process of Isolation and Purification of lipoxygenase iso enzyme from wheat germ [24]

The process involved in the isolation and purification of this enzyme is chromatography. This purified enzyme is characterized based on various parameters *i.e.* enzymatic properties like pH activity, thermal sensitivity etc., and amino acid composition.

Application: The lipoxygenase, which catalyses fatty acids into hydroperoxides, has certain application in food industry both positive as well as negative [25]. Some of them are as follows:-

- It act as an ingredient for the production of bread.
- It also act as an aroma enhancer.
- It affects color, off-flavor, and anti-oxidant properties of foods in a negative way.

CONCLUSION

During digestion process food is broken down into small parts which can be used by the body in performing various functions. It has macro molecules like fats, protein and carbohydrates which are broken down into small parts like glucose, amino acids and fatty acids. The digestive process is done right from mouth to anus. Digestive enzymes are diverse and found in the saliva secreted by salivary gland.in the stomach secreted by cells which are covering the stomach, pancreatic juice secreted by the pancreas. Irregular time of eating and fast food consumption create the problem of indigestion. In order to face these problems we need to consume those foods which are a source of these enzymes to get a natural remedy.

Conflict of interest: Nil

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How to Cite this article: Bandana Chatterjee, Anshuman Sharma. Fruit enzymes and their application: A review. *Int. j. clin. biomed. res. 2018;4(2):* 84-88.