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Review Study on Enzymes in Fruits

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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.

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. Specific genes present in the cell's nucleus determine this sequence of amino acid in enzymes. On each enzymes, an active site is present where reagents meet and react. The active site on an enzyme can only a specific enzyme [2] can catalyze accommodate certain types of reagents and only one type of reaction. For example, during the manufacturing of hemoglobin, the oxygen carrying green pigment in red blood cells (RBCs) becomes functional by inserting a single atom of iron at its centre. An enzyme named ferrochelatase brings the molecules together and catalyses them. This is the only reaction catalyzed by ferrochelatase.

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 metabolized after ingestion and are considered safe. They are important for the quality of fresh fruits i.e. growth and ripening of fruits and maintaining the same during the transportation & storage.

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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].

Table I

S.No	Fruit Enzyme	Source
1	Papain	Papaya
2	Bromelain	Pineapple Fruit & Stem
3	Ficain	Fig
4	Lipoxygenase	Soya bean

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 utilize. 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 remain active from 3 to 9 pH range [8]. Papain as a crystalline suspension is stable at 50C 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, which 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. In addition, 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 metabisulphate. Now, the cleaned latex is collected and spreaded over the trays for drying under vacuum conditions at a temperature of 550C for 4-5 hrs. The entire manufacturing process can also be described as follows

Extraction of latex from unripe fruit

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Collection of latex

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Cleaning of latex by passing through sieves

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Mixing the cleaned product with potassium meta-bisulphate

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Vacuum drying of mixture

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Packaging

The dried product is packed in the form of dried flakes in airtight 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 200C. 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

1) 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].

2) Papain shows features similar to cysteine protease and the folding pattern around active site that has its own use for drug manufacturing [11].

3) Papain can be used as meat tenderizer. This enzyme can also be used extensively as a common ingredient in brewery [12].

4) nPapain 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

Bromelain 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 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 transfered 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

1) It can be used for meat tenderization, grain protein solubilization, beer clarification, baking cookies etc.

2) It has been studied and verifies that bromelain acts as enzymatic browning inhibitor in fresh apple juices [18].

3) It helps to hydrolyze fish protein to generate fish protein

hydrolysate.

4) It also helps to treat acne, wrinkles, and dry skin [19].

Ficain

Ficain also known as Ficin, is a proteolytic enzyme extracted from fig, which belongs to a class of proteinases known as sulfhydryl 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 40C in order to separate gums and other impurities. A supernatant solution is prepared from the obtained extract by ultracentrifugation for 60 min at 40C. The obtained supernatant extract is mixed with 0.01M phosphate buffer (pH 7.5). Then, the prepared solution is transfered 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 catalyzed by lipoxygenase at different locations, are linoleic and linoleic acids.

Origin

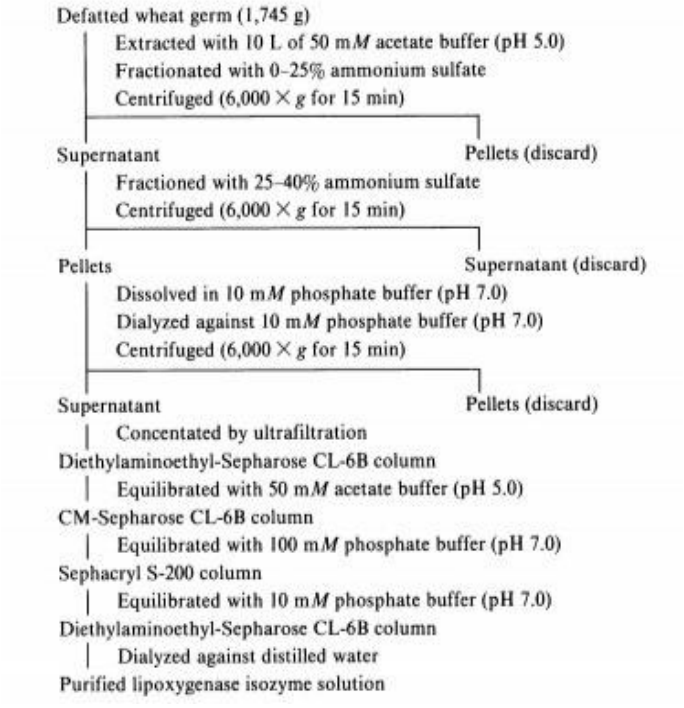
Lipoxygenase is found in vegetative tissues and plays an important role in plant defence system, but it is yet not identified about their presence in vegetative tissue in large quantity. This enzyme in tissue provides hydroperoxides substrate that helps the plants in defend themselves. Rance *et. al.* 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 assonate 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].

Figure 1

Figure 1: Isolation and Purification of lipoxygenase isozyme from wheat germ



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

1) It act as an ingredient for the production of bread.

2) It also act as an aroma enhancer.

3) It affects color, off-flavor, and anti-oxidant properties of foods in a negative way.

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