Review Article

Review on "scope and opportunities of papain as food tenderizing agent for food processing in Ethiopia"

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Abstract: The basic goal of food preservation is to limit microbial development during storage, which promotes a longer shelf life and lowers the risk of food poisoning. Fruits and vegetables are a crucial addition to the human diet because they include the essential vitamins, minerals, and fiber needed to sustain good health. Papaya contains a proteolytic enzyme known as papain, which is used in a variety of food processing procedures to break down proteins. The objective of this review is to carefully study the extraction methods for the papain enzyme that employ grinding and ultra-sonication extraction techniques and determine the application of papain as a preservative in future studies. Because the papaya fruit contains cysteine proteinases, it can be used as a significant instrument in pharmacology and medicine. The papaya fruit and seeds all contain carpaine, an alkaloid with a bitter taste that has a potent heart-depressant effect. Food preservation primarily aims to maintain the nutritional value of food, while also preserving its appearance and increasing its shelf life. Due to papaya's widespread popularity and reputation for its high nutritional value, it is necessary to fortify the fruit with the best preservation enzyme. Enzymes have evolved into critical tools for numerous industries, most notably food, animal feed, biofuel, detergent, textile, pulp, and paper industries.

Keywords: bio-catalysis; cysteine proteases; enzyme biotechnology; immobilization; industrial processes papain

1. Introduction

In contrast to chemical catalysts, enzymes are biological catalysts that are naturally occurring. They are polymers that catalyze chemical processes necessary for life, including the quick synthesis of complex chemicals, the breakdown of high-molecular-weight structures, and the conversion of biomolecules into their active form. Enzymes outperform all other chemical catalysts in terms of catalytic power, stereospecificity, and the ability to transform non-chiral substrates into chiral products. The food business makes use of these advantages. All of the enzymes employed in the food business are proteins by nature, and food technologists typically use them for food preservation while producing, processing, preparing, and treating food. The papaya is a lovely and delicious tropical fruit with a variety of health benefits. In a number of nations, papaya is grown for both domestic and international markets. Papaya is produced in about 60 different countries, the majority of which are poor nations. There are various significant responsibilities for fruits and vegetables in maintaining human health. They offer antioxidants, which are crucial in combating free radicals that are known to cause diabetes, cataracts, heart disease, hypertension, stroke, and cancer. They are also the main suppliers of vitamin A, a substance crucial for various bodily metabolic processes, in addition to its antioxidant function[1–3].

The papaya fruit is 85% water, 10%–13% sugar, and 0.6% protein and includes significant amounts of vitamins A and B1, B2, and C. Unripe papaya is a rich source of papain (a digestive enzyme), which is a vegetable pepsin that helps with digestion in an acidic, alkaline, or neutral medium. As shown in **Figure 1**, the papaya fruit has a range of aging: unripe, medium ripe, and ripe[4]. Papain is also used in the pharmaceutical, food acquisition, leather tanning, paper, and adhesive industries, as well as in sewage disposal and plastics for the dairy, bakery, fish, and perfumery industries. The plant's latex, fruit, leaves, and roots can all be used to

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extract papain, an endolytic cysteine protease enzyme. It is a 23.4kDa monomeric protein with a maximal activity temperature of 37 °C that is stable and active in a wide range of conditions. The Bradford method is used to identify papain by measuring the amount of protein present in a sample during purification[5–7].

Figure 1. Papaya fruits with a range of aging[4].

1.1. Composition of papain

An unripe papaya is actually an excellent source of carbs, proteins, and vitamins but the amount of these nutrients diminishes as the fruit ripens. **Table 1** below indicates the approximate composition of the papaya fruit at different stages of ripening[8]. For a ripe fruit, it can be seen that the papaya fruit's protein, lipid, and carbohydrate contents fall sharply. Nitrogen (1.49%) and carbon (38.10%) are present, according to the final analysis. When the ratio of carbon (C) to nitrogen (N) in the raw fruit is between 25 and 30, microbes can grow and thrive. The finding indicates that the papaya fruit has a great C-to-N ratio (26:1), indicating that microbial interactions can happen easily on the surface of the papaya peel and fruit[7,8].

Table 1. Approximate composition of papaya fruit at different stages of ripening[8].

Sample of papaya fruit	MC (%)	DM (%)	CF (%)	Ash (%)	CP (%)	Fat (%)	Carbohydrate (%)
Unripe	54.48	45.52	14.52	5.25	10.56	0.23	30.35
Hard ripe	58.22	41.78	13.67	4.84	9.04	0.31	27.87
Very ripe	68.39	31.61	9.67	3.15	6.89	0.33	20.04

At different stages of ripening, from an unripe papaya to one that is quite ripe, the vitamin composition and non-nutritive components of the papaya fruit peel decrease. **Table 2** shows stages of papaya maturity according to the skin color when stored at room temperature. A sulfhydryl group, three disulfide bridges, and a single-chained polypeptide called papain are absolutely necessary for the enzyme's function. Prepropapain, an inactive precursor of papain, is expressed.

To make active papain, many cleavage events must occur, including the initial cleavage of the 18 amino acid preregion and a second cleavage of the glycosylated 114 amino acid proregion. This proregion functions as a natural inhibitor and a folding model[9].

Table 2. Stages of papaya maturity according to skin color when stored at room temperature[10,11].

Maturity stage	Score range
0	Fruit has completely developed. 100% green skin color.
1	Represents papaya with a yellow-colored cover area of between 1% and 25% of the skin surface.
2	Represents papaya with ¼ mature. Fruit skin with up to 50% to 75% yellow-covered area.
3	Represents papaya with ¾ mature. The fruit surface is covered with up to 50% to 100% yellow color.
4	Mature. The fruit is covered with 76% yellow or totally yellow color, and the area near the stem is green.

1.2. Application in food and its methods

All foods contain food preservatives, with the exception of those from kitchen gardens. Foods are processed by producers by adding food preservatives. In general, the objective is to prevent food from rotting during transit. The basic goals of food preservation are to maintain the food's nutritional content, maintain its look, and lengthen its shelf life. The industrial applications are shown in **Figure 2**.

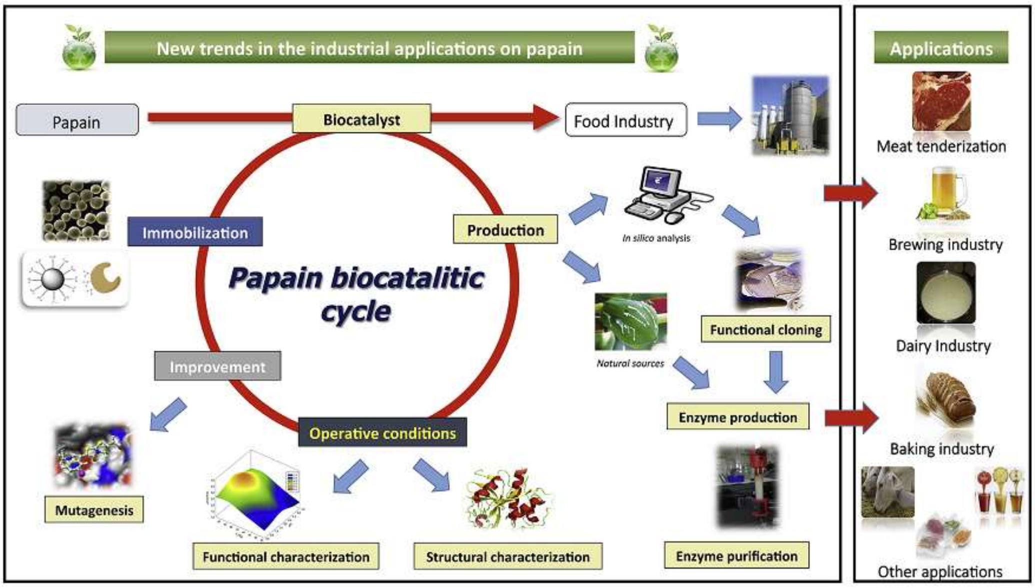

Figure 2. Complete bio-catalytic cycle of papain[4].

One of the earliest human innovations is the ability to preserve food, since this keeps food from rotting, which is necessary for survival. For this reason, various methods of preservation (the traditional ones include boiling, freezing, refrigeration, pasteurization, dehydration, smoking, and pickling) have been discovered and refined. Nuclear radiation and modified packaging methods, such as vacuum and hypobaric packing, are examples of artificial food preservation methods. Sugar, salt, alcohol, and vinegar are all common food preservatives. Some of the most common techniques are treatments using antimicrobial agents (which inhibit or destroy the growth of bacteria, molds, insects, and other organisms), chelating agents, and antioxidants[12].

Both the meat business and consumers are currently concerned about the serious issue of the quality of meat products. Tenderness, juiciness, flavor, fragrance, color, and texture are all qualities that influence how well meat and other foods are received by consumers. Tenderness is typically regarded as the most significant palatability feature. In addition to beer chill-proofing, cheese manufacturing, flavor and color extraction, and meat tenderizing, papain is applied to food using plant compounds as well[11,13].

1.3. Basic objectives

The main objectives of the study include reviewing the utilization of the papain enzyme, an inexpensive and easily accessible food preservative, to maintain the nutritional value, aesthetic appeal, and shelf life of food. Other objectives of the project are reviewing the manufacturing of the papain enzyme from inexpensive, locally available papaya fruits for food stabilization and meat tenderization and the preparation of fresh papaya fruits for use as a stabilizer. It is possible to characterize the papain enzyme using GC-MS, UV spectroscopy, and HPLC techniques. To avoid or reduce food waste, a product must be smoothed and softened before being soaked with hard food. This is done to make tough meat softer creation and evaluation of papain enzymes from the papaya fruit.

1.4. Significance of this review

The basic significance is applying papain enzyme as an active preservative, which allows food, basically meat, to have a long shelf life and prevents spoilage, as well as differentiating the preservatives applied to common and special foods. The review also dealt with other minor importance of the papain enzyme in applications besides preservation, such as aiding digestion in acidic, alkaline, or neutral medium. It also exhibits pain-relieving properties. This work aimed to open researchers' eyes to similar investigations to further spread food science-related studies and investigations.

2. Literature review

2.1. Origin and application area of papain enzymes

Enzymes are biological catalysts that speed up normally slow reactions by lowering the activation energy of the reaction without any noticeable structural changes at the end of the reaction. Enzymes are more selective than chemical catalysts. One of the primary benefits of enzymes is high selectivity, which results in fewer side reactions and hence easier separations. Wurtz and Bouchut discovered papain in the late 19th century after partially purifying the substance from papaya sap. When papain was named, it was merely known as a proteolytically active component in the latex of the tropical papaya fruit. In the 1980s, the active site geometry was updated, and the three-dimensional structure was calculated to a resolution of 1.65 Å[9,11].

Enzymes have been employed since ancient times, which has led to a greater understanding of enzymes as well as their increased demand and use. Many academic and corporate researchers, however, are still looking for new uses and better technologies. Despite extensive research, the relationship between structure and function remains one of the most important current challenges in enzyme engineering[14].

2.1.1. Enzymes in detergent industry

Enzymes have been included in detergent compositions to prevent the eutrophication of water brought on by phosphorus-containing detergents. Proteases, amylases, lipases, and celluloses have all been used to break down protein, carbohydrate, and lipid stains on clothing. In the detergent sector, enzymes offer a number of benefits, including the following:

- Lower cost, since enzymes are used at low detergent concentrations.
- Acceptable to the environment: Enzymes are biodegradable and have no harmful impact on sewage treatment processes.
- Higher efficiency in stain removal.
- Less use of pollutants, such as phosphate, bleach, and caustic substances[14].

2.1.2. Enzymes for bioenergy

Even though bioethanol and biodiesel are now produced and used as popular bioenergy, research on additional bioenergy is essential. Cyanobacteria, which include nitrogenase and hydrogenase, can create hydrogen. The enzymes involved in the effective synthesis of bioenergy from cyanobacteria must be thoroughly researched and, if necessary, engineered[15].

2.1.3. Enzymes for biomedical analysis

Clinical indicators, such as cholesterol, glucose, glutamate, lactate, and urea, are detected using enzymatic biosensors, which are widely utilized in the biomedical field. Because of the huge number of people with diabetes and other metabolic disorders, disposable blood glucose sensors have been actively explored and commercialized. Enzymatic biosensors for real-time detection of dopamine in the brain have also been reported as an alternative to conventional fast-scan cyclic voltammetry[14,15].

Because the papaya fruit contains cysteine proteinases, it can be used medicinally or pharmacologically. The papaya fruit, seeds, and leaves contain carpaine, an alkaloid with a bitter taste that has a strong depressant effect on the heart. The papaya fruit contains a lot of nutrients and is rich in vitamins and minerals. The combination of dietary fiber, phenolic antioxidants, minerals, and vitamins in the papaya fruit may be what gives it its protective effects against pathological and physiological abnormalities, such as cardiovascular disease, inflammation, and aging. Papaya may help improve the lung condition of people who smoke or are frequently exposed to secondhand smoke. Consuming foods high in vitamin A, such as papaya, can protect people's lives and maintain the health of their lungs[4,8,16].

2.1.4. For agricultural and food industries

Biosensors are used to monitor the freshness of raw foods as well as to manage their quality and safety during the manufacturing process. Sensing devices are utilized in these circumstances to test various components of the food and evaluate the food's rancidity, maturity, decline, and shelf life[14].

2.1.5. For pesticide detection

Herbicides, insecticides, fungicides, and rodenticides are widely utilized in agriculture and need to be strictly regulated by the sector, medical professionals, and regulatory organizations. Two techniques govern enzymatic bio-sensors for pesticides: the direct methodology, which tracks changes in the number of detectable chemicals caused by the enzymatic reaction, and the indirect strategy, which tracks enzyme inhibition[14,15].

2.1.6. Application in food as preservatives

Papain's useful properties have generated increasing attention in a range of industrial uses, particularly in the fields of meat tenderization and in the food, animal feed, brewing, and textile industries. Additionally, papain has been mentioned as an ingredient in skincare and tooth-whitening dentifrices. The main uses of the enzyme are outlined in this subsection. The production of nutrient-dense foods, or foods that are rich in nutrients but low in calories, can be aided by the use of enzymes. This could be achieved by enzymes hydrolyzing anti-nutritive compounds, such as phytates and enzyme inhibitors, and breaking down rigid plant-based foods to release nutrients, such as vitamins and minerals, as well as transforming substrates, such as allicin, or simply concentrating or stabilizing beneficial components in foods[15,16].

Meat tenderization

Exogenous proteases are increasingly used to encourage meat suppleness. The ability of the meat industry to both satisfy the rising demand for assuredly tender meat and increase the value of lower-grade meat cuts must be given top priority. There are many techniques for improving post-mortem softness, including mechanical tenderization, water content enhancement, and various enzymatic treatments. The typical method for softening meat is auto-proteolysis, which is mostly mediated by cathepsins and capains. Meat is then refrigerated at 4 °C for 7–10 days. Papain is a powerful meat tenderizer because it can hydrolyze virtually any protein found in muscle tissues, tendons, and ligaments when functioning under ideal conditions (pH around 7–8 and temperature between 60 °C and 65 °C). Natural substances that contain proteolytic enzymes, such as numerous fruits and vegetables, are referred to as natural tenderizers. These natural proteolytic enzymes can be utilized to effectively break down tough meat. The most frequently discussed of these plant proteolytic enzymes is papain. The enzyme is injected into the animal before slaughter, allowing for a homogeneous distribution of papain in the animal's meat, and numerous trials have been carried out to examine the influence of papain's meat-softening effect.

This approach is still used today. Because the injection of active papain can cause suffering and stress in animals, this procedure is no longer employed[15,17,18].

Another method is to inject inactive papain. This is accomplished by first oxidizing the catalytic cysteine in papain with hydrogen peroxide before administering it to the animal. Following the slaughter, the anoxic conditions lead to a drop in catalytic cysteine, which causes papain to reawaken. The main drawback of this antemortem method is the difficulty in predicting the degree of tenderization, which depends on a number of the animal's physiological factors. Textural inconsistencies compared with acceptable-quality beef slices, over-tenderization, disagreeable tastes or odors, or organ degeneration that may be of economic relevance are a few possible problems. For lower-grade beef slices, post-mortem application is often permissible. Papain is commercially available in powder and liquid forms (e.g., PANOL LIQUIPANOLT100), as well as in combination with other proteases, such as bromelain (e.g., ENZECO DUAL PROTEASE). Other components (salt, phosphates, or taste enhancers, such as sodium glutamate) may be present in some commercial recipes[7].

Tenderizing effect of papaya

Papaya is a natural supply of proteolytic enzymes, according to Branen et al.[19]. According to Kang and Warner[20], the combined effects of papain, chymopapain, and papaya peptidase A caused papaya to tenderize meat. Because of its more advantageous activity at neutral pH, chymopapain was the main factor in tenderization. Meat tenderization can be improved by administering this papain, which affects the structural component of muscles[21]. The papaya fruit, they claimed, was tapped to a maximum depth of two millimeters, and the latex that was collected in a container was dried to produce powder at temperatures below 70 °C. It was dissolved in water, which boosted the activity of certain enzymes[17,22].

Factors affecting tenderness

Meat tenderness is influenced by both pre- and post-slaughter variables. Species, breed, age, sex, nutrition and care, genetic influence, and stress conditions are all elements to consider. Species is the most important factor influencing tenderness among the often-cited preslaughter factors[17].

2.2. Properties and basic constituents of papain enzyme

The principal properties of the papain enzyme are as follows:

- Alternate name = Papaya peptidase I
- Specificity = Cleaves somewhat nonspecifically at exposed residues
- Source = Carica papaya latex
- Storage conditions = Store at 4 °C
- Molecular weight = 23.000 Da
- Inhibitors = Heavy metals, Carbonyls, NEM, p-Chloromercuro-benzoate
- Extinction coefficient = 76,630 $cm^{-1}M^{-1}$

- Isoelectric point = pH 9.6

The catalytic residues of the enzyme are:

- Cysteine (C158)
- Histidine (H292)
- Asparagine (N308)

Papain, a single-chained polypeptide, has a sulfhydryl group and three disulfide bridges. The activity of the enzyme is essential. It expresses prepropapain, an inactive papain precursor. The cleavage of the 18 amino acid preregion followed by the subsequent cleavage of the glycosylated 114 amino acid proregion are two of the six stages needed to produce an active papain, as shown in **Table 3**.

Table 3. Complete amino acid constituents of papain.

Amino acid	**No.**	**Amino acid**	**No.**
Lysine	10	Glycine	28
Histidine	2	Alanine	14
Arginine	12	Valine	18
Aspartic acid	7	Isoleucine	12
Asparagine	12	Leucine	11
Glutamine	8	Tyrosine	19
Threonine	13	Phenylalanine	4
Acid	12	Half cysteine	6
Serine proline	10	Cysteine	4
Glutamine	8	Tryptophan	5

Note: No. in the table above stands for the number of residues assigned to the papain molecule.

This proregion serves as a folding template and an intrinsic inhibitor. The surface of the cleft houses the active site, which is made up of a cysteine and a histidine. With the exception of four short helical segments and one short structural segment, the conformation of the chain is uneven[9,17].

2.3. Principal characteristics of purified papain

A white or grayish-white powder, which is just mildly hygroscopic, is purified papain. It is almost completely insoluble in other organic solvents but completely soluble in water and glycerol. Depending on how it is prepared, papain has varying degrees of effectiveness. Papain may break down into 35 times as much lean mass as it weighs. The finest papain digests egg albumin 300 times its own weight. It should be stored in a tightly sealed container. The optimal pH for its activity is 5.0; however, it also works in neutral and alkaline conditions. High-quality papain should meet the following criteria:

- The color should be creamy white
- Moisture content should be above 10%
- Total ash content should not be greater than 11.1% on a moisture-free basis
- Should not contain any foreign substances
- Should possess a proteolytic activity not less than that of the Ceylon reference[23]

2.4. Structure of papain

Residues of 212 amino acid make up the single chain of papain. At least three amino acid residues, including Cys25, His159, and Asp158, can be found in the papain active site. The enzyme activity is diminished when Cys25 is oxidized or bound to a metal ion; however, the reducing agent cysteine (or sulfite) or EDTA can restore enzyme activity. Three pairs of disulfide connections are made by the remaining six cysteine residues; however, only one pair is present in the active site, as shown in **Figure 3**.

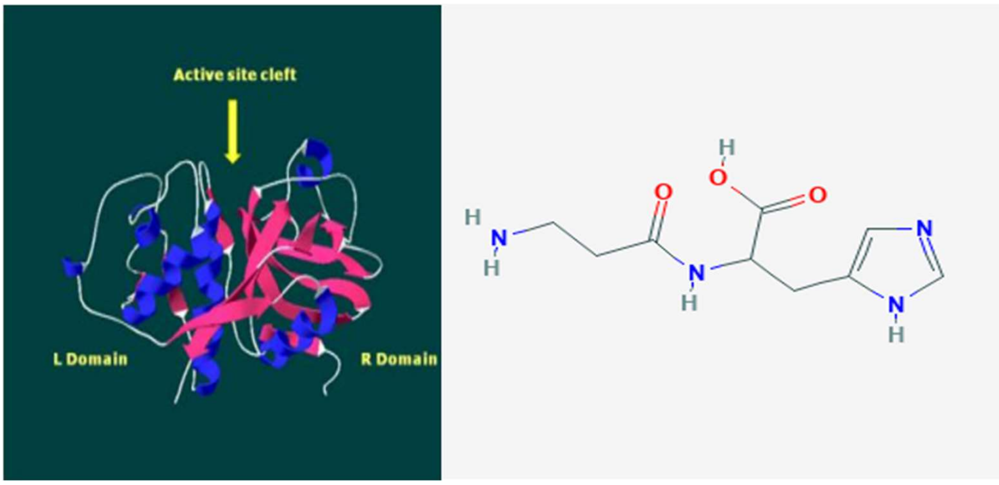

Figure 3. Structure of papain enzyme.

2.5. Extraction and analysis of papain

Selecting an enzyme source is the first step in the production of enzymes. Through fermentation methods, enzymes can be obtained from microorganisms, plants, and animals. Papain is obtained by longitudinally slicing a mature, unripe fruit and collecting the abundant latex in a container positioned beneath the fruit while it is still on the tree. The latex is taken to the lab right away and diluted with distilled water to the necessary concentrations to prevent coagulation. There are four main steps to the process:

1) Obtaining environmental samples
2) Isolation of DNA and manipulation of the genetic material
3) Construction of metagenomic library
4) Screening of new function enzymes and sequencing of genetic material from the metagenomics[8,14]

Traditionally, papain enzyme is made from the milky latex extracted from the green fruit's skin, as shown in **Figure 4**. Fruits that have nearly achieved full size but are still green provide the most latex, and latex yields are typically maximum in the first twelve months of tapping. The yields in the second year are approximately 65% of the yields in the first year, declining further in each subsequent year. This is due to the fact that the size and number of fruits, as well as latex yield, decrease with the age and height of the tree[24].

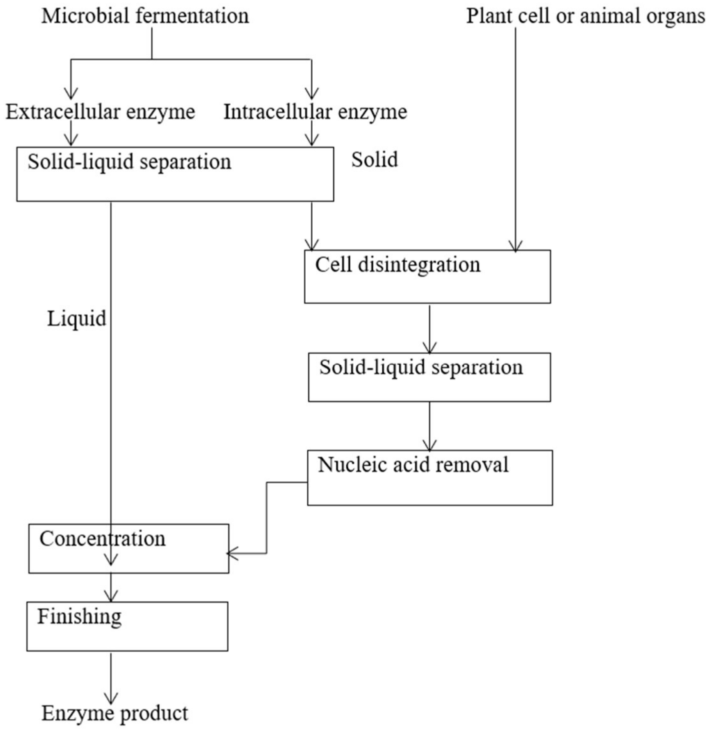

Figure 4. Preparation steps of enzymes[15].

Endopeptidases chymopapain (EC 3.4.22.6), caricain (EC 3.4.22.30), and glycyl endopeptidase (EC 3.4.22.25) are all found in the latex of the papaya plant (Carica papaya), which also produces papain. In actuality, papain makes up just 5–8% of papaya endopeptidases. Papain can be extracted from papaya latex using precipitation techniques, which can produce high yields of up to 53 g of crude enzyme per kilogram of latex. Despite being frequently used in industries, the methods can only produce papain that is up to 39% purity. To avoid oxidation of the free cysteine thiol groups and maintain its protease activity, crude papain is commonly subjected to reducing agents after purification. When necessary, the free thiol groups of papain can be regenerated by adding low-molecular-mass thiols, such as cysteine or dithiothreitol[7].

2.6. Determination of enzymatic activity

If papain is to be commercialized for usage in the export market or the local food sector, its enzymatic activity must be determined. To certify the result, the method used to assess papain activity must be validated. The hydrolysis of natural proteins or synthetic substrates, such as esters or low-molecular-weight amides, can be used to test papain enzymatic activity.

Dyes, chromogenic compounds, or fluorogenic compounds may be released during such reactions, which can be detected using a spectrophotometer. In a study, this entailed employing casein as a protease substrate and dissolving dried samples of 0.05 g in 5mL sodium acetate buffer at 10 mM (pH 7.5) and 5mL calcium acetate buffer at 10 mM (pH 7.5). For each sample, 455 L of casein at 65% w/v was warmed in a thermal bath at 371 ℃ for 10 min before adding another 20 L. After 10 min, the processes were halted with 455 L trichloroacetic acid at 110 mM and maintained in the thermal bath for another 30 min. To discard the solid created, the two form phases were separated by centrifugation at 9000 rpm and 4 ℃ for 20 min (Fresco 17 Thermo). The supernatant was removed. Protease assays were performed on the supernatant. Aliquots of 625 L of supernatant were mixed with 1570 L of 500 mM sodium carbonate and 250 L of Folin-Ciocalteus reagent.

Since the released tyrosine exhibited a blue tint, the protease activity was assessed using spectrophotometry. Each sample was read using a spectrophotometer set to 660 nm. The quantity of protein in each sample was calculated using the Biuret method. Whether papain is to be economically exploited for an export market or for usage in the local food industry, the ability to assay the amount of enzyme activity is essential. National Standards offices, for instance, could carry it out. The enzyme that hydrolyzes (or breaks down) proteins is called papain. As a result, measuring a hydrolysis byproduct is the foundation of papain activity assays. Assays can be carried out in two primary ways:

Method 1

This approach is based on papain's capacity to coagulate milk. It is a low-cost method, but it takes time. Furthermore, the lack of a consistent procedure for determining the clotting point, combined with variances in the milk powder used, might lead to inaccuracies.

Method 2

The second method is based on light absorption science, often known as absorptiometry. The amount of radiation (or "color" of light) absorbed by a chemical solution is measured using this technique. A yellow-colored solution, for example, is known to absorb blue light (blue is the complementary hue of yellow). The more yellow there is in the solution, the more blue light is absorbed, and the relative importance of each quality parameter for enzymatic activity depends upon the commodity or the product and whether it is fresh (with or without flavor modifiers, such as dressings and dips) or cooked[9,17,21,25].

3. Conclusion

This review shows that the majority of commonly used enzymes in food processing are regarded as "natural" and biodegradable, and their use is associated with more environmentally friendly results and less detrimental impacts on the environment. Enzymatic reactions are selective, reproducible, rapid, and efficient, and they almost never produce unwanted byproducts. Enzymes have become a crucial tool for many industries as a result, particularly those that rely on preservation, such as food, animal feed, biofuel, detergent, textile, pulp, and paper industries. Fortification with the best preservation enzyme is required due to papaya's extensive consumption and the fact that it is well-known throughout the world for its nutritional and dietary benefits.

Conflict of interest

The author declares no conflict of interest.

Abbreviations

CP	Crude Protein
CF	Crude Fiber
MC	Moisture Content
DM	Dry Matter
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Dimethylene Tetraamine
NEM	Net Energy Metering
pH	Power of Hydrogen
PPE	Papaya Peel Extraction

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