

**THE EXTRACTION OF AMINO ACIDS FROM PAPAYA LEAVES
USING SUPERCRITICAL CARBON DIOXIDE WITH WATER**

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ABSTRACT

In this study, a new extraction method for the isolation papain from papaya leaves (*carica papaya sp.*) is being developed using supercritical carbon dioxide (SC-CO₂) with water. The parameters of temperature, extraction time and comparison yield with hot water extraction were studied. Results indicated that temperature and extraction time had significant effect on the extraction yield. The highest extraction yield (0.56mol/liter) amino acid was obtained at 300 bar, 50 °C and 2hours. Extracted yields were then analysed by UV-vis spectrophotometer at 660nm and casein as a substrate. The composition of the extracted yields was greatly impacted by the operating conditions. At the best extraction condition (300 bar, 50 °C and 2 hours), amino acid had the highest concentration (0.56mol/liter) among all the other detected samples. The present findings showed that papaya leaves is a potential source of papain enzymes compounds. In addition, supercritical carbon dioxide extraction is a promising a high yield and alternative process for construct the papain compounds from papaya (*carica papaya sp.*) leaves which is by-product raw material. For further study, High Performance Liquid Chromatography (HPLC) should be using to analyses the enzyme to more accurate result.

ABSTRAK

Dalam kajian ini, kaedah mengekstrak papain daripada daun betik (*Carica papaya sp.*) dikaji dengan menggunakan superkritikal karbon dioksida (SC-CO₂) dengan air. Parameter untuk ekstrak ini ialah suhu pengekstrakan, waktu pengekstrakan dan hasil daripada ekstrak dibandingkan dengan mengekstrak dengan menggunakan air panas. Keputusan kajian menunjukkan bahawa suhu dan masa pengekstrakan mempengaruhi keputusan pengekstrakan. Hasil pengekstrakan tertinggi (0.56mol/liter) asid amino diperolehi pada 300 bar, 50°C dan 2jam. Keputusan hasil ekstrak kemudian dianalisis dengan menggunakan spektrofotometer UV-vis pada 660nm dan casein digunakan sebagai substrat. Susunan keputusan ekstrak sangat dipengaruhi oleh keadaan eksperimen. Pengekstrakan yang terbaik ialah pada keadaan 300 bar, 50 °C dan 2 jam, asid amino mempunyai kepekatan tertinggi (0.56mol/liter) di antara semua sampel yang diuji. Penemuan ini menunjukkan bahawa daun betik merupakan sumber yang berpotensi menghasilkan enzim papain. Selain itu, ekstrak dengan menggunakan supercritical karbon dioksida adalah proses yang menjanjikan hasil yang tinggi dan alternatif untuk menghasilkan enzim papain dari daun betik (*Carica papaya sp.*) yang juga bahan buangan. Untuk kajian akan datang, High Performance Liquid Chromatography (HPLC) perlu digunakan untuk mendapatkan keputusan yang tepat.

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LIST OF SYMBOLS/ ABBREVIATIONS

| | | |
|-------------------|---|--|
| C | - | Degree celcius |
| % | - | Percent |
| Ea | - | Activation energy |
| Kg | - | Kilogram |
| P | - | Pressure |
| V | - | Volume |
| T | - | Temperature |
| mg | - | Miligram |
| ml ⁻¹ | - | Per mililiter |
| min ⁻¹ | - | Per minute |
| CO ₂ | - | Carbon Dioxide |
| MPa | - | Megapascal |
| w/v | - | Weight per volume |
| H ₂ O | - | Water |
| EDTA | - | Ethylenediaminetetraacetic acid |
| BAEE | - | Benzoyl-L-arginine ethyl ester |
| HPLC | - | High performance Liquid Chromatography |

CHAPTER 1

INTRODUCTION

1.1 Background of study

Papaya is a fruit native to eastern Central America and was cultivated long before the arrival of the Europeans. Spanish and Portuguese invaders took the fruit and quickly spread it to their other settlements. It was found growing in the West Indies by 1513 and by 1583; it found its way to the East Indies via the Philippines. It had also made its way into Africa at an equally early date, and spread through the Pacific islands as Europeans discovered it. By 1800, papaya was grown in all tropical regions with Hawaii and South Africa now the main exporters.

The papaya plant is a large herb that grows rapidly, reaching heights of more than twenty-five feet and producing a soft wood. The huge fingered leaves form a spiral similar to those of the palm tree. The plant grows quickly from seed and bears fruit within a year, continuing to do so for another two years before the tree is cut down. The tree grows best in temperatures of 25 C and does not like storms winds. It also requires good drainage as the roots will rot if they become water-logged. Frost also kills the tree.

Papaya latex is a thixotropic fluid with a milky appearance that contains about 15% of dry matter. Forty percent of this dry matter is constituted by enzymes, mainly cysteine endopeptidases since altogether they account for more than 80% of the whole enzyme fraction. Although papain is a minor constituent (about 8%)

among the papaya endopeptidases, this enzyme, more easily purified, has been the most extensively studied (Mohamed Azarkan *et al.*, 2002).

The papain is a natural proteolytic enzyme that is extracted from latex in the leaf, the stem and the papaya's unripe fruit. It is a food grade, highly active endolytic cysteine protease from papaya and is one of the widely used industrial enzymes. It is also less expensive than microbial enzymes and has a wide range of specificity and good thermal stability among other proteases. Molecular weight of papain is 23,000 Da and an isoelectric point of 9.5. Papain molecules consist of a single peptide chain of 211 amino acid residues folded into two parts that form a cleft and having 11 lysine residues. It also has an esterase activity (J. Chamani, 2008).

The application of papain enzymes for industries are clarifying beer, meat tenderization, preservation of spices, contact lens cleaners, detergents, pet food palatability, digestive aids, blood stain remover, blood coagulant and cosmetic. Papain is also widely used for many medical and pre-medical purposes such as to assist protein digestion in chronic dyspepsia, gastric fermentation, gastritis, removal of necrotic tissues, preparation of tyrosine derivatives for the treatment of Parkinsonism, preparation of tetanus vaccines, skin cleansing agents, acne treatment, cancer, etc (Debi Choudhury *et al.*, 2009).

1.2 Problem statement

Papain enzyme has been extensively studied and is an enzyme of industrial use and high research interest (Rubens Monti *et al.*, 2000). The latex of carica papaya is a rich source of the cysteine endopeptidases, including papain, glycylic endopeptidase, chymopapain and caricain, which constitute more than 80% of the whole enzyme fraction. Papain is a minor constituent (5-8%) among the papaya endopeptidases (Sarote Nitsawang, 2006).

The traditional methods for the extraction of plant materials include steam distillation and organic solvent extraction using porcelain, maceration or Soxhlet techniques. These procedures however have distinct drawbacks such as time-consuming and labour-intensive operations, handling of large volumes of hazardous solvents and extended concentration steps that can result in the loss or degradation of target analytes (L.Casas *et al.*, 2008).

Supercritical fluids extraction is alternative extraction technologies that consume smaller quantities of organic solvent. The combined liquid-like solvating capabilities and gas-like transport properties of supercritical fluids make them particularly suitable for the extraction of plant tissue. In addition, the solvent strength of this can be easily tuned by simply changing the applied pressure or/and temperature. Furthermore, the stabilization by lyophilization of the papain is also found out so as to make sure the enzymes are not rapidly inactivated by oxidation and autocatalytical degradation.

1.3 Objectives

The objective of this study is to extract amino acids from papaya leaves

1.4 Scope of study

In order to achieve the objectives, the following scopes have been identified:

1. Effect of time and temperature of the supercritical extraction which is time extraction are 1, 2 and 3 hours and the temperature of vessel are 40 C, 50 C and 60 C.
2. Comparison of high yield extraction method between hot water extraction and supercritical extraction method.

1.5 Rationale and Significance

Papaya leaves are produced as a by-product from removal of plants at the end of their productive cycle. High transport costs seriously limit any secondary uses and in most cases this waste is left to rot, producing phytophathogens that cause ecological problems and pose a risk to human health. Furthermore, one potential alternative use for this waste is extraction of proteolytic enzymes such as papain and not just in the fruit. These also apply waste to wealth concept hence reducing amount of waste. On the other hand, traditional methods use organic solvent for the extraction of plant. The organic solvents have a large volume of hazardous and precarious for pharmaceutical and food processing. Furthermore, the best extraction method that wants to minimize the using of organic solvent in extraction.

CHAPTER 2

LITERATURE REVIEW

2.1 Papain

2.1.1 Overview

Carica papaya or common name is papaya contains many biologically active compounds. The important compound is papain. Besides, cysteine protease or papain is obtained from unripe papaya, papaya leaves or papaya roots by drying papaya latex by suitable method which is either sun drying, tray or oven drying or spray drying. Pure papain is apparently as strong a proteinase as any of the well-known enzymes. The fresh latex is extremely powerful proteolytically, and perhaps more than half of the total protein contained in it is at first active, though easily inactivated by oxidation. Therefore, commercial preparations of papaya latex do not keep indefinitely and often lose their natural activity within a few months. Nevertheless, papain is perhaps the cheapest form of proteolytic activity on the market today, and its use has increased greatly in recent years (A. K. Balls, 1940).

Cysteine proteases constitute an important class of proteolytic enzymes found in prokaryotes as well as eukaryotes like plants and animals. Structurally they belong to the papain family where individual members accomplish catalysis employing a common mechanism involving the highly conserved catalytic triad (Cys-His-Asn) residues. Although the overall topological features and mechanism of catalysis are conserved, unique active site residues define substrate specificities for

individual members (Min Zhang *et al.*, 2006). On the other hand, the quantity of fresh papaya latex and dried papaya latex as known as crude papain also vary with the sex of the tree and the age of the tree. Female and hermaphrodite trees yield cruder papain than male trees also older fruits yields more than younger fruit. By the way, the activity of the papain is higher in the extracts from the younger fruit than the older fruit because latex comes from unripe fruit.

Papain from papaya was one of the earliest substances used for debridement. Both papaya as fruit and papain as a commercially available powder are used in wound care. Green papaya is rich in two enzymes that have very strong digestive properties that are papain and chymopapain. Papain, the enzyme used in commercial meat tenderizers, has the ability to dissolve dead tissue without damaging living cells. The stems, leaves and fruits contain copious amount of latex. Accuzyme, a debriding ointment that contains papain and urea is available to debride necrotic tissue and liquefy slough in a variety of acute and chronic lesions. Chymopapain was also used in the healing and recovery of surgical wounds. Arvigo and Balick reported that sliced fruit or crushed papaya seeds applied to wounds, curs and infections assisted with healing. Carpaine is an alkaloid compound is also found in green papaya and has been shown to have antibacterial properties (Mahmood A.A, 2005).

Production of papain requires considerable technical parameters starting from the plant. The latex is collected from the unripe papaya, leaves or roots. The collected latex is dried and treated with suitable solvents in order to validate the activity of papain enzyme to be used in the different applications. Yield of papain depends on cultivator, time of taping, nutritional status of plant and the region (Krishnaiah D., 2002). One novel of separation technique with the ability to be scaled up easily, to be operated continuously and to be highly selective is liquid-liquid extraction using microemulsions (Daliya S. Mathew, 2005).

2.1.2 Characteristic of papain

Papain is the natural bio-enzyme extracted from white latex of unripe carica papaya skin through modern biological engineering technology. It is –SH containing peptide chain endonuclease, which has the activity of prolease and esterase, wide specificity, and relatively strong hydrolysis ability to animal and plant protein, polypeptide, ester, and acid amide; it also has synthesis ability, which can synthesize protolysate into proteinoid. It is soluble in water and glycerine, the colour of solution is either transparent or light yellow, sometimes milky white. It is almost insoluble in such organic solvents as ethanol and chloroform; it is best used when PH=5.7 (useful between 3 to 9.5), and also functional at neutral or slight acid PH value; isoelectric point is 18.75, most suitable temperature is 55-60 C but it is functional between 10 C to 85 C, good heat resistance, will not completely lose activity even at 90 C, suppressed by oxidants and activated by deoxidizers. Papain has the characteristic of high enzyme activity and strong thermal stability, it is safe and sanitary to use as well.

This enzyme has the property of substantial breaking the molecules. The use of its property is estimated in the commercial application essentially in the soap and detergent powder manufacture. Present market products are made mostly by raw papain. The effectiveness of the enzyme can be improved by modifying and activating. Papains have wide specificity. Arnon (1970) has indicated that it will degrade most protein substrates more extensively than pancreas protease. Papain is activated by cystein, sulphide and sulphite. It is enhanced when heavy metal binding agents such as EDTA are also present. Kirschenbau (1971) indicated that N-bromosuccinimide enhances the activity. Substances which react with sulfhydryl group including heavy metals and carbonyl reagents. Aldehydes exhibited and benzoylamidoacetonitrile as an inhibitors have considerable effect when the papain is used in pharmaceutical applications. The general characteristic of the two derived enzymes vary to some extent since they all have different temperature of inactivation and operate with different kinetics when applied (Krishnaiah D, 2002).

2.1.3 Enzyme

Enzymes are proteins that catalyze, for example increase the rates of chemical reactions. In enzymatic reactions, the molecules at the beginning of the process are called substrates, and the enzyme converts them into different molecules, called the products. Almost all processes in a biological cell need enzymes to occur at significant rates. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell.

Like all catalysts, enzymes work by lowering the activation energy (E_a) for a reaction, thus dramatically increasing the rate of the reaction. Most enzyme reaction rates are millions of times faster than those of comparable un-catalyzed reactions. As with all catalysts, enzymes are not consumed by the reactions they catalyze, nor do they alter the equilibrium of these reactions. However, enzymes do differ from most other catalysts by being much more specific. Enzymes are known to catalyze about 4,000 biochemical reactions. A few RNA molecules called ribosome also catalyze reactions, with an important example being some parts of the ribosome. Synthetic molecules called artificial enzymes also display enzyme-like catalysis.

Enzyme activity can be affected by other molecules. Inhibitors are molecules that decrease enzyme activity; activators are molecules that increase activity. Many drugs and poisons are enzyme inhibitors. Activity is also affected by temperature, chemical environment like pH and the concentration of substrate. Some enzymes are used commercially, for example, in the synthesis of antibiotics. In addition, some household products use enzymes to speed up biochemical reactions which are enzymes in biological washing powders break down protein or fat stains on clothes; enzymes in meat tenderizers break down proteins, making the meat easier to chew.

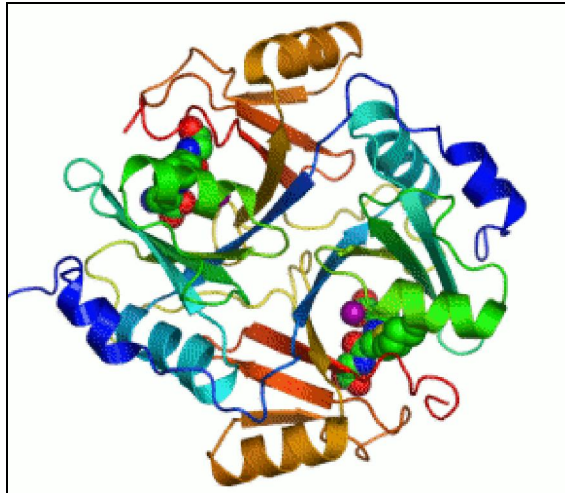


FIGURE 2.1 (Grisham & Garrett, 1999)

2.1.3.1 Characteristics of enzyme

Enzymes can be isolated and are active outside the living cell. They are such efficient catalysts that they accelerate chemical reactions measurably, even at concentrations so low that they cannot be detected by most chemical tests for protein. Like other chemical reactions, enzyme-catalyzed reactions proceed only when accompanied by a decrease in free energy; at equilibrium the concentrations of reactants and products are the same in the presence of an enzyme as in its absence. An enzyme can catalyze an indefinite amount of chemical change without itself being diminished or altered by the reaction. However, because most isolated enzymes are relatively unstable, they often gradually lose activity under the conditions employed for their study.

2.1.3.2 Chemical nature of enzyme

All enzymes are proteins. Their molecular weights range from about 10,000 to more than 1,000,000. Like other proteins, enzymes consist of chains of amino acids linked together by peptide bonds. An enzyme molecule may contain one or more of these polypeptide chains. The sequence of amino acids within the polypeptide chains is characteristic for each enzyme and is believed to determine the

unique three-dimensional conformation in which the chains are folded. This conformation, which is necessary for the activity of the enzyme, is stabilized by interactions of amino acids in different parts of the peptide chains with each other and with the surrounding medium. These interactions are relatively weak and may be disrupted readily by high temperatures, acid or alkaline conditions, or changes in the polarity of the medium. Such changes lead to an unfolding of the peptide chains as denaturation and a concomitant loss of enzymatic activity, solubility, and other properties characteristic of the native enzyme. Enzyme denaturation is sometimes reversible.

Many enzymes contain an additional, non-protein component, termed a coenzyme or prosthetic group. This may be an organic molecule, often a vitamin derivative, or a metal ion. The coenzyme, in most instances, participates directly in the catalytic reaction. For example, it may serve as an intermediate carrier of a group being transferred from one substrate to another. Some enzymes have coenzymes that are tightly bound to the protein and difficult to remove, while others have coenzymes that dissociate readily. When the protein moiety the apoenzyme and the coenzyme are separated from each other, neither possesses the catalytic properties of the original conjugated protein the holoenzyme. By simply mixing the apoenzyme and the coenzyme together, the fully active holoenzyme can often be reconstituted. The same coenzyme may be associated with many enzymes which catalyze different reactions. It is thus primarily the nature of the apoenzyme rather than that of the coenzyme which determines the specificity of the reaction. The complete amino acid sequence of several enzymes has been determined by chemical methods. By x-ray crystallographic methods even the exact three-dimensional molecular structure of a few enzymes has been deduced.

2.1.3.3 Classification and nomenclature of enzyme

Enzymes are usually classified and named according to the reaction they catalyze. The principal classes are as follows. Oxidoreductases catalyze reactions involving electron transfer, and play an important role in cellular respiration and energy production. Some of them participate in the process of oxidative phosphorylation, whereby the energy released by the oxidation of carbohydrates and fats is utilized for the synthesis of adenosine triphosphate (ATP) and thus made directly available for energy-requiring reactions. Transferases catalyze the transfer of a particular chemical group from one substance to another. Thus, transaminases transfer amino group, transmethylases transfer methyl groups, and so on. An important subclass of this group is the kinases, which catalyze the phosphorylation of their substrates by transferring a phosphate group, usually from ATP, thereby activating an otherwise metabolically inert compound for further transformations.

Hydrolases catalyze the hydrolysis of proteins which is proteinases and peptidases, nucleic acids (nucleases), starch (amylases), fats (lipases), phosphate esters (phosphatases), and other substances. Many hydrolases are secreted by the stomach, pancreas, and intestine and are responsible for the digestion of foods. Others participate in more specialized cellular functions. For example, cholinesterase, which catalyzes the hydrolysis of acetylcholine, plays an important role in the transmission of nervous impulses.

Lyases catalyze the nonhydrolytic cleavage of their substrate with the formation of a double bond. Examples are decarboxylases, which remove carboxyl groups as carbon dioxide, and dehydrases, which remove a molecule of water. The reverse reactions are catalyzed by the same enzymes. Isomerases catalyze the interconversion of isomeric compounds. Ligases, or synthetases, catalyze endergonic syntheses coupled with the exergonic hydrolysis of ATP. They allow the chemical energy stored in ATP to be utilized for driving reactions uphill.

2.1.3.4 Specificity of enzyme

The majority of enzymes catalyze only one type of reaction and act on only one compound or on a group of closely related compounds. There must exist between an enzyme and its substrate a close fit, or complementarity. In many cases, a small structural change, even in a part of the molecule remote from that altered by the enzymatic reaction, abolishes the ability of a compound to serve as a substrate. An example of an enzyme highly specific for a single substrate is urease, which catalyses the hydrolysis of urea to carbon dioxide and ammonia. On the other hand, some enzymes exhibit a less restricted specificity and act on a number of different compounds that possess a particular chemical group. This is termed group specificity. A remarkable property of many enzymes is their high degree of stereo specificity, that is, their ability to discriminate between asymmetric molecules of the right-handed and left-handed configurations. An example of a stereo specific enzyme is L-amino acid oxidase. This enzyme catalyses the oxidation of a variety of amino acids of the type $R-CH(NH_2)COOH$. The rate of oxidation varies greatly, depending on the nature of the R group, but only amino acids of the L configuration react.

2.1.3.5 Enzymatic Action

Like all catalysts, enzymes accelerate the rates of reactions while experiencing no permanent chemical modification as a result of their participation. Enzymes can accelerate, often by several orders of magnitude, reactions that under the mild conditions of cellular concentrations, temperature, pH , and pressure would proceed imperceptibly or not at all in the absence of the enzyme. The efficiency of an enzyme's activity is often measured by the turnover rate, which measures the number of molecules of compound upon which the enzyme works per molecule of enzyme per second. Carbonic anhydrase, which removes carbon dioxide from the blood by binding it to water, has a turnover rate of 10^6 . That means that one molecule of the enzyme can cause a million molecules of carbon dioxide to react in one second.

Most enzymatic reactions occur within a relatively narrow temperature range usually from about 30°C to 40°C, a feature that reflects their complexity as biological molecules. Each enzyme has an optimal range of *pH* for activity; for example, pepsin in the stomach has maximal reactivity under the extremely acid conditions of *pH* 1-3. Effective catalysis also depends crucially upon maintenance of the molecule's elaborate three-dimensional structure. Loss of structural integrity, which may result from such factors as changes in *pH* or high temperatures, almost always leads to a loss of enzymatic activity. An enzyme that has been so altered is said to be denatured. Consonant with their role as biological catalysts, enzymes show considerable selectivity for the molecules upon which they act called substrates. Most enzymes will react with only a small group of closely related chemical compounds; many demonstrate absolute specificity, having only one substrate molecule which is appropriate for reaction.

Numerous enzymes require for efficient catalytic function the presence of additional atoms of small nonprotein molecules. These include coenzyme molecules, many of which only transiently associate with the enzyme. Nonprotein components tightly bound to the protein are called prosthetic groups. The region on the enzyme molecule in close proximity to where the catalytic event takes place is known as the active site. Prosthetic groups necessary for catalysis are usually located there, and it is the place where the substrate and coenzymes, if any bind just before reaction takes place. The side-chain groups of amino acid residues making up the enzyme molecule at or near the active site participate in the catalytic event. For example, in the enzyme tryrosine, its complex tertiary structure brings together a histidine residue from one section of the molecule with glycine and serine residues from another. The side chains of the residues in this particular geometry produce the active site that accounts for the enzyme's reactivity.

2.1.4 Application of papain

The Papain has many uses and functions in a variety of industries like clarifying beer, meat tenderization, preservation of spices, contact lens cleaners, detergents, pet food palatability, digestive aids, blood stain remover, blood coagulant and cosmetics. Papain also widely used for many medical and para-medical purposes such as to assist protein digestion in chronic dyspepsia, gastric fermentation, gastritis, removal of necrotic tissue, preparation of tyrosine derivatives for the treatment of Parkinsonism, preparation of tetanus vaccines, skin cleansing agents, acne treatment and etc. (Choudhury *et al.*, 2008) to effectively improve product's quality and grade and lower production cost at the same time.

Application of papain in different industries such as pharmaceutical Industry, medicines that contain papain are resistance to cancer, tumor, lymphatic leukemia, bacteria and parasite, tubercle bacillus, and inflammation; they are good for gall, and they can kill pains and help with digestion. They are also helpful to treat gynecopathy, glaucoma, and hyperosteogeny, heal wounds from gunshots or knife cuts; identify blood type, and treat insect stings. For food Industry, hydrolyze protein from food's giant molecules into peptide or amino acid through enzymatic reaction. Applied for hydrolyzing animal and plant's protein, making tenderizer, hydrolyzing placenta and soybeans, making cracker swelling agent, noodle stabilizer, beer and beverage clarifier, premium oral medicine, health care products, soy sauce, and wine starter. It can effectively convert protein, greatly improve food's nutrition value, and lower cost; it is also beneficial for body digestion and absorption. In beauty and cosmetic industry, add papain into protein and grease included cosmetic products can whiten and smooth skin, lighten freckles and remove grease, accelerate blood circulation, improve skin function, and enhance product quality. It can also be integrated into diet tea and beauty products. For household chemical product, used in soap, washing agent, detergent, and hand soap; it can eliminate dirt, grease, bacteria, and it is safe to use. In feeds industry, used for feeds additives to explore protein source; it helps with absorption, improves feeds utilization rate, saves cost, and aids animal digestion and growth. It can be added into feeds for pig, cow, sheep, chicken, duck, goose, fish, and shrimp; it can also be mixed into premium compound

fertilizer for vegetables and fruits. Leather made by using the depilating agent that contains papain has small pores and shinny grain for leather industry. Lastly for textile industry, it can be applied in wool processing, degumming of silkworm chrysalis, and silk refining. It is soft, comfortable, and resistance to contraction.

2.1.5 Market Potential

Papain is used in many industries for variety of reasons. Some of the end-users are breweries, pharmaceuticals, food, leather, detergents, meat and fish processing etc. Thus, the end use segments are many. Most of these industries are growing. Good quality papain has export demand as well. In spite of very good domestic as well as export demand, papain manufacturing has not yet picked up in the North-East and hence there are good prospects for new entrants.

2.1.6 Manufacturing process

Typically, for manufacturing process of papain the white milky latex of green and fully grown papaya fruits is collected in the early morning by making deep longitudinal cuts by stainless steel or wooden sharp knives. Latex is collected in stainless steel trays while latex coagulated in the surface of the fruits is scrapped and collected in the trays. A fruit is tapped about 6 times in the course of 16 days. This latex is passed through 50 mesh sieves to remove dirt and then it is mixed with potassium metabisulphate and spread on trays and dried in a vacuum shield drier at a temperature of about 55 C for 4 until 5 hours.

The dried product is packed in air-tight containers and stored in a cool, dry place. It should be kept in flake form as powdering decreases the stability of the product during storage. Dried flakes are powdered and diluted with lactose powder to get papain. Plastic containers should be used to pack crude papain flakes or powder

as metal containers would result in loss of enzyme activity. Transportation is also very critical as papain has to be kept below 200 C temperature or else its shelf life is reduced. With proper storage and handling, its shelf life is 5-6 months. Recovery of papain is in the range of 25% to 30%. In other words, 100 kg of good quality latex is required to produce 25 until 30 kg of papain. The process flow chart is as under:

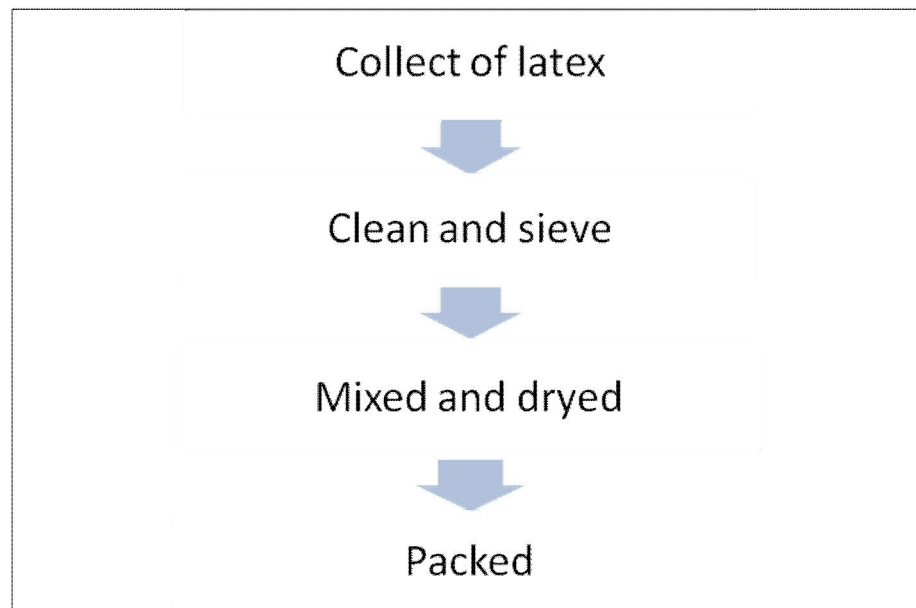


Figure 2.2: Process flow chart of manufacturing process

2.2 Extraction

2.2.1 Supercritical Fluid Extraction

Fluids become supercritical when they are compressed beyond their critical pressures (P_c) and simultaneously heated beyond their critical temperatures (T_c). Supercritical fluids cannot be liquefied even with extreme compression. However, one may increase pressure to change the density of a fluid continuously from a gas-like state to a liquid-like state. Near the critical region small change in pressure can give rise to large changes in density. In addition to density, diffusivity of the