

THE EXTRACTION OF PAPAIN FROM PAPAYA LEAVES

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ABSTRACT

The use of papaya leaves to extract papain enzyme was evaluated for different extraction technique mainly using grinding method, enzyme-assisted extraction and ultrasonication. Enzyme concentration was then determined by measuring the amount of amino acid released in the reaction of protease assay. The combination of grinding method, enzyme-assisted extraction was proven to give highest yield of papain by 36.75% when compared to grinding alone. Then further purification of the extracted papain was done by using ammonium sulfate precipitation. The optimized concentration of ammonium sulfate saturation was achieved at 55% concentration in order to fully precipitate the protein. The combination of grinding, enzyme-assisted extraction and ultrasonication can be considered as a potential extraction technique to extract papain as it is more delicate towards protein extraction. Furthermore, the investigation on other source of papain such as from the petioles, stalks and stem could also be done.

ABSTRAK

Penggunaan daun betik dalam pengeskrakan enzim papain telah dinilai melalui teknik-teknik yang berbeza iaitu terutamanya melalui pengisaran, penambahan enzim dan ultrasonikasi. Kemudian kepekatan enzim ditentukan dengan mengukur kadar asid amino yang terhasil di dalam tindak balas ujian protease. Hasil kombinasi teknik pengisaran, penambahan enzim dan ultrasonikasi telah terbukti menghasilkan ekstrak tertinggi sebanyak 36.75% lebih tinggi apabila dibandingkan dengan teknik pengisaran semata-mata. Seterusnya, pemurnian larutan tersebut terus dijalankan melalui kaedah penganapan ammonium sulfate. Kepekatan ammonium sulfate yang optimum telah dicapai pada kadar tepu 55% untuk menghasilkan mendapan protein sepenuhnya. Kombinasi teknik pengisaran, penambahan enzim dan ultrasonikasi boleh dipertimbangkan sebagai satu teknik yang berpotensi untuk mengekstrak enzim papain untuk kaedah pengekstrakan protein yang lebih halus. Tambahan lagi, kajian mengenai sumber lain untuk mendapatkan papain boleh dijalankan sama ada menggunakan batang dan dahan betik.

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LIST OF SYMBOLS/ABBREVIATIONS

<i>l</i> μ	-	1 x 10 ⁻⁶
°	-	Degree
%	-	Percent
<i>A</i>	-	Absorbance
<i>BAEE</i>	-	Benzoyl-L-arginine-ethyl-ester
<i>C</i>	-	Celcius
<i>EDTA</i>	-	Ethylenediaminetetraacetic acid
<i>g</i>	-	gram
<i>g</i>	-	gravity
<i>GPC</i>	-	Giant Capillary Conjunctivitis
<i>HPLC</i>	-	High Performance Liquid Chromatography
<i>Kg</i>	-	Kilogram
<i>kHz</i>	-	kilohertz
<i>L</i>	-	Liter
<i>MIC</i>	-	Minimum Inhibitory Concentrations
<i>min</i>	-	Minute
<i>mM</i>	-	MiliMolar
<i>ml</i>	-	milliliter
<i>mm</i>	-	Millimeter
<i>nm</i>	-	Nanometer
<i>TCA</i>	-	Trichloroacetic Acid
<i>US</i>	-	United States of America
<i>USD</i>	-	US Dollar
<i>UV</i>	-	Ultraviolet

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CHAPTER 1

INTRODUCTION

1.1 Background

1.1.1 Enzyme

Enzyme is known for its powerful attribute in catalyzing reactions. It is present in animal, plant and bacteria. You may find up to 4000 different kind of enzymes in an animal cell. It performs by lowering the activation energy of a given reaction, helps in speeding up the reaction identical to the catalyst. Enzymes are protein specialized to catalyze reaction. When an enzyme is present, the reaction occurs at a much higher rate and the enzyme itself is not consumed in the activity. For example, the saliva in our mouth contain amylase, the amylase helps in breaking down the starch to simpler unit making the food easier to digest in the stomach. In the food science application, enzyme has been acknowledged as a digestive supplements and have been added in the nutrition of the athletes. It had been shown that enzyme provide various benefits in improving human health. It helps in digestive disorders and also in weight management problems. Studies and researches should be done more in proving enzymes benefits and convince consumer in the advantages of enzyme in their health improvement. Besides, with advancement in the enzyme industry, it is not impossible that enzyme could be a potential investment in the future.

1.1.2 Origin

Papaya belongs to Caricaceae family and the plant is originated from Caribbean Coast of Central America. This small botanical family, native to tropical and subtropical America is represented by 31 species. *Carica papaya* is the most economically important and widely cultivated species. It is usually cultivated in the tropical and neo-tropical regions of the world between 32° North and South (Pantoja, Follett, & Villanueva-Jiménez, 2002). Even though papaya is believed to have been cultivated by early civilizations; no records are available prior to the arrival of Columbus in America (Morton, 1987). This herbaceous plant is also known as papaw, paw paw, kapaya, lapaya, tapaya, papayao, papaya, papaia, papita, lechosa, fruta bomba, mamon, mamona, mamao and tree melon. Dated back to 1600s, papaya is believed to found its way and reached Malacca (Litz, 2004). There are several local cultivars in Malaysia include Subang, Setiawan and Taiping (Krishnaiah D, 2002). Despite its long presence in the agricultural land of Malaysia, no significance improve in the use of the plant to be commercially grown widely besides enjoying its melon like fruits or served as fruit salads and cooked stews.

1.1.3 Morphology

The shape of papaya plant resembles palms leaves with large monoaxial palms. Young plants are single stemmed during the first 1-2 years of growth. However, in some highly fertile orchards, heavy lateral branch might also develop early on juvenile plants. Mature papaya leaves are palmate with deep lobes, supported by smooth, hollow petioles. This petiole gives highly textured leaf scars on the smooth, light tan, hollow stem. The persistent leaf scar enlarges as the plant grows in circumference. On the other hand, ripe papaya fruit resemble melons and are rich in vitamin A and C. The latex contains protease that is papain and is collected from unripe green fruit (Litz, 2004).

1.1.4 Papain

In the group of cysteine protease, papain family accounts the largest of it all. Papain may be obtained from bacteria, plant, vertebrates and invertebrates. Commercial papain available in the market is a plant derived preparation coming from the latex of papaya. Papain or cysteine protease hydrolase was first extracted from crude dried latex of papaya (Balls & Thompson, 1940). The term papain was first introduced by Wurtz and Brochut in describing the proteolytic principle in papaya latex (Boyer, 1971). Proteolytic enzyme is a group of enzyme capable of hydrolyzing the peptide bond in a protein molecule (Sandhya, Sumantha, & Pandey, 2008). Papain consists of a single polypeptide chain with three disulfide bridges and sulfhydryl group necessary for activity of the enzyme. The molecular weight of papain is 23,406 Da and its optimum pH for maximum activity is around 6.0 to 7.0. It will digest most protein substrate more extensively than the pancreatic proteases. Papain exhibits broad specificity, cleaving peptide bonds of basic amino acids, leucine or glycine. It also hydrolyzes esters and amino acids (IUBMB Enzyme Nomenclature, 2009).

1.1.5 Stability

Papain is soluble in water at 10mg/ml. Prior to use, the enzyme is typically diluted in buffer containing 5mM L cysteine. Its activation or stabilizing agents include EDTA, cysteine and dimercaptopropanol (Arnon, 1970). Although papain solution has good temperature stability, the solution stability is pH dependent. Papain solutions are unstable under acidic condition such as pH 2.8 and will result in significant loss of activity. Previous research had shown that papain extracted from latex will exhibit at low pH a partially structured state known as the molten globule state and A-states (Huet *et al.*, 2006). Papain solutions are stable to several denaturing and its full activity can be maintained after re-crystallization in 70% methanol and in 8M urea solution (Klein, 1969).

1.1.6 Application

Papain is commonly used in cell isolation procedures where it has proven more efficient and less destructive than other proteases on certain tissues. A study shows that papain has been used to isolate viable, morphologically intact, cortical neuron from postnatal rats (Heuttner, 1986). Hence, it is not surprising that papaya leaves have been used traditionally to wrap around meat and act as meat tenderizer. The other uses of papain have been reported in the use of clarifying beer, in tanning in cosmetic industry, manufacture of chewing gum and as a digestive medicine in the pharmaceutical industry (Litz, 2004). Papain has also been used in the contact lens solution. Patient of Giant Capillary Conjunctivitis (GPC) is advised to at least clean their contact lens with proteolytic enzyme papain solution once a week to remove deposits and quieting GPC (National Research Council, 1991). Traditionally, some of the plant extracts including papaya have been used to treat infections by gastrointestinal nematodes that have consequences on human and also in livestock farming. Recently, it is proven that this traditional method is really effective against the nematodes due to the presence of papain that is able to cause damage to the cuticles of the nematodes (Huet *et al.*, 2006). However in medicinal area, the use of papain together with other proteolytic enzyme in enzyme therapy for treating cancer patient has been useful. It is shown that papain can digest the protein fibrous coating or the blocking factor so that our body can kill the cancer.

1.1.7 Measurement of activity

The enzymatic activity of papain can be determined using benzoyl-L-arginine-ethyl-ester (BAEE) as the substrate. BAEE was dissolved in 0.1 M pH 6.1 papain solutions which contains 1mM EDTA, then 5mM L-cysteine was added. The reaction rate was determined by UV absorbance at 258nm with a UV-Vis spectrophotometer (Miyamoto, Watanabe, & Ishihara, 2004). One unit enzyme activity was defined as the activity that hydrolyzed 1 μ mole/min of BAEE at 37 $^{\circ}$ C (Arnon, 1970). Furthermore the enzyme activity could also be measured by HPLC method by terminating the activity with the addition of acetic acid. The reaction is

then filtered and the filtrate can be further analyzed by HPLC. The amount of product will be linearly related to the peak height and the rate of enzyme reaction can be accurately determined from the peak height of benzoyl arginine (Chen, Wu, & Wang, 1982). A simpler method used casein as a substrate and the reaction will be terminated by trichloroacetic acid. This colorimetric method also used Folin-Ciocalteu's reagent.

1.1.8 Antimicrobial activity of papain

The bioactive compound of leaf extracts can be investigated for antibacterial activity against some human pathogenic bacteria using the diffusion method. The standard organism which is the pathogen will be introduced separately and thoroughly mixed with molten nutrient agar in Petri dish. By punching holes on the agar to create wells, leaf extract sample would be left for several hours to diffuse. After that, the minimum inhibitory concentrations (MIC) would be determined by broth dilution technique. It helps to measure more exactly the concentrations of an antibiotic necessary to inhibit growth. Low MIC value is a good indication of high efficacy against the test organism (Anibijuwon & Udeze, 2009).

1.2 Problem statement

Papain, a cysteine protease is usually obtained from the latex of *Carica papaya*. Incisions will be made on a young green fruit of papaya to collect the latex from it. This is the well known approach and widely use in the commercialization of papain. However it is not only the latex that contains this valuable enzyme. In fact the whole parts of the papaya tree including its stems, petioles and leaves can be utilize for the extraction of papain except it's root (Sanchez, Serrano, & Rodriguez, 2009). One of the potential resources is the papaya leaves. Leaf tissue yielded the largest amount of lyophilized extract and protein (Sanchez, Serrano, & Rodriguez,

2009). Further purification and better extraction method to obtain higher yield and proteolytic activity is necessary to get the most out of papaya leaves.

Traditionally, in order to obtain intracellular products of plant cell, the leaves specifically would be ground or blended first. This is to make sure that large cells are broken by chopping action. Another alternative would be the incorporation of other extraction process of the material. Such of that is the use of ultrasonication and enzyme digestion. Ultrasonication is applied to provide mechanical shear force to break the cell wall of the wall. In addition, some of the cell wall degrading enzyme such as cellulase and glucanase can weaken or breakdown the cell wall leading to osmotic disruption of the cell hence more protein is release to the solution. Furthermore, there's also been an urge in food and medicinal processing to avoid or minimize the use of organic solvent in extraction process. The proposed method of using enzyme assisted extraction has not been tested before in extracting papain.

1.3 Objectives of study

The proposed research was studied to achieve the following objectives:

- 1) To investigate the extraction method of using ultrasonication and enzyme assisted extraction.
- 2) To measure the amount of papain concentration in the extracted material.

1.4 Scope of study

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

- 1) The type of extraction applied namely grinding, enzyme-assisted extraction and ultrasonication

- 2) The recovery of papain achieved by addition of different amount of ammonium sulfate to the solution from 40%, 45%, 50%, 55% to 60% saturation

1.5 Rationale and significance

Traditionally, papain will be only known to be extracted from its latex, no further study was done on the potential ability of extracting papain from the leaves. Other source of raw material would increase the utilization of the papaya tree and not just the fruit only is valuable. Besides, the employment of leaves that is considered to be a by-product of papaya cultivars would result in the waste to wealth application hence reducing the amount of waste.

Furthermore, the inquiry of the best extraction method that wants to minimize the use of organic solvent would open a room for more improvement of a readily available method. Besides, the potential ability of papain to be used in the cancer treatment would provide an opportunity for further investigation and research done in this area.

CHAPTER 2

LITERATURE REVIEW

2.1 Papain from papaya latex

Commercial products of papain available in the market were extracted from the latex of papaya. Previously, further purification of the extracted papain will undergo salt precipitation and drying (Balls & Thompson, 1940). Other modern technique to purify papain from fresh latex include aqueous two phase extraction (Nitsawang, Hatti-Kaul, & Kanasawud, 2006) and resulted in higher purity of papain obtain in much shorter time compare to salt precipitation. This technique also claims obtaining pure papain without any contaminating protease activity. Furthermore, some improvement have been made in the purification of papain without the use of high concentrations of salt or thiol containing substances reported by using natural inhibitors existent in latex resulting in crystallized papain (Monti *et al.* , 2000). It is reported that the latex of the green papaya contains much papain and higher enzymatic activity (Balls & Thompson, 1940). Classic method of extracting papain from the papaya involve the collection of latex by making incision on the unripe fruit and further processing of drying the latex will result in papain powder. In the recent suggested method for commercialization, spray dryer has been incorporated to assist in drying the latex of the papaya (Krishnaiah D, 2002). However, the effect of making incision on unripe green fruit would leave scars on the fruit resulting in lower market value.

2.2 Papain from papaya leaf

Analysis show that leaves, petioles and stalks of papaya also contain papain. It is also stated that in the earlier study, the methods of obtaining leaf papain are chemically possible (Balls & Thompson, 1940). Proteolytic study of the enzymatic extracts from leaf shown that leaf tissue exhibit the highest overall extract yield, extract protein content and total yield. Besides, leaves of papaya can be obtained throughout the years of cultivation and the quality only differs in the quantity of juice acquired (Balls & Thompson, 1940). In a large planting area, when leaves are eliminated during the end of productive cycle or from infected plants it is usually disposed in large area and left to rot. Some of them are occasionally used as animal feed if only the forage is scarce. Furthermore, the utilization of leaves would mean the utilization of the by-product of the papaya tree, hence maximizing the benefits of the papaya tree.

2.3 Commercialization of papain

Papain has a lot of beneficial use in the industries. Brewery, pharmaceuticals, food and household appliances are some of the sectors that have made use of papain. Thus, the end use of papain are varies and can be commercialize. In Malaysia, there have been no manufacturing of papain despite the suitable climate where papaya can be grown. Globally, papain is purchased and distributed by food ingredient specialist located at Europe and US (Agribusiness Development Centre , 2000). However, the major raw material used was from latex and there have been no reports on the manufacturing of papain from the leaf of papaya. Latex in no doubt is a rich source of papain but the benefits of papaya leaf as a by-product cannot be neglected. Furthermore, leaf is available throughout the season and raw material availability is important in the production of papain. Prices for papain has been quoted as low as USD 10/kg and as high as USD 80/kg for papain with a high TU measurement.

2.4 Production of papain

In India only, the production of papain annually is around 150 tones. About 35% is BPC grade papain and the rest is purified papain, 55% of the BPC grade papain is consumed internally and the rest is exported, while 90% of the purified papain is exported. The source of the production of papain solely relies on latex. The common process of production of papain would include the harvesting of the latex and drying. The detailed process is almost the same everywhere. The unripe papaya is first given a longitudinal cut in the evening and it is wrapped by polyethylene bag for collection of latex during next day morning. After collection, the latex is mixed with potassium meta-bisulfite (Kms) and kept in cold storage to avoid fermentation. Subsequently the latex is spread in trays and the drying takes about 4 hours. After drying the latex is scratched from the trays. It is again mixed with Kms and ground in hammer mills to get fine powder. Then its proteolytic activity is tested in laboratory. Subsequently, lactose is added to the latex powder to get BP of IP grade papain. The papain is finally packed in airtight polyethylene bags or metal containers (Viridi, 2009).

2.5 Papain as an intracellular product

Eventually, plant tissues do pose some specific problems which must be taken into account when developing methods for extraction. Plant tissues have rigid cellulose cell wall and this must be sheared to release the content of the cell. Besides that, it should be aware that papain might be exposed to oxidation during the extraction which would decrease the enzymatic activity. Apparently the loss of activity of enzyme during extraction process has been of great concern. Lower activity of the papain enzyme would result in lower value of the commercial product. Therefore, extraction method should be optimized to obtain the highest amount of enzyme.

2.6 Extraction of raw material

In developing the method for the extraction of leaves, other preparation of biologically active compound from leaves involve in the grinding or crushing of the leaves first. The samples would be ground in a blender. By grinding cell disruption will take place hence releasing the intracellular product. Cell wall disruption, which accelerates mass transfer and extraction kinetics, can be obtained by mechanical or enzymatic treatment, or a combination of both (Jung & A.Mahfuz, 2009). Some of other used method of extracting plant material is hot water extraction, subcritical water extraction (Ghoreishi & Shahrestani, 2009), supercritical fluid extraction (Floch *et al.*, 1998), solvent extraction (Fakeye *et al.*, 2007), microwave-assisted extraction (Pan, Niu, & Liu, 2003) and also bubble column extraction (Zhao, Zu, & Li, 2009). In order to minimize the use of chemical compound in the extraction process, the suggested method involves grinding using distilled water, ultrasonication and enzyme assisted extraction. In addition, extraction of enzyme or protein requires a more delicate or softer technique than mentioned previously. Because enzyme and protein is prone to denaturation and thermo labile, a high temperature operating condition or excessive use of organic solvent would affect the efficiency of the extraction process.

2.7 Ultrasonication

Ultrasound technology has been widely used in many areas such as food processing, process improvement and in the biological disintegration, it is the most important technology at present as a laboratory method to extract extracellular component (Valero *et al.*, 2007). Most of the research done in this area implemented ultrasonication as an extra tool in extraction process. Ultrasonication is used most commonly to disrupt various types of cell including prokaryotes and eukaryotes (Ahmed, 2005). This process will create vibrations that cause mechanical shearing of the cell wall or known as the effect of cavitation (Jap'on-Luj'an, Luque-Rodríguez, & Castro, 2006). Cavitation is the formation of tiny bubbles cause by fast adiabatic compression and expansion which give rise to high temperatures and

pressures within the bubbles but only minimal effect to the temperature of overall system (M.D. Luque de Castro, 2004). In the ultrasonic extraction of Epimedium C from Epimedium fresh leaves, cell damage was assessed using transmission electron microscopy where plasmolysis occurs in the leaf cells after ultrasonic-assisted extraction (Zhang *et al.*, 2009). From the literature, it is suggested that ultrasound has destructive effects on the structures of leaf tissue, the cell walls and the chloroplast. This mechanical action of ultrasonic is very important to facilitate swelling and hydration process which is vital in extraction improvement (Vinatoru, 2001). At the moment, sonication had been employed to extract various active compounds including flavonoids, saturated hydrocarbons, fatty acid esters, methyl xanthine, and steroids from plant material. The mechanism of which ultrasonication work that is the collapse of the cavitation bubbles near cell walls is expected to produce cell disruption which is exactly the target of rendering ultrasonication. In one work comparing the use of ultrasonication and maceration, the utilization of ultrasound has proven to be much simpler, faster and more effective method than traditional maceration to extract organic compound from plant matrices (Jacques, *et al.*, 2007). In that case, extraction of enzyme can also be done using ultrasonic extraction. In one particular work of enzyme extraction from sludge flocs, ultrasonication has been successful to provide a mean of extraction without inactivating the enzyme and fully extracted it from the sludge (Guanghai *et al.*, 2009). Moreover, integrating ultrasonication in the enzyme extraction from plant material can be fully realized if further research done such as the effect of localized temperature produced from the cavitation effects at larger scale and also the feasibility of ultrasonication to be implemented in industrial use (Vinatoru, 2001).

2.7.1 Mechanism of ultrasonication

During ultrasonication, liquids are sonicated at high intensities, the sound waves that propagate into the liquid media result in alternating high-pressure and low-pressure cycles, with rates depending on the frequency (Suslick, K.S, 1998). During the low-pressure cycle, high-intensity ultrasonic waves create small vacuum bubbles or voids in the liquid. When the bubbles attain a volume at which they can

no longer absorb energy, they collapse violently during the high-pressure cycle. This phenomenon is termed cavitation. During the implosion, very high temperatures and pressures are reached locally. The implosion of the cavitation bubble also results in liquid jets of up to 280m/s velocity. The resulting shear forces break the cell envelope mechanically and improve material transfer. Ultrasound can have either destructive or constructive effects to cells depending on the sonication parameters employed.

2.7.2 Condition for ultrasonication extraction

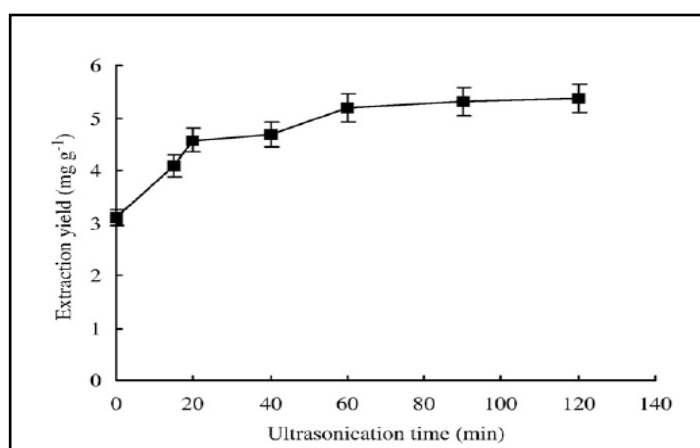


Figure 2.1 Effect of ultrasonication time on the extraction yield of Epimedin C from fresh Epimedium leaves (Zhang *et al.*, 2009).

The ultrasonication time proposed is between 20 min- 60 min and from the figured above the extraction yield of Epimedin C clearly levitates as ultrasonication time is extended as from 20 min to 60 min. However the effects are diminished at certain value and additional time in ultrasonication might not affect the yield of the extraction. Furthermore, it must be noted that the ultrasonication time may not exceed 60 min when time consumption is considered. So the highest extraction would be expected in 60 minutes.

In view of the power applied to the system during ultrasonication, the values are varied between 20 KHz and 500 KHz. Ultrasound increases the swelling index by

vegetated material during sonication. The extracted value is much greater under sonication compared with mechanical stirring. The mechanical effects of ultrasonication during extraction have been demonstrated in the use of marigold leaves when sonicated at 20 kHz and 500 kHz. The external oil glands were observed and the lower frequency was found to destroy all excretion hair as well as part of the leaves, whereas high frequency left tissue unaffected. Figure 2.2, 2.3 and 2.4 show the observations that have been made to the marigold leaves at different sonication power.

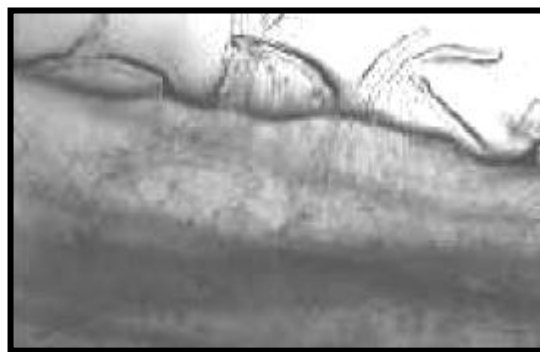


Figure 2.2 Control, non-sonicated, shows a high density of excretion hairs (Vinatoru, 2001).



Figure 2.3 Pot marigold petal sonicated at 20 kHz. All excretion hairs were destroyed by ultrasound (Vinatoru, 2001).



Figure 2.4 Pot marigold petal sonicated at 500 kHz. Only top of excretion hairs are destroyed (Vinatoru, 2001).

2.8 Enzyme assisted extraction

Enzymes are extremely versatile compounds that can catalyze a wide range of chemical reaction. Because of their characteristics, they are, in principal, an ideal catalyst for use in analytical applications as they act in aqueous solutions at ambient temperature and atmospheric pressure. Furthermore, the application of enzyme is not limited to that but also has been used in extraction of intracellular material from plant cell. Besides the mechanical action applied to any plant cell such as grinding, milling and ultrasonication the use of enzyme as a tool in extraction process is extensive. This is due to the fact that enzyme extraction is more environmental friendly and economical (Zu, *et al.*, 2009). In the industrial process, enzyme assisted extraction has been applied in the extraction of oil from a seed or fruit. Compare to the conventional method, the incorporation of enzyme treatment in the process has proven to yield a good quality of extraction oil, higher protein content and also a reduction in the operation time (Dominguez, Nunez, & Lema, 1995).

In addition, in one work observing the enhancement of extraction from grape seed oil with enzymatically pre-treated seed, it is shown that extraction yield is higher than the application of the supercritical fluid extraction alone (Passo *et al.*, 2009). In cosmetic and pharmaceutical industry in which the use of enzyme is more promising, the cost of associated with enzyme treatment can be neglected and use to extract more valuable oils. In addition, although enzyme extraction often be treated

as extra process or as a pre-treatment of raw material, the incorporation of enzyme treatment can provide a valuable method to reduce the use of conventional methods which are presents some industrial disadvantages such as plant security problems, emissions of volatile organic compounds up to the atmosphere, high-operation costs and poor quality products caused by high processing temperatures (Zuñiga *et al.*, 2003). Hence, enzyme extraction can be perceived as a tool for more delicate extraction and also valid at industrial level.

2.8.1 Mechanism of enzyme action

Our target protein, papain is located in the intracellular of the plant cell. Hence it is desired to make sure papain is released to the solution for better extraction and higher yield obtained. The use of cell wall degrading enzyme has been suggested to be able to degrade the cell wall of plant tissue containing cellulose, hemi-cellulose and pectin (Fu, *et al.*, 2008). The mechanism for enzyme-assisted extraction is that enzyme such as glucanase and pectinase can weaken or break down the cell wall rendering the intracellular materials making it more accessible for extraction (Li, Smith, & Hossain, 2006). The enzyme will treat the cell wall as the substrate and digest the material comprising the cell wall. However different enzyme may give different yield as some enzyme comprises the activities of other protein they encounter during cell wall degradation (Fu, *et al.*, 2008) and also by their characteristic in nature. Hence it is of the utmost important to see which enzyme or mixture of enzyme to give better extraction by doing selection of enzymes first (Fu, *et al.*, 2008).

Although the field of interest of enzyme assisted extraction is in degrading the cell wall of plant the use of enzyme is not limited to extraction of plant material process only. In the analysis of catechin a type of flavonoids in the milk-tea beverages, the use of enzyme hydrolysis is capable to release the catechins from their conjugated forms. From the study, the short time pepsin treatment sufficiently digested the protein fraction to disrupt the catechin-protein interaction and eventually enhance their recovery from the milk matrices (Ferruzi & Green, 2006). In another

invention to improve a process for obtaining instant tea, the treatment of the tea leaves with the tannase and cell wall digesting enzyme leads to a soluble tea with a unique biopolymer profile. The use of enzymes release and modify plant cell wall biopolymers thus creates a product which contribute to flavor and acid stability of the instant tea (Lehmberg *et al.*, 1999). Moreover, in the extraction of oil it is well established that oil bodies in plant are trapped in the meshwork of proteins and cellulose/hemicelluloses structures, thus the treatment of enzyme will eventually assist in disbanding the structures (Shah, Sharma, & Gupta, 2004).

2.8.2 Type of enzyme used

Different desired material may affect the type of enzyme to be use for pre-treatment. When it comes to extracting oil from oil seed or kernels, the enzymes used are usually enzyme cocktail or a mixture of enzyme. The most common ingredient of enzyme cocktail includes cellulase, protease and pectinase (Zuñiga *et al.*, 2003) (Shah, Sharma, & Gupta, 2004) (Passos *et al.*, 2009) (Dominguez, Nunez, & Lema, 1995). In the enhancement of grape seed oil extraction, 4 types of enzyme were used namely the cellulase, protease, xylanase and pectinase (Passos *et al.*, 2009). Other works relating to the use of plant leaves applied the use of cellulase, beta-glycosidase and pectinase (Fu, et al., 2008) (Zu, *et al.*, 2009). However in the work of tea protein extraction the use of the enzymes cocktail is quite different, incorporating the use of alcalase and protamex (Shen *et al.*, 2008).

The type of enzyme used would depend greatly on the working material. If the desired material contains inside a rigid cellulose cell wall, the perfect matching enzyme will be the combination of cellulase and pectinase. Cellulase will randomly splits cellulose chains into glucose while pectinase will breakdown or transform the pectin that stabilizes the cell wall (Cinar, 2005).