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REVIEW ON ISOLATION AND PURIFICATION OF PAPAIN ENZYME FROM PAPAYA FRUIT

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Abstract **- Papain is one of leading enzyme from papaya plant which has many applications in medical field. So collection, isolation and purification of papain enzyme is necessary from papaya plant to use. In this review paper, isolation and purification of papain from papaya peels, latex of papaya fruit and commercially available papain is discussed with some optimized parameters explained by previous researcher. Whereas purification with Precipitation method, Aqueous-Two Phase system (ATPS), Reverse-Micelles System (RMS), Three Phase Partitioning (TPP) are also discussed with result and future scope. This all techniques are very selective for purification of enzyme or protein one of which salt precipitation is one of easiest method for purification. TPP and ATPS had showed better results in activity and purity of papain enzyme respectively and RMS has also showed better result but disadvantage in large scale production. This paper also gives some importance of purification and small introduction to papain enzyme.**

Keywords**- papain, papaya, purification, isolation, peels, latex**

I. INTRODUCTION

Enzymes which are found in plant and its product are called plant protease. Enzyme may formed simultaneously with proteins, as for maintaining balance between protein formation and denaturation [1]. There are many plant parts from which enzyme can be extracted. But mainly, the activity of a particular enzyme is found in latex producing plant [1].

Papain is one of plant enzyme, extracted from papaya fruit, as it is found in larger quantity. It is globular protein with 212 amino acid and weight of 23 kDa. Papain is cysteine protease and comes with high stability at wide range of condition even at high temperature [2].

Papain has many medicinal properties such as curing allergies, healing of sport injuries [2], wound debridement [3], degradation of toxicity or adverse effect of drug [4], meat tenderization [5], antibacterial, etc. Extraction of papain from plant is necessary due to wide application in medicinal uses, pharmaceutical, food industries, etc. As papain has many scientific applications, it needs to be extracted in small quantity for research work or large quantity for industrial purpose.

Extraction and purification of enzyme is still a major concern for researcher, as stability of enzyme need to be maintain and making environment according to enzyme is bit difficult. Many methods had been employed to isolate and purify papain enzyme from various part of papaya plant. Some of methods are Precipitation, Three-Phase Partitioning (TPP), Reverse Micelles System (RMS), Aqueous Two-Phase System (ATPS), etc. and selectivity of technique should be needed when we talk about protein extraction. Every technique has its own advantage and disadvantage but as all depends on results. In this review paper, different techniques are discussed for isolation and purification of papain from papaya peels, latex even commercially available crude papain enzyme.

II. RELATED WORK AND METHODOLOGY

2.1. Collection and Isolation of crude papain enzyme:-

2.1.1. From latex of papaya fruits:-

Latex is fluid material present in fruit. This latex helps to nourish fruit, help in ripening of fruit, etc. Latex of papaya fruit contain papain enzyme which is collected from fruit by making cut/incisions on the fruits with a sharp stainless steel apparatus [6]. During collection of latex from papaya fruit, some points are need to remember that during incision on fruit it should not cut more than 2mm, as different juice get mixed with latex and lowers the quality of crude papain enzyme [7]. After incision latex

should be collected within 1-2 min(s) as after it rapidly dry [8], and during of latex collection, foreign dirt, and other contamination should be avoided [3]. Before storing latex, 0.3M NaOH should be added as it helps from avoiding oxidation [7]. Commercially available papaya latex is also available as a spray dried papaya latex.

2.1.2. From peels of papaya fruits:-

Peels are one of major waste producing part from every fruit. Papaya peels contain papain enzyme which can be isolated by different method. In 2007, Chaiwut, Nitsawang, Shank and Kanasawud showed that isolation of peels can be done by removing peel of papaya and chopping into suitable small pieces followed by drying at 55ºC until 10% w/w obtained. Then dried peels were grounded in a blender and soaked in distilled water. After that filtration and centrifugation was done to obtain a clear solution of papaya peel crude extract. In 2010, Chaiwut, Pintathong and Rawdkuen showed comparison between fresh and dried peels, and also used two extractant (water and 50mM phosphate buffer of pH 7) at ratio 1:9 (w/v) followed by centrifugation and filtration. Collection of papain can also be done by molding whole plant in mortar and pestle in which better activity was at pH 6.5 with volume of 2ml[11].

2.2. Purification of papain:-

Purification is one of major concern for many researchers to get purified final product especially when we talk about protein or enzyme. As this also needs for many industries to purify plant product and increase in revenue. Purification helps to reduce contamination and concentrating desired analyte to increase specific activity.

Once crude extract is ready, various methods are employed or available to purify enzyme. The extract is subjected to treatment that separate proteins into different fraction and process is called fractionation. Early fractionations steps utilize difference in protein solubility, which is a complex function of pH, temperature, salt or reagent concentration, and other factors [12].

2.2.1. Purification by precipitation methods:-

Precipitation is of the simple and very easy technique to purify protein. In precipitation method, commonly ammonium sulfate salt is used as it is very soluble in water and has an ability to interact with more water molecules and other remain as precipitate. Nitsawang, Hatti-Kaul and Kanasawud performed two-step salt precipitation method in 2006, where first 45% saturated of ammonium sulfate used and secondly sodium chloride. In this method initial salt shows protease activity of papain was maximal of 39% with lower purified. Whereas second salt gives protease activity low and purity of papain enzyme became 86-90%.

Other than ammonium sulfate salt and sodium chloride, some chemical reagent can also been employed for precipitation of enzyme. Chaiwut, Nitsawang, Shank and Kanasawud (2007) has also showed that precipitation can also be done with help of alcohol solution. This experiment showed that ethanol is good precipitating agent for enzyme than ammonium sulfate salt. They used 70% ethanol whereas A. Manosroi, Chankhampan, Pattamapun, W. Manosroi and J. Manosroi (2014) used 95% ethanol with sodium chloride and ammonium sulfate, which showed 82.31% purity of papain.

2.2.2. Purification by ATPS:-

Aqueous two-phase extraction is used to purify protease from crude extract as it is a selective technique in downstream processing. Nitsawang, Hatti-Kaul and Kanasawud (2006) had shown that ATPS is better purification process for papain than salt precipitation. This experiment had also shown purification using different amount of ammonium sulfate and polyethylene glycol.

Li et al. (2010) has performed an experiment on purification of papain by making ATP system in tube which consist of enzyme solution, polyethylene glycol, salt solution and deionized water. They had experimented on commercially available dried latex papain with Response Surface Methodology (RSM) used to optimize the ATPS process.

In 2013, Rathnasamy and Kumaresan showed purification by using Ionic Liquid based ATPS (IL-ATPS). Forward extraction was carried out by mixing enzyme solution with appropriate amount of Tetra Butyl Phosphonium Bromide (TBPB) plus dipotassium phosphate directly in the equal amount. Backward extraction was performed for enzyme recovery. NaCl of around 0.5 M was added in ATPS to modify ionic strength. According to optimization study of IL based ATPS, for better purification, concentration, temperature, and pH of system should be 150mM, 30oC, and 7.5 respectively.

2.2.3. Purification by Reverse Micelles (RMs) System

All protein or enzyme can be separated by RMs system as this help to solubilize protein in aqueous environment while other in organic phase. This is due to RMs are heat stable, nano-sized of

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surfactant which are encapsulated small amount of H_2O in organic solution [16]. Commercially available papain can be purified by RMs technique. This experiment was shown by Mathew and Juang (2005) and purified papain and also recovered papain activity in greater extent. They took commercially available papain and subjected to forward extraction with sample and same amount of organic and aqueous solution followed by centrifugation. The upper phase of solution was then subjected to back extraction where new aqueous phase was added which consisting of 10% (v/v) isopropyl alcohol or counter-ionic surfactant tri-n-octylmethyl-ammonium chloride (TOMAC) (0.01-0.07 mol/dm^3).

2.2.4. Purification using TPP

Three-Phase Partitioning is one of simple, rapid method for purifying and concentrating protein. In 2010, Chaiwut, Pintathong and Rawdkuen showed purification of papain from papaya peels. In first extraction, ammonium sulfate and t-butanol was used. Then bottom phase was subjected to second stage TPP with t-butanol and ammonium sulfate solution to obtain concentrations around 55% (w/v) (result in Table 1). Latex can also be purified by using TPP [18].

III. RESULTS AND DISCUSSION

3.1. Isolation and purification of papain from papaya peels:-

3.2.

From Chaiwut, Nitsawang, Shank and Kanasawud experiment in 2007, isolation was done to make crude sample solution for reagent precipitation experiment followed by optimal activity of papain at various factors. This experiment shows that on crude sample of papain enzyme, 70% (v/v) Ethanol reagent gave good proteolytic yield as well as specific activity. From the Table 1, it can be said that ethanol is better precipitating agent than ammonium sulfate salt. In Table 1 there is increase in specific activity so here we can say that purity of papain is also increasing and separation of papain from different protein is also occurred. After checking optimal activity of papaya peel extracted sample at different temperature, pH, and concentration, it shown that at 75^{o}C, pH at 8 and concentration of 5mM has higher papain activity. In 2010, Chaiwut, Pintathong and Rawdkuen conducted experiment with fresh and dried papaya peels and shown that dried peels results in major specific activity with water as an extractant with lower conc. which was at pH 4.3. Whereas fresh peels shows major concentration with lower specific activity with buffer of pH 7. So here, theoretically, we can say that using dried papaya peel with water as an extractant and maintaining pH at 7, can improve specific activity with higher protease conc. of papain enzyme. Water is can be used as an extractant because it maintain structural stability of papain and also it has very good extracting property of polar substance [19]. As Chaiwut, Pintathong and Rawdkuen concluded that using water can decreased protease activity of papain. This result can be due to different part of plant contain different structural papain enzyme. Also to maintain stability of papain in papaya peels, 0.75% sodium meta-bisulfate can be added [5]. After that Chaiwut, Pintathong and Rawdkuen (2010) conducted TPP method for purify papain and showed that intermediate phase of second TPP extraction gives better specific activity of papain (as given in Table 1).

3.3. Isolation and purification of papain from commercially available product:-

Commercially available papain was purified with help of ATPS and RMS. In 2005, Mathew and Juang used commercially available papain and purified by using bis(2-ethylhexyl)sulfosuccinate (AOT) reverse micelles. In this experiment, first forward extraction was carried out for papain and purity found to be 60-70 %. As to get more pure papain, second backward extraction was performed with counter ionic surfactant and purity was found to be 80 – 90 %. On other hand back extraction was performed with isopropyl alcohol and also got 80 – 90% purity but with low activity recovery than TOMAC. Whereas, Li et al. (2010) had shown experiment, in which commercial available latex was purified with ATPS. In this experiment optimized condition were applied and purity was found to be 96 – 100% (data given in Table 2). Both experiments contain commercially available papain material which is derived from latex of papaya fruit. So after collection and isolation of latex from papaya fruit we can separate or purify papain from crude by ATPS or RMS.

3.4. Isolation and purification of papain from papaya latex:-

Purification of papain from latex was performed with precipitation method and ATPS [13]. In this two-step salt precipitation method was employed for thawed latex and found to be 86 – 90% purity of papain but with lower activity. Whereas in ATPS, with 8%PEG – 15% ammonium sulfate, it gives 88% purity of papain was with higher activity than salt precipitation. Rathnasamy and Kumaresan (2013) used IL-ATPS to purify thawed latex of papaya fruit. And in this experiment, purity was found to be 96.22% pure papain enzyme (data in Table 3). Three phase

partitioning technique can also used in papain purification from latex and was showed by Urgessa, Itana and Raga in 2019 under optimized parameter of TPP where purification fold was found to be 8 in intermediate phase and 6.61 in bottom phase with activity recovery 94.14% and 75.15% respectively (data in table not showed).

If papain is used as collagenolytic activity, or use in wound debriment, meat tenderization, then simple precipitation method is enough. Whereas when we need papain as a drug or in various drug applications, it needs to be pure enzyme. When we talk about peels of papaya fruit then using waste peels can help to reduce waste as well as it help to extract papain enzyme from it as also it is used in cattle food. For latex collection, raw papaya fruit will be beneficial as it contain more amount of latex and therefore latex is used in many industries for papain enzyme.

Table 1:- Purification of papain from peel

Method	Total protein content (mg)	Specific activity (U/mg)
60% saturated $(NH_4)_2SO_3$	14.17	1.15
70% Ethanol	15.09	1.68
TPP (1^{st} step)	1.44	480.6
TPP (2^{nd} step)	0.62	727.4

Table 2:- Purification of papain from commercially available papain

Methods	Amount of papain (%)
RMS (forward extraction)	60-65
RMS (backward extraction)	80-90
ATPS	96-100

Table 3:- Purification of papain from latex

Methods	Amount of papain (%)
Two-Step Salt precipitation	86-90
ATPS	88
IL-ATPS	96.22

IV. FUTURE SCOPE

Analyzing of papain from different geographical area from different selectivity technique with optimized parameter and for various impact factors would be beneficial. As different part of plant contain different protein as contamination with papain enzyme. Making ATPS technique for larger production is little bit hazardous in purification process as PEG is little bit toxic. Development needed for more economical technique for purification and to maintain activity of enzyme.

V. CONCLUSION

Although when we talk about application of enzyme then activity of enzyme should be maintain and if to reduce adverse effect of various protein content with enzyme, purity comes into the picture. ATPS, RMS, TPP, Precipitation, all this techniques help to increase selectivity of enzyme or protein in extracting process. For purity of papain, ATPS is consider as good extracting technique as it shows better results in latex purification. Whereas in activity of papain, TPP shown better results in both parts i.e. peel and latex of papaya fruit. TPP and ATPS can be used in large scale whereas RMS comes in lower scale production as many issues occurs to make it difficult process in large scale production. Other than above listed method, for purification of papain, chromatography method is also used.

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