Korean J. Chem. Eng., **23**(6), 972-976 (2006)

Hydrolysis of raw hide using proteolytic enzyme extracted from papaya latex

Sittiruk Pitpreecha and Siriporn Damrongsakkul†

Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University, Bangkok 10330, Thailand
(*Received 30 May 2006 • accepted 27 June 2006*)

Abstract−Crude proteolytic enzyme was extracted from papaya latex using two solvents, water and phosphate buffer pH 6. The yield of extracted enzyme using water as a solvent was similar to that using phosphate buffer. Following the solvent extraction, the extracted enzyme was precipitated in 45 wt% saturated ammonium sulfate solution. The yield and activity of precipitated enzyme considerably decreased. Crude proteolytic enzyme extracted using water as an extracting liquid was, therefore, selected to use in gelatin production from raw hide hydrolysis, comparing to the use of commercial papain. The effects of hydrolysis conditions on gelatin recovery and properties of obtained gelatin were investigated. The optimum conditions for the activities of both crude extracted enzyme and commercial papain were at 75 °C and pH 7. At this condition, the highest percentages of gelatin recovery were obtained from raw hide hydrolysis reactions. The gelatin recovery and gel strength of gelatin obtained from crude extracted enzyme and commercial papain hydrolysis were similar. This proved that crude extracted enzyme from papaya latex could be effectively used in gelatin production, instead of the use of commercial papain, with a comparatively low cost.

Key words: Gelatin, Enzymatic Hydrolysis, Papaya Latex, Papain, Proteolytic Enzyme

INTRODUCTION

Gelatin is used in many traditional applications such as in the food, pharmaceutical, photographic, and cosmetic industries. Gelatin is a large protein obtained from partial hydrolysis of collagen, the most common fibrous protein found in skin, hide, bone, horn and white connective tissue of animals. There are two typical methods of gelatin production: acid and liming processes. But these two typical methods have many disadvantages such as long period of processing time, and considerable waste generated. In the past decades, the enzymatic hydrolysis of several natural materials has been extensively studied and several substantial improvements, such as yield, were reported [Oh et al., 1987; Park et al., 2001; Guerard et al., 2001]. The enzymatic hydrolysis for gelatin production is also of interest since the processing time is short and a small amount of waste is generated. There are many works on enzymatic hydrolysis for protein waste treatment [Taylor et al., 1989; Simeonova et al., 1996; Aspmoa et al., 2005]. They reported the methods of protein waste hydrolysis using various type of commercial proteolytic enzymes. The most recent work of Ratanathammapan [2005] on the uses of papain in the gelatin production from raw hide hydrolysis reported that papain could be used to produce low gel strength gelatin. However, the production of commercial papain is not available in Thailand and the imported papain is expensive. It is well known that papain is a plant-derived proteolytic enzyme from papaya, which is a local plant of Thailand. This work, therefore, aimed to utilize proteolytic enzymes extracted from local papaya latex in the production of gelatin from raw hide hydrolysis. The crude extraction of proteolytic enzymes from papaya latex was first investigated. Then, the obtained crude extracted enzyme was used in the hydrolysis of raw hide to produce gelatin. The performance of crude extracted enzyme and the properties of obtained gelatin were investigated and compared with the case of using commercial papain.

†To whom correspondence should be addressed.
E-mail: siriporn.d@chula.ac.th

EXPERIMENTAL

1. Crude Extraction of Proteolytic Enzymes from Papaya Latex

Local papaya latex used in this work was collected from the unripe papaya (*Carica papaya*), C.V. Khag Dam. The solvents used to extract proteolytic enzymes from papaya latex were distilled water and phosphate buffer pH 6. Papaya latex and solvent were mixed at the volumetric ratios of latex to solvent=1 : 1. The mixture was stirred at 4 °C for 10 minutes and centrifuged for 30 minutes. The supernatant was separated from residue by vacuum filtration. A part of extracted enzyme solution was lyophilized prior to characterization, i.e., protein determination and enzyme activity assay. The other part of extracted enzyme solution was precipitated in 45 wt% saturated ammonium sulfate solution at 4 °C. After that, the precipitate was separated by centrifugation prior to lyophilization.

2. Enzymatic Hydrolysis of Raw Hide

Raw hide used in this work was limed split, provided by World Pet International Co., Ltd. The dried split was ground into an average size of 1.18 mm (mesh number 16). The hydrolysis reaction was carried out at the ratio of raw hide to phosphate buffer solution =1 : 4 [Ratanathammapan, 2005]. Raw hide was well mixed with phosphate buffer solution at the desired pH and the slurry was then heated to the desired temperature. When the desired temperature (hydrolysis temperature) was reached, the enzyme was added. The samples were periodically collected and heated to 90 °C for 15 minutes to deactivate enzyme. Centrifugation and filtration techniques were used to separate gelatin solution from hide residue. The filtered gelatin solution was brought to determine the protein concentration by Lowry method. The rest was dried prior to gel strength measurement.

3. Enzyme Characteristics

The activities of crude extracted enzyme and commercial papain

(supplied by Fluka) were assayed according to the method modified from the assay of endo-protease using azo-casein (Megazyme). The yield of enzyme activity was defined as follows:

$$Y = \frac{S}{L} \times 100 \quad (1)$$

where

Y : The yield of activity of crude extracted enzyme
S : The total activity of sample
L : The total activity of papaya latex

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine the molecular weight of proteolytic enzyme, using 12.5% polyacrylamide gel. The samples were prepared in 5X Sample buffer (bromopheno).

4. Protein Determination

Lowry method was used to determine the percentage of protein content in gelatin solution. The absorbance of the sample at the wavelength of 750 nm was measured with a UV-VIS spectrophotometer. The standard curve was prepared by using bovine serum albumin (BSA). The percentage of gelatin recovery was calculated based on the following equation:

$$P = \frac{G}{K\left(\frac{H}{V}\right)} \times 100 \quad (2)$$

where

P : Percentage of gelatin recovery [wt%]
G : The protein concentration of gelatin solution [g/ml]
K : The protein fraction of raw hide=0.6362 [Pitpreecha, 2006]
H : The weight of raw hide [g]
V : The volume of buffer solution [ml]

5. Gel Strength of Gelatin

The gel strength of gelatin was determined on a 12.5% gel (w/v), formed by dissolving the dried gelatin in distilled water at 60 °C, and cooling the solution at 4 °C (maturation temperature) for 16-18 h. The gel strength of gelatin was determined by using a texture analyzer, equipped with 1.27 cm diameter hemisphere head cylindrical at a cross-head speed of 0.8 mm/s. The gel strength of the gelatin sample was determined by measuring the force required to depress the center of gel surface vertically to a depth of 4±0.01 mm. The measured gel strength was in the double bloom standard.

RESULTS AND DISCUSSION

1. Crude Extraction of Proteolytic Enzymes from Papaya Latex

Fig. 1 presented the yield of activity of proteolytic enzyme from solvent extraction step (before precipitation) and precipitation step. The yields of crude proteolytic enzymes extracted using water and buffer as extracting liquids were similar. After a precipitation step in 45 wt% ammonium sulfate, it was found that the yield and enzyme activity of enzyme significantly decreased. This was generally known that as an extracted substance was purified, the extracted yield would be decreased [Belter et al., 1998; Wuttiwiangtham, 1993]. The enzyme activity of crude enzyme solution was extracted using water (before precipitation) and after precipitation in ammonium sulfate, depicted in Fig. 2, demonstrated that after precipitation the activity

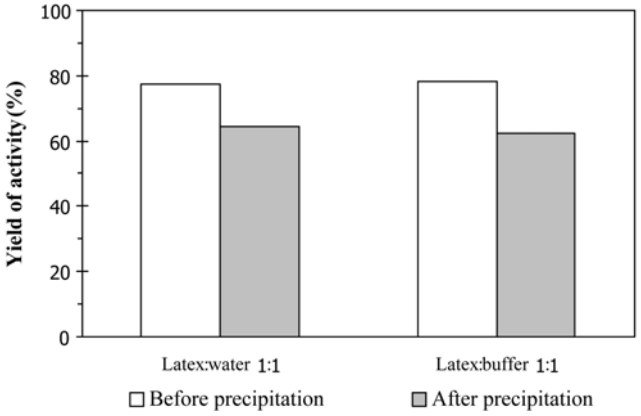

Fig. 1. Yield of activity of crude proteolytic enzyme extracted using water and phosphate buffer pH 6 as solvents (referred as "before precipitation"), and yield of activity of precipitated enzyme in 45 wt% ammonium sulfate (referred as "after precipitation").

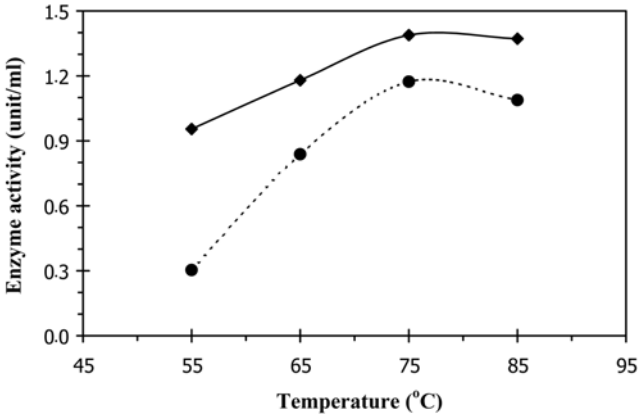

Fig. 2. Comparison of the activities of 1% w/v crude proteolytic enzyme solution at various temperatures, pH 6. Proteolytic enzyme was extracted from papaya latex using water as a solvent at a latex : water ratio=1 : 1 (referred as "before precipitation") and precipitated in 45 wt% ammonium sulfate. (-◆-) before precipitation, (-●-) after precipitation.

of crude proteolytic enzyme decreased at all temperatures. The result suggested that the precipitation step might not be necessary for crude extraction of proteolytic enzyme from papaya latex.

Crude proteolytic enzyme extracted using water as a solvent without precipitation in ammonium sulfate (further referred as crude extracted enzyme) was further compared to commercial papain in terms of activity and molecular weight. Fig. 3, presenting the activity of crude extracted enzyme and commercial papain as a function of temperature and pH, shows that the activity of crude extracted enzyme increased as the temperature increased from 55 °C to 75 °C, then started to decrease as the temperature was higher than 75 °C. The trend of the activity of crude extracted enzyme was similar to that of commercial papain, but the activity of crude extracted enzyme was slightly lower than that of commercial papain.

Fig. 4 reveals the molecular weight analysis of crude extracted enzyme and commercial papain by SDS-PAGE. It could be noticed that for crude extracted enzyme, there were at least three main mole-

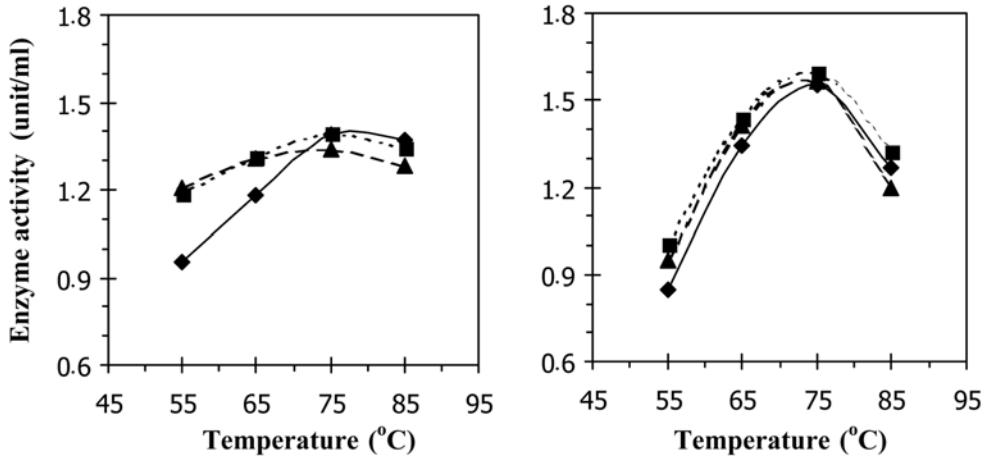

Fig. 3. Activity of 1% w/v crude extracted enzyme using water as a solvent (latex : water ratio=1 : 1), and 1% w/v commercial papain: (-◆-) pH 6, (-■-) pH 7, (-▲-) pH 8.

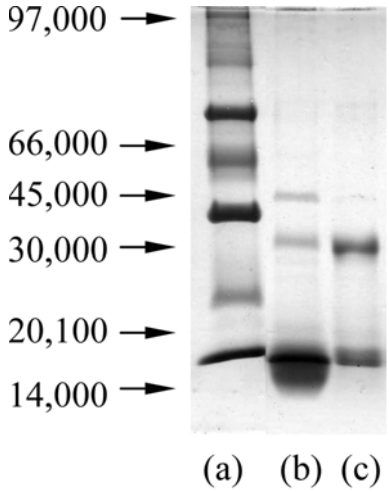

Fig. 4. SDS-PAGE analysis of: (a) low molecular weight standard marker, (b) crude proteolytic enzyme extracted using water, and (c) commercial papain.

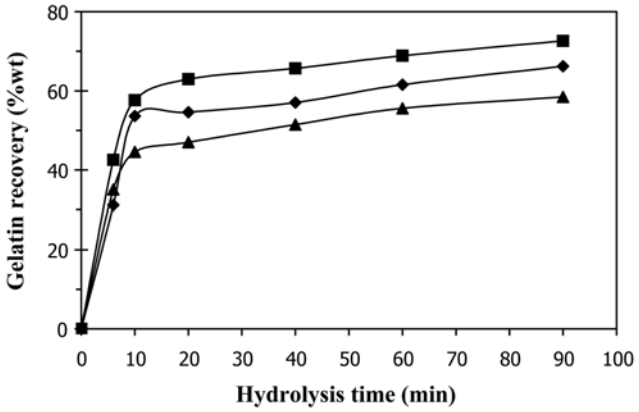

Fig. 5. Gelatin recovery from raw hide hydrolysis using crude extracted enzyme at pH 7 and various temperatures: (-◆-) 65 °C, (-■-) 75 °C, (-▲-) 85 °C.

cular weight bands; 35 kDa, 21 kDa, and 14 kDa. The approximate molecular weight of 35 kDa and 21 kDa was identified as chymopapain and papain, respectively. The lowest molecular weight band, 14 kDa, was dye marker from the 5× sample buffer. In case of commercial papain (lane c), 2 protein bands; 21 kDa and 14 kDa appeared on polyacrylamide gel. This elucidated that crude extracted enzyme still contained chymopapain which was a less active enzyme, compared to papain. As a result, the activity of crude extracted enzyme was less than that of commercial papain.

2. Enzymatic Hydrolysis of Raw Hide

The hydrolysis of raw hide was carried out using the condition at which the activities of crude extracted enzyme and commercial papain were highest (75 °C, pH 7). The optimal ratios of enzyme to raw hide of crude extracted enzyme and commercial papain were fixed at 0.035 : 100 and 0.15 : 100, respectively [Pitpreecha, 2006]. This provided a fair comparison as the total activity used in both hydrolysis reactions was the same.

The percentage of gelatin recovery from crude extracted enzyme and commercial papain hydrolysis reactions is depicted in Figs. 5 and 6, respectively. From both hydrolysis reactions, the initial rate of gelatin recovery was greatly increased (first 10 minutes of hydrolysis time), then the rate of gelatin recovery was slowly increased when the hydrolysis reaction proceeded. The previous works by Guerard et al. [2001] and Ratanathammapan et al. [2006] indicated that the slowdown of the enzyme hydrolysis reaction could be mainly attributed to a decrease in the concentration of peptide bonds (substrate) available, not a decrease in an enzyme activity and a product inhibition. When the hydrolysis reaction continued to 90 minutes, the percentage of gelatin recovery from crude extracted enzyme hydrolysis at 75 °C, 65 °C, and 85 °C reached 72 wt%, 66 wt%, and 58 wt%, respectively. In the case of commercial papain, the percentage of gelatin recovery at 75 °C, 65 °C, and 85 °C were 71 wt%, 66 wt%, and 61 wt%, respectively, which were very similar to those from crude extracted enzyme hydrolysis.

The gel strength of gelatin samples obtained from two different hydrolysis reactions was presented in Fig. 7. The gel strength of gelatin solution obtained from crude extracted enzyme and commercial papain hydrolysis were similar. The gel strength of gelatin solution at 85 °C was highest, followed by the one at 65 °C and 75 °C. The lowest gel strength was observed at 75 °C, which corresponded

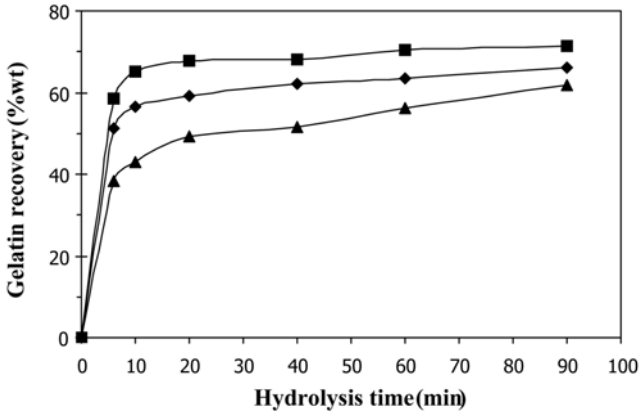

Fig. 6. Gelatin recovery from raw hide hydrolysis using commercial papain at pH 7 and various temperatures: (-◆-) 65 °C, (-■-) 75 °C, (-▲-) 85 °C.

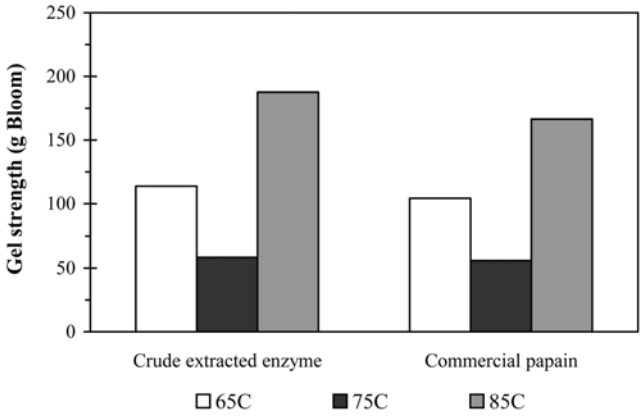

Fig. 7. Gel strength of gelatin obtained from hydrolysis reactions using crude extracted enzyme and commercial papain at pH 7 and various temperatures.

to the condition for the highest enzyme activity. In other words, at the condition for highest enzyme activity, short chain gelatin or low molecular weight gelatin was obtained from the hydrolysis. On the other hand, high molecular weight gelatin could be obtained from the hydrolysis of which the condition corresponded to low enzyme activity. This was because peptide bonds of collagen were extremely cleaved by enzymatic hydrolysis reaction much more than by acid and lime [Simeonova et al., 1996]. Enzymatic hydrolysis of collagen sources resulted in either low gel strength gelatin or collagen hydrolysate compared to typical hydrolysis by acid and lime. The results suggested that crude extracted enzyme and commercial papain was suitable to be used in enzymatic hydrolysis of raw hide to produce a range of gelatin having relatively low gel strength. The gel strength of gelatin obtained could be controlled by manipulating the hydrolysis conditions.

CONCLUSIONS

Proteolytic enzymes contained in papaya latex can be simply extracted by either water or phosphate buffer pH 6. The activities of crude extracted enzyme were comparable to those of commercial papain. The highest activity of crude extracted enzyme from papaya latex and commercial papain at 75 °C, pH7 was 1.4 and 1.6 unit/ml, respectively. The results indicated that crude proteolytic enzyme from simple extracting using water as a solvent could be effectively used to produce gelatin from raw hide hydrolysis as well as the use of commercial papain which was more expensive. The gelatin recovery from both hydrolysis reactions related to the gel strength of the obtained gelatin. The gel strength of the obtained gelatin from both hydrolysis reactions was relatively low.

ACKNOWLEDGMENTS

This research receives financial support from The Thailand Research Fund-Master Research Grants (TRF-MRG) 2004. Papaya latex was kindly obtained from Kasetsart University Research and Development Institute, Thailand.

REFERENCES

Aspmo, S. I., Horn, S. J. and Eijsink, V. G. H., "Enzymatic hydrolysis of Atlantic cod (*Gadus morhua* L.) viscera," *Process Biochem.*, **40**, 1957 (2005).

Belter, P., Cussler, E. L. and Hu, W., *Bioseparation*, John Wiley & Sons, New York (1998).

BS EN ISO 9665:2000. "Adhesives-animal glues-methods of sampling and testing (ISO 9665:1998)" (2000).

Chomarat, N., Robert, L., Seris, J. L. and Kern, P., "Comparative efficiency of pepsin and proctase for the preparation of bovine skin gelatin," *Enzyme Microb. Technol.*, **16**, 756 (1994).

Damrongsakkul, S., Ratanathammapan, K., Komolpis, K. and Tanthapanichakoon, W., "Enzymatic hydrolysis of raw hide using papain and nuetrase," to be submitted to *Bioresource Technol.* (2006).

Friess, W., "Collagen - biomaterial for drug delivery," *Eur. J. Pharm. Biopharm.*, **45**, 113 (1998).

Gomez-Guillen, M. C., Gimenze, B. and Montero, P., "Extraction of gelatin from fish skins by high pressure treatment," *Food Hydrocolloid*, **19**, 1 (2005).

Guerard, F., Dufosse, L., De La Broise, D. and Binet, A., "Enzymatic hydrolysis of proteins from yellowfin tuna (*Thunnus albacares*) wastes using alcalase," *J. Mol. Catal. B-Enzym.*, **11**, 1051 (2001).

Oh, K. D. and Kim, C., "A study on enzymatic hydrolysis of cellulose in an attrition bioreactor," *Korean J. Chem. Eng.*, **4**, 105 (1987).

Park, C. Y., Ryu, Y. W. and Kim, C., "Kinetics and rate of enzymatic hydrolysis of cellulose in supercritical carbon dioxide," *Korean J. Chem. Eng.*, **18**, 475 (2001).

Pitpreecha, S., *Gelatin production from large animal raw hide using proteolytic enzyme extracted from papaya latex*, Master Dissertation, Department of Chemical Engineering, Faculty of Engineering, Graduate School, Chulalongkorn University (2006).

Ratanathammapan, K., *Effects of enzymatic conditions on gelatin production from raw hide*, Master Dissertation, Department of Chemical Engineering, Faculty of Engineering, Graduate School, Chulalongkorn University (2005).

Sharphouse, J. H., *Applied protein chemistry*, Applied Science Publishers, London (1980).

Simeonova, L. S. and Dalev, P. G., "Utilization of a leather industry waste," *Waste Manage.*, **16**, 765 (1996).

Taylor, M. M., Diefendorf, E. J., Foglia, T. A., Bailey, D. G. and Feair-

heller, S. H., "Enzymatic treatment of offal from fleshing machines," *JALA*, **84**, 71 (1989).
Walker, J. M., *The protein protocols handbook*, Humana Press, New Jersey (1996).
Walsh, G., *Proteins: Biochemistry and biotechnology*, John Wiley & Sons, New York (2002).
Wuttiwiangtham, T., *Purification and characterization of papain from latex of papaya (Carica papaya Linn.)*, Master Dissertation, Department of Biotechnology, Faculty of Science, Graduate School, Chulalongkorn University (1993).