10.7603/s40837-014-0002-8
GSTF International Journal of Chemical Sciences (JChem) Vol.1 No.2, May 2014

Application of Adsorption Chromatography in the Papain Processing

Effect on Recovery of Papain from Aqueous Solutions

HE YUHAI, SYED MOHD SAUFI BIN TUAN CHIK, and CHONG FUI CHIN*
Faculty of Chemical and Natural Resources Engineering
Universiti Malaysia Pahang
Kuantan, Pahang, Malaysia.
**E-mail: fcchong@ump.edu.my*

Received 19 Feb 2014 Accepted 13 Mar 2014

***Abstract* - A direct recovery of papain enzyme from unclarified *Carica papaya* juice was developed successfully using a reversed phase expanded bed adsorption chromatography (RP-EBAC). The dynamic binding capacity for the RP-EBAC at 10% breakthrough of 810.52 µg papain/g adsorbent was achieved at a linear flow velocity of 936 cm/h, bed expansion of two and feedstock viscosity of 2.68 mPa·s. Two-step elution was employed to enhance the purity of papain in RP-EBAC which gives high papain purity of 74.98% and high purification factor of 7.04. This work shows a great potential of using RP-EBAC with elution optimization step to purify papain from unclarified *Carica papaya* juice.**

Key words - Papain, Chromatography, RP-EBAC, Purification

I. INTRODUCTION

Papain is an enzyme of high value which has been applied in many areas such as food industry and pharmaceutical. Papain is used for pain and inflammation as well as fluid retention following trauma and surgery. Nevertheless, the application of papain in the pharmaceutical area requires the papain of high purity. Papain's purification has been done by many methods such as salt precipitation, aqueous two phase extraction and chromatography. The most effective method on the promotion of the purity was used is chromatography. Unfortunately, chromatography has its disadvantage that is need to prior clarify the feedstock in order to avoid the particulate inside of the feedstock may block the chromatography bed and lower the efficiency of the bed, it may also reduce the frequency of use of the adsorbent. This is not help to the industrious application. The selection of appropriate purification method in order to achieve high purity of papain and high efficiency of purification is important due to the industrious requirement. Expanded bed adsorption chromatography (EBAC) is a novel method which allows the feeding of the particulate-containing feedstock [1,2]. This feature solved the problem of bed blocking that happens in the conventional chromatography. At the same time, it forms a direct recovery of proteins by a very efficient purification process which integrates the clarification, concentration and purification in one unit operation with many advantages such as generates robust, easy to scale up and biocompatible. Reversed phase liquid chromatography (RPLC) is a separation based on the hydrophobicity of the protein. RPLC is an important technique of the characteristic of high purification resolution, which exploits the separation of proteins based on hydrophobic interactions between the stationary phase ligands and hydrophobic regions on the protein surface. Papain has high hydrophobic region on the surface which enabled the application of RPLC. In this study, EBAC and RPLC was combined together to purify papain from crude

DOI: 10.5176/2339-5060_1.2.10

Published online: 01 July 2014

papaya fruit juice which shows a great efficiency. The overall steps involved in the study are depicted in Figure 1.

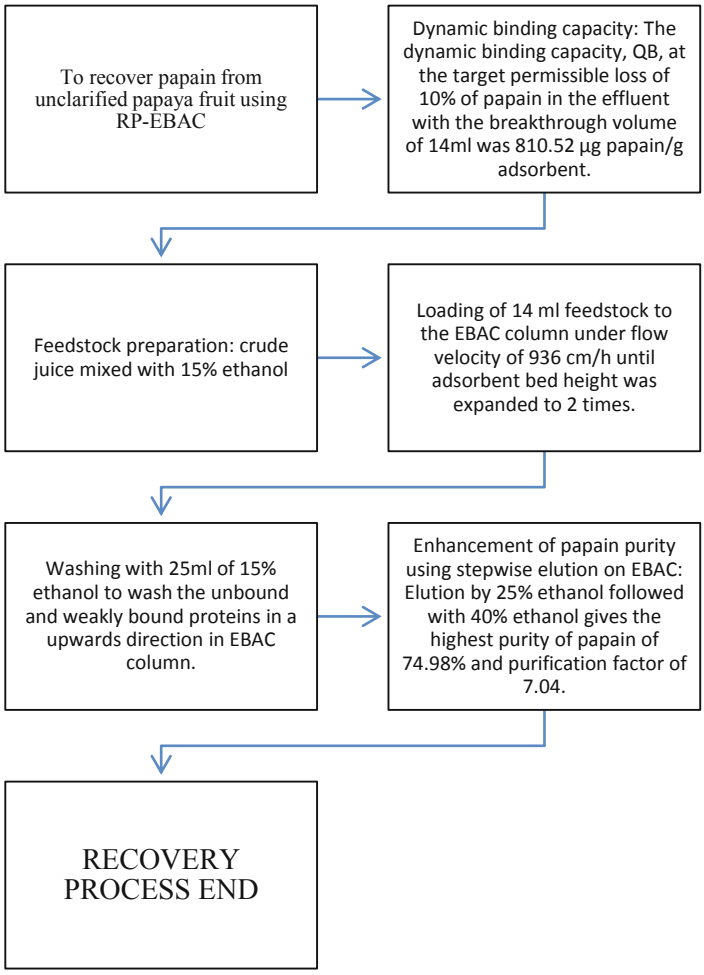

Figure 1. Process scheme for the recovery of papain from papaya fruit juice using the RP-EBAC

II. MATERIALS AND METHODS

A. ADSORBENT AND CHEMICALS

Amberlite™ XAD7HP (Rohm and Haas, USA) was studied to perform RPLC to recover papain from the unclarified papaya juice. Folin & Ciocalteus Phenol reagent, casein, L-cystein, L-tyrosine, papain standard (catalog no.: P4762), protein assay agents and chemicals for electrophoresis were purchased from Sigma-Aldrich (USA). Trichloroacetic acid (TCA) was supplied by Merck (Germany). Protein marker used was Precision Plus Protein™ All Blue Standards (#161-0373) from Bio-Rad (USA). All the chemicals used were of analytical grade.

B. EQUIPMENTS

Fastline™ 10 EBAC system had a glass column of inner diameter of 1 cm, height 40 cm and maximum expanded height of 35 cm. EBAC system employed localized stirring method for fluid

distribution. The system was purchased from UpFront Chromatography (Copenhagen, Denmark). A unit of peristaltic pump (520S, Watson Marlow, UK) was used for the feedstock feeding. The viscosity measurement was performed by using the spindle (SC14-18, rotating at a speed of 200 rpm) of the viscometer (DV-III, Brookfield Eng. Lab., USA). Briefly, sample was loaded into the viscometer cone and the viscosity of the sample was taken as the mean of three measurements.

C. PREPARATION OF CRUDE SAMPLES

Unripe green papaya fruits (*Carica papaya*) were collected from a selected papaya tree at a local farm, Gambang, Malaysia. For each batch of the experiment, papaya was used is collected at the same time and the size is similar. After washed the freshly harvested unripe papaya fruit and the seeds was removed (if any), the pulp was blended using the juice extractor (MJ-68M, Panasonic, Japan). The unclarified juice was added with L-cystein to make the final concentration of 1 mM, this was done to enhance and stable the papain activity [3].

D. DIRECT RECOVERY OF PAPAIN FROM FEEDSTOCK SUSPENSION USING EBAC

The Fastline™ 10 EBAC column was filled with 4 ml of Amberlite™ XAD7HP adsorbent corresponding to a settled bed height of 5 cm. Bed expansion and equilibration was done by 10 ml of binding buffer through the mixing chamber and onto the column at a flow rate of 19.35 ml/min (peristaltic pump 520S, Watson Marlow, UK, linear flow velocity of 1066.5 cm/h) until a constant bed height was obtained. The bed was allowed to expand to a bed height of 10 cm (corresponding to a two-fold expansion) and the adaptor was positioned at 12 cm prior to the loading phase. 14 ml of the feedstock suspension of 243.15 µg/ml papain was loaded into the column with flow velocity of 936 cm/h to maintain the bed expansion degree of 2 and then followed by washing with 25 ml 15% ethanol to remove weakly or unbound proteins from the voids of bed. Then 25 ml of 40% ethanol was used as the elution buffer. The elution of papain was also performed in the upwards flow expanded bed mode due to the design of Fastline™ 10 EBAC column without filter and net equipped at the bottom of column. Elution in the expanded mode was also reported with the advantage of avoiding potential problems that was the bed clogged due to the presence of residual solids in the voids between adsorbent particles if elution was operated in a packed bed mode [4,5]. Elution fractions were collected and subjected to protein analysis and quantification.

III. RESULTS AND DISCUSSIONS

A. DYNAMIC BINDING CAPACITY

Frontal analysis observed from the breakthrough curve is important to estimate the maximum loading amount of target protein passed through adsorbent bed. In most of the industrial practice, loading stage of feedstock was terminated at C/C_0=0.1 in in order to reduce the loss of target product in flowthrough [6,7]. The breakthrough curve of the papain adsorbed onto the Amberlite™ XAD7HP described above is shown in Figure 2. The dynamic binding capacity, Q_B, at the target permissible loss of 10% of papain in the effluent with the breakthrough volume of 14 ml calculated from (1) was 810.52 µg papain/g adsorbent.

$$Q_B = \frac{C_0 V_b}{V_s} \quad (1)$$

Where C_0 is the initial concentration of papain in the feedstock suspension (mg/ml), V_b is the volume at 10% breakthrough (ml) and Vs is the settled volume of the adsorbent (ml).

Dynamic binding capacity was influenced by applied liquid velocity and viscosity of the mobile phase [8]. In this case, low dynamic binding capacity was caused by insufficient time for solid-liquid phase to reach equilibrium stage. The flow rate used in the test of breakthrough curve was at 936 cm/h and the settled bed height was at 5 cm. The bed was expanded to a degree of 2 with the bed height of 10 cm, thus, the residence time of feedstock in the column was only 38 s. The equilibrium binding time obtained in the batch adsorption was 15 min (data is not shown). Apparently, 38 s was not enough to reach the adsorption equilibration. This phenomenon was also observed by other researchers [6,7]. The adsorption of papain by the EBA process could be improved by increasing the settled volume of adsorbent used to form the expanded bed with a consequential increase in the residence time of liquid in contact with the bed. The increasing of the adsorption by increasing the bed height was performed by some researches and they observed that adsorption of target protein was significantly improved. Nevertheless, the maximum expanded height of Fastline™ 10 EBAC column is limited to 35 cm, it is not possible to run a bed height in expanded mode which can be adapt to the requirement of 15 min equilibration in order to obtain a high adsorption. Although the improvement of adsorption by increasing the residence time in this research cannot be fulfilled, the front analysis about the breakthrough curve and bed height is of instructive significance for the future work.

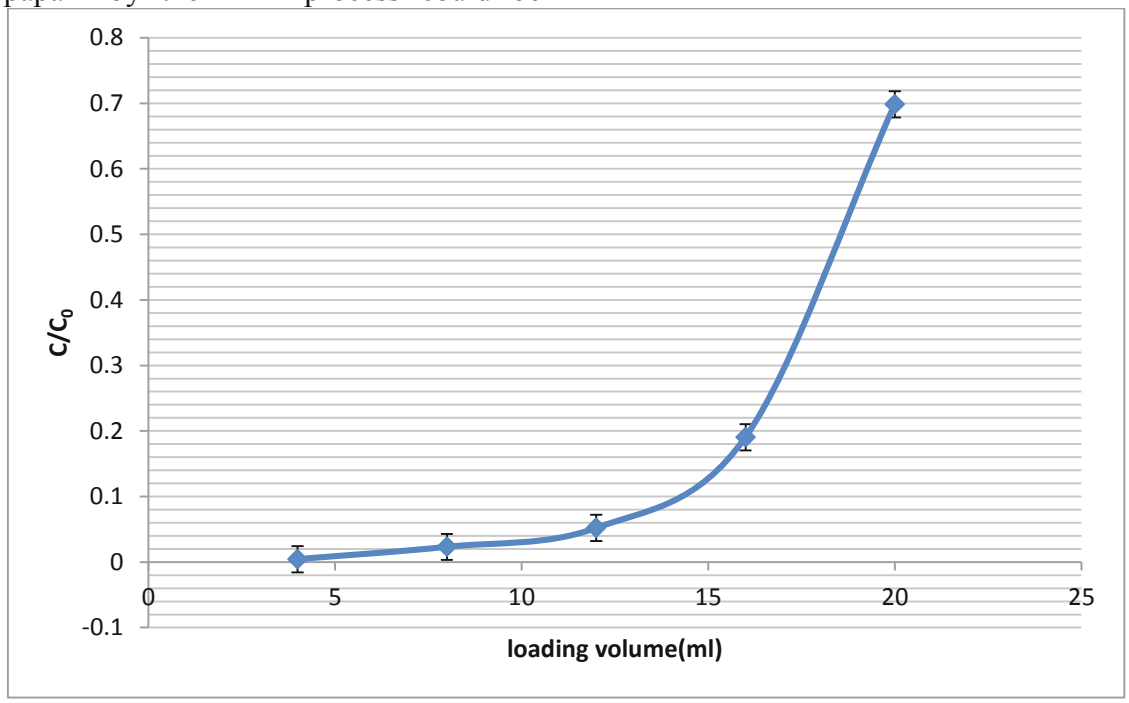

Figure 2. Breakthrough curve of the papain from unclarified feedstock onto Amberlite™ XAD7HP in RP-EBAC

B. OPERATION OF RP-EBAC

The RP-EBAC process for the adsorption and elution of papain from feedstock suspension was then investigated. The settled bed height was fixed to 5 cm as used in the frontal analysis of breakthrough curve. When the bed expansion was stable at a degree of 2, the unclarified feedstock suspension with 243.15 μg/ml of papain was applied onto the column. The solid particles flowed through the bed voids and left the column through the outlet pipe. A successful elution scheme from the study involved of two major elution steps: the first step is elution of impurities at a lower ethanol concentration followed by elution of papain with 40% ethanol.

Table 1 shows that two-step elution with 25% ethanol as the first-step elution buffer result in the highest purity of papain in the second-step elution, the purity and purification factor obtained as high as 74.98% and 7.04 respectively, and the yield of papain was 35.39%. The optimization of first-elution step was carried out in two ways: (i) the use of 20% ethanol to obtain a higher yield of papain or (ii) the use of 25% ethanol to obtain a higher purity of papain. It should be emphasized that it was considered more importantly to achieve a highly pure papain rather than a high yield since the *Carica papaya* is an abundant cheap starting material, thus, favoring the second of the two options described above. The RP-EBAC allowed the adsorption of papain (a hydrophobic molecule) onto the Amberlite (a hydrophobic solid support) in the ethanol solution (a polar mobile phase). In the study, decreasing the mobile phase polarity by adding more organic solvent reduced the hydrophobic interaction between the solute and the solid support resulting in desorption. It was observed that the more hydrophobic the molecule the more time it spent on the solid support and the higher the concentration of organic solvent was required to promote desorption [9].

Figure 3 is the graphical illustration of SDS-PAGE of the pooled protein fractions collected in RP-EBAC operation using two-step elution. Lane 2 of Figure 3 is the pooled fraction of elution. Lane 2 of Figure 3 is the pooled fraction of elution using stepwise elution strategy. This demonstrates that 25% ethanol is favoring to elute the protein which has a smaller molecular weight than papain, and also because of this, the papain purity was enhanced. In RPLC system, the smaller protein was resolved in earlier elution and proteins above about 40 kDa was not removed from the column [10].

TABLE 1. THE PURIFICATION PERFORMANCE OF PAPAIN FROM FEEDSTOCK SUSPENSION USING RP-EBAC WITH TWO-STEP ELUTION

Elution conditions	Purification stage	Volume (ml)	Yield of Protein (%)	Yield of papain (%)	Purity (%)	Purification factor	Loss of protein (%)	Papain lose in whole process (%)
20%, 40%	Flow through	15	35.21±0.45	6.96±0.30	2.14±0.06			
	15% washing	25	19.88±0.29	3.92±0.10	2.13±0.02			
	20% elution	15	2.65±0.21	6.52±0.06	26.7±1.87			
	40% elution	25	8.32±0.52	48.37±0.23	62.95±7.05	5.81±0.65	33.94±0.10	34.23±1.99
25%, 40%	Flow through	15	35.65±0.62	6.65±1.23	1.99±0.40			
	15% washing	25	19.21±0.74	3.89±0.33	2.16±0.10			
	25% elution	15	6.74±0.20	23.89±0.01	37.78±1.13			
	40% elution	25	5.03±0.34	35.39±023	74.98±5.67	7.04±0.53	33.37±0.42	30.18±1.79
30%, 40%	Flow through	15	37.36±0.81	6.03±0.92	1.84±0.24			
	15% washing	25	20.04±0.06	4.07±0.25	2.31±0.13			
	30% elution	15	9.73±0.06	35.95±0.01	42.07±0.24			
	40% elution	25	6.54±0.67	25.56±0.08	44.5±4.49	3.91±0.39	26.33±0.01	28.39±0.56
35%, 40%	Flow through	15	35.78±0.42	6.49±0.46	1.98±0.11			
	15% washing	25	18.66±0.58	4.41±0.12	2.57±0.15			
	35% elution	15	14.61±0.52	59.45±1.2	44.38±0.69			
	40% elution	25	3.44±0.05	9.78±0.32	30.97±1.5	2.84±0.14	27.51±1.59	19.87±1.2

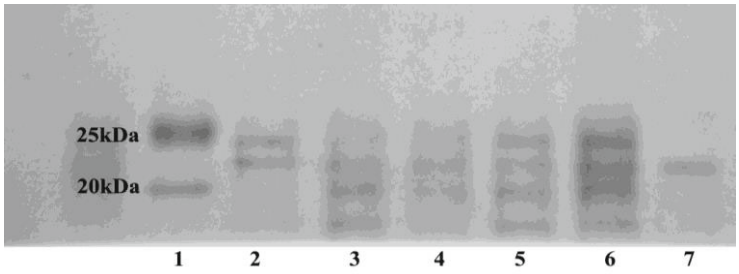

Figure 3. Graphical illustration of SDS-PAGE of the pooled protein fractions collected in RP-EBAC operation using two-step elution. Lane 1: protein molecular weight marker. Lane 2: second-step elution by 40% ethanol. Lane 3: first-step elution by 25% ethanol. Lane 4: washing with 15% ethanol. Lane 5: flowthrough. Lane 6: feedstock. Lane 7: papain standard.

IV. CONCLUSION

Reversed phase expanded bed adsorption chromatography (RP-EBAC) has been applied successfully to purify the papain from an unclarified *Carica papaya* juice. The EBAC mode combined clarification, capture and purification of the papain in a united process. In RP-EBAC with two-step elution strategy, the elution of the papain using ethanol solution of 25% and 40% (v/v) separately which led to a papain purity of 74.98% and purification factor

of 7.04. The first-step elution of 25% ethanol removed the contaminant proteins efficiently and thus the purity of papain was enhanced.

ACKNOWLEDGMENT

This study was funded by the Postgraduate Research Scheme (GRS 110356) from the Universiti Malaysia Pahang. Y. H. He was financially supported by Universiti Malaysia Pahang.

REFERENCES

[1] Chase, H.A. 1994. Purification of Proteins by Adsorption Chromatography in Expanded Beds. *Trends in Biotechnology*, **12**: 296-303.

[2] Anspach, F.B., Curbelo, D., Hartmann, R., Garke, G. and Deckwer, W.-D. 1999. Expanded-Bed Chromatography in Primary Protein Purification. *Journal of Chromatography A*, **865**: 129-144.

[3] Homaei, A., Sajedi, R., Sariri, R., Seyfzadeh, S. and Stevanato, R. 2010. Cysteine Enhances Activity and Stability of Immobilized Papain. *Amino Acids*, **38**: 937-942.

[4] Hjorth, R. 1999. Expanded Bed Adsorption: Elution in Expanded Bed Mode. *Bioseparation*, **8**: 1-9.

[5] Li, J. and Chase, H.A. 2009. Use of Expanded Bed Adsorption to Purify Flavonoids from Ginkgo Biloba L. *Journal of Chromatography A*, **1216**: 8759-8770.

[6] Ng, M.Y.T., Tan, W.S., Abdullah, N., Ling, T.C. and Tey, B.T. 2007. Direct Purification of Recombinant Hepatitis B Core Antigen from Two Different Pre-Conditioned Unclarified Escherichia Coli Feedstocks Via Expanded Bed Adsorption Chromatography. *Journal of Chromatography A*, **1172**: 47-56.

[7] Ramos, A., Acien, F.G., Fernandez-Sevilla, J.M., Gonzalez, C.V. and Bermejo, R. 2011. Development of a Process for Large-Scale Purification of C-Phycocyanin from Synechocystis Aquatilis Using Expanded Bed Adsorption Chromatography. *Journal of chromatography. B- Analytical technologies in the biomedical and life sciences*, **879**: 511-519.

[8] Chase, H.A. and Draeger, N.M. 1992. Affinity Purification of Proteins Using Expanded Beds. *Journal of Chromatography A*, **597**: 129-145.

[9] Buszewski, B. and Noga, S. 2012. Hydrophilic Interaction Liquid Chromatography (Hilic)--a Powerful Separation Technique. *Analytical and Bioanalytical Chemistry*, **402**: 231-247.

[10] Scopes, R.K. 1993. *Protein Purification: Principal and Practice.* Berlin: Springer Press.