Dissolution profile of partially purified bromelain from pineapple core encapsulated in alginate-guar gum with in vitro study of antiplatelet activity

A A Irfan, S Setiasih and S Hudiyono
Department of Chemistry, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Kampus UI Depok, Depok 16424, Indonesia
Corresponding author’s e-mail: siswati.setiasih@sci.ui.ac.id

Abstract. The aim of this study is to maintain the antiplatelet activity of pineapple core bromelain from degradation in gastric fluid by encapsulating the enzyme in alginate-guar gum crosslinked with glutaraldehyde. Bromelain isolation was followed by purification process such as ammonium sulfate precipitation and dialysis. The specific activity obtained showed an enhancement started for crude enzyme (20.11 U/mg), ammonium sulfate fraction (190.64 U/mg), and dialysis fraction (229.77 U/mg). Dialysis fraction was encapsulated by in situ loading method in alginate-guar gum hydrogel with 1.25% (v/v) glutaraldehyde concentration. The swelling ratio of the hydrogel is 234.37% in acidity of 1.2; and 1375.91% in acidity of 7.4; while the encapsulation efficiency amount was 60.42%. In addition, maximum concentration of bromelain released in dissolution test was higher in artificial intestinal environment (1.279 mg/L) than in artificial gastric fluid (0.13 mg/L), with maximum proteolytic activity of 1.2 U/mL and 0.11 U/mL, respectively. In vitro study of antiplatelet activity showed a good inhibition for dialysis fraction (50.6%) and dissolution fraction (34.86%), indicating that the hydrogel is good enough to maintain the activity of bromelain.

Keywords: pineapple core, bromelain, alginate-guar gum hydrogel crosslinked with glutaraldehyde, encapsulation, dissolution, antiplatelet activity

1. Introduction
The frequency of death due to blockage of blood vessels in the brain and heart is one of the issues that became the center of attention in the field of health. World Health Organization (WHO) data in 2012 showed that 17.5 million people worldwide died from cardiovascular disease, equivalent to 31% of 56.5 million of all deaths worldwide. Blockage of blood vessels can be caused by irregular eating patterns, smoking, and complications of certain diseases caused by increasing of platelet aggregation [1]. Aspirin and clopidogrel are often used as agents of antiplatelet aggregation, but their use causes side effects of nausea, headache, stomach cramps, and stomach ulcers [2]. Therefore, it takes another antiplatelet alternatives with fewer side effects, one of which is bromelain contained in pineapple plants.

Bromelain has been studied since 1894 [3] and has been proven to be useful in the therapeutic field as an antithrombotic, fibrinolytic, anticoagulant, and anti-inflammatory agent [4]. However, the application of bromelain as an oral drug tends to be less than optimal. According to the study conducted by Setiasih et al. [5], the proteolytic activity of bromelain enzymes decreases with time as they are placed in artificial gastric juices that have a pH of 1.2. The decrease in activity is caused by the interaction of bromelain with fluid and enzymes in the stomach, causing deactivation and degradation of the bromelain [6]. In fact, the intestine can absorb the enzyme bromelain intact without making the enzyme losing the activity or even degraded [7–8].
Encapsulation is the process by which a particular active ingredient is caught in a polymer matrix [9]. This method can be used to protect bromelain from the influence of the stomach environment so that the release of bromelain in the intestine is optimal. In this research, the isolated bromelain from pineapple core is encapsulated into the glutaraldehyde crosslinked alginate-guar gum hydrogel [10–12].

2. Materials and methods

2.1. Materials

The source of bromelain enzyme was pineapple core obtained from a seller of Palembang pineapple in Pasar Induk, Kramat Jati. Synthesis of hydrogel used sodium alginate powder (medium viscosity), guar gum powder, and glutaraldehyde solution 25 % (v/v) (Sigma Aldrich).

2.2. Isolation, ammonium sulfate precipitation, and dialysis of bromelain

The pineapple core were weighed, cut and crushed with a blender in Pasar Induk, Kramat Jati. Synthesis of unloaded alginate-guar gum hydrogel was synthesized with 3 variations of glutaraldehyde concentration, i.e. 1.25 %; 2.5 %; and 5 % (v/v). The synthesis began by dissolving 1.5 g of sodium alginate and 0.5 g of guar gum in 80 mL of water. The mixture was stirred and then added with glutaraldehyde to the desired concentration variation. After homogeneous, the solution was diluted with distilled water up to 100 mL and kept undisturbed for ±12 hours. Then, the hydrogel solution was suspended into a 0.5M CaCl₂ solution with syringe (0.1 mm) and let undisturbed for 1 hour. The mixture was filtered and washed with aquadest 3 times. Hydrogels were then dried in an oven at 50 °C until completely dry.

2.3. Synthesis of bromelain loaded alginate-guar gum hydrogel

The bromelain loaded alginate-guar gum hydrogel was also synthesized with the same variation of glutaraldehyde concentrations and a method similar to the unloaded hydrogels. The difference was in the addition of 1 mL of bromelain standard 100 ppm for optimum glutaraldehyde concentration determination process and 1 mL of dialysis fraction of bromelain for dissolution test, after the solution was homogenous with glutaraldehyde. Drying process was done using freeze dry for 4 days. The encapsulation efficiency was determined by dissolving the weighed hydrogel in 10 mL of phosphate buffer 0.2M pH 7.4 and then let undisturbed for 1 day. Subsequently, the solution was sonicated for 1 hour and centrifuged. The obtained filtrate was determined for its protein content. The encapsulation efficiency is determined by the equation [11]:

\[
\text{Encapsulation efficiency (\%)} = \frac{[\text{Loaded bromelain}] \times \text{Total mass of all synthesized hydrogel}}{[\text{Added bromelain}] \times \text{Total mass of test hydrogel}} \times 100\%
\]

2.5. Dissolution test of isolated bromelain loaded alginate-guar gum hydrogel

Dissolution test of bromelain loaded hydrogel was done by dissolution apparatus type 1. The bromelain loaded hydrogel was weighed and then fed into the dissolution apparatus (100 rpm, 37 ± 0.5 °C). For 2 hours, the hydrogel was immersed in KCl-HCl buffer solution pH 1.2 as an artificial gastric fluid medium. Every 30 minutes, 10 mL of filtrate was taken. The hydrogel was then transferred into phosphate buffer 0.2M pH 7.4 as an artificial intestinal environment medium for 8 hours. In the first 2
Table 1. Bromelain activity from each purification process

| Samples                        | Volume (mL) | Total proteolytic activity (U) | Total protein (mg) | Specific activity (U/mg) | Purity (times) | Yield (%) |
|--------------------------------|-------------|--------------------------------|--------------------|--------------------------|----------------|-----------|
| Crude enzyme                   | 100         | 351,6                          | 17,48              | 20,11                    | 1              | -         |
| Ammonium sulfate precipitation |             |                                 |                    |                          |                |           |
| F1 (0-20)%                     | 4           | 29,66                          | 0,33               | 88,77                    | 4.41           | 8.43      |
| F2 (20-50)%                    | 5           | 50.5                           | 0.26               | 190,64                   | 9.47           | 14.36     |
| F3 (50-80)%                    | 2           | 2,33                           | 0.02               | 78,9                     | 3.92           | 0.66      |
| Remaining filtrate             | 78          | 3,9                            | 1,12               | 348                      | 0.17           | 1.1       |
| Dialysis                       | FDialysis   | 256,3                          | 0,22               | 229,77                   | 11.42          | 14.78     |

3.6. In vitro study of antiplatelet activity
Determination of antiplatelet activity was performed on dialysis fraction and dissolution fraction with the highest activity. Determination method refers to Born's method with 1 mg/mL aspirin as positive control and aquadest as negative control. The aggregation percentage was determined by the formula [13].

2.7. Hydrogel characterization
The unloaded and bromelain loaded alginate-guar gum hydrogel was characterized using Fourier Transform Infrared (FTIR) to see any changes in functional groups in the hydrogel and to determine the crosslinking and bromelain interaction with the matrix.

3. Results and discussion

3.1. Isolation, ammonium sulfate precipitation, and dialysis of bromelain
The source of bromelain was a Palembang pineapple core, which was selected because it has a relatively larger amount of bromelain than other pineapple waste. The obtained crude enzyme was then purified by precipitation with ammonium sulfate and dialysis. The precipitation stage was principally referred to salting out process in which the water that solvated the protein will be attracted by ammonium sulfate salt which has a higher solubility than the protein. As a result, the interaction between proteins with their solvents will be weaker than their interactions with other proteins, so that proteins will aggregate to form large molecules with lower solubility. Then, at the dialysis stage there was a concentration gradient between the environment inside the sac and the environment outside the sac, so the molecules inside that are smaller than the pore size of the sac will diffuse out. In contrast, the solvent in the outside environment of the sac will go inside to balance the change in concentration due to the molecular diffusion process. The bromelain activity of each purification stage is tabulated in table 1. From the table, it can be concluded that the specific activity of the enzyme increases with purification. The highest specific activity is the dialysis fraction of 229.77 U/mg with a purity level of 11.42 times the crude enzyme. This fraction was encapsulated into the hydrogel matrix and determined for its antiplatelet activity.

3.2. FTIR spectra and visual appearance of unloaded and bromelain loaded alginate-guar gum hydrogel
Visually (figure 1), the unloaded and bromelain loaded hydrogels have a non-uniform spherical shape. The difference lies in the brown color of unloaded and white color on loaded hydrogels, which are the effect of different drying methods. The Fourier Transform Infrared (FTIR) spectra (figure 2) of the hydrogel matrix exhibit several specific spectrum of sodium alginate and guar gum, among which are broad peaks of 3400-3000 cm⁻¹ waves typical for the vibration mode of hydroxyl groups (O-H), peak at
Figure 1. Visual appearance of (a) oven dried unloaded alginate-guar gum hydrogel and (b) freeze dried bromelain loaded alginate-guar gum hydrogel

Figure 2. FTIR spectra of (a) alginate-guar gum hydrogel, alginate-guar gum hydrogel (b) crosslinked with glutaraldehyde and (c) loaded with bromelain

1579 cm$^{-1}$ and 1400 cm$^{-1}$ which are the asymmetric and symmetric anionic carboxylic acid groups (COO-) vibration, peak at 1032 cm$^{-1}$ which is a marker of the carbonyl groups (C=O) and the peak at 960 cm$^{-1}$ and 828 cm$^{-1}$, respectively, denote the C-H bond for mannuronic acid and guluronic acid in the sodium alginate chain [14].

Crosslinked hydrogel spectra showed a decrease in absorption at wave number 3400–3000 cm$^{-1}$. This is due to the acetyl formation, which converts 2 equivalents of the hydroxy group from sodium alginate and guar gum to ether bonds. In addition, there is an increase in absorption at wave number 1250 cm$^{-1}$, which also can be associated with the formation of acetal rings and ether bonds, the product of the reaction between the hydroxy groups of sodium alginate and guar gum with aldehyde groups of glutaraldehyde [15].

Crosslinked hydrogel spectra showed a decrease in absorption at wave number 3400–3000 cm$^{-1}$. This is due to the acetyl formation, which converts 2 equivalents of the hydroxy group from sodium alginate and guar gum to ether bonds. In addition, there is an increase in absorption at wave number 1250 cm$^{-1}$, which also can be associated with the formation of acetal rings and ether bonds, the product of the reaction between the hydroxy groups of sodium alginate and guar gum with aldehyde groups of glutaraldehyde [15].

FTIR spectra between unloaded and bromelain loaded hydrogels showed the same absorption peaks. This indicates that there is no chemical interaction between bromelain and the hydrogel matrix. However, there is a change of intensity in the wave number 3400–3000 cm$^{-1}$ indicating a physical interaction of hydrogen bond between bromelain and matrix [16].
3.3. Encapsulation efficiency of alginate-guar gum hydrogel

From figure 3, it can be seen that the greater the concentration of glutaraldehyde added, the smaller the encapsulation efficiency. This is relevant to the structure of the hydrogel matrix, which becomes increasingly dense due to the addition of glutaraldehyde, resulting in the trapped bromelain to become smaller. This rigid structure also causes the trapped bromelain to have no chance of escaping the matrix when immersed in phosphate buffer [17], so its release tends to decrease with the increasing of glutaraldehyde concentrations. The highest efficiency of 88.42 % is owned by hydrogel without the addition of glutaraldehyde (0 %, v/v), but the swelling ratio is still too high in both artificial environments, which is lead to a large release in the gastric fluid and uncontrolled release in the intestinal environment. Therefore, hydrogel with glutaraldehyde concentration of 1.25 % (v/v) is considered to be the best variation and selected to encapsulate the isolated bromelain. Encapsulation of isolated bromelain into the alginate-guar gum hydrogel crosslinked with glutaraldehyde concentration of 1.25 % generate an efficiency of 60.42 %.

3.4. Dissolution test of isolated bromelain loaded alginate-guar gum hydrogel

Based on figure 4, the dissolution fraction in artificial gastric fluid has a relatively lower protein content than the artificial intestinal environment. The maximum measured protein content in artificial gastric fluid was 0.13 mg/L, much smaller than the maximum protein content in the artificial intestinal environment of 1.79 mg/L. The proteolytic activity of dissolution fraction also tends to be higher in the artificial intestinal environment rather than artificial gastric fluid. The maximum proteolytic activity in artificial gastric fluid occurred at 120 minutes at 0.11 U/mL, while in the artificial intestinal environment occurred at 420 mins of 1.2 U/mL. The dissolution fraction with the highest proteolytic activity was then determined for its antiplatelet activity.
Table 2. Antiplatelet activity of bromelain fraction

| Samples               | Absorbance with ADP Addition | Aggregation (%) | Inhibition (%) |
|-----------------------|------------------------------|----------------|---------------|
|                       | Before                       | After          |               |
| Aquadest              | 0.494                        | 0.025          | 94.93         | -             |
| Aspirin               | 0.493                        | 0.485          | 1.62          | 98.29         |
| Dialysis fraction     | 0.499                        | 0.265          | 46.89         | 50.6          |
| Dissolution fraction  | 0.467                        | 0.187          | 59.95         | 36.84         |

3.5. In vitro study of antiplatelet activity

Based on table 2, the dialysis fraction before the encapsulation shows an inhibition percentage of 50.6%. After encapsulation, the percent of inhibition was relatively unchanged at 36.84%. Therefore, it can be concluded that the encapsulation method is good enough in maintaining the antiplatelet activity of bromelain from degradation in gastric fluid.

4. Conclusions

Bromelain can be encapsulated in alginate-guar gum hydrogel crosslinked with glutaraldehyde. The maximum protein content of bromelain in the artificial intestinal environment is greater than in the artificial gastric fluid. Furthermore, the maximum proteolytic activity of dissolved bromelain in the artificial intestinal environment is greater than in the intestinal environment. The inhibition percentage of antiplatelet aggregation is relatively unchanged between bromelain in before and after encapsulation process. This suggests that the activity of bromelain can be maintained up to the intestine.

Acknowledgements

This research was funded by Hibah Publikasi Internasional Terindeks untuk Tugas Akhir Mahasiswa Universitas Indonesia (PITTA) 2018.

References

[1] Musfiroh F F, Setiasih S, Handayani S, Hudiyono S and Ilyas N M 2017 *IOP Conf. Ser.: Mater. Sci. Eng.* **299** 012017
[2] Kurnia S D, Setiasih S, Handayani S and Hudiyono S 2017 *AIP Conf. Proc.* **2023** 020077
[3] Febriani K, Wahyuni I, Setiasih S and Hudiyono S 2017 *AIP Conf. Proc.* **1862** 030095
[4] Manzoor Z, Nawaz A, Mukhtar H and Haq I 2016 *Braz. Arch. Biol. Technol.* **59** e16150010
[5] Setiasih S, Adimas A C D, Dzikria V and Hudiyono S 2017 *IOP Conf. Ser.: Mater. Sci. Eng.* **299** 012016
[6] Shiew P S, Fang Y L and Majid F A A 2010 *Proc. 3rd Int. Conf. Biotechnology for the Wellness Industry* 8-9 October 2010 Kuala Lumpur (Kuala Lumpur: Universiti Teknologi Malaysia)
[7] Chobotova K, Vernallis A B and Majid F A A 2010 *Cancer Lett.* **290** 148–56
[8] Castell J V, Friedrich G, Kuhn C S and Poppe G E 1997 *Am. J. Physiol.* **273** G139–46
[9] Chen Y E and Chi H 2017 *Mater. Sci. Eng. C* **83** 233–46
[10] Bosio V E, Basu S, Abdullha F, Villaiba M E C, Güida J A, Mukherjee A and Castro G R 2014 *React. Funct. Polym.* **82** 103–10
[11] George M and Abraham T E 2007 *Int. J. Pharm.* **335** 123–9
[12] Roy I, Sardar M and Gupta M N 2005 *Biochem. Eng. J.* **23** 193–8
[13] Moriyama H, Hosoe T, Wakana D, Itabashi T, Kawai K, Iizuka T W, Hoshi K, Fukushima K and Lau F C 2009 *J. Health Sci.* **55** 103–8
[14] Helmiyati H and Syarifudin A 2018 *AIP Conf. Proc.* **2023** 020080
[15] Yeom C K and Lee K H 1997 *J. Appl. Polym. Sci.* **67** 209–19
[16] Setiasih S, Prabowo H A, Budianto E and Hudiyono S *Journal of Applied Pharmaceutical Science (JAPS)* **8(10)** 17–24
[17] Eswaramma S and Rao K S V K 2017 *Carbohydr. Polym.* **156** 125–34