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Extraction of Crude Acid Protease from Goat (*Capra aegagrus*) Abomasum

Tanalyna Hasna[1], Sri Mulyani[2], Umar Santoso[3], Yudi Pranoto[4]

[1]Department of Food Science and Technology, Brawijaya University, Jl. Veteran, Malang, Jawa Timur 65145 Indonesia

[2]Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia

[3, 4]Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta, Indonesia

Abstract: ***The present study reports the extraction of crude acid protease from goat abomasum (CAPGA). The CAPGA was partially purified using different levels of ammonium sulphate precipitation and dialysis with dialysis sack MWCO 12 KDa. Protein concentration was determined by Bradford method using bovine serum albumin as the standard. The result shows that CAPGA extracted by ammonium sulphate precipitation of 75-100% has highest protolithic activity. CAPGA activity and specific activity after being dialyzed was higher than before being dialyzed.***

Keywords: ammonium sulphate, CAPGA, enzyme activity, extraction

1. Introduction

Acid protease is an enzyme having roles in a reaction that involves protein breakdowns. Commercially, protease occupies the highest rank of all enzymes and includes more than 60% of the total enzyme trading [1]. Protease in food industries is targeted to reduce turbidity in beer industries, reduce gluten in bakery industries, and agglomerate milk in cheese industries [2].

Goat (*Capra aegagrus*) is one of ruminants exist in Indonesia. Goat has complex structures and functions of digestion organ. The pascadiaphragm digestion organs are consisted of stomach with several segments that are the rumen of 80%, reticulum of 5%, omasum of 7-8%, and abomasum of 7-8% [3].

A purification and characterization of pepsinogen and pepsin from sheep has been conducted [4]. The research resulted single bands of pepsinogen and pepsin in electrophoresis results with molecular weights of 34 KDa that produced the highest activity if conditioned in pH of 3. Extraction process of crude acid protease from Indonesian local goat's abomasum has not been analyzed much and the characteristics have not identified yet. Hence, research on how the extraction process and characterization of crude acid protease from goat is conducted is required.

2. Material and Methods

Materials

Ammonium sulphate, glycine base, tris base and NaOH were purchased from Merck. *Bovine blood hemoglobin* and trichloroacetic acid were obtained from Sigma Aldrich. Coomassie Brilliant Blue G-250,

Preparation of abomasum

Abomasum of 1.5 years-old goat was obtained from Solo, Indonesia. The samples were packed in polyethylene bags containing shaved ice and transported to the research laboratory within 45 minutes. Upon arrival, it was washed with tap water to eliminate remaining dirt and blood. Samples that were already clean were collected and stored in sealed plastic bags at -20ºC until they were used for extraction and analysis.

Preparation and partial purification of CAPGA

Preparation of CAPGA was prepared and extracted as references methods [5] [6] with a slight modification. Frozen abomasum was thawed and cut into pieces in thickness of 1-1.5 cm and then homogenized for one minute with 10 mM of Tris-HCl buffer, pH of 7.5, and at a ratio of 1:2 (w/v). The homogenate was centrifuged at 10,000 x *g* for fifteen minutes at 4ºC using Sigma Sartorius of 3-30 K centrifuge. The residue was discarded and the supernatant was collected to be adjusted to pH of 2.0 with 1 M HCl and allowed to stand at 25ºC for thirty minutes. The suspension was centrifuged at 5,000 x *g* for thirty minutes at 4ºC. The supernatant was then fractionated with ammonium sulphate (0-25, 25-50, 50-75, and 75-100% of saturations) then centrifuged at 10,000 x *g* for ten minutes at 4ºC. The residue was suspended in 50 mM of Glycine-HCl buffer (pH of 2.0) and dialyzed for 24 hours at 4ºC using the MWCO dialysis sack of 12 KDa. Dialysate was used as a crude acid protease extract. Proteolithic activity was assayed using bovine blood hemoglobin as a substrate [6]. Protease activities of each fraction were calculated. The concentration of ammonium sulphate giving the highest protease activity was chosen for further analysis.

Protein determination

Protein concentration was determined by Bradford method [7] using bovine serum albumin as the standard by measuring the absorbance at 595 nm.

Assay for protease activity

Potential crude acid protease's activities as well as pepsin's activities against hemoglobin were determined [6]. Fifty

microliters of appropriately diluted enzyme samples were mixed with 100 µl of solution consisting of 2.0% acid-denatured bovine hemoglobin and 350 µl of 100 mM glycine–HCl buffer (pH of 2.0). After incubating the mixture at 37ºC for fifteen minutes, the reaction was immediately stopped by adding 500 µl of 8.0% trichloroacetic acid (TCA). The mixture was centrifuged at 10,000 x *g* for fifteen minutes and the absorbance of the supernatant at 280 nm was measured. One unit pepsin's activity against hemoglobin was defined as the amount of enzyme that catalyzed an increase of 1.0 in the absorbance at 280 nm per minute under the assay conditions.

3. Result and Discussion

3.1 Initial Extraction of CAPGA

CAPGA was initially fractionated using four levels of sulphate ammonium concentration with some volumes of goat CAPGA, each of which was 20 ml and the activities were tested. Result data of CAPGA testing in four levels of ammonium sulphate fraction are shown in table 1.

Table 1: Result of CAPGA fractionation.

Ammonium Sulphate Concentration (%)	*CAP Activity (U/ml)*
0	260.67^{a}
0-25	1258.58^{b}
25-50	1378.22^{b}
50-75	1438.04^{b}
75-100	1699.51^{b}

Different notations of small letter superscript in the same column show that there are real effects/ differences ($p \geq 0.05$).

Ammonium sulphate saturation of 75-100% produces the highest CAP activity that is 1699.51 U/ml defined as 1699.51 CAP activity units in 1 ml (Table 1). Hence, the concentration of 75-100% was used as the chosen ammonium sulphate concentration in next processes of CAPGA production with bigger volumes.

3.2 Extraction of CAPGA

Ammonium sulphate's precipitant saturation of 75-100% was resuspended using 50 mM of Glycine-HCl, pH of 2. The resuspension result still had ammonium sulphate salt content so that another process was demanded to clean it. The process was a dialysis using molecular weight cut off (MWCO) of 12 KDa as the dialysis membrane. This membrane was functioned to separate protein constituting CAPGA and ammonium sulphate salt by passing solution resulted from the resuspension through semipermeable membranes. Results of before-and-after-being-dialyzed CAPGA extraction is presented in table 2.

Table 2: CAPGA before and after being dialyzed

	Protein Content (mg/ml)	*CAPGA Activity (U/ml)*	*CAPGA Specific Activity (U/mg)*	*CAPGA Specific Activity (U/g)*
Before being dialyzed	1674.27^{a}	1174.22^{a}	0.01^{a}	701.33^{a}
After being dialyzed	1277.85^{b}	1434.22^{b}	1.12^{b}	1122.38^{b}

Different notation of small letter superscript in the same column shows there are real effects/differences ($p > 0.05$)

Protein content of CAPGA from goat abomasum after being dialyzed was lower than before being dialyzed. This might have caused by the existence of protein whose molecular weight was 12 KDa was dissolved so that it affected values of protein content to become less after being dialyzed. CAPGA activities after being dialyzed showed a higher result than the activities before being dialyzed. Successful purification stages give higher enzyme activity values than previous stages [8].

4. Conclusion

Crude acid protease is isolated from goat abomasum (CAPGA) by ammonium sulphate precipitation of 75-100% to obtain highest protolithic activity. CAPGA activity and specific activity after being dialyzed was higher than before being dialyzed.

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