Isolation and Utilization of Protease Lactic Acid Bacteria as Meat Tenderizer

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***Abstract*— The objective of this research was to observe the potency of protease of Lactobacillus LBP 1 as meat tenderizer. The study included the proteolytic enzyme activity and the effect of the enzyme concentration to meat tenderizing. The isolation of protease used ammonium sulphate of 15, 30,40, and 60%, to be determined their specific activities. The enzyme activity was calculated as the number of protease which catalysed the releasing 1 µmol of tyrosin per minute and was measured by spectrophotometer at wave length of 274 nm. Fraction which has the highest activity then was chosen for dialysis process and the specific activity was determined. Meat tenderizing was done by immersed the meat into the enzyme extract in concentration of 0.25, 0.50, 0.75 and 1.00% respectively for 30 min. The meat tenderize was measured by penetrometer. Fraction 0f 45% ammonium sulphate showed the highest activity and has specific activity of 0.794 U/mg. Partial purificationby ammonium sulfate of this fraction by dialysis process showed a specific activity of 1.202 Unit/mg. Tenderizing of meat score correlated with the enzyme concentration and give the tenderize score of 5.3 mm/g/10s, 5.8 mm/g/10s, 6.9 mm/g/10s for immersing of enzyme concentration of 0.25, 0.50, 0.75 and 1.00% respectively for 30 min.**

Keywords—Lactic acid bacteria, proteolitic enzyme, specific activity, meat tenderizer

I. INTRODUCTION

Meat tenderizing is one of the problems for meat consumption. Protease from plant like papaya and pineapple Exploration of the protease sources showed that protease derived bacterially could be used as meat tenderizing. Production of protease derived from microorganism has some advantages, i.e. produced in a large amount, has an equal quality, cheaper cost, produced in a short time and the growth can be managed easily[1]. Protease derived bacterially more were produced more than plan or animal derived protease [2].

Protease enzyme will digest meat protein like collagen, and elastin to be shorter fibers which lessen the toughness of meat. Lactic acid bacteria mostly was found in fermented food and showed characteristic as non pathogenic bacteria. Some research showed that some species of this bacteria potentially as proteolytic bacteria [3][4] and many of these enzymes were known have activities to degrade the myofibrilar and sarcoplasma proteins in meat [5][6][7] *Bekasam* is one of fish fermented foods which are produced traditionally in Indonesia. We has isolated some of proteolytic lactic acid bacteria from it product which included some strains of *Pediococcos* and *Lactobacillus*, which have been identified qualitatively using MRS-Casein Agar based on the clear zone area. The highest proteolitic was given by a bacteria namely of LBP 1[8]. In this research the specific activity of the proteolytic derived enzyme of LBP 1 was evaluated and then developed as an alternative of meat tenderizer. The effect of enzyme concentration to meat tenderizing were observed at a definite time.

II. METHODS

A. Culturing of LBP1

The isolate bacteria stock was subcultured twice. About 1% of isolate was inoculated to MRS broth (Oxoid) and incubated at 37^{0}C for 20 h. The suspension then was centrifuged (Eppendorf) for 15 minutes at 3500 rpm, supernatant were discarded and the pellet were suspended in by50 mL of sterilized NaCl 0.85%. This mixture then was used as a culturing starter of LAB bacteria as enzyme producer.

B. Production of Protease Derived Bacterially

20 mL of inoculum were inoculated in 180 mL MRS Broth containing of 5% casein (b/v), then was shake at 120 rpm at 37^{o}C for 16h. After that the mixture then was centrifuged at 5000 rpm at temperature 4^{o}C for 15 min. The supernatan contained protease enzyme were collected and isolated using ammonium sulfate

C. Isolation of Protease

About of 50 mL of supernatant was added by15%, 30%, 45% and 60% (b/v) of amonium sulfate respectively. The mixture then were centrifuged, the residue were collected and about 15 mL of phosphate buffer pH7 then was added and keep at temperature of 4^{o}C. Each of some fractions then were measured their activity and the protein content

D. Purification of Protease

About 7 mL of the highest activity fraction from isolation process was put into a dialysis pocket and was dialyzed in 70 mL phosphate buffer pH 7 at 4^{o}C. Dialysis process was done until the solvent has no $BaSO_4$ containing that were tested using 1% $BaCl_2$

E. Determination of enzyme activity

About 2 mL of sample was added to 2 mL 0.05M of phosphate buffer pH 7. After 5 min, the mixture then was added by 2 mL 0f 2% casein (in 0.05 M phosphate buffer pH7) casein as a substrate and incubated for 10 min at 37ºC Put 4 mL of 0.4M TCA to each sample and the mixture were incubated again for 30 min. The mixture were centrifuge at 500 rpm at 4ºC for 10 min. The supernatan then were collected and measured their absorbance at wave length of 274 nm. Protease activity was expressed in unit (U) which is defined as the number of protease which catalysis the lost of 1 µmol tirosin per min. Enzyme activity was calculated by equation below.

$$\text{Activity} = \frac{[\text{Tyrosin}]}{\text{Mm tyrosin}} \times \frac{V}{p \times q} \times fd$$

V : volume of sample (mL)
p : `volume of enzyme (mL)
q : incubation time (minute)
fd : dilution factor
Mm tirosin: 181.19 (gram/mol)

F. Determination of Protein

About of 0.5 mL sample was added with 5 mL Biuret reagent. Homogenized the mixture and stand at room temperature for 10 min. About of 0.5 mL Follin Ciocalteu reagent then was added to the mixture, stand at room temperature for about 30 min. The solution was measured its absorbance by spectrophotometer at wave length of 708 nm. Standard curve was made using BSA as a standard.

G. Production of protease derived LBP-1 used for meat tenderizer

About 10 mL of stock culture of LBP-1 were inoculated in 90 mL of growth media (80 mL MRS broth + 10 mL 5% casein) then were incubated for 16 j at 37℃. After that, about 100 mL of culture mixture were inoculated into 900 mL of the growth medium (800 mL MRS Broth + 100 mL of 5% casein), incubated for 16 h at 37℃. The mixture then was centrifuged at 5000 rpm for about 10 min at 4℃. The supernatant were collected and were precipated using ammonium sulphate 45%. The residue was separated using centrifugation at 5000 rpm at 4℃, then was filtered using Whatman No. 1, washed using acetone and dried at room temperature. The dried crude enzyme then was dissolved into phosphate buffer pH 7.

H. Measurement the effect of enzyme concentration to meat tenderizing

Meat was cut into 2x2x2 cm, then were immersed in enzyme solution for concentration of 0.25, 0.50,0.75 and 1.00% (b/v) respectively for about 30 minutes. Measurement of meat tenderizing was done using penetrometer.

III. RESULTS AND DISCUSSION

A. Enzyme activities of fraction precipitation with ammonium sulphate and dialysis

Protease enzyme which was produced by inoculation of LBP 1 in MRS medium containing 5% casein was precipited using 15%, 30%, 45% and 60% ammonium sulfate respectively and were evaluated their activity as is shown at Table 1.

TABLE I. ENZYME ACTIVITIES OF SOME FRACTION OF PRECIPITATION WITH AMMONIUM SULPHATE

Fraction precipated of ammonium sulfate	Enzyme activity (U/mL)	Protein (mg/mL)	Specific activity (U/mg)
15%	0.230	0.587	0.392
30%	0.339	0.813	0.417
45%	0.697	0.880	0.794
60%	0.319	1.585	0.201

Precipitation at 45% ammonium sulfate gives the highest specific enzyme (0.794 U/mg), it means that 45% ammonium sulfate gives the best condition of salting out for protease enzyme derived from LBP-1. In salting out process, the solubility of protein decreases caused by a competition of salt ions and protein in binding the water molecule. Ammonium sulfate has ability to pull the water molecule on the surface of enzyme, so that enzyme molecule will form an aggregate. Precipatation of protease enzyme isolated from *Lactobacillus acidophilus* was also found that precipitation with 45% ammonium sulfate gave the highest specific activity, but LBP-1 protease gave a lower activity (0.794 U/mg) then L.acidophilus protease (1.24 U/mg) [9]. LBP-1 protease enzyme has a higher activity than Bacillus substilis 1012M15 (0.475 U/mg) [10].

The best fraction of enzyme which gave the highest specific activity was purified using dialysis process and gave an increasing of specific activity as is shown at Tabel II.

TABLE II. ENZYME ACTIVITIES OF PROTEASE DERIVED LBP-1

Enzyme	Enzyme activity (U/mL)	Protein (mg/mL)	Specific activity (U/mg)
Crude extract	0.316	3.021	0.045
Ammonium sulfate 45%	0.697	0.080	0.794
Dialysis	0.877	0.729	1.202

B. The effect of enzyme consentration to meat tenderizing

The toughness of meat closely related to sarcomere protein. Degradation of meat sarcomere including a breakdown of myofibril and connective tissue i.e. collagen and elastin, and therefore produced a more tender meat [11]. Heat treatment and degradation of these proteins by protease enzyme will produce a tenderize meat. In this research, LBP-1 protease enzyme was used as meat tenderize and the effect of it protease consentration were evaluated (Tabel III).

The result of this treatment showed that increasing of enzyme concentration was followed by increasing of meat tenderizing. At enzyme concentration of 0.25%, 0.50%, 0.75%, and 1.00%, the tenderize score are 5.3 mm/g/10s, 5.8 mm/g/10s, 6.9 mm/g/10s and 8.0 mm/g/10s respectively. Based on standard score of meat tenderizing, the best treatment was immersing of meat in 0.25%- 0.50% protease enzyme for 30min, which represented from tender (6 mm/g/10s) to very tender (7mm/g/10s)

TABLE III. THE EFFECT OF ENZYME CONCENTRATION TO MEAT TENDERIZING

Enzyme cons % (b/v)	Meat tenderizing (mm/g/10s)
0.00	4.6
0.25	5.3
0.50	5.8
0.75	6.9
1.00	8.0

IV. CONCLUSION

Protease derived LPB-1 was potentially to be develop industrially especially for meat tenderize because of its activity and the ability to tender meat.

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