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Submission date: 11-Jun-2023 05:16PM (UTC+0700)
Submission ID: 2113512918
File name: 1800-Article_Text-6261-1-10-20191227.pdf (600.56K)
Word count: 2606
Character count: 13193

ISBN : 978-602-14917-9-9

EXTRACTION OF PROTEASE ENZYMES FROM GINGER FOR MEAT MIXING PROCESS

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Abstract. Protease is an commerce enzyme that is important in the world as meat mixing process, because in general the consumption of meat generally from old livestok. Protease has potential from an economic standpoint and in 2018 had taken control of 63% of the total sales of enzymes in the world. One unexplored enzyme is a protease from ginger. The analysis showed that Protease Activity (PA) obtained from the best treatment drying time of 40 hours at 50 °C followed by 60 hours drying time at 50 ° C, respectively at 0.2817 U / ml and 0.1089 U / ml. The results of molecular weight determination and the enzyme active site found that ginger protease enzyme has a cluster of serine residue at the active site with an alkaline pH of about 9.2 to 11.5 and a molecular weight of 167.74 Da after being confirmed by electrophoresis. The resulting enzyme activity can be enhanced by the addition of $FeCl_2$ and experienced no inhibition with EDTA. Ginger protease enzyme used in this study confirmed the inhibition by inhibitors HgCl experience.

Keywords: Ginger, Protease

Introduction

Tenderness is a quality criteria become the main reference, because the meat consumed in Asian countries is derived from an old meat. Softening meat can actually be done with the withering and by placing the carcass in cold temperatures. But it does not always achieve the expected results. Sometimes tenderness may occur during the process of withering / cooling carcass caused by the degradation / hydrolysis of proteins in the meat muscle structure (Foegeding and Lanier, 1996). The use of protease enzyme is the most effective way to process the meat softening and in developing countries and is commonly done.

Based on statistical data, imports increased enzyme Indonesia each year. In 2013 imports totaled 56,564,899 kg enzyme at a price of US $ 123 132 576 (CBS, 2013). Of the total imports of these enzymes, as much as 40% is a protease enzyme. Even the United States imported 200,000 kg per year papaya latex specifically used for meat tenderizer (Word, 1983) yet to produce protease enzyme of plant tissue that has been commonly known such as papain, bromelain many obstacles fisin and continuity in terms of the number and effectiveness. It is therefore necessary to find sources other protease enzymes.

Research on the enzyme protease from ginger rhizome has not been made in Indonesia that is still little data on the ginger rhizome protease. This study is expected to provide information about the characteristics and use of protease enzymes extracted from the ginger plant. Results are expected to be widely applicable and can increase the economic value and the value of the benefits of ginger.

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Research and Method

Based on the above problems, the orientation of this research was conducted in two phases. At the first phase this research divided into two factors, first factor is longer drying ginger (20, 40, 60, 80 hours) and the secord factor is a drying temperature (40, 50 and 60 ºC), this treatment was repeated 10 times. At the final phase is about the observation yield and activity of protease enzymes and also the enzymes molecular weight determination and determination of a reactive group to use some specific inhibition of the enzyme and electrophoresis. Besides, as additional observations made of protease enzyme activity assay is based on the best treatment of dry ginger third stage stored in a sealed container at room temperature conditions and applied to the beef

Treatment Method

Extraction of Protease Enzymes from Ginger Rhizome

The ginger rhizome is sliced transversely with a thickness of 3-4 mm and is homogenized with 0.2 M phosphate buffers pH 7.4 (1: 3) containing 0.025 M Na2EDTA and 0.15 M NaCl using an electric blender at 4 ºC. Rhizome slices are immersed in extracting buffers for 30 minutes at 4 ºC before homogenization. Homogenization was carried out for 15 minutes, with the first 5 minutes using low speed and the next 10 minutes with maximum speed. The obtained homogenate was filtered with a filter cloth, the filtrate obtained was allowed to stand in the refrigerator for 15 minutes. The precipitate is then discarded and then centrifuged at 10,000 xg for 30 minutes at 4□C. Supernatant is a crude extract of the protease enzyme and then tested for its activity.

Precipitation of the protease enzyme extract was carried out by the salting out method using ammonium sulfate $(NH_4)_2SO_4$ technical salt until 50% saturation. The addition of ammonium sulfate is carried out little by little and stirred slowly using a magnetic stirrer and the temperature is maintained at 4 °C and then allowed to stand for 30 minutes in the refrigerator. The solution was centrifuged again at 10,000 xg for 15 minutes at 4 ° C. The deposited enzyme was used for further testing.

The drying of the deposited protease enzyme was carried out using an oven with variations in temperature according to treatment. The oven is heated first according to the treatment temperature, 1 hour before the drying process begins. Samples are placed in aluminum foil, and drying is terminated if the moisture content of the material is 10 + 4%. The dried protease enzyme is put in a sealed plastic bag, and stored in a desiccator until the protease activity is tested. Before being tested for proteolytic activity, dry enzymes were dissolved in phosphate buffers 20% (w / v)

Electrophoresis

To find out the fractionation of protease enzymes extracted from tempeh by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Meanwhile, to find out the location of protein migration that occurs, it can be colored with amidoblack or coomasieblue (CBB). With this coloring you can see colored bands when there is a protein fraction, so that the purity of an enzyme can be estimated. The procedure is to remove the gel from the glass slab and put it in a CBB solution (staining solution), and gently shake it until the blue color can be bound by protein. Then just wash the CBB which is not bound by protein with a destaining solution until the protein band is clearly visible. To eliminate the remaining CBB you can use a shaker for 15 minutes.

To find out the functional group of an enzyme, various kinds of inhibition are used which correlate directly with the active group of the enzyme. Inhibitors provided include DFP (Diisopropyl Fluoro Phosphate), EDTA, IAA (Iodoasetamid), PCMB (Parakloromercuribenzoat), diazoacetyl norleucine, and so on.

Result and Discussion

Based on the statistical data in the field, the production of ginger in Jember reached 45.8 tonnes per quarter harvest in 2013. If the harvest is abundant, the price will drop ginger 1/3 of the market price. Utilization of ginger as a medicinal plant also is less promising and raised the value of

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selling ginger. Therefore the use of ginger as a source of protease enzymes is expected form of fact and supported by Ginger Growers Association and the local government.

The results of protease enzyme activity for the combination of drying time and temperature drying is done, is given in the following table.

Table 1. Protease Enzyme Activity Results

Drying Time	Drying temperature (°C) and Protease Enzyme Activity		
	40	50	60
20	0.11371	0.11281	0.11031
40	0.28216	0.28171	0.18981
60	0.07141	0.10891	0.10101
80	0.00991	0.00671	0.00581

The results of further testing with Minitab for treatment residuals obtained diversity data as shown below

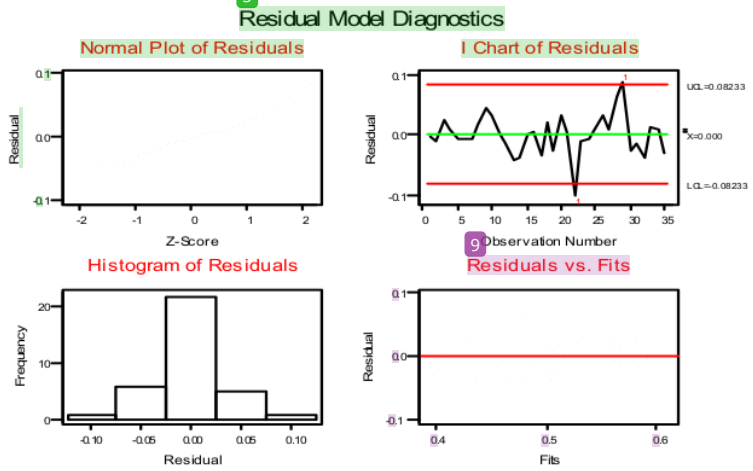

Figure 1. Residual Model Diagnostics of Protease Enzyme Activity

The results of the above analysis shows that Protease Activity (PA) obtained from the best treatment drying time of 40 hours at 50 ° C followed by 60 hours drying time at 50 ° C, respectively at 0.2817 U / ml and 0.1089 U / ml. Allegedly protease enzyme instability at high temperatures with a long exposure time. This is because a change or modification of the enzyme leads to not only take place on serine residues that exist on the surface, so that citraconic anhydride interact with the active center of the enzyme

The above research results are in line with the study of enzyme activity related to temperature, that the higher the drying temperature and the drying time of the ginger rhizome that is carried out, the lower the activity of the protease enzyme produced. The decrease in activity that occurs due to the protease enzyme itself is a protein that when under increasing temperature effects the energy of the enzyme molecules becomes so strong that they go beyond the energy barrier to break the secondary bonds that maintain the enzyme in its original state (active catalytic state). As a result, tertiary and secondary structures are lost (enzyme inactivation occurs) accompanied by decreased enzymes. Apart from denaturation, it is suspected that enzyme damage (which is marked by a

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decrease in activity) is caused by the oxidation of phenol compounds which are indeed present in the fresh ginger rhizome into quinone form.

The drying process carried out in fresh ginger rhizomes can increase the speed of oxidation, both due to the drying temperature which can stimulate the work of the polyphenolase enzyme and due to the availability of oxygen needed in the oxidation. As you know, polyphenolase is an enzyme that plays a role in the oxidation of phenol compounds. Inactivation of the protease enzyme extracted from the dried ginger rhizome occurs when the oxidized quinone of the phenol compound interacts with the enzyme protein. It is suspected that quinones will react with protein amino acids and can damage the physico-chemical properties of proteins.

The ginger protease enzyme from the results of this research study is a serine group protease enzyme that has an SH group on its active side. The group can undergo oxidation which is triggered by factors such as oxygen and heat, both of which occur during enzyme drying. Oxidation of these groups will produce disulfide bonds which will cause interactions of protein molecules so that the active side is no longer free and binds to the substrate so that enzyme activity decreases.

Decreased activity at 80 °C is caused by an enzyme undergoing irreversible denaturation which causes the enzyme to decrease its catalytic power due to changes (expansion or development) of the polypeptide chain structure. The mechanism of heat in inducing protein denaturation is very complex and involves destabilizing the interactions of hydrogen bonds, electrostatic bonds, hydrophobic bonds, and Van Der Walls bonds so that the stability of proteins due to the influence of heat varies.

As additional observation data, protease fractionation was also carried out with 15% sodium dedosil sulfate polyacrylamide gel electrophoresis (SDS-PAGE). In SDS-PAGE the movement (migration) of the protein depends on the size of the molecule (mass) of the polypeptide ie the smaller the polypeptide the faster the movement of the protein, so that it is separated based on its molecular weight (MW) with molecules that have a small MW will go down first.

Low voltage is used at the stacking gel stage (the initial stage) and is increased after the sample reaches the resolving gel (the final stage). The use of different voltages (from the results of the study) apparently does not cause different protein patterns and the voltage function is thought to only affect the duration of the electrophoresis process (the speed of movement of proteins due to electrical charges). The electrophoresis results were visualized by gel painting using CBB followed by silver staining to get good results which can be seen in Figure 1 below.

The results of molecular weight determination and the enzyme active site found that ginger protease enzyme has a cluster of serine residue at the active site with an alkaline pH of about 9.2 to 11.5 and a molecular weight of 167.74 Da after being confirmed by electrophoresis. The electrophoresis is performed are given in the following figure.

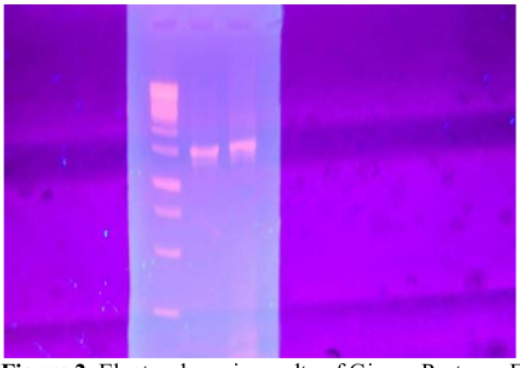

Figure 2. Electrophoresis results of Ginger Protease Enzyme (Molecular Weight)

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The line that was obtained from SDS-PAGE above shows a thin band, allegedly due to the concentration of protein in the very low range effect on the thickness and concentration of protein bands on SDS-PAGE results. The thickness of the protein bands were formed on SDS-PAGE results showed the content or the amount of protein that has the same molecular weight, which are at the same tape. The protease acid specificity is determined by its amino acid residue close to the splitting point (primary specificity). The enzyme substrate will bind to the serine group of the enzyme so that changes in the active site will cause enzyme inactivation. Serine type enzymes have amino acids histidine and tryptophan around the amino acid of cysteine which is the active side of the enzyme. These amino acids have the same role as amino acids that bind to the substrate.

The resulting enzyme activity can be enhanced by the addition of $FeCl_2$ and experienced no inhibition with EDTA. Ginger protease enzyme used in this study confirmed the inhibition by inhibitors HgCl experience.

Conclusion

Protease enzyme ginger grown in Jember has a cluster of serine residue at the active site with an alkaline pH of about 9.2 to 11.5 and a molecular weight of 167.74 Da after being confirmed by electrophoresis. Protease Activity (AP) obtained from the best treatment drying time of 40 hours at 50 ° C followed by 60 hours drying time at 50 ° C, respectively at 0.2817 U / ml and 0.1089 U / ml.

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Acknowledgments

Thank you to Politeknik Negeri Jember for funded the implementation activities, as well as to our partners: TDC UNAIR who helped implement the research

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