ECONOMIC METHODS OF GINGER PROTEASE'S EXTRACTION AND PURIFICATION

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Abstract: This article reports the ginger protease extraction and purification methods from fresh ginger rhizome. As to ginger protease extraction, we adapt the steps of organic solvent dissolving, ammonium sulfate depositing and freeze-drying, and this method can attain crude enzyme powder 0.6% weight of fresh ginger rhizome. The purification part in this study includes two steps: cellulose ion exchange (DEAE-52) and SP-Sephadex 50 chromatography, which can purify crude ginger protease through ion and molecular weight differences respectively.

Keywords: ginger protease, extraction, purification, DEAE-52, SP-Sephedax 50

1. INTRODUCTION

There are several kinds of vegetal protease used in food industry nowadays, such as papain, bromelin etc, and all of them have similar proteolytic activity. It is proved that, compared with papain, ginger (Zingiber officinale roscoe) protease has 10-folds activity in meat tenderization. And furthermore, it can significantly improve not only the flavor but also the

Please use the following format when citing this chapter:

Qiao, Y., Tong, J., Wei, S., Du, X. and Tang, X., 2009, in IFIP International Federation for Information Processing, Volume 295, *Computer and Computing Technologies in Agriculture II, Volume 3*, eds. D. Li, Z. Chunjiang, (Boston: Springer), pp. 1619–1628.

quality of meat by increasing its nutritious value (Naveena，B.M.，2001; Yan Zhou et. al.,1996; Zhang Pingping et. al.,2001; Jin-neng Lin,1991), which makes it a valuable meat tenderization. It also can significantly improve the clarifying degree of wine and beer (Tang Xiaozhen et.al. 2002). Ginger protease can improve the quality of food processing on the ground of not reducing nutritious value. With the proteolytic of ginger protease, those proteins which are difficult to digest, such as casein, bean separate protein (Yuan-lin Song et.al., 2001; Ming-fa Zhang et.al., 1991), can be decomposed into small molecules as peptides or amid acids. Some of those small molecule materials have bio-activity, and belong to functional food. Also, ginger protease can be used in the proteolysis of animal and vegetal proteins, or as dough adjuster in baking, or milk solidification factor, or addictive in cosmetic. Our Chinese traditional food, Ginger Freezing Milk, is solidified by ginger juice, and the milk solidification factor here is proved to be ginger protease (Zhang Pingping et.al., 1999). Ginger protease consists of some complicated components, which have similar structures and properties, and make it difficult to attain highly purified ginger protease. Hai-yan Cai et, al have researched the distributing of ginger protease in fresh ginger and its activity changing tendency during storing, and the method of crude ginger protease extracting by using AOT-isooctane and CTAB heptane/octanol anti-micelle extracting (Cai Haiyan et, al., 2004), Jing-quan Dai have purified ginger protease by cellulose ion exchange chromatography (DEAE-52), by the following steps: dissolve ginger juice in acetone, then dry it into powder. Before purification, first dissolve the powder again by buffer, then deposit it by various concentrations of ammonium sulfate, then dialysis, and at last purified those materials by chromatography column (Dai Jingquan et al., 2003). Researchers throughout the world had tried many methods to purify this enzyme (Hompson ,E.H et al.,1973; Zhao Zhiping et.al.,1995; Ohtsuki,Kozo; et al.,1995; Kyung H. Choi et.al.,2000), and had got the crystal structure and X-ray structure as well (Kyung H. Choi et.al.,2000). But the process of extraction and purification are too complicated to be extended the scale from laboratory to industry. Therefore, finding some economic methods to market it needs further researches, especially on how to extract and purify it by a simple and effective method which can be used in industry has been highlighted.

2. MATERIAL AND METHODS

2.1 Material and samples preparation

Ginger rhizome is high quality yellow ginger of LaiWu, Shangdong.

Tyrosine, supplied by Bio-Chemical College of Science Institution of China; Casein and Bovine serum albumin, supplied by Shanghai Chemical Reagents Station (with N: 14.5—15.5%); Commassie Blue G-250, repacked import by Shanghai Chemical Reagents Station. All the above are G. R. grade.

The chromatography column was obtained from Shanghai Ya-Long Biochemical Apparatus Company (16mm*20cm).

2.2 Methods of assays

2.2.1 Ginger protease activity determination (Qian Yuanze 1991)

The definition of protease activity is under a certain temperature (40℃) the mass of protease which is used to produce 1μg tyrosine by hydrolyze casein. The steps of determining the activity of ginger protease is as following:

Mix the solutions of enzyme (1.0ml) and casein (1.0ml 0.5%), and incubated for 10min at 40℃. The assay is stopped by adding 2.0ml trichloroacetic acid (0.4mol/l), and then the solution is filtered after 10min standing. The color reaction is to mix 1.0ml filtrate with 5.0ml sodium carbonate (0.4mol/l) and 1.0ml Flionhydroxybenzene, incubated for 20min at 40℃. The comparision reaction was same as the assay above except for the adding sequence was reverse between casein and trichloroacetic acid. And then using the solution of the blank group to adjust the extinction, and measure the absorbance at 280nm.

The formula of enzyme activity calculation is:

U=A*K*N

U: the unit of enzyme activity

A: the value of absorbance

K: calculated by dividing 100 with the corresponding value of absorbance of 280nm at the 100μg/ml tyrosine standard curve.

N: the multiple of enzyme dilute

2.2.2 Extraction of crude ginger protease

Ginger rhizomes are cleaned and extracted juice, and then the juice is deposit in low temperature and centrifuged at 4800 r/min for 20min to get rid of starch. Then it is suction filtrated to remove some large contaminants. The filtrate is mixed with acetone which is pre-cooled in refrigerator (1:1), and then the sediment is collected after centrifuged at 30000r/min for 10min. The sediment is dissolved in phosphoric acid buffer and centrifugated again. This time, the supernatant is collected and mixed with ammonium sulfate to saturation of 30%. The solution is place in low temperature for 24h, and then centrifuged and filtrated. The supernatant is collected again and mixed with ammonium sulfate to saturation of 60%, and again place in low temperature for 24h, and centrifugated to get the sediment. Then it is dissolved by phosphoric acid buffer (pH=6), and dialyzed in dialysis bag. At last, crude ginger protease powder is attained after freeze-drying.

2.2.3 DEAE-52 Sepharose assay

According to the result of primary assays, the crude ginger protease was dissolved in Tris-HCl buffer (pH 7.5) with the concentration of 30 mg/ml, and then was absorbed on a column of DEAE-52, which was first washed by Tris-HCl buffer (pH 7.5) with sodium chloride (0.1mol/l) in order to get rid of the un-combined protein. Then, a gradient concentration eluting buffer began with an initial speed of 1ml/min with 0.2~0.5mol/l sodium chloride and Tris-HCl buffer (pH 7.5). Then the optimization wash concentration and volume were determined by measuring the OD value of protein in washing, and the optimization wash speed also could be determined by comparing the protein curve in the washing under the optimization condition that mentioned above. The efficiency of purification of DEAE-52 was measured by testing the enzyme activity in the washing. The cellulose ion exchange chromatography purify ginger protease through the difference of ion between compounds, and because of the hydrophilic nature of cellulose, it has little tendency to denature proteins which is very important for enzymes.

2.2.4 SP-Sephadex 50 assay

The enzyme purified through DEAE-52 is taken into bag to be dialyzed in order to get rid of the ions and condense it. Then diluted it into20mg/ml by phosphate buffer (pH6.0, with 0.1mol/l sodium chloride), and then refined it by SP-Sephadex 50. During this process, the volume of the dissolve was 4ml, and the wash speed 0.4ml/min, collect speed 7ml per tube which meant 18 ml/min. The Sephadex gel chromatography column purifies ginger protease

from the difference of molecular weight of components, which can get rid of contaminants with different molecular weight.

3. RESULTS AND DISCUSSION

3.1 Extraction of crude ginger protease

In this study, the method used to extract crude ginger protease can get higher enzyme activity compared with former ones (extracted by acetone powder), the higher rate is 43% according the experimental data. After freezing and drying, the crude enzyme powder weight is 0.6% of fresh ginger rhizome, with the activity of 1.2×105U/g.

3.2 Cellulose ion exchange chromatography(DEAE-52 Sepharose) (Jian Lu et,al.,2005)

3.2.1 Solution concentration and volume determination

It is shown in fig1 that when the solution concentration is lower than 30mg/ml, nearly all of the protein is absorbed when the volume is 0.1ml or 0.3ml. While the volume becomes 0.5ml, protein appears in the supernatant as the concentration higher than 30mg/ml, and has a positive correlation trend of the volume and concentration of the solution. While the volume becomes 0.7ml, the same trend appears at the concentration of 20mg/ml. According to the assay, two groups of optimization of concentration and volume are 30mg/ml, 0.5ml and 20mg/ml, 0.7ml respectively. Concerned the length of the column, the former group is determined as the best one. (According the column contains 40ml DEAE-52 homogenized solution, a result of volume to it can be calculate as 4ml)

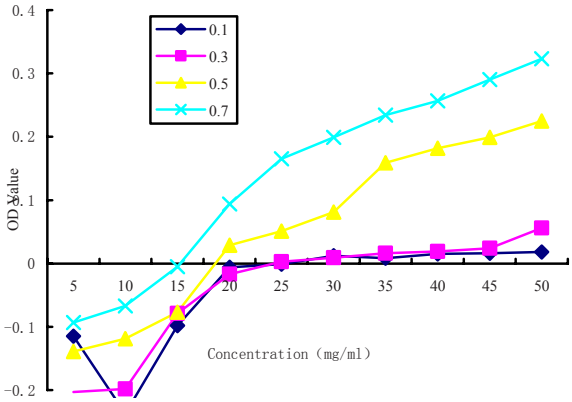

Fig 1. Effects of solution concentration and volume on the protein concentration in supernatant

3.2.2 Wash speed determination

There is hardly any protein available in the washing while the wash speed is 0.5ml/min as shown in fig2. It is probably that the wash speed is too slow to wash down the enzyme which is firmly absorbed by DEAE, and an incompletely wash process is made. However, when the wash speed rises to 1.5ml/min, the protein concentration in the washing becomes very high, but with no obvious peak. The reason for this may lies in the too fast speed, which makes the enzyme cannot exchange completely with exchanger in the column and cannot be absorbed, therefore, the enzyme is washed out into the foregoing tubes. With the wash speed of 1.0ml/min, three distinct protein peaks are attained, with OD values of 0.495, 0.549, and 0.771 respectively, and this speed is determined as the best wash speed.

3.2.3 Purifying effects of DEAE

There are three protein peaks in fig2, but compared by fig3, no enzyme activity is found in the peaks except of the second one. After measuring the purified enzyme solution in the second peak, the OD value is 0.955, and compared with the crude lyophilization ginger protease, the effects of DEAE-52 purification are listed below in table1.

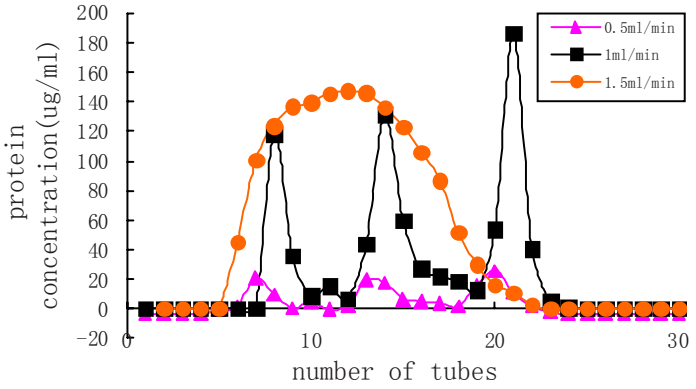

Fig 2. Effects of wash affected by speed

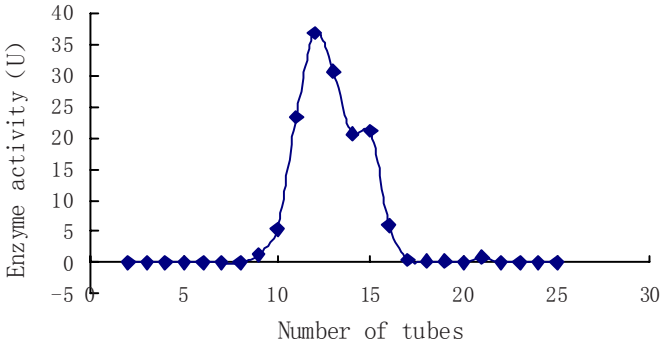

Fig 3. The curve of enzyme activity

Table 1. Effects of DEAE-52 purification

Items	Protein μg/mL	Enzyme activity U	Specific activity U/μgPr	Multiple Purification
Lyophilization protease	58.84	21.19	0. 36	——
DEAE-52	45.00	26.42	0. 59	1.64

3.3 SP- Sephadex (Ming-fa Zhang et.al., 1991)

Collecting the washing of the second peak, refine it by SP-Sephadex, and protein concentration and enzyme activity are shown in fig4 and fig5 respectively.

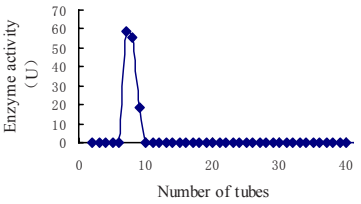

Fig4 Washing Curve of protein concentration by SP-Sephadex

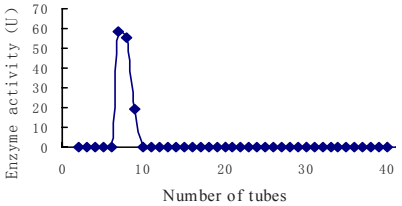

Fig5 Enzyme proteolytic activity curve

It is obvious that there is a solo protein as well as enzyme peak, and the tube with high protein concentration also with high enzyme activity and purifying effect. The effects of SP-Sephadex are recorded in table2.

Table 2. Effects of SP-Sephadex purification

	Protein μg/mL	Enzyme activity U	Sepecific activity U/μgPr	Multiple of purification
Crude enzyme	58.84	21.19	0. 36	1
DEAE-52	45.00	26.42	0. 59	1.64
SP-Sephadex50	43.00	31.91	0.74	2.06

It is shown in table2 that refined by DEAE and SP-Sephadex, the enzyme solution with high activity and low protein concentration, and the multiple is 2.06.

4. CONCLUSION

4.1 Extraction of ginger protease

There are many methods of enzyme extraction, such as organic solvent deposit, super filtrate concentration, dialysis, adsorption and freeze-drying. Those mentioned here are methods of crude enzyme extraction, used to concentrate and enrich crude proteases. Refining methods used to purifying proteases include polypropylene amide gel, dextran gel, DEAE-cellulose, DEAE-gelose, and electric chromatography etc. The methods such as spray-drying and freeze-drying adapt to large extent extraction, for example industrial extraction. The method used in this study can not only attain a comparable higher yield but also transfer to industrial procession.

Enzyme additives used in food industry are always crude enzymes (National Standard of PR, 1986), because the high rate of purification is just a luxury in common application.

4.2 Purification of ginger protease

One enzyme washing peaks after DEAE, and the multiple of purification is 1.64. This result is different from the report of Jing-quan Dai (Zhang Pingping et.al.,1999)that two enzyme washing peaks was attained. The reason may lies behind the different working condition and outer environment. According to the experimental result of this study, the purification of SP-Sephedax only attains a non-significant effect. If it were the industrial extraction, this step can be omitted.

4.3 Components differences of Crude enzyme and Purified enzyme

The term of crude enzyme is defined by OD value in 280nm, which indicate the material is protein. After the testing of enzyme activity, the crude enzyme is attained which includes a lot of impurities such as contaminated proteins, organic and inorganic salt etc. After purification of DEAE and SP-Sephadex, most of the contaminants are got rid, which is exhibited by the higher enzyme activity.

ACKNOWLEDGEMENTS

Funding for this research is provided by Nature Science Project Funds of Shandong Province (Q2004B01); and Scientific Technology Research Projects of Shandong Province (2007GG30009002).

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