Study on preparation and stability of antioxidant peptides from acer truncatum seed

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Abstract. The acer truncatum seed protein was hydrolyzed by alcalase, neurase, papain and trypsin, respectively, to get four kinds of peptide fractions. Ant oxidative activities of all peptide fractions were evaluated by degree of hydrolysis and DPPH radical scavenging activity. Trypsin peptide (TP) exhibited the highest ant oxidative activity compared to other peptide fractions. Single factor test was applied to optimize the hydrolysis condition. The optimum conditions obtained were substrate concentration of 4 %, enzyme amount of 16000 U/g, enzymatic hydrolysis time of 6 h, and pH of 7.8.

1. Introduction
Bioactive peptides with special physical function have attracted much attention [1]. However, many artificial antioxidants have potential side effects, such as liver damage and carcinogenesis, limiting their applications. Thus, it is essential to develop and utilize effective and natural antioxidants to protect the human body from ROS [2].

Acer truncatum is a unique species of maple tree in China. It was found that the acer truncatum seed contained about 40 percent oil, in which the amount of unsaturated fatty acids is up to 90 %. Therefore, the objective of the present work was to produce bioactive peptides from defatted acer truncatum. In order to obtain the highest yield of peptides, the hydrolysis condition was optimized according to experimental design. Moreover, the antioxidant activities of antioxidant peptides were also investigated by means of in vitro free radicals scavenging tests [3].

2. Materials and methods

2.1. Materials
Acer truncatum oil were supplied by Weifang Ivyuan biological technology co., LTD. Trypsin, papain, alcalase, alcalase were purchased from Novo Company. All other chemicals and reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co, Ltd (Shanghai, China).
2.2. Preparation and extraction of protein fractions
Fold the seed meal into the filter tube, the upper end of the filter tube shall not exceed the opening of the siphon. Oil was separated by soxhlet extraction. After the residue was broken in the crusher, it was dried in a constant temperature oven under 40°C conditions to obtain the protein [4].

2.3. Screening of protease
In the present study, Acer truncatum seed protein was separated hydrolyzed by alcalase, neutrase, papain and trypsin, for the production of antioxidant peptides. Acer truncatum seed meal protein preparation of five copies, five enzymes were added to the protein solution of Acer truncatum seed with the mass fraction of 2%, and the amount of enzyme was 15000 U/g. In their respective optimum temperature and pH of the enzyme solution under 4 h (see Table 1 for details).

Table 1. Optimal reaction conditions of five enzymes used for hydrolysis

| Enzyme   | pH   | temperature /°C | time /h | amount of enzyme/U·g·l |
|----------|------|-----------------|---------|------------------------|
| trypsin  | 8.0  | 37              | 4       | 15 000                 |
| papain   | 7.0  | 55              | 4       | 15 000                 |
| alcalase | 9.5  | 50              | 4       | 15 000                 |
| neutrase | 7.0  | 45              | 4       | 15 000                 |

2.4. Single-factor test design
Effect of pH, amount of enzyme, enzymatic hydrolysis time, and substrate concentration, on enzyme amount on hydrolysis degree of sample and DPPH clearance rate of product were investigated using single factor experiments. Variables and experimental levels for single-factor testing were designed with pH of 7.5, 7.8, 8.1 and 8.4; enzyme of 8 000 U/g, 12 000 U/g, 16 000 U/g, 20 000 U/g and 24 000 U/g; enzymatic hydrolysis time of 5 h, 6 h, 7 h, 8 h and 9 h; substrate concentration of 4%, 5%, 6%, 7% and 8% [5].

2.5. Determination of index
Determination of degree of hydrolysis (DH):

\[
DH(\%) = \frac{B \times N_b}{\alpha \times M_p \times h_{tot}} \times 100
\]

Where B was the amount of NaOH (ml), N_b was the concentration of NaOH (mol/l), M_p was the quality of Acer truncatum protein (g), h_tot was the number of peptide bonds in Acer truncatum protein, \( \alpha \) was the average dissociation degree of protein amino group of Acer truncatum seed can be calculated by formula \( \alpha = \frac{1}{1+10^{pH-pK}} \) (where pH was the pH of solution, pK was the dissociation constant \( \alpha-NH_3^+ \) (pK=7).

Determination of DPPH radical scavenging activity:

\[
\text{DPPH radical scavenging activity (\%)} = \left[ 1 - \frac{A_l - A_f}{A_c} \right] \times 100
\]

Where \( A_c \) is the absorbance value of blank (ethanol substituted samples), \( A_i \) is the absorbance value of the tested sample, \( A_j \) is the absorbance value of background (ethanol substituted DPPH).

2.6. Statistical analyses
Statistical analysis was performed by the SPSS statistic program for Windows (SPSS17.0). All tests were repeated three times. The data obtained were subjected to analysis of variance (ANOVA) and mean differences evaluated by Duncan test (p<0.05).
3. Result and discussion

3.1. Effect of different proteases on peptide of acer truncatum seed

Table 2. Effects of proteases on hydrolysis degree, DPPH radical scavenging rate of peptides

| Enzyme   | pH  | temperature/°C | time/h | amount of enzyme/U.g-1 | Hydrolysis degree (%) | DPPH radical scavenging activity (%) |
|----------|-----|----------------|--------|------------------------|-----------------------|-------------------------------------|
| trypsin  | 8.0 | 37             | 4      | 15 000                 | 4.935                 | 38.845                              |
| papain   | 7.0 | 55             | 4      | 15 000                 | 6.749                 | 11.923                              |
| alcalase | 9.5 | 50             | 4      | 15 000                 | 18.041                | 4.868                               |
| neutrase | 7.0 | 45             | 4      | 15 000                 | 18.082                | 38.597                              |

The enzymatic products of the same substrate protein may have different biological activities after enzymatic hydrolysis by different proteases, so the screening of enzyme is one of the important links in the preparation of antioxidant peptides. From table 2, according to the hydrolysis degree and DPPH radical scavenging activity of enzyme, the order of hydrolysis ability of the four proteases from strong to weak is neutrase > alcalase > papain > trypsin. The highest DPPH radical scavenging activity of trypsin was 38.845 % [6].

From table 2, according to the conditions of hydrolysis degree and antioxidant value, trypsin was selected as the hydrolytic enzyme prepared from the antioxidant peptide of acer truncatum seed.

3.2. Single-factor experiment

3.2.1. The effect of pH on the hydrolysis degree and DPPH radical scavenging activity

Figure 1. Effect of pH on the hydrolysis degree and DPPH radical scavenging activity

Figure 1 showed the effect of pH on the hydrolysis degree and DPPH radical scavenging activity. The hydrolysis degree and DPPH radical scavenging activity increased over the increase in pH from 7.5 to 7.8. The hydrolysis degree decreased when pH increased from 8.4 to 8.7 and the DPPH radical scavenging activity sharply declined when pH increased from 7.8 to 8.7. Because each protease has its own optimal pH. When the pH is above or below optimal pH, the protease may denaturation or inactivated. Therefore, the pH of 7.8 was chosen for the further research.
3.2.2. *The effect of enzyme amount on the hydrolysis degree and DPPH radical scavenging activity*

![Figure 2](image_url)

From Figure 2, the hydrolysis degree increased over the increase in amount of enzyme from 8000 U/g to 12000 U/g and the hydrolysis degree tended to be flat when the amount of enzyme increased from 12000 U/g to 24000 U/g. The DPPH radical scavenging activity increased over the increase in amount of enzyme from 8000 U/g to 16000 U/g and the DPPH radical scavenging activity decreased when the amount of enzyme increased from 16000 U/g to 24000 U/g. The reason for the decrease of DPPH radical scavenging activity may be that excessive enzyme catalysis can further hydrolyze and inactivate the peptides with antioxidant activity [7]. Therefore, the enzyme amount of 16000 U/g was chosen for the further research.

3.2.3. *The effect of enzymatic hydrolysis time on the hydrolysis degree and DPPH radical scavenging activity*

![Figure 3](image_url)

**Figure 3.** Effect of enzymatic hydrolysis time on the hydrolysis degree and DPPH radical scavenging activity
Figure 3 showed that the effect of enzymatic hydrolysis time on the hydrolysis degree and DPPH radical scavenging activity. The hydrolysis degree and DPPH radical scavenging activity increased rapidly in enzymatic hydrolysis time from 5 h to 6 h. The hydrolysis degree tended to be flat when the enzymatic hydrolysis time increased from 7 h to 9 h. And the DPPH radical scavenging activity decreased rapidly after 6 h and tended to be flat in enzymatic hydrolysis time from 7 h to 9 h. Because protease digestion process is carried out step by step, peptides with DPPH radical scavenging activity are partially enzymized, and resulting in reduced activity. The antioxidant activity of polypeptides is due to that the special groups can react with free radicals, namely, hydrogen donor groups. Only when these polypeptides have appropriate molecular weight, the specific hydrogen donor groups can maximize their contact with free radicals, and showing strong antioxidant properties [8]. Therefore, the enzymatic hydrolysis time of 6 h was chosen for the research.

3.2.4. The effect of substrate concentration on the hydrolysis degree and DPPH radical scavenging activity

![Figure 4](image)

**Figure 4.** Effect of substrate concentration on the hydrolysis degree and DPPH radical scavenging activity

From Figure 4, the hydrolysis degree decreased over the increase in substrate concentration from 2% to 5%. The DPPH radical scavenging activity increased when the substrate concentration increased from 2% to 4% and the DPPH radical scavenging activity decreased in substrate concentration increased from 4% to 10%. Because the viscosity of the enzymatic hydrolysate increased over the increase in solution substrate concentration. And this affected the diffusion of protease and decreases the water activity, thus inhibiting the hydrolysis reaction [9]. Therefore, the substrate concentration of 4% was chosen for the further research.

4. Conclusion

In this paper, according to the conditions of hydrolysis degree and antioxidant value, trypsin was selected as the hydrolytic enzyme prepared from the antioxidant peptide of acer truncatum seed.

In single-factor test experiment, the optimum conditions obtained were substrate concentration of 4%, enzyme amount of 16000 U/g, enzymatic hydrolysis time of 6 h, and pH of 7.8.

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