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Proteolysis of burley tobacco-leaf extracts and antioxidant activity of the hydrolysates

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Abstract

Burley tobacco-leaf protein extracted by alkali and precipitated by acid was hydrolyzed enzymatically, and optimum protease and hydrolysis parameters were determined. Pepsin was best and gave the highest hydrolysis rate (15.55%). As per results of the single-factor test, optimum hydrolysis conditions were: temperature 37 °C, pH 2, and an [E]/[S] ratio of 5%. These hydrolysis conditions were optimized by an orthogonal experiment that rendered the same results as the single factor experiment. The ratio of essential to total amino acids and that of essential to non-essential amino acids contained in the burley tobacco-leaf protease hydrolysate were higher than the FAO/WHO standard values of 0.4 and 0.6, among which hydrophobic amino acid content was the highest, followed by negatively and positively charged amino acid contents. In *vitro* antioxidant experiments showed that the scavenging rates of DPPH, superoxide anion, and hydroxyl radicals of tobacco-leaf protein hydrolysates were higher than those before hydrolysis. Further, the hydroxyl radical-scavenging rate was the most significant, and total antioxidant capacity was improved. The nutritive value of the burley tobacco-leaf protein hydrolysate improved upon pepsin-mediated hydrolysis, and the antioxidant activity was better. These results provide a theoretical basis for further development and utilization of tobacco protein resources.

Keywords: burley tobacco leaf; protein; proteolysis; hydrolysate; amino acid; antioxidant activity; pepsin; radical scavenging.

Practical Application: 1. Test five proteases for their effects on the hydrolysis of burley tobacco-leaf protein and optimize hydrolysis conditions. 2. Pepsin is the best protease to use and the corresponding optimal conditions were effectively determined. 3. The amino acid species diversity and composition of the hydrolysate of burley tobacco-leaf protein are excellent, and its nutritional value improved with hydrolysis. 4. The hydrolysate of burley tobacco-leaf protein showed high antioxidant capacity, which is of great value in the pharmaceutical, food and cosmetics industry.

1 Introduction

Food industry is a life industry related to the national economy and people's livelihood. The development of food industry plays an important role in improving people's income, maintaining reasonable economic growth and maintaining social security and stability. China, in particular, has the largest population, which means that China needs food more than any other country in the world, and food has more profound significance for China. The development of China's food industry is the main driving force of agricultural industrialization and plays an important role in effectively solving the problems of rural development, agricultural efficiency and farmers' income.

Plant leaf protein, also known as green protein concentrate, is the protein extracted from plant stems and leaves (Santamaría-Fernández & Lübeck, 2020). Plant leaf protein is rich in diverse amino acids, whereby it can be used as protein feed or as a dietary protein-supplement for humans (Pérez-Vila et al., 2022; Ghaly & Alkoaik, 2010). Plant leaf protein is a new protein resource with high utilization value. It has wide material sources and great development potential, and has broad application prospects in the food, feed, pharmaceutical, and other industries (Dawodu & Abdulsalam, 2015; Zhang et al., 2015). Tobacco is an important special cash crop in China, whose planting area and yield rank first in the world. In addition to being used as raw material for the tobacco industry, tobacco leaf protein content is also very rich. In particular, the soluble protein content in tobacco leaves is very high (Shi et al., 2019; Vansuyt et al., 2003). Tobacco leaf proteins have many special functions in addition to the basic functional properties of proteins. Previous studies have shown that tobacco leaf protein has high application value in the feed, food, and pharmaceutical industries (Zhang et al., 2013; Teng & Wang, 2012). Therefore, tobacco leaf protein is a plant protein resource with great utilization potential and development prospects. In recent years, many studies on plant leaf proteins have pointed that the efficiency of direct utilization of leaf proteins is low, but its function is enhanced and its utilization efficiency is improved after hydrolysis. Compared with crude leaf protein, proteolysis products often have better bio-absorbability and bioactivity (Teixeira et al., 2014; Navarro-Peraza et al., 2020).The active peptide extracted from plant leaf protein by enzymatic hydrolysis has antibacterial activity and protease inhibition activity, and shows high stability, which is an important aspect

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that determines the potential of the resource for the preparation of antimicrobial peptides (Yang et al., 2021; Vidal et al., 2020). Xie et al. conducted a study on the hydrolysis of alfalfa leaf protein by protease, and obtained active peptides with antioxidant effects and high nutritional value (Xie et al., 2008). Similarly, Sun et al. (2021) showed that mulberry leaf protein and its hydrolysate had good antioxidant activity (Sun et al., 2021). However, reports on tobacco protein hydrolysates are scarce at best. Tobacco resources in China are rich and diverse, thus, it is of great significance to further study and develop tobacco leaf protein technology for improving the comprehensive utilization value of tobacco, and give full play to the functionality of tobacco leaf protein.

In this study, burley tobacco-leaf protein was extracted, and the amino acid composition and antioxidant activity of the resulting hydrolysate were analyzed. The alkali soluble-acid sinking method was used to extract protein through compound protease, papain, alkaline protease, neutral and pepsin to obtain the hydrolysate from burley tobacco-leaf protein, and select the best effect of a protease enzyme solution; subsequently, an orthogonal experiment was designed to optimize the preparation method of burley tobacco-leaf protein hydrolysate, test different enzymes for the digestion of tobacco leaf protein, and determine the relationship between the rate of hydrolysis and the peptide yield. Differences in amino acid composition and antioxidant effects of protein hydrolysates of burley tobacco were analyzed. We aimed to provide theoretical and technical support to further develop and utilize of tobacco-leaf protein resources.

2 Materials and methods

2.1 Materials

Plant material: Burley tobacco-leaf protein was prepared by the method of alkali solution and acid precipitation.

Reagents: Complex protease (1.2 × 105 U/g, Beijing Solarbio Technology Co., Ltd.); Papain (8 × 106 U/g, Beijing Solarbio Technology Co., Ltd.); Alkaline protease (2.4 × 104 U/g, Beijing Solarbio Technology Co., Ltd.); Neutral protease (5 × 104 U/g, Beijing Solarbio Technology Co., Ltd.); Pepsin (3000 NFU/g, Beijing Solarbio Technology Co., Ltd.).

2.2 Proteolysis of burley tobacco leaves by different proteases

The O-phthalaldehyde (OPA) method (Spellman et al., 2003) was used to determine the rate of hydrolysis of tobacco leaf proteolysis by different proteases. The hydrolysis conditions for the five proteases tested herein are shown in Table 1.

The bicinchoninic acid method (Khramtsov et al., 2021) was used to determine the polypeptide content in tobacco leaf proteins before and after hydrolysis. Standard curves were drawn using bovine serum albumin. Polypeptide concentration in the samples was measured before and after the hydrolysis of tobacco leaf protease to calculate the increase of polypeptide after hydrolysis, i.e., the polypeptide yield.

Table 1. Reaction conditions for hydrolysis using different proteases.

Protease type	Temp °C	pH	[E]/[S]%
Protemax	50	7.5	5
Papain	55	6	5
Alkaline protease	55	9	5
Dispase	45	7	5
Pepsin	37	2	5

2.3 Single factor experiment of tobacco-leaf protein hydrolysis by pepsin

The rate of hydrolysis and polypeptide yield were used as indexes to study the effect of pepsin on proteolysis of tobacco leaf proteins. The hydrolysis conditions were pH (1, 1.5, 2, 2.5, 3, or 3.5), temperature (35, 36, 37, 38, 39, or 40 °C), and enzyme to substrate ratio [E]/[S] (2.5%, 3%, 3.5%, 4%, 4.5%, or 5%).

2.4 Orthogonal experiment of tobacco-leaf protein hydrolysis by pepsin

On the basis of the single factor experiment, the L16 (43) optimization orthogonal experiment was conducted; the rate of hydrolysis was the index, and pH (A), temperature (B) and [E]/[S] (C) were the independent variables. The factors and levels included in this orthogonal experiment are shown in Table 2, and the treatment combinations of the orthogonal experiment are shown in Table 3.

2.5 Determination of the amino acid composition of tobacco leaf protein

Tobacco leaf samples (0.2 g) were placed in 10-mL centrifuge tubes to which 5 mL of extraction solution was added to leave standing for 40 min before placing in an ultrasonic instrument

Table 2. Factors and levels included in the orthogonal experiment.

Factor	Level			
pH (A)	1	1.5	2	2.5
Temp (B)	35	36	37	38
[E]/[S]% (C)	3.5	4	5.5	5

Table 3. Treatment combinations included in the orthogonal experiment.

Treatment	A (pH)	B Temp (°C)	C [E]/[S] (%)
1	1	1	1
2	1	2	2
3	1	3	3
4	1	4	4
5	2	1	2
6	2	2	1
7	2	3	4
8	2	4	3
9	3	1	3
10	3	2	4
11	3	3	1
12	3	4	2
13	4	1	4
14	4	2	3
15	4	3	2
16	4	4	1

for centrifugation at 5000 R/min for 10 min. Then, supernatants were passed through 0.45 mm organic-phase filter membranes, transferred to injection bottles, and submitted to the amino acid automatic-analyzer for determination and analysis.

2.6 Determination of antioxidant capacity of hydrolysates of tobacco-leaf protein

The scavenging ability on DPPH, superoxide anion, and hydroxyl free radicals and total antioxidant capacity were measured for the protein hydrolysates of burley tobacco-leaf protein hydrolyzed by pepsin. The antioxidant activity of the hydrolysates was comprehensively evaluated by the following four indexes.

Determination of DPPH free radical-scavenging rate

10 μL of hydrolysate to be tested and 190 μL of DPPH ethanolic solution were mixed in a 96-well plate and kept in the dark at room temperature for 30 min. Absorbance at 515 nm and the rate of absorbance decline were used to reflect the DPPH free radical-scavenging ability of the sample. In the control group, absolute ethanol was mixed with the sample instead of DPPH ethanolic solution. The formula to calculate the scavenging rate is as follows (Equation 1):

$$D\% = \frac{A_1 - (A_3 - A_2)}{A_1} \times 100\% \quad (1)$$

Where, D% is the DPPH free radical-scavenging rate; A_1 is the absorbance of the blank; A_2 is the absorbance of the control, and A_3 is the absorbance of the sample.

Determination of the superoxide anion-radical scavenging rate

In this case, 250 ULAP-Temde system solution and 125 μL enzymatic hydrolysis solution were mixed, and then added and thoroughly mixed with 250 μL hydroxylamine hydrochloride solution. The mixture was reacted at 37 °C for 30 min, and then added with 250 μL p-aminobenzenesulfonamine solution and 250 μL α-theamine solution, and thoroughly mixed. Color development at 37 °C was recorded. The red azo compound was generated after 20 min of reaction. The absorption peak at 530 nm was measured in a 96-well plate with 200 μL. The scavenging ability of superoxide anion was negatively correlated with absorbance at 530 nm. In the blank control, deionized water was used instead of the enzymolysis sample. The formula for calculation of the scavenging rate is as follows (Equation 2):

$$D\% = \frac{A_1 - A_2}{A_1} \times 100\% \quad (2)$$

Where, A1 is the absorbance of the blank, and A2 is the absorbance of the sample.

Determination of the hydroxyl radical-scavenging rate

Each sample was placed in a centrifuge tube, added with 0.15 mL 0.2 mol/L phosphate buffer (pH 7.4), 0.3 mL 0.75 mmol/L $FeSO_4$ and 0.3 mL 0.01% H_2O_2, shaken for 1 min and thoroughly mixed. Then, 0.15 mL tobacco leaf protease was added to hydrolyze the sample, followed by 0.15 mL 1.5 mmol/L o-dinitrogen solution and 0.45 mL deionized water. For the control group, the samples were replaced with the same amount of deionized water, and the blank tube was replaced with the same amount of deionized water; then the samples were vortex and placed in a water bath at 37 °C for 60 min. The samples were centrifuged at 10000 rpm for 10 min at room temperature, and the absorbance of each supernatant was measured at 536 nm. The inhibition of absorbance decline rate at 536 nm reflects the ability of the sample to clear light free radicals. The formula for calculation of scavenging rate is as follows (Equation 3):

$$D\% = \frac{A_1 - A_2}{A_3 - A_2} \times 100\% \quad (3)$$

Where, A_1 is the absorbance of the sample; A_2 is the absorbance of the control; and A_3 is absorbance of the blank.

Determination of total antioxidant capacity (ABTS method)

ABTS (7 mmol/L ABTS) and potassium persulfate (2.45 mmol/L) were prepared as described previously. The mixed 1:1 solution was left standing in the dark at room temperature for 12-16 h to prepare the $ABTS^{+}\cdot$ mother solution, and then diluted 40 times with 10 mmol/L PBS buffer to prepare the $ABTS^{+}\cdot$ working solution. After subtracting the absorbance of the $ABTS^{+}\cdot$ working solution from the PBS blank control, the absorbance at 734 nm was 0.70 ± 0.05. Then, 200 uL ABTS working solution was added to a 96-well plate, add 10 uL of tobacco protease diluted 10 times, 10 uL PBS buffer was added to the control well, and the mixture was gently mixed. After incubation at room temperature for 6 min, absorbance at 734 nm was determined. Trolox standard solution (0, 0.15, 0.3, 0.6, 0.9, 1.2, 1.5 mmol/L) was prepared. Calculate ABTS antioxidant capacity by the following standard curve formula (Equations 4-5):

$$y = -0.1523x + 0.5438 \quad (4)$$

$$R^2 = 0.9921 \quad (5)$$

Where, y is the absorbance of the sample; x is the concentration of the Trolox; R^2 is the fitting coefficient.

3 Results

3.1 Optimization of hydrolysis conditions of tobacco-leaf proteins

Comparison of proteolytic effects of different proteases on tobacco leaves

The rate of hydrolysis and the polypeptide contents of tobacco-leaf proteins hydrolyzed by different enzymes gradually increased during the first 5 h, and then tended to remain stable (Figures 1-2). When pepsin was used to hydrolyze tobacco leaf protein, the rate of hydrolysis and peptide yield were highest at 15.52% and 35.39%, respectively, followed by compound protease, alkaline protease, neutral protease and papain, with the rate of hydrolysis at 14.73%, 12.00%, 10.33%, and 9.89%,

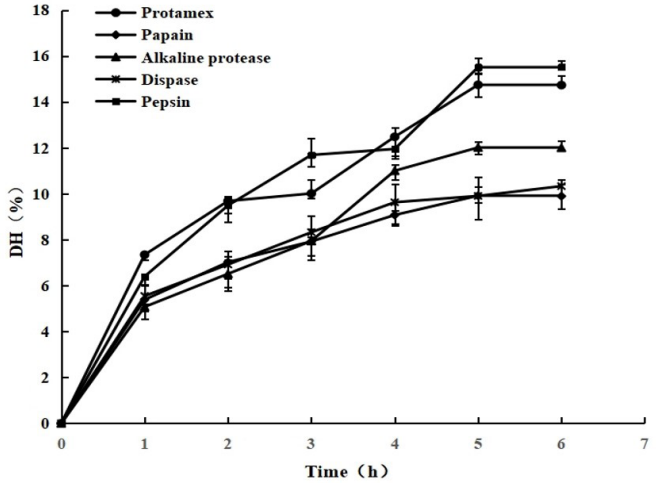

Figure 1. Effects of different proteases on the rate of hydrolysis of burley tobacco-leaf protein.

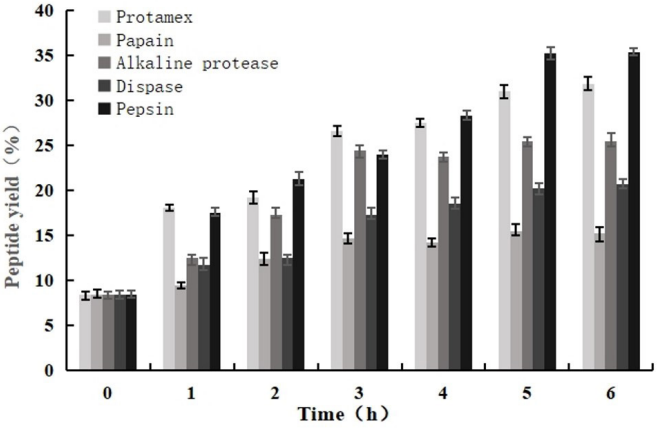

Figure 2. Comparison of peptide yield of burley tobacco leaves protein hydrolyzed by different proteases.

and polypeptide yields of 31.85%, 25.49%, 20.70%, and 15.49%, respectively.

Single factor experiment results of hydrolyzing tobacco leaves protein by pepsin

According to the comparison of the proteolytic effects of different proteases on tobacco leaves, pepsin was selected and a single factor experiment was designed to analyze the corresponding optimal hydrolysis conditions. Polypeptide yield of tobacco-leaf protein increased with rate of hydrolysis. At the beginning, the rate of hydrolysis and polypeptide yield increased with increasing pH, temperature, and [E]/[S]. When pH and temperature increased to a certain value, both the rate of hydrolysis and the polypeptide yield began to decrease, confirming that the rate of hydrolysis correlated positively with polypeptide yield. Thus, at pH 2, the highest hydrolysis rate was 15.94%, and the polypeptide yield was 32.53%. At 37 °C, the highest rate of hydrolysis was 16.31%, and the polypeptide yield was 33.11%. At [E]/[S] = 5%, the highest rate of hydrolysis attained was 15.57%, and the polypeptide yield was 31.85% (Figures 3-5).

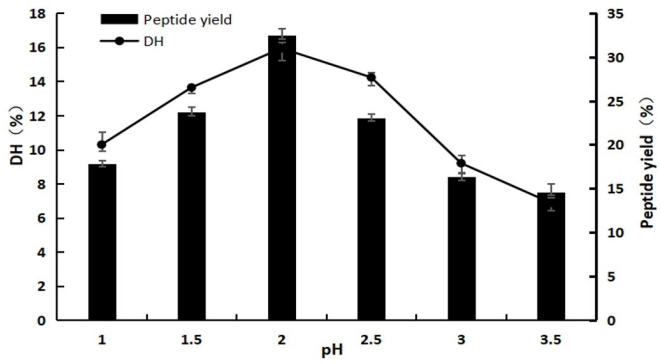

Figure 3. Effect of pH on the hydrolysis of burley tobacco-leaf protein by pepsin.

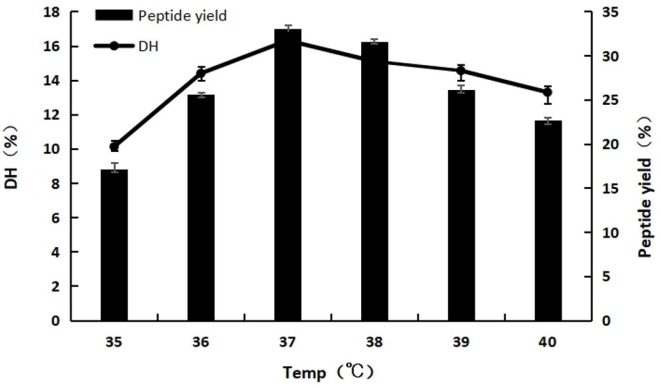

Figure 4. Effect of temperature on the hydrolysis of burley tobacco-leaf protein by pepsin.

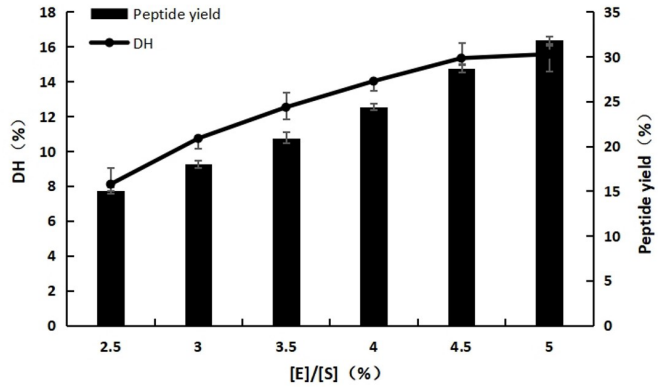

Figure 5. Effect of [E]/[S] on the hydrolysis of burley tobacco-leaf protein by pepsin.

Hydrolysis of tobacco leaf-protein by pepsin. Orthogonal experiment

Based on the results of the single factor test described above, we designed an orthogonal experiment to determine the optimal conditions for the hydrolysis of tobacco-leaf protein by pepsin. The optimal treatment combination was A3B3C4, i.e., pH 2, temperature 37 °C, [E]/[S] 5%. Further, in order to ensure the reliability of these results, three independent

experiments were performed under the optimal reaction conditions, with resulting rate of hydrolysis of 15.61%, 15.74%, 15.32%, respectively. The average value being 15.55%, which is higher than that observed in other studies. Based on the magnitude of their effects on hydrolysis, the factors affecting pepsin hydrolysis of tobacco-leaf protein followed this order: A > C > B (Table 4). Multivariate analysis of variance was used to study the significance of pH, temperature, and [E]/[S] effects on hydrolysis rate. The R-square value of the model was 0.798, implying that pH, temperature, and [E]/[S] explained 79.80% of the variation in rate of hydrolysis. The results showed that pH and temperature had a significant effect on the rate of hydrolysis ($P < 0.05$), whereas E/S did not (Table 5).

Table 4. Statistics of the results of the orthogonal experiment.

Treatment	A (pH)	B (Temp °C)	C ([E]/[S]%)	Rate of hydrolysis (%)
1	1	1	1	5.38
2	1	2	2	6.29
3	1	3	3	8.38
4	1	4	4	7.89
5	2	1	2	9.38
6	2	2	1	8.39
7	2	3	4	8.39
8	2	4	3	9.12
9	3	1	3	8.23
10	3	2	4	15.49
11	3	3	1	14.67
12	3	4	2	13.53
13	4	1	4	12.36
14	4	2	3	9.08
15	4	3	2	11.23
16	4	4	1	11.12
K1	27.94	35.35	39.56	
K2	35.28	39.25	40.43	
K3	51.92	42.67	34.81	
K4	43.79	41.66	44.13	
k1	6.99	8.84	9.89	
k2	8.82	9.81	10.11	
k3	12.98	10.67	8.70	
k4	10.95	10.42	11.03	
Optimal combination			$A_3B_3C_4$	
R	6.00	1.83	2.33	
Primary and secondary order			A > C > B	

3.2 Analysis of amino acid composition of tobacco-leaf protein hydrolyzed by pepsin

After protein of burley tobacco leaves was hydrolyzed by pepsin, total amino acid content in the hydrolysate was approximately eight times that before hydrolysis. In addition, the ratios of essential to total amino acids (E/T) and essential to non-essential amino acids (E/N) before and after hydrolysis were higher than the values of 0.4 and 0.6 stipulated by FAO/WHO standards (Table 6), thereby meeting the nutritional requirements of amino acid pattern recommended by FAO/WHO standards. These results showed that the E/T and E/N values of burley tobacco-leaf protein increased after pepsin hydrolysis, implying that hydrolysis improved the nutritional value of burley tobacco-leaf protein.

In addition, hydrophobic, negatively, and positively charged amino acids of burley tobacco-leaf protein increased after hydrolysis by pepsin, thus becoming nine, ten, and five times higher than before hydrolysis (Table 6), respectively. Some sulfur-containing amino acids among hydrophobic ones, are effective free-radical scavengers. Negatively charged amino acids mainly include aspartic acid (Asp) and glutamic acid (Glu), which are two important flavor-related amino acids. In turn, positively charged amino acids can enhance radical scavenging activity by providing protons to stabilize electron-deficient radicals while maintaining their stability through resonant structures, or by increasing interactions with radicals through hydrophobic association. Therefore, the rich and varied amino acid species after hydrolyzing burley tobacco-leaf protein with pepsin can play an important role in further improving the biological activity of tobacco-leaf protein.

3.3 Analysis of antioxidant activity of hydrolysate of burley tobacco-leaf protein hydrolyzed by pepsin

The scavenging rate of the burley tobacco-leaf protein hydrolysate on DPPH free radical, superoxide anion radical, and hydroxyl radical increased with hydrolysis time and peaked at 6, 6, and 5 h, respectively. The highest scavenging rates were 36.25%, 57.30%, and 31.69%, respectively (Figures 6-8).

Total antioxidant capacity of the hydrolysate of burley tobacco-leaf protein hydrolysate obtained by pepsin increased with hydrolysis time, and was basically stable after 6 h, after peaking at 3.11 mmol/g (Figure 9).

4 Discussion

As a high-quality protein resource, plant protein has great development potential and has been a research hotspot in the

Table 5. Multivariate analysis of variance of the results of the orthogonal test.

ANOVA	Quadratic sum	df	Mean square	F	P	Significance
Intercept	1578.67	1.00	1578.67	375.22	< 0.001	**
A (pH)	80.97	3.00	26.99	6.42	< 0.03	*
B (Temp)	7.95	3.00	2.65	0.63	0.62	
C ([E]/[S])	11.02	3.00	3.67	0.87	0.51	
Residual error	25.24	6.00	4.21			

$R^2 = 0.798$; df:degree of freedom; F: equality of variances. *p < 0.05.**p < 0.01.

Table 6. Comparison of amino acid content (mg/mL) of burley tobacco leaves before and after proteolysis using pepsin.

Type of amino acid	Burley tobacco-leaf protein	Hydrolysate of burley tobacco-leaf protein
Ile	4.24	33.12
Leu	7.33	84.86
Lys	6.52	13.62
Met	4.98	22.34
Phe	5.73	60.26
Thr	3.64	55.55
Val	3.58	30.65
Trp	3.72	40.70
Asp	3.45	90.10
Ser	5.43	40.70
Gly	6.42	20.45
Ala	4.87	56.80
Glu	17.89	118.35
Cys	0.98	7.10
Tyr	4.37	40.45
His	5.69	32.55
Arg	5.53	48.10
Pro	5.23	20.45
TAA	99.60	816.15
EAA/TAA	0.40	0.42
EAA/NEAA	0.66	0.72
HAA	39.68	349.18
NCAA	21.34	208.45
PCAA	17.74	94.27

EAA: essential amino acids; TAA: total amount of amino acids; NEAA: non-essential amino acids; HAA: hydrophobic amino acids; NCAA: negatively charged amino acids; PCAA: positively charged amino acids.

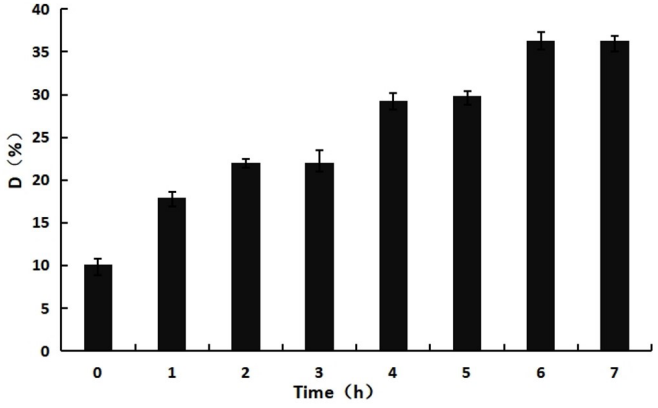

Figure 6. DPPH free radical-scavenging rate of the hydrolysate of burley tobacco- leaf protein hydrolyzed by pepsin.

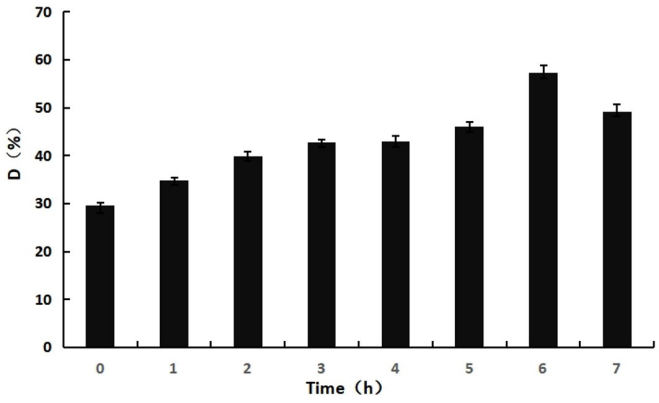

Figure 7. Superoxide anion radical-scavenging rate of hydrolysate of burley tobacco-leaf protein hydrolyzed by pepsin.

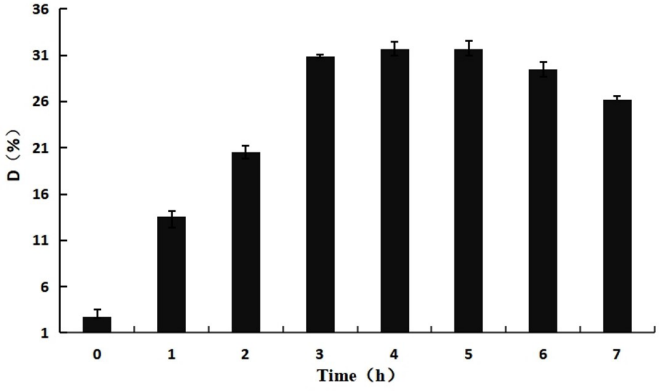

Figure 8. Hydroxyl radical-scavenging rate of hydrolysate of burley tobacco-leaf protein hydrolyzed by pepsin.

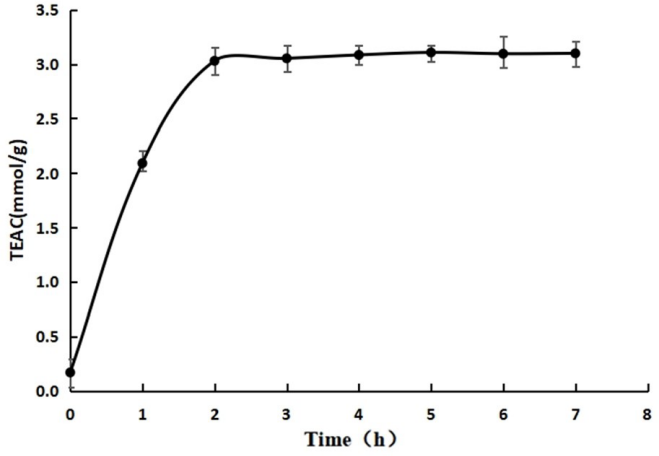

Figure 9. Total antioxidant capacity of hydrolysate of burley tobacco-leaf protein hydrolyzed by pepsin.

field of food industry science and technology (Ma et al., 2022). In order to improve the function and characteristics of plant protein, and aiming to obtain plant protein hydrolysates with high activity and high nutritional value, researchers have paid much attention to the technology of hydrolyzing plant protein by proteases (Ye et al., 2022; Rudolph et al., 2017). Different proteases usually exhibit different properties. For example, alkaline protease is a deep internal protease that can cleave peptide bonds, including those in phenylalanine, tyrosine, tryptophan and lysine carboxyl groups; in turn, neutral protease is a metalloproteinase that preferentially cleaves the peptide bond between leucine and phenylalanine; Papain can decompose peptide bonds in hydrophobic regions, including threonine, tryptophan, and

phenylalanine, among others (Zhu et al., 2019; Lorenzo et al., 2018; Shazly et al., 2019). The protein content in tobacco leaves is high, and that in burley tobacco leaves is particularly high, reaching 20.48%, which is of great value for the development of protein resources (Bokelman & Ryan, 1985). However, to date, there have been few reports on the results of proteolytic conditions of burley tobacco. In this study, five different proteases were tested for their hydrolysis effects on burley tobacco-leaf protein. According to the rate of hydrolysis and the peptide yield obtained in each case, pepsin was determined to have the best hydrolysis effects, and the optimal hydrolysis conditions were, 37 °C, pH 2, and an [E]/[S]% value of 5%.

We analyzed the antioxidant capacity of the hydrolysate of burley tobacco-leaf protein by pepsin. The results showed that the antioxidation effect of protein hydrolysates from burley tobacco leaves improved, compared with that before hydrolysis. The antioxidation ability of the hydrolysates gradually increased with hydrolysis time within a certain range. Previous studies showed that, more low-molecular-weight peptides, which tend to have higher antioxidant activity, are produced with increasing rate of hydrolysis (Wen et al., 2020; Wang et al., 2021). Additionally, the amino acid composition is an important factor for the antioxidant activity of peptides. Low molecular weight peptides contain some amino acids with special functions, such as tryptophan, histidine, phenylalanine, and lysine, among others (Lorenzo et al., 2018; Arrutia et al., 2020). The types and amounts of these amino acids play an important role in the overall antioxidant ability of peptides. Some studies seemingly suggest that peptides containing Tyr and Trp at the C-terminal can show strong free radical-scavenging ability, while amino acids such as Ser and Ile can show high free radical-scavenging ability at both the N-terminal and the C-terminal of the peptide (Li et al., 2022; Nisov et al., 2020). The increase of these special functional amino acids among the proteolysis products of burley tobacco is an important reason for the improvement of its antioxidant capacity. In addition, the results of this study showed that the content of hydrophobic, negatively, and positively charged amino acids in hydrolysates from burley tobacco-leaf protein also showed an increasing trend. Previous studies have shown that changes in the proportion of amino acids also have a great impact on the biological activity of peptides. For example, aromatic amino acids can provide free radicals with protons, such that they show a high ability to capture free radicals. At the same time, they can resonance to maintain the stability of free radicals (Kaprasob et al., 2022; Chalamaiah et al., 2019). Furthermore, hydrophobic and negatively charged amino acids can play an antioxidant role through the carbonyl or amino-chelating metal ions of the side chain. Hydrophobic amino acids can act as hydrogen donors, interacting with other amino acids to enhance the hydrophobic properties of the peptide, thereby enhancing its antioxidant capacity (Zheng et al., 2022; Laohakunjit et al., 2017). Therefore, its abundant amino acid types and balanced amino acid composition ratio in the hydrolysate of burley tobacco-leaf protein are important reasons for the improvement of its antioxidant capacity. In the future, the hydrolysates of burley tobacco-leaf protein should be separated and purified to further evaluate the biological activities of different peptide components and fully explore the potential applications of the hydrolysates of burley tobacco-leaf protein.

5 Conclusion

A comparison of the hydrolysis effects of different proteases on burley tobacco-leaf protein showed that pepsin was the best. The optimal hydrolysis conditions were: pH 2, temperature 37 °C, and [E]/[S]% value of 5%. Using pepsin to hydrolyze burley tobacco-leaf protein, the polypeptide yield in the hydrolysate correlated positively with the rate of hydrolysis. Furthermore, the amino acid species diversity and composition of the hydrolysate of burley tobacco-leaf protein are excellent, and its nutritional value improved with hydrolysis. Meanwhile, the hydrolysate of burley tobacco-leaf protein showed high antioxidant capacity. The separation and purification of the component polypeptides present in the hydrolysate of burley tobacco-leaf protein and the evaluation of their activity warrant further research.

Conflict of interest

The authors declare no conflict of interest.

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