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Functional properties, structural characteristics and biological activities of deer blood hydrolysates obtained by using different protease

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Abstract

In this study, we aimed to investigate the potential functional properties, structural characteristics and biological activities of the hydrolysates of deer blood obtained by using different protease. Deer blood hydrolysate treated by pepsin (DBH-P) and treated by alacase (DBH-A) showed higher degree of hydrolysis (DH) and smaller molecular weight (M_w). Scanning electron microscope (SEM), solubility, Fourier Transform Infrared Spectroscopy (FTIR), secondary structure, color properties, amino acids and antioxidant porperties. The results suggest that the type of protease used for the treatment significantly influences the functional properties, structural characteristics and biological activities of the resulting hydrolysates.

Keywords: deer blood; hydrolysate; protease; structures; activities.

Practical Application: The contribution of different protease is investigated as a sustainable mild route for the preparing of protein hydrolysates. The different deer blood hydrolysates treated by pepsin, trysin and alacase was investegated. The results showed that the molecular weight, micromorphology, solubility, secondary-structure, antioxidant activity, etc. were different in these hydrolysates. The results of this study can provide the preparation method of protease hydrolysates with different structures and properties for the food industry, and can also provide an effective way for the utilization of animal blood waste.

1 Introduction

There is a long history of raising deer (*Cervidae*) in Asia, Europe, America and Oceania. Deer blood is used medicinally in a few countries, such as China, Russia and Korea, and it is discarded as a waste in most areas, resulting in waste of resources and environmental pollution. In fact, deer blood rich in nutrient that is beneficial to human health. It was reported that deer blood contains complex chemical composition, there is 80%~81% water, 2%~4% inorganic constituent, 3%~4% ash content and 16%~17% organic constituent (Jiang et al., 2006). Proteins and various enzymes are main composition of nutrient, otherwise, there are lipids, fatty acid, sterols, hormones, purines, vitamins and polysaccharides, mineral element, etc (Jiang et al., 2006). Some reports showed that deer blood had the pharmacological functions of treatment of leukopenia after chemotherapy (Wang & Chen, 2019), immunomodulatory effects (Li et al., 2020), anti-melanoma effect (Pan et al., 2020), rehabilitation of acute kidney injury (Zhang et al., 2021), etc.

However, the application of deer blood is mainly by directly taking of fresh or dry products called "deer blood crystal". The main nutritional ingredient of deer blood was protein, there are a few researchers studied on its protein hydrolysates. These research mainly concentrate on enzymolysis technology (Xu et al., 2018; Liu et al., 2016), preparation of heme (Qin et al., 2016), antioxidant (Xu et al., 2014), angiotensin I-converting enzyme inhibitory activity (Yue & Zhu, 2008), etc. However, there are many kinds of proteases with different mechanism and site of action, which endows enzymatic hydrolysates different structures and properties. There are limited studies on the modification of deer blood by different proteases, and this is of great significance to the practical application of deer blood in food industry.

The aim of this study was to evaluate the properties of deer blood hydrolysates, including their molecular weight, amino acids, secondary structure, UV-vis properties, color properties, microstructure, solubility, etc. Moreover, an analysis of antioxidant activity of the hydrolysates was also carried out.

2 Materials and methods

2.1 Deer blood pretreatment

Fresh sika deer blood obtained by Shilu Co., Ltd, Changchun which had passed quarantine inspection and removed fibrous protein by stirring with a stick to avoid coagulation, then diluted two times with deionized water. The mixture was submerged in ice, then disrupted with ultrasound for 5 s by a 3 s interval for 15 min at the power of 500 watts.

2.2 Enzymatic hydrolysis of deer blood

Enzyme, including pepsin (1:3000, CAS: 9001-75-6), trysin (250 U/mg, CAS: 9002-07-7) and alacase (200 U/mg, CAS: 9014-01-1) were purchased from Shanghai yuanye Bio-Technology Co., Ltd, Shanghai. The preparation of hydrolysates were according the method of the report (Zheng et al., 2019) with some modification. Specifically, the hydrolysis with pepsin (DBH-P) (enzyme/substrate ratio, 2%) was conducted at 40 °C and pH 2.0, trysin treatment (DBH-T) (enzyme/

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substrate ratio, 2%) was conducted at 40 °C and pH 8.0, while hydrolysis with alacase (DBH-A) (enzyme/substrate ratio, 2%) was performed at 50 °C and pH 8.5. All the protease were at their suitable conditions (Verma et al., 2019a). After 6 hours reaction, the reaction mixture were heated at 90 °C for 15 min to inactivate enzymes. After cooling down, the pH of samples were adjusted to neutral. The solutions were centrifuged at 4000 rad for 20 min, the supernatants were collected and frozen-dried. The unenzymatic sample (DB) was treated with the same method as prepared.

2.3 Determination of degree of hydrolysis

The determination of degree of hydrolysis (DH) was followed the method of Sarabandi et al. (2019) with some modification. Suspension of deer blood hydrolysate and TCA (0.44 M) were mixed in equal ratio, then leaved it at 4 °C for 10 min. After that, the mixture were centrifuged at 8000 rad for 10 min. Bradford method (Bradford, 1976) was used to calculated the resulted soluble protein. The DH was the percentage of TCA soluble protein in total protein.

2.4 Molecular weight distribution

The different molecular weight (M_w) of deer blood hydrolysates was measured by using the method reported (Ashraf et al., 2020) with modification, conducted on HPLC (SHMADZU, Japan) equipped with a column 7.8 mm × 30.0 cm (TSK-GEL G2000SWXL, TOSOH Bioscience, Japan). Mixture of acetonitrile/water/TFA (20:80:0.1) was used as a mobile phase. In brief, the freeze-dried hydrolysates were solubilized into mobile phase (0.25 mg/mL) and filtered by using 0.45 μm membrane. The elution time was 30 min with a speed of 0.5 mL/min at 30 °C and detected at a wavelength of 220 nm. The standard substance used for molecular weight correction curve are bovine serum albumin (M_w 66.446 kDa), cytochrome C (M_w 2.588 kDa), bovine insulin (M_w 5.734 kDa), bacitracin (M_w 1.423 kDa), glutathione (M_w 0.310 kDa), and hippuric acid (M_w 0.179 kDa).

2.5 FTIR analysis

Infrared spectroscopy and secondary structure analysis was carried out according the method of Zhan et al. (2021) with some modification. Samples accompanied with KBr were subjected to FTIR analysis using a spectrometer (Bruker vertex70, Coventry, Germany). Spectra were acquired in the region from 4000 to 500 cm^{-1}.

2.6 Colour measurements

The colour properties were determine referring to the method of Verma et al. (2018a) using a colorimeter (Ci60, x-rite, USA). The calibration was done by a standard plate. The CIELAB color space was employed to get the values of L^*, a^*, and b^*.

2.7 Amino acids

The amino acid composition analysis was carried out using the procedure described by previous work (Liu et al., 2020a) with an amino acid analyzer (L-8900A, Hitachi). The samples were hydrolyzed at 110 °C for 22 hours in 6 mol/L HCl.

2.8 UV-vis properties

Uv-vis properties was measured by the method of Zhong et al. (2015) with some modification. Samples were dissolved in 100 mL deionized water and scanned with an UV-1601 UV-vis spectrophotometer (Shimadzu, Tokyo, Japan) in the range of 200-800 nm.

2.9 Measurement of solubility

The solubility of the samples was conducted using the method of Tkaczewska et al. (2019) from pH 2 to pH 12.

2.10 SEM

The surface morphology of samples were observed according to the method of Dara et al. (2020) and Akman et al. (2022) by scanning electrom microscope (SEM) Jsm-6510LA SEM (SHIMADZU., Japan). Lyophilized samples were ground into powder. The conductive double-sided adhesive was used to fix the samples on the specimen table and then sputter coated with gold.

2.11 Antioxidant activity

The DPPH, ABTS, and FRAP radical scavenging activities were determined using previous method (Liu et al., 2020b) with minor modification. The DPPH, ABTS, FRAP working solutions were prepared according the report. Then, 0.2 mL of the sample solution was mixed with 3.6 mL of the working solution and stored in the dark. After 30 min incubation, the absorbance was tested at 525 nm, 734 nm, and 593 nm.

2.12 Statistic analysis

All results were expressed as the mean ± standard error and evaluated for statistical significance using SPSS software (Version 19.0). One-way analysis of variance (ANOVA) were used to determine the differences among treatments ($p < 0.05$).

3 Results and discussion

3.1 Dgree of Hydrolysis (DH)

As showed in Figure 1A, there was no significant difference about DH after hydrolysis with pepsin and alacase ((DBH-P and DBH-A) for the same treatment time, but their DHs were significantly higher than that of trysin treatment group (DBH-T) ($P < 0.05$).

The DH of protein is affected by factors such as protease types. The reduction of specific peptide bonds during enzymatic hydrolysis may be responsible for the difference in degree of hydrolysis (Bao et al., 2017). Different proteases may cause different DH, size and conformation of hydrolysates, which ultimately affect the functional properties of hydrolysates (Chalamaiah et al., 2012).

3.2 Size-Exclusion High Performance Liquid Chromatography (SE-HPLC)

As showed in Table 1, molecular weights and their distributions of all the samples showed differences. The molecular weight of DB was mainly distributed from 10 kDa to 30 kDa, accounting for 66.59% of the total. After enzymolysis, the molecular weight decreased and the amount of smaller molecules increased, especially DBH-P and DBH-A.

The molecular weights of samples obtained by SE-HPLC were consistent with the result of hydrolysis degree (DH). Trysin appeared less effect on reducing molecular weight of deer blood. This is consistent with the result of Orcajo et al. (2013), which showed that the apolipoprotein cores of egg yolk protein remained largely intact after trysin hydrolysis. It has been reported that peptides with high molecular weights possess anti-inflammatory effect (Jang et al., 2017). Other wise, small molecular peptides have important physiological functions in human absorption and metabolism. For example, milk peptides can transport trace elements and improve body immunity. Soybean peptides, corn peptides and sardine peptides can lower blood pressure (Zhou et al., 2006). Another study showed that the protein hydrolysates with molecular weights under 13 kDa possess antioxidant activity (Nguyen et al., 2017).

3.3 Determination of FTIR

Figure 1D showed the FTIR spectra of the samples. The peak of 3200 cm^{-1}-3400 cm^{-1} appeared in spectrum of all samples. The amide I, II and III bands corresponded to the peak at 1655.34 cm^{-1}, 1538.14 cm^{-1} and 1240.51 cm^{-1}.

The secondary structure of different hydrolysates were obtained by simulation as showed in Table 2, all the samples exhibited the highest β-turn proportion from 41.97-58.45%. The proportion of secondary structures were showed in Table 2.

The region of 3200 cm^{-1}-3400 cm^{-1} didn't change much before and after hydrolysis, this results were different from the report of Ai et al. (2019), which showed higher peak height and broader peak widths in pepsin hydrolysates than those of other

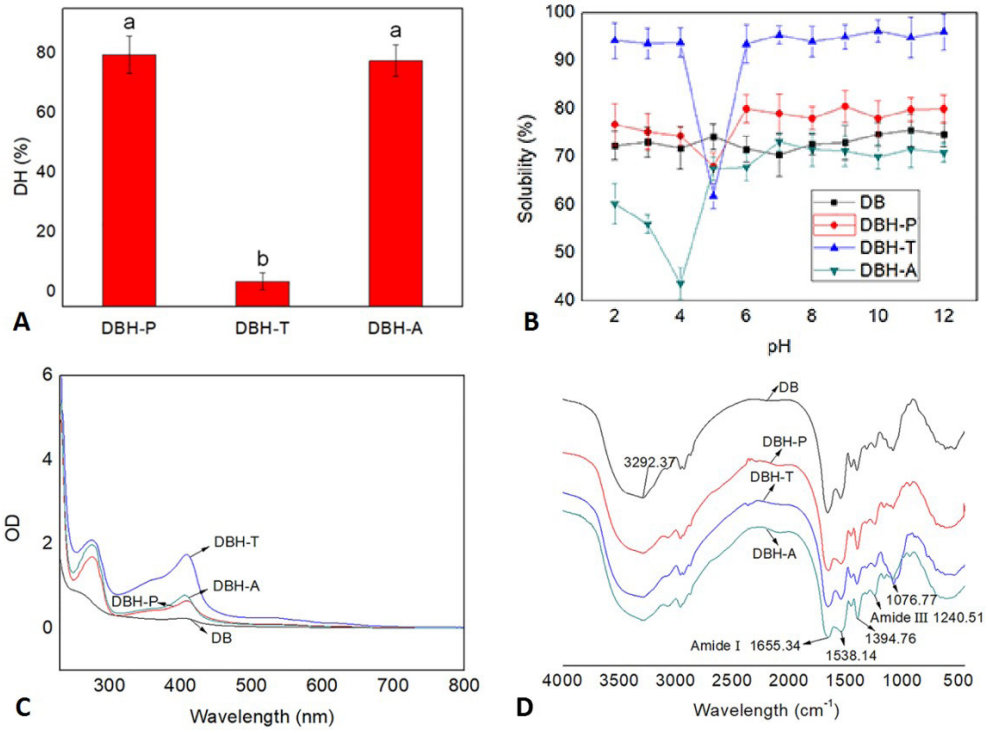

Figure 1. A: Degree of hydrolysis (DH); B: Solubility; C: UV-Vis wavelength scanning; D: FTIR spectra of deer blood hydrolysates. In Figure 1A, different letters in each column indicate significant difference ($p < 0.05$).

Table 1. Molecular weight and the distribution of deer blood hydrolysates.

Molecular weight (Da)	> 30 k	30 k-10 k	10 k-5 k	5 k-3 k	3 k-1 k	1 k-0.5 k	< 0.5 k
DB	0.02 + 0.00^a	66.59 + 0.55^a	0.27 + 0.03^a	0.34 + 0.01^a	2.02 + 0.01^a	18.97 + 0.06^a	11.82 + 1.09^a
DBH-P	0.01 + 0.00^a	4.84 + 0.23^b	27.82 + 1.21^b	31.32 + 1.22^b	19.82 + 0.98^b	13.62 + 0.32^b	2.58 + 0.04^b
DBH-T	0.00 + 0.00^a	64.00 + 1.25^c	5.48 + 0.51^c	5.62 + 0.11^c	8.70 + 0.06^c	5.12 + 0.31^c	11.09 + 0.75^a
DBH-A	0.00 + 0.00^a	5.41 + 0.15^d	0.00 + 0.00^d	26.41 + 1.25^d	36.88 + 1.01^d	25.42 + 1.11^d	5.88 + 0.02^c

All data were obtained from three groups of samples in the same treatment after three replications and the experiments were conducted in triplicate. Different letters in each column indicate significant difference ($p < 0.05$).

Table 2. The changes of secondary structure of different hydrolysate proteins.

Enzyme	β-sheet (%)	α-helical (%)	Random structure (%)	β-turn (%)	β-sheet + α-hilical (%)
DB	15.77 + 0.02[a]	13.09 + 0.16[a]	13.25 + 0.02[a]	57.88 + 0.43[a]	28.86 + 0.22[a]
DB-P	17.26 + 0.23[b]	12.57 + 0.11[b]	11.72 + 0.02[b]	58.45 + 0.28[a]	29.83 + 0.12[b]
DB-T	26.57 + 0.18[c]	19.63 + 0.02[c]	-	53.80 + 0.25[b]	46.20 + 0.54[c]
DB-A	21.72 + 0.09[d]	19.54 + 0.33[c]	16.76 + 0.02[c]	41.97 + 1.06[c]	41.26 + 0.02[d]

All data were obtained from three groups of samples in the same treatment after three replications and the experiments were conducted in triplicate. Different letters in each column indicate significant difference ($p < 0.05$).

proteases treated. The result showed no obvious hydrogen bonds formation before and after hydrolysis.

Amide I peak is the most intense absorption band in proteins, which is mainly associated with the C=O and C-N stretching vibrations and also relates to the backbone conformation (Tekle et al., 2022). Another prominent absorption band is the amide II peak, which is associated with in-plane N-H bending, and the C-N and C-C stretching vibrations (Van Wyk & Amonsou, 2022). Amide III derives largely from the feature of C-N stretching and N-H bending (Maji et al., 2019).

Generally, the characteristic absorption bands occurred at 3200-3400 cm^{-1} and 2800-3000 cm^{-1} are considered the stretching vibration of the -OH and -CH groups, respectively (Wang et al., 2014). The amide I band is commonly used to analyze the secondary structure of the protein. High β-turn proportion indicated large amount of glycine and proline on the corner (Palau et al., 1982).

The degree of tightness of protein structure can be expressed by the sum of β-sheet and α-helical, it can also reflect the total amount of intermolecular hydrogen bonds (Ai et al., 2019). DBH-T showed highest total amount of intermolecular hydrogen bonds, while DB showed lowest, which implied that DBH-T had relatively stable secondary structure and DB had weak secondary structure.

3.4 Color properties

The color properties are presented in Table 3. The different color of hydrolysates depend on the composition of raw materials and hydrolysis conditions (Thiansilakul et al., 2007). The brightness, redness and yellowness of samples had significant differences ($p < 0.05$). DBH-P produced a red powder with higher brightness (65.94 ± 0.61). The redness and yellowness of DBH-A were higher.

Color is an important quality indicator in food industry, sometimes it reflects the changes in product quality (Verma et al., 2018a) and involves the sense of the product (Rafieian et al., 2015). This result consists with Rajabzadeh et al. (2018), who showed pepsin hydrolysate had higher brightness. There were some researchers showed that the Maillard reaction is responsible for yellowness of hydrolysates (Thiansilakul et al., 2007). In this work the reaction temperature of DBH-A was highest at 50 °C, this result consists with Chalamaiah et al. (2010) and Rajabzadeh et al. (2018).

3.5 Amino acid analysis

Table 4 showed the amino acids composition of deer blood and its hydrolysates. A total of 17 amino acids were determinded.

Table 3. Color properties of deer blood hydrolysates.

	Color L value	Color a value	Color b value
DB	42.49 ± 0.24[a]	4.15 ± 0.59[a]	6.11 ± 0.87[a]
DB-P	65.94 ± 0.61[b]	4.99 ± 0.20[a]	10.85 ± 0.10[b]
DB-T	51.87 ± 0.13[c]	6.03 ± 0.64[b]	10.97 ± 0.17[b]
DB-A	57.38 ± 0.80[d]	7.84 ± 0.40[c]	11.69 ± 0.12[c]

All data were obtained from three groups of samples in the same treatment after three replications and the experiments were conducted in triplicate. Different letters in each column indicate significant difference ($p < 0.05$).

Tryptophan was not detected because it was destroyed by acid hydrolysis during pretreatment of the amino acids. Of all the samples, the highest content of amino acids was asparaginic acid, glutamic acid and leucine. All the three protease treatments result in the increase of essential amino acids, DBH-A had the highest content, the ratio of essential amino acid is 504.78 ± 0.35 quality per 1000 parts. As showed in Table 4, the contents of hydrophobic amino acids in the three hydrolysates were increased. DBH-A contained most content of hydrophobic amino acids of 442.26 ± 0.21 quality per 1000 parts.

Glutamic acid and aspartic acid are major excitatory neurotransmitters incentral nervous system of the mammalian and are found to be vital in learning, memory, movement and brain functions (Skvortsova et al., 2000; Skerry & Genever, 2001; Pradhan et al., 2014). The deficiency of them may cause neurological or psychiatric disorders (Choi, 1988; Lipton & Rosenberg, 1994). Leucine supplementation can increase the muscle protein fractional synthetic rate, so as to help the age-related decline in muscle mass in elderly individuals (Xu et al., 2015). The ratio of essential amino acid in DBH-A was higher than the ideal protein value recommended by the FAO). Hydrophobic amino acids play an important role in scavenging free radicals (Samaranayaka & Li-Chan, 2011). It is reported that the antioxidant properties of peptides were related to the presence of hydrophobic amino acids (Zhan et al., 2021), and hydrophobic amino acids are also related to the ACE inhibitory activity in structure-activity relationship (Yang et al., 2020).

3.6 UV pectra analysis

Figure 1C showed the UV-Vis spectra of samples. There were two main characteristic absorption in the spectrum, one was at 275 nm, another was at 408 nm. The absorption strength were different in each sample, DB showed the weakest intensity and DBH-T showed the strongest intensity.

The characteristic absorption at 275 nm was mainly due to the presence of tyrosine, tryptophan and phenylalanine (Zhong et al.,

Table 4. Amino acid composition of deer antler base gelatin and gelatin hydrolysate.

Amino acids	DB	DBH-P	DBH-T	DBH-A
Asp	114.59 ± 0.08[a]	113.80 ± 0.05[a]	110.84 ± 0.08[b]	113.07 ± 0.06[a]
*Thr	54.29 ± 0.04[a]	58.50 ± 0.02[b]	57.92 ± 0.06[b]	60.45 ± 0.08[c]
Ser	46.42 ± 0.03[a]	50.20 ± 0.04[b]	49.22 ± 0.01[b]	48.82 ± 0.01[b]
Glu	146.71 ± 0.08[a]	92.92 ± 0.05[bd]	130.09 ± 0.14[c]	92.57 ± 0.03[d]
Gly	39.37 ± 0.02[a]	46.52 ± 0.01[b]	43.12 ± 0.05[c]	41.11 ± 0.07[d]
#Ala	68.38 ± 0.09[a]	84.73 ± 0.03[b]	77.72 ± 0.03[c]	85.09 ± 0.01[d]
Cys	13.05 ± 0.01[a]	7.71 ± 0.01[b]	12.21 ± 0.02[c]	6.79 ± 0.01[d]
*,#Val	69.21 ± 0.05[a]	76.66 ± 0.09[b]	72.72 ± 0.08[c]	78.41 ± 0.11[d]
*,#Met	2.28 ± 0.02[a]	1.78 ± 0.01[b]	0.74 ± 0.00[c]	3.45 ± 0.01[d]
*,#Ile	28.60 ± 0.01[a]	10.09 ± 0.06[bd]	24.61 ± 0.01[c]	10.59 ± 0.08[d]
*,#Leu	116.45 ± 0.10[a]	119.73 ± 0.12[b]	111.95 ± 0.06[c]	127.81 ± 0.02[d]
#Tyr	30.46 ± 0.04[a]	25.75 ± 0.08[bd]	29.79 ± 0.02[c]	24.87 ± 0.06[d]
*,#Phe	61.96 ± 0.07[ac]	66.81 ± 0.09[b]	61.62 ± 0.05[c]	71.04 ± 0.09[d]
*Lys	76.67 ± 0.01[a]	90.07 ± 0.10[bd]	84.94 ± 0.06[c]	90.50 ± 0.04[d]
*His	41.23 ± 0.00[a]	64.20 ± 0.05[b]	44.60 ± 0.04[c]	62.52 ± 0.01[d]
Arg	48.49 ± 0.06[a]	47.70 ± 0.01[a]	43.30 ± 0.07[b]	41.91 ± 0.03[c]
#Pro	41.86 ± 0.03[ad]	42.84 ± 0.02[b]	44.60 ± 0.01[c]	40.99 ± 0.08[d]
*Essential amino acids	450.68 ± 0.28[a]	487.84 ± 0.36[b]	459.10 ± 0.26[c]	504.78 ± 0.35[d]
#hydrophobic amino acids	419.19 ± 0.34[a]	428.38 ± 0.29[b]	423.76 ± 0.33[c]	442.26 ± 0.21[d]
Total amino acids	1000	1000	1000	1000

*Essenial amino acids. #Hydrophobic amino acids. Results are expressed as the mean ± SD from triplicate determinations. Different letters in the same row indicate significant differences ($P < 0.05$).

2015). The absorption peak at 408 nm was the characteristic absorption peak of heme porphyrin ring (Hosseinzadeh & Moosavi-Movahedi, 2016). It can be seen that the absorbance intensity of hydrolysates was strengthened compared with DB, indicating the exposure of the buried hydrophobic groups by hydrolysis, this is similar with the result of Aziz et al. (2021). In fact, different proteases have different cleavage sites and hydrolysis capacities, which may lead to residual secondary or even tertiary structures in the hydrolysates. When the polypeptidic chain folds into a secondary or tertiary structure, aromatic amino acids with ultraviolet absorption can be buried within the molecule, and these hidden aromatic amino acids can not participate in UV-vis absorption (Zhao et al., 1995). The same explanation applies to heme porphyrin ring.

3.7 Solubility properties

Solubility properties of samples were shown in Figure 1B. All the samples had higher solubility (> 65%) under neutral and alkaline conditions. DBH-P and DBH-T showed enhanced solubility in aqueous compared to DB and had the lowest solubility at pH 5, while DBH-A had the lowest solubility at pH 4.

Solubility property is one of the most important functional characteristics of protein hydrolysates. Each hydrolysate had a lowest solubility pH, probably due to the least repulsive force between proteins at their isoelectric points, it caused the protein deposition.

DBH-P had more acidic lowest solubility (pH 4), this may due to it prepared by alkaline protease and had more negative charge (Li et al., 2014). Otherwise, the solubility of hydrolysates also affected by degree of hydrolysis (DH) and the surface hydrophobicity of hydrolysate (Ai et al., 2019).

3.8 Scanning Electron Microscopy (SEM)

The microstructures of samples are shown in Figure 2A-2D. The morphology of deer blood and hydrolysates were irregular in their particle shape and size. They all had a crystalline and broken glass-like structure.

The result is consistent with the report of croaker gelatin protein hydrolysate-rohu protease (Dara et al., 2020). Different shape, cracks and particle size were mainly cause by freeze-drying process (Chen et al., 2012). The distribution of particle size seemed relate to the degree of hydrolysis (Chi et al., 2014). The DH of trysin hydrolysate (DBH-T) is lower of 3.4%, so DB and DBH-T presented a larger granularity, and DBH-P and DBH-A with DHs of 79.5% and 77.5% (Figure 1A) exhibited smaller size of the particles. This result also corresponds to the molecular weight (Table 1). DBH-P and DBH-A had significantly smaller molecular weights and has a wider distribution, which corresponds to smaller particles and more uneven particle size distribution in SEM. Meanwhile, most particles of the DB had fussy surface, while the other three samples had more particles with smooth surface, the reason remains to be explored.

3.9 Determination of antioxidant activities

DPPH, ABTS and FRAP radical scavenging assay of deer blood and its hydrolysates were showed in Figure 3, the antioxidant activity of the samples showed doze-dependent in all the three assays.

As showed in Figure 3A, with the increase of sample concentration, the free radical scavenging activity increased rapidly, when the concentration was higher than 30 mg/mL, the DPPH free radical scavenging activity increased slowly.

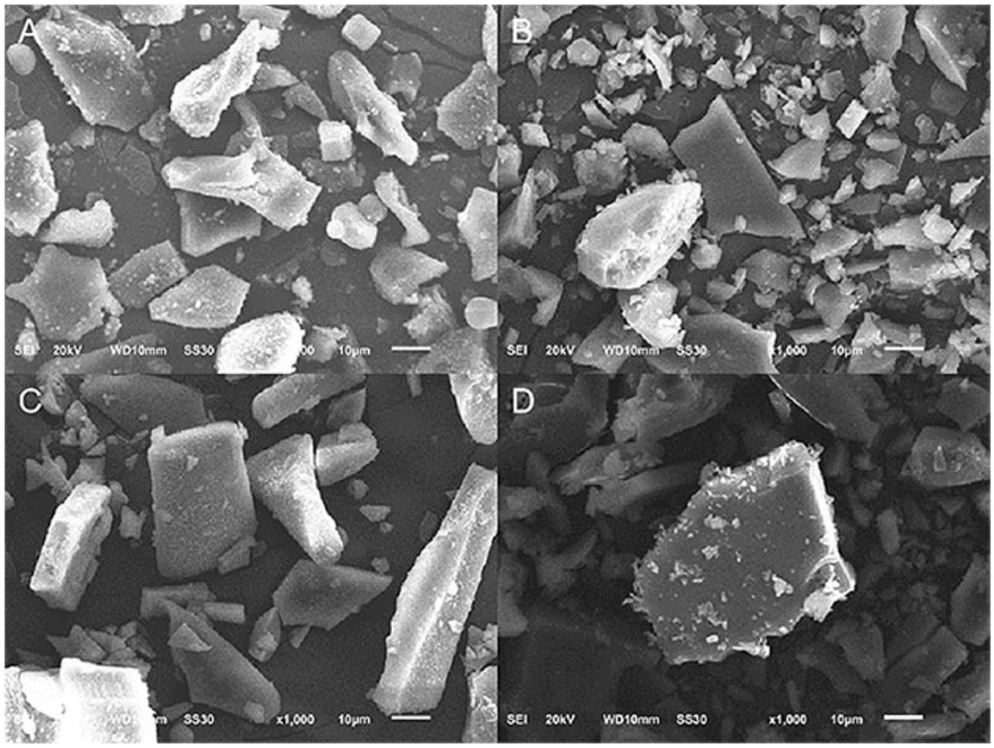

Figure 2. SEM images of (A) DB (B) DBH-P (C) DBH-T (D) DBH-A with 1000 times magnification.

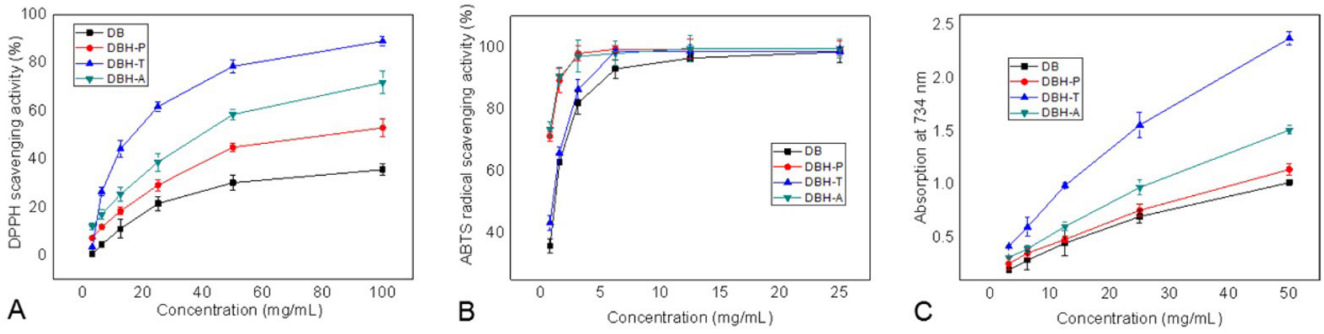

Figure 3. The antioxidant activity of deer blood hydrolysates: (A) DPPH assay, (B) ABTS assay and (C) FRAP assay.

DB showed the lowest activity of 35.6% at the concentration of 100 mg/mL and DBH-T showed the highest activity of 88.9% at the same concentration.

The ABTS assay was showed in Figure 3B, all the samples showed doze-dependence and high ABTS free radical scavenging activity. The clearance rate of DB was close to 100 percent when the concentration was 25 mg/mL, as to the hydrolysates, the concentration was 6.25 mg/mL. As showed in Figure 3C, the ferric reducing activity in FRAP assay exhibited a doze-dependence. DBH-P, DBH-T, DBH-A had higher ferric reducing antioxidant power. DBH-T showed the highest ferric reducing antioxidant power.

All the samples showed excellent antioxidant activity after the hydrolysis. The DPPH radical is widely used for evaluating the antioxidant activity of natural compounds, and its a relatively stable nitrogen-centered free radical (Verma et al., 2018b). The DPPH assay results are consistent with those of Wang et al. (2021), wherein a DPPH scavenging activity increased with the hydrolysate concentration. There was a big difference in antioxidant activity of each sample. There were researchers confirmed that some amino acids, especially hydrophobic amino acids were related with DPPH scavenging activity, such as tyrosine and phenylalanine (Li et al., 2008). However the results of amino acid analysis did not showed such a trend. This may because besides amino acids, the hydrophobic peptide segments and the structures with electron-donating properties could improve DPPH free radical clearance activity (Jemil et al., 2014).

ABTS assay is based on the presence of electron donor or hydrogen donor (Re et al., 1999), the free radical scavenging activity depends on the capacity of antioxidant peptides to participate in hydrogen transfer (Olagunju et al., 2018). It always used to evaluate the antioxidant capacity of both lipophilic and

hydrophilic molecules. The ABTS assay result is similar with our report earlier, which showed deer antler base gelatine and its hydrolysate had excellent performance in scavenging ABTS free radical, superior to DPPH and FRAP (Liu et al., 2020b).

Ferric reducing antioxidant power (FRAP) was used to measure the reducing power of molecules, it can reflect the antioxidant ability of substance. The better antioxidant ability of hydrolysates showd in FRAP assay may because hydrolysis increased quantity of the lower molecular weight moiety (Verma et al., 2019a). Otherwise, some researchers indicated that there was a direct correlation between antioxidant activities and reducing power of peptides, because enzymatic hydrolysis exposed more internal groups, resulting in higher electron or hydrogen contributions, which related to the ability of FRAP (Li et al., 2010; Tang et al., 2012). DBH-T had the highest activity compared with the other samples, this result was consist with the report of Verma et al. (2019b), which 5~10 kDa fraction showed higher FRAP activity. However 5~10 kDa fraction in DBH-T sample was less. This may because the sample of this report was deer blood, which had more complex components except for protein, the reducing power of ferric iron might be affected by other factors such as components as well as collective action of peptide, free amino acid, structural, functional groups, concentration of the peptides and their molecular weights (Verma et al., 2019b).

4 Conclusion

The hydrolysates of deer blood possessed different physicochemical and functional characteristics, which were attributed to the different effects of three proteases on the structure of deer blood. However, before utilizing of deer blood and its hydrolysates, i.e., in food additives or nutraceuticals, the hydrolysates have to undergo more detailed studies.

Conflict of interest

The authors declared that they have no conflict of interest for this article.

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