Quality-grade analysis of velvet antler materials using ultra-weak delayed luminescence combined with chemometrics

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ORIGINAL ARTICLE

Abstract

The pharmacological properties of velvet antler, a well-known animal-derived traditional Chinese medicine material and functional food, are extensively acknowledged globally. However, there are significant restrictions on the quality standard and control system of velvet antler. The four quality grades of velvet antler slices taken from *Cervus nippon Temminck* and *Cervus elaphus Linnaeus* were evaluated in this study using delayed luminescence (DL) and chemical analysis of protein and amino acid content. Our results demonstrated a significant degree of consistency in the assessment impacts of the measurements for identifying velvet antler slices of different quality grades from *Cervus nippon Temminck* and *Cervus elaphus Linnaeus*. Additionally, it is possible to distinguish velvet antler slices of Rangifer tarandus from those of other deer species by exploiting DL parameters (i.e., I0, Beta, Tau, and T). Moreover, there is a strong correlation between the DL parameters and particular amino acids detected in velvet antler slices. As an innovative, quick, and systematic methodology, DL enables the examination of the overall property of various grades of velvet antler slices. The integrated assessment using DL and chemical components can give a novel method for determining the quality of animal-derived herbs and functional foods.

Keywords: quality grades; velvet antler; delayed luminescence; chemometrics; herbal quality control

Introduction

Delayed luminescence (DL), if lit with an excitation light source, is an ultra-weak emission of optical photons (wavelength: 400–800 nm) alighted from a number of materials (Scordino *et al.*, 2014). DL has a far lower intensity than well-known fluorescence or phosphorescence, but with a much longer decay time (from milliseconds to seconds) (Sun *et al.*, 2016b). DL was recently utilized to determine the quality features of dry powders generated from Chinese herbal ingredients sourced from plants (Jia *et al.*, 2020; Sun *et al.*, 2016a, 2016b, 2017b, 2018, 2019a, 2019b, 2020). DL is a novel approach for quantifying plant-derived herbal materials that may be used to determine the medicinal characteristics of a wide variety of herbs prepared in a diverseness of growth habitats, periods, processing stages, and formulations (Jia *et al.*, 2020; Sun *et al.*, 2016a, 2017b, 2018, 2019a, 2019b, 2020). As a consequence, DL is a promising method for herbal quality control and a unique tool for combining with other analytic technology platforms, such as the high-performance liquid chromatography (HPLC) fingerprint platform, which is utilized to determine bioactive component content (Sun *et al.*, 2020).

One of the best-known animal-derived traditional Chinese medicine (TCM) material and functional food is velvet antler (Sun, 2017). The term "velvet antler" refers to a densely haired but not yet ossified male deer antler (Luo *et al.*, 2017), commonly used globally in herbal remedies (Figure 1) (Sui *et al.* 2014; Zhang *et al.*, 2019), dietary supplements and functional foods (Lee *et al.*, 2015). The velvet antlers of *Cervus nippon* Temminck and *Cervus elaphus* Linnaeus are officially included in the Chinese Pharmacopeia (2020 edition; Chinese Pharmacopoeia Commission, 2020). The entire antler is typically split into velvet antler slices for the commercial sale of TCM decoction pieces. China's agriculture industrial standard (NY/T 1162-2006) establishes four quality grades for velvet antler slices depending on their degree of ossification, because it is believed that the degree of ossification is related to the pharmacological activity of velvet antler slices. Owing to the lowest degree of ossification, the segment of the upper part of the velvet antler, known as "LaPian (LP)," is believed to have the best pharmacological action, as well as expensiveness (Figure 1) (Gong and Li, 2014; Liu, 2019). According to their degree of ossification and position on the antler, velvet antler slices are further classified as "Fenpian (FP)," "Xuepian (XP)" and "Gupian (GP)" by quality grades (Figure 1) (Liu, 2019). The market value of GP is the lowest one, as it has the highest amount of ossification. FP and XP are the most often available materials for medical use; however, FP and XP are frequently used interchangeably in the market (Liu, 2019). Therefore, quality evaluation of velvet antler is crucial not only because of the vast amount of velvet antler produced on the Chinese mainland each year (about 400 tons) and a variety of therapeutic uses but also because of the requirements of authenticity identification. However, just a few investigations are discovered on the quality of animal-derived TCM materials. The Chinese Pharmacopoeia (2020 edition; Chinese Pharmacopoeia Commission, 2020) suggests microscopic identification and a thin-layer chromatography technique using glycine as a spotting indicator for determining the quality of velvet antler (Xu *et al.*, 2015). However, there are practically limited regulations or criteria for grading the quality of velvet antlers. Therefore, a new quality evaluation method and approach would help enhance the quality control of velvet antler product.

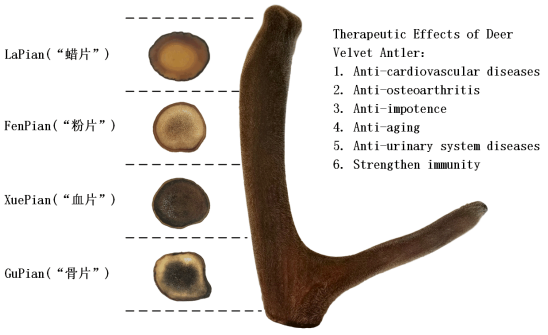

Figure 1. Medicinal properties and quality grades of velvet antler are described schematically.

The present study determined whether DL could be utilized to provide a quality evaluation for TCM material derived from animals. We determined the DL properties of dry powders of various grades of velvet antler slices. Additionally, the same velvet antler samples were evaluated for determining protein and amino acid content, because DL is an effective technique for evaluating quality grades of velvet antler slices. Combination of DL and chemical component analysis could provide a novel method for assessing the quality of animal-derived herbs and functional foods.

Materials and Methods

Velvet antler materials

Cervus nippon Temminck, *Cervus elaphus* Linnaeus and *Rangifer tarandus* velvet antler samples (five samples each of LP, FP, XP and GP slices) were purchased from herbal medicine markets in the Chinese region of Jilin. Before submitting to Changchun University of Chinese Medicine in Changchun, China, Jianxun Zhu validated all samples based on their appearance features. LP has a compact structure, an oily consistency, a yellowish hue, high wax content, and little to no sedimentation. FP has dense, rigid, and tough tissues, deficient oiliness, fine pores on one side, silt-sized pores in the middle, and wax

rings on the other side. Surface pores of XP are larger than those of LP, and XP possesses the same hue as that of blood. In addition, there are honeycomb-shaped ossification circles on the surface of GP. All velvet antler samples were crushed using a grinder, and 150- and 300-μm particles were selected using a standard sieve. At room temperature, the samples were stored in a dark box containing 3–5-mm silica gel.

DL analysis

The powdered velvet antler samples (150-μm particles) were kept in a dark box containing 3-5-mm silica gel for 16 h prior to DL detection (Sun *et al.*, 2016b). Meluna Research's (the Netherlands) DL measuring platform included a dark sample chamber equipped with a photomultiplier tube (model 9558QB; Electron Tubes Enterprises, Ruislip, UK). The chamber was maintained at a temperature of 22°C. The cathode end of the photomultiplier tube was 51 mm in diameter and was sensitive between 300 nm and 800 nm. To maintain a dark count rate of 10 counts/second, the tube was chilled to -25°C. A fast preamplifier was used to amplify DL photon emission signal (model 9301; ORTEC, Oak Ridge, TN, US). A personal computer equipped with a counting card (model 6602; National Instruments, Austin, TX, US) was utilized to acquire signal data. We created 1-g samples of velvet antler powder. Each 1-g sample was put in a 1-cm-diameter Petri dish and electrified for 10 s using a white light emitting diode source. The DL of each sample was determined for three times in a row. The total number of 15 measurements of each velvet antler slice samples was used for analyzing the DL characteristics of that particular velvet antler slice. DL kinetics was determined by measuring the number of counts over a period of 30 s in sequential 0.05-s intervals, providing 600 data points.

Protein and amino acid examination

Analysis of total protein content

Weighed 2-g samples of velvet antler (300-μm particles) were put in a tube. Then, 0.4-g copper sulfate, 6-g potassium sulfate, and 20-mL sulfuric acid were added to the tube for digestion. When the tube reached 420°C, the digesting process was further maintained for an hour or until liquid in the tube turned green. After cooling the liquid, 50-mL water was added for Kjeldahl analysis. The mixed liquid was then injected into a Kjeldahl nitrogen analyzer (model K1100; Hanon Instruments, Jinan, China), which was pre-loaded with sodium hydroxide solution, sulfuric acid standard solution, and boric acid solution containing mixed indicator for automatically testing the total protein content of velvet antler samples.

Analysis of amino acid content

The amino acid content of velvet antler samples (2-g, 300-μm particles) was determined using an automatic amino acid analyzer (Model L-8900; Hitachi Co., Osaka, Japan) equipped with a visible light detector. For determining 16 amino acids, analytical 2622 (4.6 × 60 mm) and guard 2650 (4.6 × 40 mm) columns were used. An auto-sampler was used immediately after injecting the sample into columns for NIN post-column derivatization. At 440 nm, NIN-derivatized proline was detected, while the other amino acids were detected at 570 nm. The amino acid analyzer automatically expressed the content of 16 amino acids.

Data processing and statistical analysis

Chemical data statistics

The total protein content of LP, FP, XP and GP of velvet antler slices was determined for statistical analysis. Following this, a two-tailed, unpaired Student's *t*-test was used to compare various grades of velvet antler slices (SPSS version 23.0; IBM, Armonk, NY, US); differences were considered significant at $P < 0.05$. The heat map technique was used to analyze variations in the amino acid composition of 16 amino acids between velvet antler slices LP, FP, XP and GP.

DL data statistics

To derive four DL parameters (i.e., I0, Beta, Tau and T), the characteristics of DL photons were calculated using the hyperbolic function (Sun *et al.*, 2020). The average of all the measures was used to determine the DL characteristics of each velvet antler sample. A two-tailed, unpaired Student's *t*-test (SPSS version 23.0) was used to compare different grades of velvet antler slices; differences were considered significant at $P < 0.05$. Additionally, the correlation between 16 amino acids and four DL characteristics was quantified using Spearman's rank correlation. $|\rho| > 0.70$ was recognized as a strong linear association by Spearman (He *et al.*, 2017). Following this, a network view was created using Cytoscape version 3.2.1 (www.cytoscape.org) (Cline *et al.*, 2007).

Results

The velvet antler samples collected from *Cervus nippon* Temminck and *Cervus elaphus* Linnaeus were determined by DL measurements and chemical analysis. DL was used to determine LP, FP, XP and GP samples. The DL decay curves of LP, FP, XP and GP powders from *Cervus nippon* Temminck are shown in (Figure 2A). To extract the four parameters, the DL decay curves were

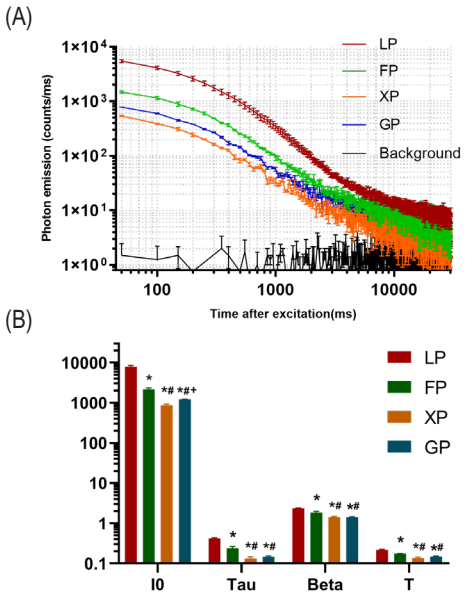

Figure 2. Velvet antler samples (*Cervus nippon* Temminck) were measured using the DL method. (A) Curves of DL decay for LP, FP, XP and GP. The data are presented as mean ± standard error of mean (SEM). Take note of the log–log scale used to visualize the data. (B) Comparing the DL attributes of LP, FP, XP and GP using histograms. *FP, XP or GP versus LP, $P < 0.05$. #XP or GP versus FP, $P < 0.05$. +XP versus GP, $P < 0.05$. Beta is the decay rate of DL, I0 denotes the beginning intensity of DL curve, and T and Tau denote decay time and DL features, respectively.

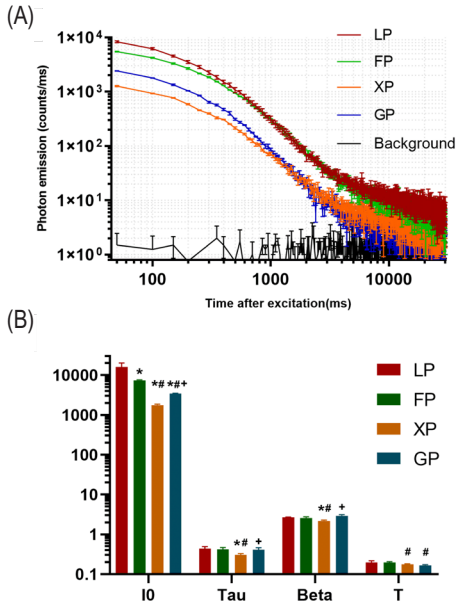

Figure 3. Velvet antler samples (*Cervus elaphus* Linnaeus) were measured using the DL method. (A) Curves of DL decay for LP, FP, XP and GP. The data are presented as mean ± standard error of mean (SEM). Take note of the log–log scale used to visualize the data; (B) Comparing the DL attributes of LP, FP, XP and GP using histograms. *FP, XP or GP versus LP, $P < 0.05$. #XP or GP versus FP, $P < 0.05$. +XP versus GP, $P < 0.05$. Beta is the decay rate of DL, I0 denotes the beginning intensity of DL curve, and T and Tau denote decay time and DL features, respectively.

fitted with a hyperbolic function. To further analyze the differences between four DL characteristics, a two-tailed, unpaired Student's *t*-test was used to compare them between LP, FP, XP and GP samples. The results indicated that the parameter I0 was considerably different across all velvet antler samples; T, Beta and Tau were significantly different across LP, FP and XP samples but were unable to differentiate between XP and GP powder (Figure 2B). Following this, we focused on the experimental findings of *Cervus elaphus* Linnaeus. The decay curves of LP, FP, XP and GP slices varied in the findings of DL measurements, although their tails overlapped partly (Figure 3A). The parameter I0 was capable of distinguishing between LP, FP, XP and GP powder, but the other DL parameters were unable to demonstrate a meaningful difference between them (Figure 3B).

Numerous investigations have revealed that the primary bioactive components of velvet antler are proteins and polypeptides (Gong *et al.*, 2019). As a result, we tested the total protein and 16 amino acid content of LP, FP, XP and GP samples from *Cervus nippon* Temminck to determine their quality. According to the data, the total protein content came down steadily and dramatically from LP to GP (Figure 4A).

A heat map is an information visualization in which values are assigned to certain colors to indicate differences between samples. In general, hues represent distinctions in values, with warmer colors representing greater values and cold colors representing smaller ones. The heat map in Figure 4B depicts the values of amino acids content analyzed in LP, FP, XP and GP samples. The nearly uniform dark red hue of amino acids in LP samples suggests that the content of these amino acids in LP is relatively high. In contrast, the predominant hue of amino acids in GP samples is blue, indicating that the concentration of these amino acids in GP is extremely low. Moreover, the hues of the majority of amino acids in FP and XP samples are between those of the two preceding samples, indicating that their amino acids content is in the middle of the four velvet antler samples.

In LP, FP, XP and GP samples, certain amino acids with distinctive contents were discovered based on the results of heat map. For instance, arginine (Arg) and leucine (Leu) had the highest and lowest contents, respectively, in LP; proline (Pro) and valine (Val) had the respective highest and lowest contents in FP; tyrosine (Tyr) and alanine (Ala) had the respective highest and lowest contents in XP; and Pro and lysine (Lys) had the respective highest and lowest contents in GP.

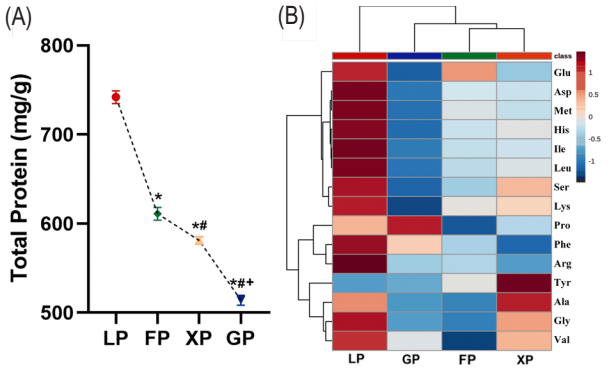

Figure 4. Velvet antler samples (*Cervus nippon* Temminck) were measured for total protein and specific amino acid contents using chemical tests. (A) Variation in total protein content between LP, FP, XP and GP. *FP, XP or GP versus LP, $P < 0.05$. #XP or GP versus FP, $P < 0.05$. +XP versus GP, $P < 0.05$. (B) Heat map of amino acid content in LP, FP, XP and GP samples.

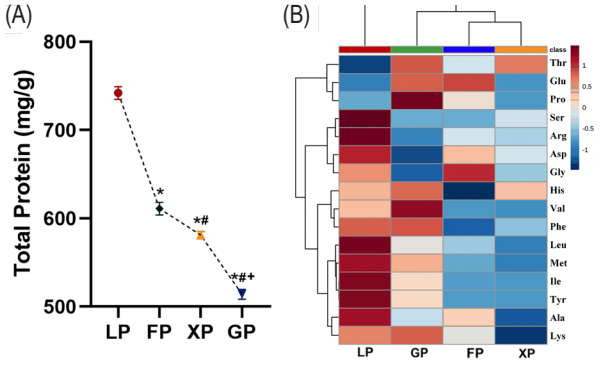

Figure 5. Velvet antler samples (*Cervus elaphus* Linnaeus) were measured for total protein and specific amino acid contents using chemical tests. (A) Variation in total protein content between LP, FP, XP, and GP. *FP, XP or GP versus LP, $P < 0.05$. #XP or GP versus FP, $P < 0.05$. +XP versus GP, $P < 0.05$. (B) Heat map of amino acid content in LP, FP, XP and GP samples.

Next, we focused on the results of samples from *Cervus elaphus* Linnaeus. It was discovered that the total protein content decreased gradually and significantly from LP to GP slices (Figure 5A), and the total protein content of each slice was very similar to that of the corresponding slice from *Cervus nippon* Temminck. Additionally, LP samples had the highest concentrations of half of the detected amino acids.

Next, GP samples, compared to FP and XP slices, contained a considerable number of high-content amino acids (Figure 5B). Some amino acids with different properties in velvet antler materials were discovered, including the highest content of serine (Ser), Leu and Arg in LP samples; the highest content of glycine (Gly) and the lowest content of histidine (His) in FP samples; the lowest content of Ala and Lys in XP samples; and the highest content of Pro and Val in GP samples (Figure 5B).

Following this, we aggregated data on DL and amino acids from LP, FP, XP and GP samples to form a network depicting a link between DL and particular compounds in velvet antler slices of *Cervus nippon* Temminck. The DL parameters had a positive and significant correlation with fhenylalanine (Phe), methionine (Met), glutamic acid (Glu), aspartic acid (Asp), and arginine (Arg) (Figure 6A). In addition, a correlation network identified nine significant positive and strong connections between

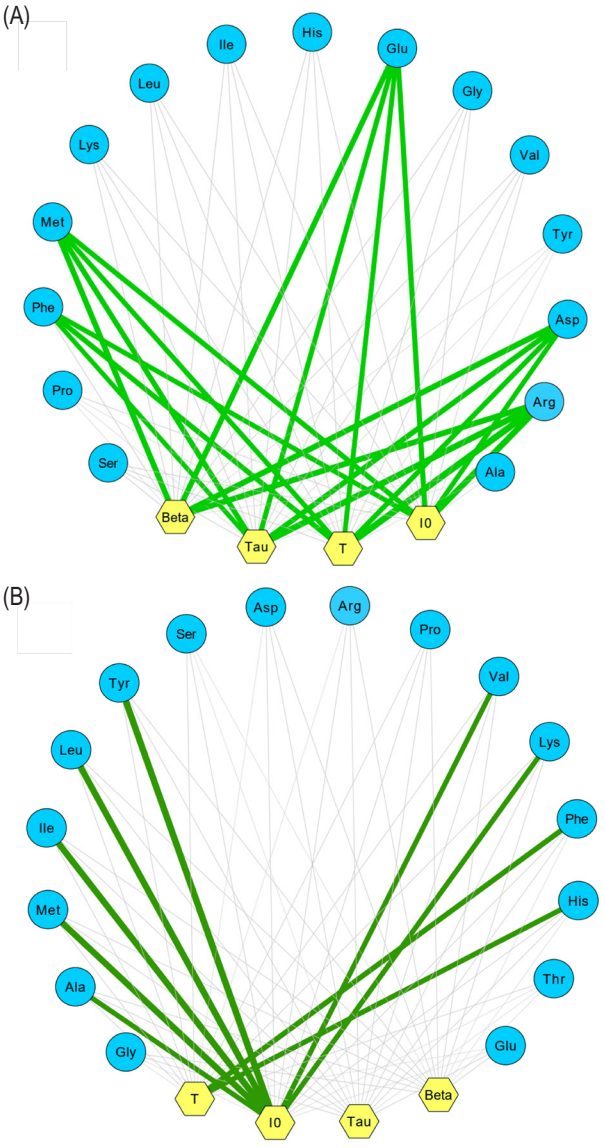

Figure 6. Correlation network between amino acids identified and DL characteristics determined in LP, FP, XP and GP samples. Green lines indicate positive and strong correlations ($|\rho| > 0.70$). The remaining correlations ($|\rho| < 0.70$) are denoted by gray lines. The thicker the line, the greater the link, and the length of each line is irrelevant. (A) *Cervus nippon* Temminck. (B) *Cervus elaphus* Linnaeus.

amino acids and DL data in velvet antler slices of *Cervus elaphus* Linnaeus. (Figure 6B).

Many velvet antler products from different species of deer are available on the Chinese market; however, only two species of deer (i.e., *Cervus nippon* Temminck and *Cervus elaphus* Linnaeus) are listed in the latest version of Chinese Pharmacopoeia (2020 edition; Chinese Pharmacopoeia Commission, 2020). Therefore, the velvet antler samples of *Rangifer tarandus* were examined to check whether DL measurements and chemical analysis could distinguish between samples that were not officially included in the Chinese Pharmacopoeia (2020 edition; Chinese Pharmacopoeia Commission, 2020). FP and XP samples from *Rangifer tarandus* were used in this investigation. The DL decay curves of FP slices indicated considerable differences in the kinetics of photon emission between the three deer species (Figure 7A). All DL parameters demonstrated a statistically significant difference in FP slices between the three deer species examined (Figure 7B). However, the DL decay kinetics of XP slices demonstrated only a slight difference (Figure 7C), and no single DL characteristic of XP slices was able to completely distinguish the three deer species (Figure 7D).

The chemical data analysis revealed no significant difference in total protein concentration between *Cervus nippon* Temminck and *Cervus elaphus* Linnaeus FP samples; however, *Rangifer tarandus* FP samples contained considerably less total protein than *Cervus nippon* Temminck and *Cervus elaphus* Linnaeus FP samples (Figure 8). In XP samples, no discernible difference in total protein level was observed between the three deer species (Figure 8). Additionally, the heat map analysis revealed substantial differences in the concentration of certain amino acids of FP and XP samples across the three deer species (Figure 9).

Discussion

Pharmacological properties of velvet antler are well known globally. However, quality standard and control system of velvet antler continue to have shortcomings. The traditional way of identifying velvet antlers is through the morphological examination of appearance, shape, size and color of velvet antler samples (Yao *et al.*, 2016). However, if the velvet antler samples are powdered or processed, recognizing the same using an experience-based observation method is challenging (Luo *et al.*, 2017). Additionally, because different quality grades of velvet antler slices are available on the Chinese market, it is difficult to ensure the accuracy and stability of identification solely based on morphological

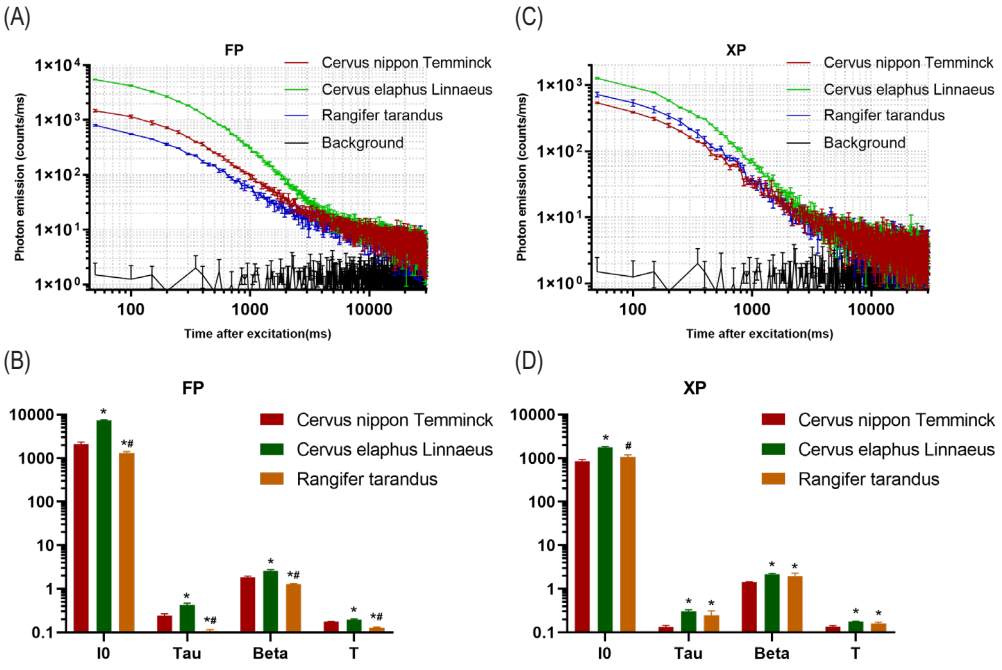

Figure 7. Velvet antler FP and XP samples of the three deer species were measured using the DL method. (A) and (C) Curves of DL decay for FP and XP. The data are presented as mean ± standard error of mean (SEM). Take note of the log–log scale used to visualize the data. (B) and (D) Comparison of the DL attributes of FP and XP using histograms. ****Cervus elaphus* **Linnaeus or *Rangifer tarandus* versus *Cervus nippon* Temminck, $P < 0.05$. #*Cervus elaphus* Linnaeus versus *Rangifer tarandus*, $P < 0.05$. Beta is the decay rate of DL, I0 denotes the beginning intensity of DL curve, and T and Tau denote decay time and DL features, respectively.**

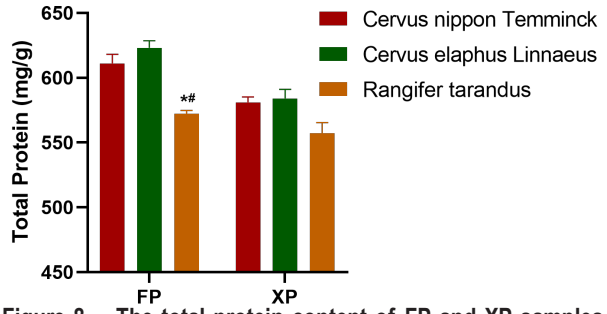

Figure 8. The total protein content of FP and XP samples among the three deer species. ****Cervus elaphus* **Linnaeus or ***Rangifer tarandus*** **versus** ***Cervus nippon*** **Temminck,** $P < 0.05$. **#***Cervus elaphus* **Linnaeus versus** ***Rangifer tarandus***, $P < 0.05$.

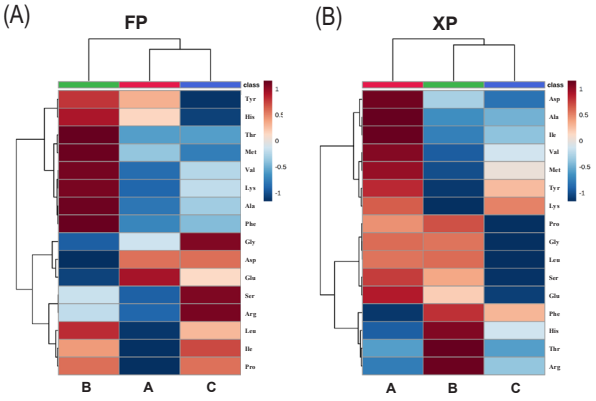

Figure 9. Heat map of amino acid content in FP and XP samples of the three deer species. "A" denotes ***Cervus nippon*** **Temminck samples. "B" denotes** ***Cervus elaphus*** **Linnaeus samples. "C" denotes** ***Rangifer tarandus*** **samples. (A) Comparison of amino acid content in FP samples. (B) Comparison of amino acid content in XP samples.**

observation, and research on the quality assessment of various grades of velvet antler slices is still limited. As a result, we used chemical analysis, which is recommended in Chinese pharmacopoeia (2020 edition; Chinese Pharmacopoeia Commission, 2020), as well as a systemic and rapid DL assessment, to exanimate various slices (LP, FP, XP and GP) of velvet antler from three different deer species.

The DL technique is a novel way of determining the quality of Chinese herbal medicine. This research employed DL to assess the quality of herbal medications derived from animals. We discovered that DL decay curves and characteristics had a strong identification effect in identifying LP, FP, XP and GP samples of *Cervus nippon* Temminck as well as FP samples between the three deer species. However, the effect of DL in identifying different graded slices of *Cervus elaphus* Linnaeus, as well as XP samples, was slightly small.

In general, DL had a significant assessment effect on velvet antler samples in our investigations. Numerous investigations have established a strong correlation between DL variability and the chemical structure of materials, particularly long-chain macromolecules, such as polysaccharides (Grasso *et al.*, 2019). This is because changes in the conformation and structure of the molecules of a sample affect the kinetics of luminescence (Barenboĭm *et al.*, 1969). Polypeptides and proteins are examples of bio-macromolecules with a lengthy chain.

We discovered that LP and FP samples of *Cervus nippon* Temminck had much higher DL decay kinetics and characteristics, as well as significantly larger total protein content, than XP and GP samples. Despite a slightly higher total protein content of XP samples, compared to GP samples, there was no significant change in the majority of DL parameters of XP and GP of *Cervus nippon* Temminck. This was because protein content was not able to describe accurately the distinctive shapes of protein molecules, and the structures of protein molecules have a significant role in DL emission (Ho *et al.*, 2002; Scordino *et al.*, 2010). This could be a reason that neither the protein content nor the DL characteristics of velvet antler XP samples could be adequately compared across the three deer species.

Substantial and strong correlations were observed between DL parameters and amino acids. The bulk of these amino acids exhibited a discernible variation in LP, FP, XP and GP slices in heat map analysis. As a result, DL characteristics could have a great potential to depict grade differences in velvet antler slices objectively. In a word, DL is a thorough and methodical calculation that accurately reflects the total information of chemical profile of samples. DL classification of velvet antler products could be a realistic solution in the lack of distinct chemical quality markers. Recently, spectral technology, such as ultraviolet and near-infrared, was employed to investigate the quality of velvet antler materials (Luo *et al.*, 2017). Owing to the fact that DL has a different range of wavelength, compared to the mentioned two technologies, as well as has the benefits of rapid, low-cost, holistic measurements, non-extraction requirements, and being pollution-free, it is possible that DL measurements would contribute new knowledge to the quality evaluation of velvet antler as well as to the quality control of other animal-derived TCM materials.

Proteins, amino acids and polypeptides are the most abundant bioactive compounds in velvet antlers, accounting for more than half of the velvet antler's content (Ding *et al.*, 2019). The total protein content of velvet antler slices declined dramatically from LP to GP in our investigations, which validated the quality grades of velvet

antler slices. Gly is the single chemical indicator about the quality of velvet antler in the Chinese Pharmacopoeia (2020 edition; Chinese Pharmacopoeia Commission, 2020). The heat map results revealed a degree of gradient variation in the Gly content of LP, FP, XP and GP samples of both *Cervus nippon* Temminck and *Cervus elaphus* Linnaeus, as well as a comparison between FP and XP samples of the three deer species.

Our findings demonstrated that the existing assessment index (Gly) in the Chinese Pharmacopeia (2020 edition; Chinese Pharmacopoeia Commission, 2020) could be expanded to characterize the quality grades of velvet antler slices. For instance, variations in the content of various amino acids, such as Tyr, His, Glu, Ser, Arg and Leu, could be used to characterize differences in FP samples from the three deer species (Figure 9A). Similar distinctions could be made between XP samples of the three deer species based on the levels of Asp, Val, Met, Tyr, Ser, Glu, Phe and His (Figure 9B). Additionally, Glu, Ser, Lys, Pro, Phe and Val are potential amino acids that may be used to differentiate between four distinct qualities of velvet antler slices of *Cervus nippon* Temminck (Figure 4B). Similarly, Asp, Gly, Leu, Met, Ala and Lys could be introduced to distinguish the four quality grades of velvet antler slices from *Cervus elaphus* Linnaeus (Figure 5B). These results strongly suggest that regulations on the quality of velvet antler to the Chinese Pharmacopoeia (2020 edition; Chinese Pharmacopoeia Commission, 2020) have a room for development in terms of amino acid content.

The exhaustive index of amino acid content could play a crucial role in enhancing the velvet antler quality standards. The data suggest that a profile composed of numerous distinctive amino acids may have a stronger identifying effect. Indeed, different studies (Liu *et al.*, 2020; Sun *et al.* 2019c) used metabolomics to examine various classes of velvet antler slices and presented an integrated evaluation score for grading velvet antler slices by incorporating other metabolites, such as fatty acids and organic acids.

According to few studies, polypeptides could be the most significant bioactive indicators of velvet antler (Xia *et al.*, 2022; Zhao *et al.*, 2016). However, the highly specific polypeptide that could be employed as a velvet antler quality measure has not been extracted satisfactorily. As a result, in future, determining the quality grades of velvet antler based on the profiles of comprehensive chemical compounds could be a practical direction.

Moreover, benefits of deer antler on the human health are likely the result of bioactive peptides (Xia *et al.*, 2022; Zhao *et al.*, 2016). Peptides extracted from deer antlers possess antioxidant, anti-inflammatory, anti-aging, and bone regeneration-promoting properties. The efficacy of deer antlers in TCM is intimately linked to these biological processes (Xia *et al.*, 2022). More than two amino acid residues are required for a peptide to exert its biological action. Based on their amino acid makeup and peptide sequence, bioactive peptides may have a wide range of unique biological effects (Xia *et al.*, 2022). Since LP samples have the maximum amount of amino acids, they have the potential to produce a greater variety and number of bioactive peptides, leading to greater bioactivity. That is why LP gets the best overall quality grade. In addition, the amount of individual amino acid in FP samples is also relatively high, resulting in good biological action of FP. However, further research is needed to confirm the truth of these hypotheses.

Currently, the Chinese Pharmacopoeia (2020 edition; Chinese Pharmacopoeia Commission, 2020) has more than 50 types of TCM materials originating from animals and over 400 types of Chinese patent medicines incorporating animal-derived pharmaceuticals. However, for the purpose of quality assessment, no more than 10 different categories of animal-derived pharmaceuticals may have their exclusive active components determined (Qiu *et al.*, 2019). As a result, all methods for evaluating medications originating from animals must be further examined. This proof-of-concept study has the potential to pave way for the future research. Additionally, the findings could be reproduced using a larger sample size of velvet antlers. Owing to the fact that DL provides a novel technique for evaluating velvet antler quality, it also has a great potential for application in evaluating the quality of other animal-derived herbal medications.

Conclusion

We graded the quality of velvet antler slices in this study using delayed luminescence and chemical analysis of protein and amino acid content. These methods could discriminate to a certain extent between various grades of velvet antler slices. DL has a considerable potential as a fast detection tool for testing of velvet antler materials. This indicates that DL may be a useful tool for evaluating TCM drugs obtained from animals. Finally, DL enables the examination of the overall properties of various grades of velvet antler slices, and the combination of DL and chemical components enables a novel method of determining animal-derived herbs and functional food quality control.

Availability of data and material

The datasets used in this study are available from the corresponding author upon reasonable request.

Competing interests

Authors declared no conflict of interest. The paper was published in a preprint website "Research Square" (Fan *et al.* 2020).

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Author contributions

Jianxun Zhu, Shasha Fan and Mengmeng Sun designed the study. Mengmeng Sun, Jingxiang Pang, Yu Yan and Jinxiang Han conducted DL measurements and statistical analyses. Jianxun Zhu and Xiaoru Xu carried out data analysis. Li Li, Ye Zhao and Nan Wang performed chemical analysis. Shasha Fan drafted the manuscript and Min He prepared illustrations. Wen-Te Chang, Tung-Ti Chang and Jiale Yang contributed to revisions of the manuscript. All authors read and approved the final manuscript.

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