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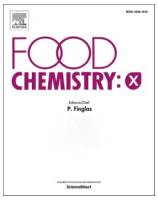

Properties and characteristics of steam-exploded donkey bone powder and corresponding whole wheat cookies

Yue Li [a], Xinru Qiu [b], Yuanshuai Jiang [b], Feng Kong [a,b,*], Guiqin Liu [a,*]

[a] School of Agriculture and Biology, Liaocheng University, Liaocheng 252000, China
[b] School of Pharmaceutical Sciences and Food Engineering, Liaocheng University, Liaocheng 252000, China

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ABSTRACT

Steam explosion was found to be the most effective preparation method for donkey bone powder, compared with non-treatment, autoclave and steam processing. And the effect of steam-exploded donkey bone powder (SEDBP) on the quality characteristics of whole wheat cookies was evaluated. Compared with untreated powder, particle size of SEDBP was reduced by 55.60 %, while oil- and water-holding capacity, water solubility and ABTS radical-scavenging rate significantly increased by 13.94 %, 26.86 %, 298.26 % and 91.47 %, respectively. Steam explosion enhanced protein digestibility from 32.17 % to 71.43 %, increased the Ca^{2+} release rate from 37.47 % to 47.82 %, and increased the release of 11 amino acids during digestion. SEDBP reduced the solvent retention capacity of flour blends and improved the color, hardness and spread ratio of whole wheat cookies. Cookies with 30 % SEDBP addition had better flavor, texture and scored the highest on overall acceptability. The results will help expand animal bone applications and develop nutrition-fortified foods.

1. Introduction

Donkey bone is one of the main by-products of the donkey meat processing, accounting for approximately 10 % of the total weight of the animal (Zhou et al., 2021). Donkey bone is rich in various nutrients such as proteins, polysaccharides, and minerals (Liu et al., 2022), with demonstrated health benefits such as the ability for replenishing the blood and nourishing the skin, regulating gastrointestinal function, enhancing immunity, strengthening the muscles and bones, and preventing osteoporosis (Fu et al., 2021).

Despite the recognized health-promoting factors linked to donkey bone, there are technological and nutritional challenges that have thus far limited the applications of bone in the preparation of food products. Powder is the main form required to obtain the efficient use of raw materials; however, the hard and ductile properties of donkey bone pose a challenge in crushing to obtain a fine powder for processing. In addition, donkey bone is filled with hydroxyapatite tightly bound to a collagen fiber network, which affects the bioaccessibility of calcium and protein (Qin et al., 2022). Given their poor nutritional and processing performance, donkey bones are often discarded, which represents a substantial waste of resources and contributes to environmental pollution. Therefore, it is necessary to develop an efficient strategy to overcome these processing limitations and realize the high-value utilization of donkey bone.

Hydrothermal treatments could improve the nutritional and technological benefits of various materials (Kong et al., 2024). Autoclave and steam processing are common methods of pre-treating raw materials. Autoclave treatment decreases the particle size of fish bone powder, which facilitates the *in vitro* digestion of calcium and protein (Wijayanti et al., 2021). Steam explosion is a rapidly developing raw material pretreatment technology that involves the use of high-temperature saturated steam as a medium to pressurize and penetrate the raw material within a sealed reaction vessel. Under controlled time and pressure conditions, the pressure is suddenly released and the heat energy is converted into mechanical work to exert a sudden explosion of steam onto the raw material. Throughout this process, the raw material is subjected to thermal degradation, acid hydrolysis, mechanical fracture, hydrogen bond disruption, and structural reorganization, which can effectively disrupt a dense structure in a short period of time, resulting in significant changes in the physical structure and chemical composition of the material (Kong, Li, et al., 2022; Kong, Zeng, et al., 2022). Cui et al. (2021) found that steam explosion treatment reduced the average particle size of tuna bone powder by 92.23 % and resulted in better calcium bioavailability than the untreated tuna bone powder. In

* Corresponding authors.
E-mail addresses: wykf2012@126.com (F. Kong), guiqinliu@lcu.edu.cn (G. Liu).

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addition, steam explosion treatment has been shown to promote the extraction of collagen peptides and chondroitin sulfate from animal bones (Zhang, Liu, et al., 2023). Therefore, these pretreatment techniques have a high application potential in the processing of animal bones. However, few studies have investigated the applications of these technologies in the preparation of donkey bone powder and corresponding food products. Whole wheat cookies are popular among consumers as they are enriched in dietary fiber and bioactive components with health benefits, which can serve as a carrier for donkey bone powder to develop food products with retained nutrients of the bone.

In this study, donkey bone was pretreated by steam explosion, autoclave, and steam processing, and the effects of these methods were compared with respect to the resulting particle size distribution, color profiles, structural properties, physicochemical properties, and protein digestibility of the obtained donkey bone powder. Given the relative advantages of steam explosion, steam-exploded donkey bone powder (SEDBP) was prepared at various concentrations (0–50 %) and added to whole wheat cookies for quality evaluations, including the solvent retention capacity (SRC) of the flour blends, color profiles, hardness, spread ratio, and sensory characteristics. This study provides new research methods and ideas for the efficient use of animal bone, laying the foundation for the further development of animal bone-based products.

2. Materials and methods

2.1. Materials

Donkey leg bones with a protein, fat, ash, and Ca^{2+} content of 12.79 %, 7.19 %, 61.43 %, and 14.08 %, respectively, were provided from Yucheng Huimin Agricultural Technology Co., Ltd. (Shandong, China). Whole wheat flour with a protein, fat, carbohydrate, and dietary fiber content of 12.5 %, 2.1 %, 58.7 %, and 12.2 %, respectively, was purchased from Xinxiang Liangrun Whole Grain Food Co., Ltd. (Henan, China). Peanut butter with a protein, fat, and carbohydrate content of 26.2 %, 41.5 %, and 25.2 %, respectively, was purchased from Nanyang Hesheng Food Co., Ltd. (Hernan, China). Soybean oil was obtained from Yihai Kerry Foodstuffs Marketing Co., Ltd. (Shanghai, China); unsalted butter was purchased from Shandong Baoyuan Grain, Oil and Food Co., Ltd. (Shandong, China); salt was purchased from Jiangsu Ruifeng Salt Industry Co., Ltd. (Jiangsu, China); sugar was obtained from Guangzhou Fuzheng Donghai Food Co., Ltd. (Guangzhou, China); and baking powder was purchased from Guilin Kesheng Food Co., Ltd. (Guangxi, China). The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), calconcarboxylic acid, sodium sulfide, and pepsin (>3000 U/mg) were obtained from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Trypsin (porcine pancreas, 250 USP units/mg) and pig bile powder were obtained from Yuanye Biotechnology Co., Ltd. (Shanghai, China).

2.2. Bone samples preparation and processing treatments

The meat, fascia, and marrow were removed from the donkey leg bones and then the bones were crushed into 0.5–2-cm pieces using a bone breaker (Langfang Guantong Machinery Co., Ltd., Hebei, China). The steam explosion treatment was carried out in a self-designed laboratorial vessel (Fig. S1, Supplementary Material) as described previously (Kong, Zeng, et al., 2022). Briefly, donkey bones were loaded into a reactor (WY19, Big Soldier Food Machinery, Henan, China), which was charged with high-pressure saturated steam to reach a pressure of 0.5 MPa that was sustained for 7 min and then the system was immediately depressurized to terminate the reaction. The selection of pressure and time was based on the preliminary trial for optimum steam explosion. The autoclave treatment was carried out with reference to the method of Nawaz et al. (2020), in which the donkey bones were heated in an autoclave sterilizer (LDZX-50 KBS, Shanghai Shen'an Medical Appliance Factory, Shanghai, China) at 121 °C for 1 h. The steam treatment was carried out with reference to the method of Jia et al. (2022) with slight modifications. In brief, the donkey bones were heated for 1 h on a steam rack and then the processed donkey bones were dried in an electrothermal blast drying oven (101-3AB, Beijing Zhongxing Weiye Century Instrument Co., Ltd., Beijing, China) at 60 °C for 14 h. The dried donkey leg bones (10 g) were then pulverized in a high-speed pulverizer (LG-30, Ruian Baixin Pharmaceutical Machinery Co., Ltd., Zhejiang, China) for 2 min and the resulting powder was passed through a 100-mesh sieve. The donkey leg bone powder was collected in a self-sealing bag and stored at 4 °C for future use. The scheme of the preparation and analysis workflow is presented in Fig. S2 (Supplementary Material).

2.3. Particle size distribution of donkey bone powder

The particle size distribution of donkey bone powder was measured using a laser particle size analyzer (NKT3100-H, Shandong Nike Analytical Instrument Co., Ltd., Shandong, China) at room temperature. The donkey bone powder was added to distilled water and dispersed using ultrasound. Particle size characteristics of donkey bone powder were assessed according to the cumulative distribution of 50 % of particles (D_{50} value), volume-weighted particle size (D[4,3]), and volumetric specific surface area (S/V).

2.4. Color of donkey bone powder

The surface color of the donkey bone powder was measured using a colorimeter (CR-10, Minolta, Japan), which included the L* (lightness), a* (red-green), and b* (yellow-blue) values. The white index (WI) of the donkey bone powder was calculated using the Hunter whiteness formula (Ge et al., 2023).

2.5. Fourier-transform infrared (FTIR) spectroscopy of donkey bone powder

The chemical structure of the donkey bone powder was analyzed using an FTIR spectrometer (IRAffinity-1, Shimadzu Corporation, Japan) with reference to the method of Kong et al. (2024). The donkey bone powder was processed using the KBr tableting method and scanned in the range of 400–4000 cm^{-1}. The range of 1600–1700 cm^{-1} was selected to calculate the proportions of α-helix, β-fold, β-turn, and random coil structures using Peak Fit software (Version 4.12, SeaSolve Software Inc., USA).

2.6. X-ray diffraction (XRD) of donkey bone powder

The XRD patterns of donkey bone powder were obtained using an X-ray diffractometer (XRD-6100, Shimadzu Corporation, Japan) with a scanning range (2θ) of 5–90° at a scanning rate of 10°/min referring to the method of Kong et al. (2024).

2.7. Physicochemical properties of donkey bone powder

2.7.1. Hydration properties

The oil-holding capacity of the donkey bone powder was determined with reference to the method of Kaur et al. (2015). In brief, the donkey bone powder (1 g) was mixed with soybean oil (10 mL), kept at room temperature for 30 min, and centrifuged at 3000 rpm for 20 min. The soybean oil was discarded and the weight of the residue was weighed. The water-holding capacity and water solubility were determined with reference to the methods of Gularte and Rosell (2011). The donkey bone powder (1 g) was mixed with distilled water (10 mL) and stored at room temperature for 24 h. The mixture was centrifuged at 3000 rpm for 20 min, the supernatant was dried, the dissolved substance was weighed to calculate water solubility index, and the residues was weighed to calculate the water-holding capacity.

2.7.2. ABTS radical-scavenging rate of donkey bone powder

Donkey bone powder was weighed to 0.5 *g* in a centrifuge tube and 10 mL of distilled water was added. After shaking and mixing, the solution was heated in a boiling water bath for 10 min, cooled down, and centrifuged to obtain the supernatant. The ABTS radical-scavenging rate of the donkey bone powder was then determined according to the method of Dudonné et al. (2009) by reacting the ABTS working solution (2.7 mL) with the supernatant (0.3 mL) for 30 min in the dark.

2.8. Protein digestibility of donkey bone powder

The *in vitro* protein digestibility of the donkey bone powder was determined with reference to the method of Kong, Zeng, et al. (2022). Briefly, 2 g of donkey bone powder was weighed and added to 20 mL of distilled water, the pH was adjusted to 2.0 using 5 mol/L HCl, then 2 mL of pepsin solution was added, and placed in water bath at 37 °C for 2 h. Subsequently, the pH was adjusted to 7.0 using 1 mol/L $NaHCO_3$, and 1 mL of trypsin-bile solution was added, and then reacted at 37 °C for 2 h. After that, the enzyme was inactivated in the boiling water bath for 10 min, cooled down and centrifuged at 5000 rpm for 20 min, the precipitates and supernatants were collected separately and the protein content of the precipitate was determined using the Kjeldahl method (GB 5009.6–2016).

2.9. Calcium release

The calcium content of the digestive solution was determined using the ethylenediaminetetraacetic acid titration method (GB 5009.92–2016).

2.10. Determination of amino acids

The amino acid contents of the digestive solution were determined using an automatic amino acid analyzer (L8900, Hitachi, Tokyo, Japan) according to the manufacturer instructions.

2.11. ABTS radical-scavenging rate of digestive solution

The ABTS radical-scavenging rate of the digestive solution was measured using the same method as described in Section 2.7.2. This involved reacting the ABTS working solution (2.7 mL) with the digestive solution (0.3 mL) for 30 min in the dark.

2.12. SRC of mixed powders

The SRC of the mixed powders was determined according to AACC Approved Method 56–11.02. In brief, SEDBP was added to the mixed powders at concentrations of 10 %, 20 %, 30 %, 40 %, and 50 % (*w*/w), and the SRC of distilled water, 50 % sucrose solution, 5 % sodium carbonate solution, and 5 % lactic acid solution were determined, respectively.

2.13. Cookies preparation

The cookies were prepared with reference to the method of Benjakul and Karnjanapratum (2018). Briefly, 51.7 g whole wheat flour (with SEDBP added at 0 %, 10 %, 20 %, 30 %, 40 %, and 50 % of whole wheat flour, w/w), 13.8 g unsalted butter, 6 g peanut butter, 2.6 g sugar, 1.4 g salt, 2.1 g baking powder, and 22.4 g distilled water were mixed thoroughly, kneaded into a dough, rolled to a thickness of 4 mm, baked at 150 °C for 30 min, cooled at room temperature for 1 h, and stored for further analysis.

2.14. Physical property analysis of SEDBP-added cookies

2.14.1. Color of the cookies

The color of the cookies was measured using the same method as in Section 2.4.

2.14.2. Spread ratio of cookies

The spread ratio was calculated by dividing the diameter of the cookies by the thickness (Kong, Li, et al., 2022).

2.14.3. Hardness of cookies

The hardness of the cookies was determined using a texture meter (CT3, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA), specifically with probe TA39. The pre-test speed was set to 2 mm/s, the test speed was set to 0.5 mm/s, the distance was 2 mm, and the trigger force was 5 *g* (Kong, Li, et al., 2022).

2.15. Sensory evaluation of the cookies

The sensory evaluation team consisted of 12 students majored in food science and engineering. Referring to the method of Babiker et al. (2021), various sensory attributes of the cookies were evaluated, including flavor, texture, color, interior and exterior appearance, odor, and overall acceptability, using a 5-point hedonic scale.

2.16. Statistical analysis

The experimental data were analyzed using SPSS software and the results are expressed as mean ± standard deviation. Differences were considered significant at $p < 0.05$. Correlation and principal component analyses of the various parameters of cookies were analyzed by Origin software (OriginLab Corporation).

3. Results and discussion

3.1. Appearance and morphological properties of donkey bone powder

3.1.1. Particle size characteristics

The effects of the hydrothermal treatments on the particle size of donkey bone powder are summarized in Table 1. The D_{50} of NDBP was 81.76 μm, the D[4,3] was 87.90 μm, and the S/V was 2594.69 cm^2/cm^3, whereas the D_{50} of SDBP, ADBP, and SEDBP was in the range of 36.30–67.35 μm, the D[4,3] was in the range of 61.62–74.54 μm, and the S/V was in the range of 2906.95–6273.84 cm^2/cm^3. Among the four different treatments of donkey bone powder, SEDBP had the smallest particle size and the largest specific surface area. Compared with the NDBP, the D_{50} and D[4,3] values of SEDBP were significantly reduced by 55.60 % and 29.90 %, respectively, and the S/V was significantly increased by 141.80 % ($p < 0.05$). This may be related to the hydroxyapatite in the collagen fiber network structure of the natural bone, which makes the donkey bone tough and hard so that it is not easily crushed (Qin et al., 2022). The process of steam explosion of the donkey bone occurred in four stages, including the steam displacement, penetration, cooking and explosion stage. In the steam penetration stage, saturated steam provided under pressure penetrated the donkey bone and rapidly condensed into liquid water to heat the bone. In the steam cooking stage, the steam and condensate under high temperature and pressure catalyzed the degradation of organic matter and caused structural changes to the donkey bone, resulting in softening of the bone. In the explosion stage, the instantaneous pressure relief caused a huge pressure difference between the inside and outside of the donkey bone so that the saturated steam and high-temperature liquid water existing inside the structure rapidly expanded and caused a shock wave to ultimately break the bone. Therefore, the steam explosion treatment effectively softened and fragmented the donkey bone, which provided a powder with a smaller particle size. This was consistent with the findings

Table 1
Appearance properties of donkey bone powder subjected to different treatments.

Samples	D_{50} (μm)	D[4,3] (μm)	S/V (cm^2/cm^3)	L*	a*	b*	WI
NDBP	81.76 ± 1.93^a	87.90 ± 1.97^a	2594.69 ± 105.65^c	34.83 ± 0.49^c	−24.03 ± 0.15^a	8.40 ± 0.00^b	30.04 ± 0.41^c
SDBP	67.35 ± 1.39^b	74.54 ± 1.22^b	2906.95 ± 39.14^c	36.30 ± 0.17^b	−24.93 ± 0.21^b	7.63 ± 0.06^c	31.17 ± 0.12^b
ADBP	66.58 ± 1.51^b	73.61 ± 1.58^b	4593.12 ± 393.26^b	36.13 ± 0.32^b	−24.60 ± 0.10^b	8.63 ± 0.06^a	31.02 ± 0.26^b
SEDBP	36.30 ± 1.39^c	61.62 ± 2.06^c	6273.84 ± 89.71^a	37.67 ± 0.23^a	−25.40 ± 0.26^c	6.37 ± 0.06^d	32.39 ± 0.21^a

Abbreviations: D_{50}, cumulative distribution of the size of 50 % of particles; D[4,3], volume-weighted particle size; S/V, volumetric specific surface area; L*, lightness; a*, red-green value; b*, yellow-blue value; WI, white index; NDBP, non-treated donkey bone powder; SDBP, steam-treated donkey bone powder; ADBP, autoclaved donkey bone powder; SEDBP, steam-exploded donkey bone powder. Different letters in the same column represent significant differences ($p < 0.05$).

of Kong et al. (2024), and Cui et al. (2021), on chicken bones, and tuna bones, respectively. In addition, Cui et al. (2021) showed that reducing the particle size of bone powder can effectively improve its bioavailability. Therefore, the steam explosion treatment was considered to have greater application value in the production of donkey bone powder.

3.1.2. Color analysis

The effects of different treatments on the color of donkey bone powder are also shown in Table 1. Compared to that of NDBP, the L* (lightness value) of SDBP, ADBP, and SEDBP significantly increased from 34.83 to 36.30, 36.13, and 37.67, respectively, and the WI significantly increased from 30.04 to 31.17, 31.02, and 32.39, respectively ($p < 0.05$). Therefore, the different treatments effectively increased the lightness and whiteness of donkey bone powder, and the effect of the steam explosion treatment was more significant, with the SEDBP showing a 8.15 % increase in lightness and a 7.82 % increase in whiteness compared with those of NDBP. The color of the donkey bone powder is mainly attributed to the white hydroxyapatite with a small amount of reddish-brown hemoglobin (Li et al., 2021). After the steam explosion treatment, the donkey bone was easier to crush and therefore the particle size decreased significantly, which in turn caused a reduction in the size of the hydroxyapatite particles that were uniformly dispersed in the powder. These structural changes may have masked the color of hemoglobin, resulting in significant improvement in the lightness and whiteness of SEDBP. These results therefore provide a theoretical basis for the addition of SEDBP in food.

3.2. Structural characterization of donkey bone powder

3.2.1. FTIR spectral analysis

The FTIR spectra for donkey bone powders prepared with different hydrothermal treatments are presented in Fig. 1A. NDBP, SDBP, ADBP, and SEDBP showed absorption peaks near 560, 603, 870, 1027, 1459, 1653, 1754, 2854, and 2926 cm^{-1}. The absorption peaks at 560, 603, and 1027 cm^{-1} were related to PO_4^{3-}; those at 560 cm^{-1} and 603 cm^{-1} are attributed to the bending vibrational absorption peaks of PO_4^{3-} and the peak at 1027 cm^{-1} is the stretching vibrational absorption peak of PO_4^{3-} (Zhang, Li, et al., 2023). The peak at 870 cm^{-1} is attributed to the bending vibrational absorption peak of CO_3^{2-} and the peak at 1459 cm^{-1} is the stretching vibrational absorption peak of CO_3^{2-}, suggesting that CO_3^{2-} entered the hydroxyapatite lattice replacing a portion of the PO_4^{3-} (Bonadio et al., 2013). The peak at 1745 cm^{-1} is the characteristic ester C═O stretching vibrational absorption peak for lipids (Gao et al., 2021), whereas the peaks at 2854 cm^{-1} and 2926 cm^{-1} are the C—H stretching vibrational absorption peaks linked to protein and lipid components (Boutinguiza et al., 2012). The peak at 1653 cm^{-1} represents the collagen and peptide chain C═O stretching vibration absorption peak (Cui et al., 2021), which belongs to the amide I region absorption peak (1600–1700 cm^{-1}).

The peaks at 1600–1640, 1640–1650, 1650–1660, and 1660–1700 cm^{-1} denote the β-sheet, random coil, α-helix, and β-turn, respectively, reflecting the secondary structure of the protein (Fu et al., 2022). The relative contents of the secondary structures of donkey bone powder subjected to different treatments and the corresponding fitted curves are shown in Fig. 1B–F. The β-sheet, random coil, α-helix, and β-turn structures respectively accounted for 23.49 %, 22.16 %, 24.45 %, and 29.91 % of NDBP, whereas the β-sheet, α-helix, and β-turn structures accounted for 37.80 %, 18.54 %, and 43.66 % of SEDBP, respectively. Therefore, compared with the NDBP, the random coil of SEDBP disappeared, the α-helix content decreased, and the β-sheet and β-turn contents increased. The disappearance of random coils may be due to conformational changes or transformation of the structure during the steam explosion process. The decrease in α-helix content may be due to the disruption of hydrogen bonding or the polymerization of protein side-chain groups after steam explosion treatment (Wang, Guo, et al., 2024).

In summary, donkey bone powder was mainly composed of carbonate, phosphate, and some organic matter. The FTIR spectra of donkey bone powder with different treatments exhibited similar positions of the absorption peaks, and the atmospheric pressure, high-pressure, and steam explosion treatments maintained the main functional groups of the donkey bone powder while reducing the particle size, which only had a small effect on the overall composition of the powder.

3.2.2. XRD analysis

The XRD patterns of donkey bone powder subjected to different treatments are shown in Fig. 1G. The diffraction angles (2θ) of NDBP, SDBP, ADBP, and SEDBP showed strong peaks around 25.9°, 29.0°, 32.2°, 39.8°, 46.7°, 49.5°, and 64.1°, which were the closest to the spectra of hydroxyapatite $Ca_5(PO_4)_3(OH)$ (Cui et al., 2021; Kong et al., 2024). Most of the peaks observed in the SDBP, ADBP, and SEDBP spectra were similar to those detected in the NDBP spectra, suggesting that the different treatments did not affect the crystal structure of donkey bone powder (Cui et al., 2021).

3.3. Physicochemical characterization of donkey bone powder

The oil-holding capacity of NDBP, SDBP, ADBP, and SEDBP was 71.18 %, 60.52 %, 57.72 %, and 81.10 %, and the water-holding capacity was 67.05 %, 82.76 %, 75.54 %, and 85.06 %, respectively (Fig. 2A-B). Therefore, compared with NDBP, the oil-holding capacity of SDBP and ADBP was significantly reduced by 14.98 % and 18.91 %, whereas the water-holding capacity was significantly increased by 23.43 % and 11.24 %, respectively ($p < 0.05$). This may be due to the fact that the atmospheric-pressure or high-pressure steaming treatment significantly reduced the particle size of donkey bone powder to consequently increase the specific surface area, thus exposing more hydrophilic groups, resulting in powder that is more hydrophilic and less lipophilic (He et al., 2019). Compared with those of the NDBP, the oil-holding capacity and water-holding capacity of SEDBP were significantly increased by 13.94 % and 26.86 %, respectively ($p < 0.05$). This change may be due to the hydrothermal and mechanical effects of the steam explosion treatment to promote the transformation of the dense structure of the donkey bone into a loose and porous structure, which makes SEDBP have greater capacity for the adsorption or retention of water and oil (Wang, Lin, et al., 2024). Good hydration properties can change the viscosity of food and prevent its contraction (Xi et al., 2023),

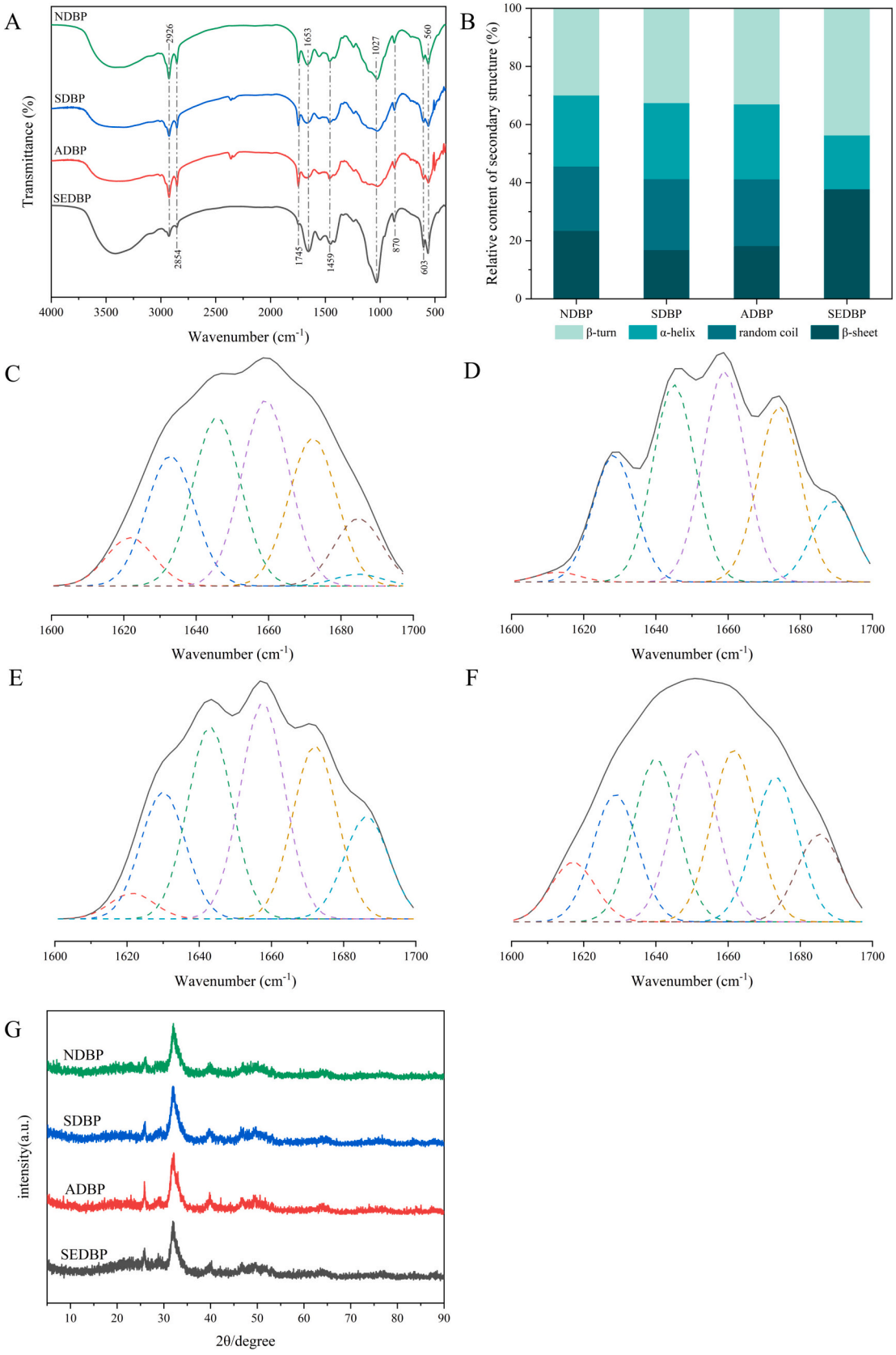

Fig. 1. Effect of different treatments of donkey bone powder on Fourier-transform infrared spectra (A); secondary structure scale diagrams (B); spectral fitting graphs for (C) NDBP, (D) SDBP, (E) ADBP, and (F) SEDBP; and XRD patterns (G). NDBP, non-treated donkey bone powder; SDBP, steam-treated donkey bone powder; ADBP, autoclaved donkey bone powder; SEDBP, steam-exploded donkey bone powder.

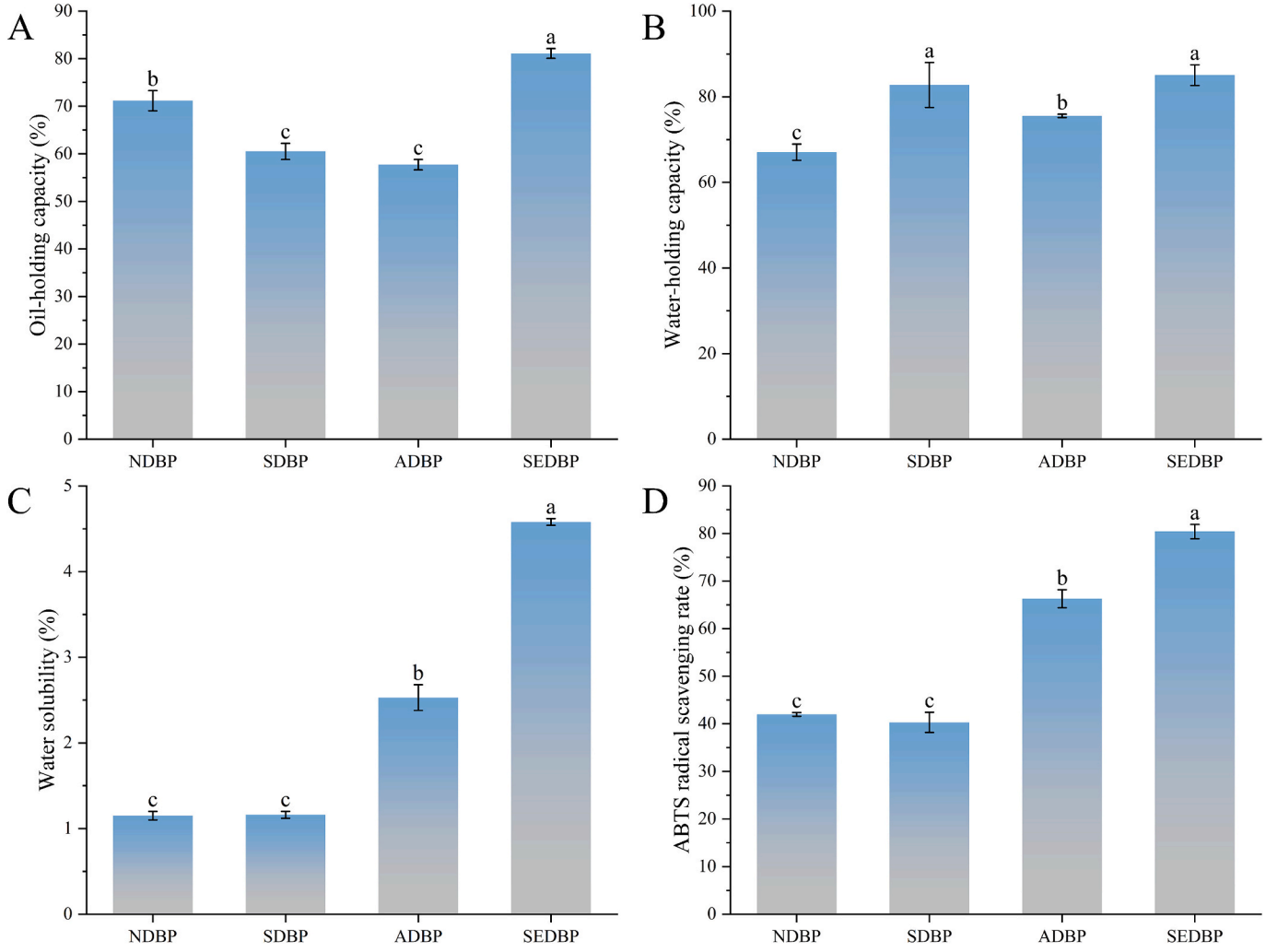

Fig. 2. Effect of different treatments on the (A) oil-holding capacity, (B) water-holding capacity, (C) water solubility, and (D) ABTS radical-scavenging rate of donkey bone powder. Different letters above bars indicate a significant difference ($p < 0.05$) for a given index.

while promoting the absorption of water and expansion in the intestinal tract to in turn promote intestinal peristalsis with an overall laxative effect (Yalegama et al., 2013). A food with a high oil-holding capacity can help to adsorb excess fat from the human body. Additionally, a high oil-holding capacity could reduce fat loss during processing or storage and maintain the flavor of the food (Xi et al., 2023). Therefore, owing to its higher water- and oil-holding capacities, SEDBP may have better physiological activity and processing properties than donkey bone powder prepared with other methods (Ouyang et al., 2023).

The water solubility of NDBP, SDBP, ADBP, and SEDBP was 1.15 %, 1.16 %, 2.53 %, and 4.58 %, respectively, and the ABTS radical-scavenging rates were 41.99 %, 40.32 %, 66.29 %, and 80.40 %, respectively (Fig. 2C-D). Therefore, SEDBP showed higher water solubility and ABTS radical-scavenging rates than SDBP and ADBP, representing a significant increase by 298.26 % and 91.47 %, respectively, compared with those of the NDBP ($p < 0.05$). This effect could be attributed to destruction of the tightly integrated donkey bone hydroxyapatite and collagen network structure under the high-temperature cooking and instantaneous explosion during the steam explosion treatment process, resulting in a large number of micropores on the surface of the donkey bone powder (Cui et al., 2021). The steam explosion treatment simultaneously reduced the particle size of the donkey bone powder to improve the specific surface area, enabling sufficient contact of the powder with water, thereby promoting dissolution of more substance by decreasing mass transfer resistance. Consequently, the dissolution of various active ingredients such as intracellular amino acids, peptides, and polysaccharides significantly increased the water solubility and ABTS radical-scavenging rate of the SEDBP.

3.4. Protein digestibility of donkey bone powder

As shown in Fig. 3A, the protein digestibility of NDBP, SDBP, ADBP, and SEDBP was 32.17 %, 31.01 %, 64.45 %, and 71.43 %, respectively, with the highest protein digestibility obtained for SDEBP, representing a significant increase of 122.04 % compared with that of NDBP. Similar to the effects on water solubility, this phenomenon may be attributed to the steam explosion disrupting the bone structure formed by the close integration of proteins and hydroxyapatite, thereby facilitating easier access for proteases to the proteins (Qin et al., 2022). And the higher specific surface area results in exposing more enzymolysis sites, which provides more opportunity for the digestive enzymes to make full contact with the donkey bone powder and ultimately increase the *in vitro* protein digestibility of the SEDBP. This increase may also reflect the ability of the steam explosion treatment to open up the super-helical structure of collagen, cut the protein chain, and dissolve the protein to destroy the overall bone structure, thus producing a more soluble protein (Qin et al., 2022). Overall, the SEDBP maintained high levels of hydration, antioxidant activity, and protein digestibility and significantly reduced particle size; therefore, this powder was further

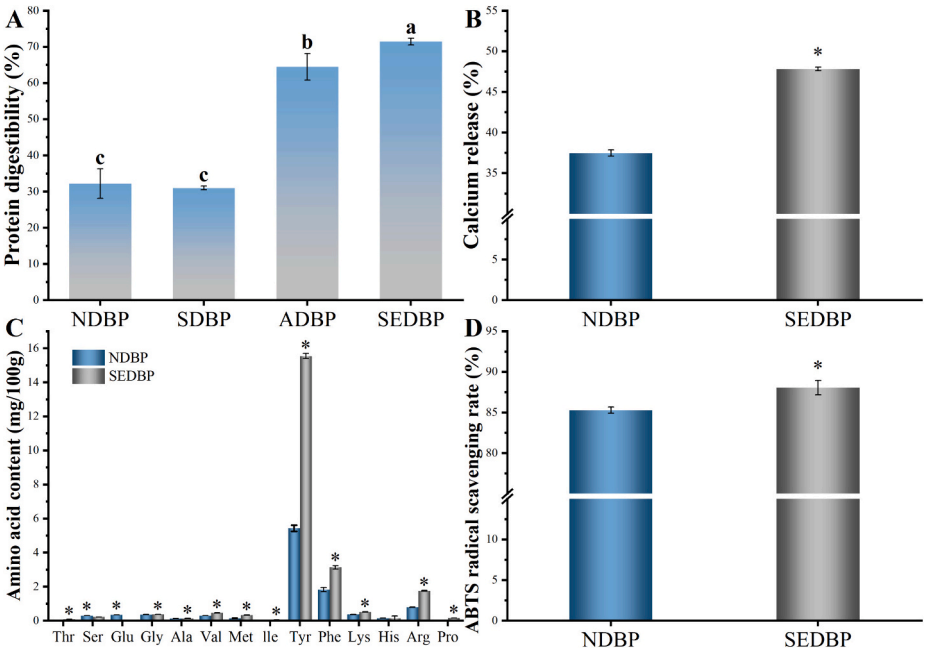

Fig. 3. Protein digestibility (A), Ca^{2+} release (B), amino acid content (C), and ABTS radical-scavenging rate (D) of *in vitro*-digested donkey bone powder. Different letters above bars indicate a significant difference ($p < 0.05$); *$p < 0.05$. NDBP, non-treated donkey bone powder; SEDBP, steam-exploded donkey bone powder.

investigated for its potential applications in food products.

3.5. *In vitro* bioaccessibility of SEDBP

The protein digestibility, Ca^{2+} release, amino acid content of the digestive solution, and ABTS radical-scavenging of the digestive solution, were measured as important indicators of the *in vitro* bioaccessibility of donkey bone powder (Kong et al., 2024). As shown in Fig. 3B, the Ca^{2+} release of SEDBP was 47.82 %, which was significantly higher than that of NDBP (37.47 %) ($p < 0.05$). The Ca^{2+} in donkey bone mainly exists in the form of hydroxyapatite in the collagen matrix, which is structurally stable and difficult to destroy (Olszta et al., 2007), therefore offering limited Ca^{2+} available for digestion and absorption. However, after the steam explosion treatment, the rigid structure of donkey bone was softened in the high-temperature steaming stage, and saturated steam could penetrate the pores of the bone and destroy the structure during the process of transient depressurization (Qin et al., 2022). Consequently, the hydroxyapatite deposited in the collagen matrix was exposed (Kong et al., 2024) and came in contact with the digestive solution to promote the release and solubilization of Ca^{2+}.

The amino acid contents of the digestive solution of NDBP and SEDBP are shown in Fig. 3C. Compared with those of NDBP, after the steam explosion treatment, the contents of glycine, alanine, valine, methionine, isoleucine, tyrosine, phenylalanine, lysine, and arginine of the digestive fluid of SEDBP were significantly increased by 1.61 %, 4.69 %, 53.84 %, 133.11 %, 40.20 %, 186.33 %, 72.22 %, 39.12 %, and 122.39 %, respectively, and the threonine and proline contents significantly increased from 0 mg/100 g to 0.09 and 0.16 mg/100 g, respectively ($p < 0.05$).

As shown in Fig. 3D, the ABTS radical-scavenging rate of SEDBP was 88.06 %, which was significantly higher than that of NDBP (85.28 %) ($p < 0.05$). After the steam explosion treatment, the total amino acid content of the digestive solution increased from 10.15 mg/100 g to 22.88 mg/100 g and the essential amino acid content increased from 2.66 mg/100 g to 4.57 mg/100 g. This suggested that SEDBP has high nutritional potential and that its dietary intake may prevent diseases related to a nitrogen imbalance caused by essential amino acid deficiency (Reis et al., 2020). In particular, arginine, threonine, glycine, proline, and isoleucine have antioxidant effects; lysine has beneficial effects on the immune system along with potential anti-tumor effects; arginine can help to improve sleep; and glycine has neuroprotective effects (Zhang et al., 2024).

3.6. *SRC of flour blends*

SRC is an effective indicator for evaluating the quality of a mixed flour and cookies (Kong, Li, et al., 2022). The lactic acid SRC correlates with the gluten strength, sodium carbonate SRC correlates with damaged starch, sucrose SRC correlates with arabinoxylan, and distilled water SRC correlates with the water-holding capacity of all polymers. The SRC values of the mixed flours with different SEDBP contents are shown in Fig. 4. The water SRC of the whole wheat flour was 82.58 g/100 g, and the lactic acid, sodium carbonate, and sucrose SRC values were 91.91, 100.08, and 114.07 g/100 g, respectively. The addition of 10–50 % SEDBP significantly reduced the solvent retention of water, lactic acid, sodium carbonate, and sucrose in the mixed flours (all $p < 0.05$). This may be due to the fact that whole wheat flour has more hydrophilic groups and is enriched in arabinoxylan and damaged starch; therefore, since the addition of SEDBP resulted in a reduced percentage of whole wheat flour, the water, sucrose, and sodium carbonate solvent retention of the mixed powder was significantly reduced. Although SEDBP is rich in protein, it does not contain gluten proteins; therefore, the addition of SEDBP damaged the spatial structure and continuity of the original gluten network, reducing its strength along with significant reduction in the lactic acid solvent retention force. The sucrose SRC of the mixed powder containing SEDBP was also reduced, which was conducive to the evaporation of water during the baking process of cookies, contributing to the production of high-quality cookies with low moisture content (Jeong et al., 2024); this has an added benefit of helping to reduce the baking time and energy cost to a certain extent (Guttieri et al., 2001). Barrera et al. (2007) demonstrated that high-quality cookies and cookie powder had a low water retention capacity. They further showed that lower water absorption by flour provoked higher water absorption by sugar that reduced the viscosity of the dough, making the dough easier to stretch and ultimately producing better quality cookies (Barrera et al., 2007).

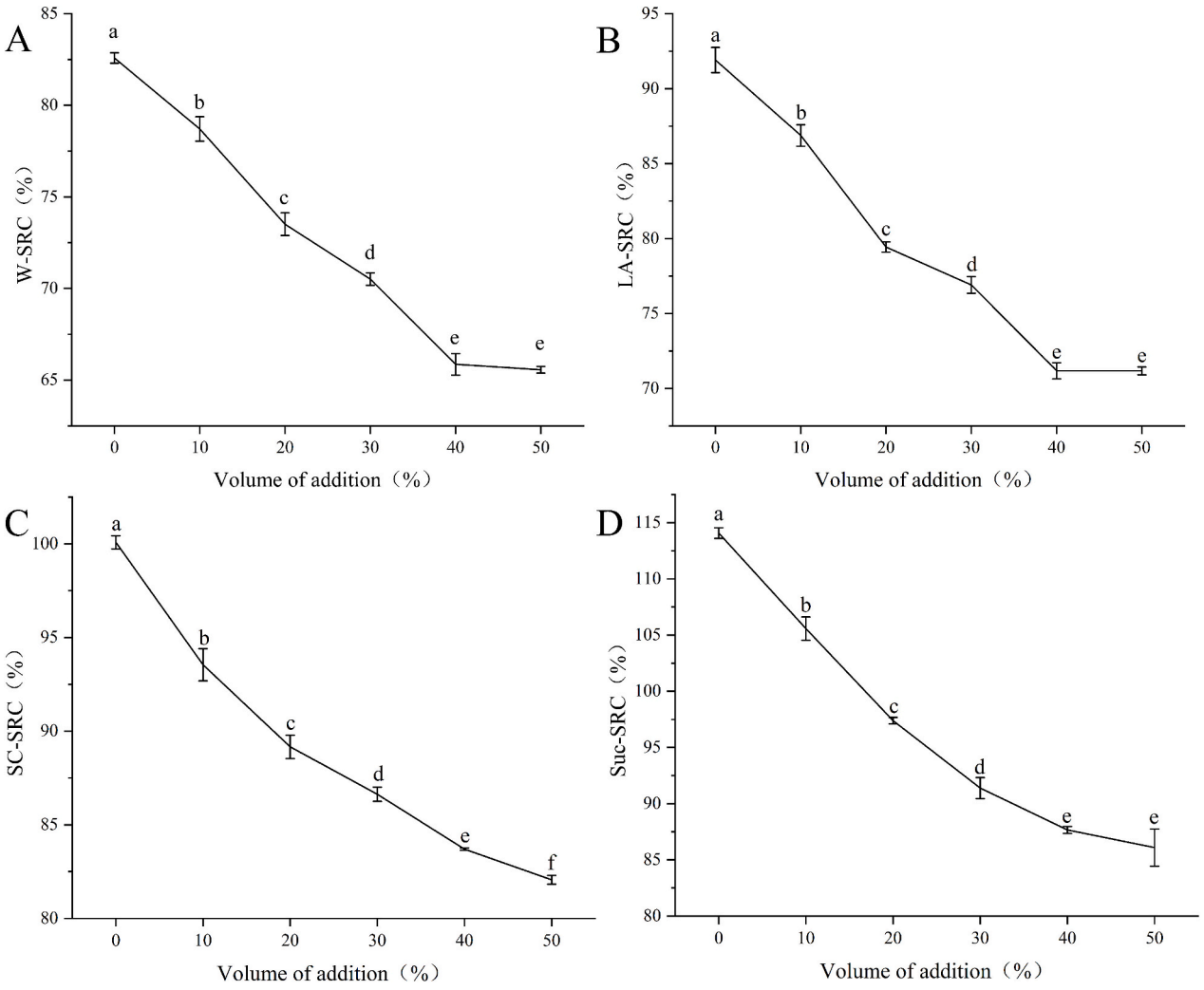

Fig. 4. Effect of steam-exploded donkey bone powder addition at different concentrations on the (A) water (W) (B), lactic acid (LA), (C) sodium carbonate (SC), and (D) sucrose (Suc) solvent retention capacity (SRC) of the mixed powders. Different lowercase letters indicate a significant difference ($p < 0.05$).

Table 2
Effect of steam-exploded donkey bone power added at different concentrations on the color, hardness, and spread ratio of cookies.

Addition (%)	L*	a*	b*	WI	Hardness (g)	Spread ratio
0	21.43 ± 0.06^{e}	−17.70 ± 0.30^{a}	17.33 ± 0.15^{b}	17.62 ± 0.05^{d}	3241.25 ± 616.25^{a}	9.78 ± 0.12^{c}
10	21.98 ± 0.17^{d}	−18.98 ± 0.26^{b}	17.93 ± 0.21^{a}	17.73 ± 0.17^{d}	2896.25 ± 216.01^{a}	9.79 ± 0.16^{c}
20	22.50 ± 0.10^{c}	−19.03 ± 0.06^{b}	17.43 ± 0.15^{b}	18.32 ± 0.05^{c}	2397.75 ± 187.09^{b}	10.04 ± 0.06^{b}
30	22.83 ± 0.15^{b}	−19.30 ± 0.00^{bc}	16.88 ± 0.21^{c}	18.68 ± 0.16^{b}	1904.80 ± 170.22^{c}	10.14 ± 0.07^{b}
40	23.10 ± 0.17^{b}	−19.53 ± 0.12^{cd}	16.73 ± 0.12^{c}	18.91 ± 0.15^{b}	1581.25 ± 227.33^{cd}	10.56 ± 0.05^{a}
50	23.77 ± 0.32^{a}	−19.83 ± 0.21^{d}	15.53 ± 0.25^{d}	19.71 ± 0.25^{a}	1444.50 ± 153.54^{d}	10.69 ± 0.15^{a}

Abbreviation: WI, whiteness index. Different letters in the same column represent significant differences ($p < 0.05$).

3.7. Physical properties of whole wheat cookies enriched with SEDBP

As shown in Table 2, the addition of SEDBP to whole wheat flour significantly increased the lightness (L*) of the cookies compared to that of the pure whole wheat cookies ($p < 0.05$); the WI of the cookies was significantly increased when SEDBP was added at levels of 20–50 % ($p < 0.05$). This is likely due to the white color of SEDBP, which can improve the lightness and whiteness of cookies. The a* values were the highest ($p < 0.05$) for the whole wheat cookies and gradually decreased with an increasing level of added SEDBP, whereas the redness of the cookies decreased with increasing addition of SEDBP. Compared to that of the whole wheat cookies, the yellowness of the 10 % SEDBP-added cookies increased significantly ($p < 0.05$) with 10 % addition of SEDBP, although there was no further change with addition of 20 % SEDBP, and then the redness decreased significantly ($p < 0.05$) with addition of 30–50 % SEDBP. This change in red color may be due to the interaction of SEDBP and the color of the whole wheat flour itself, as well as browning reactions such as caramelization and Maillard reactions during high-temperature baking.

Crispness is a desirable characteristic of cookies, and a certain reduction in hardness helps to improve the acceptability of cookies by providing consumers with a better taste experience (Lara et al., 2011). As shown in Table 2, there was no significant change in the hardness of cookies with the addition of 10 % SEDBP ($p > 0.05$), whereas there was a significant decrease in the hardness of cookies with the addition of

20–50 % SEDBP ($p < 0.05$) as compared to that of the whole wheat cookies. This may be caused by the inhibitory effect of SEDBP on formation of the gluten network, resulting in a reduction in the strength of the gluten protein network. Moreover, the addition of SEDBP reduced the percentage of whole wheat flour in the dough, along with a gradual decrease in the content of insoluble dietary fiber and gluten proteins, resulting in a decrease in the hardness of the cookies (Nguyen et al., 2021). Compared with whole wheat cookies, the addition of 20–50 % SEDBP significantly increased the spread ratio of the cookies ($p < 0.05$). This may be due to the fact that the addition of SEDBP diluted the gluten content and improved the mobility of the dough, thereby favoring dough extension. The increased spread ratio may have also been caused SEDBP weakening the water retention capacity of the mixed powder, which is in line with the findings of Yang et al. (2022). Increasing the spread ratio would increase the likelihood that the quality of the cookies would be favored by consumers (Sulieman et al., 2019).

3.8. Sensory quality evaluation of whole wheat cookies enriched with SEDBP

The effect of the addition of different levels of SEDBP on the sensory evaluation of cookies is presented in Fig. 5. In terms of flavor, cookies prepared with SEDBP had higher sensory evaluation scores compared with those of the whole wheat cookies, with the highest scores obtained for cookies with 30 % SEDBP. The addition of SEDBP also directly affected the color of the cookies. The addition of 10–30 % SEDBP improved the color of the whole wheat cookies and increased their acceptance scores, whereas the color of cookies with 30–40 % SEDBP was significantly altered compared to that of whole wheat cookies, resulting in a paler color and lower sensory evaluation scores. Compared with that of the pure whole wheat cookies, the odor was improved with the addition of 10–30 % SEDBP, with the best odor score achieved at 30 %; however, the odor deteriorated with the addition of 40–50 % SEDBP, affecting the overall sensory quality of the cookies. The addition of SEDBP changed the original texture and external and internal appearance of the whole wheat cookies to different degrees. Specifically, addition of 30 % SEDBP improved the sensory properties of whole wheat cookies, resulting in the highest overall acceptability scores.

3.9. Correlation and principal component analysis of various parameters of cookies

Correlation analyses of the SEDBP addition level, SRC of the mixed powder, physical characteristics, and sensory evaluation of the cookies were carried out to determine the key factors to consider for regulating the quality of cookies with different levels of added SEDBP. As shown Fig. 6A, the addition of SEDBP showed strong positive correlations with the L* value, WI, spread ratio, and exterior appearance of the cookies ($r = 0.9925$, 0.9798, 0.9692, and 0.9737, respectively; all $p < 0.01$), along with the cookies' interior appearance ($r = 0.8151$, $p < 0.05$). Conversely, the addition of SEBDP showed strong negative correlations with the water, lactic acid, sodium carbonate, and sucrose SRC values and the hardness value ($r = -0.9835$, -0.9744, -0.9732, -0.9719, and -0.9879, respectively; all $p < 0.01$). SEDBP also showed negative correlations with the a* and b* values of the cookies ($r = -0.9096$ and -0.8521, respectively; both $p < 0.05$).

The effect of SEDBP on the quality of the cookies was analyzed using principal component analysis. As shown in Fig. 6B, the first and second principal components accounted for 72.7 % and 20.4 % of the total variation, respectively. The first principal component was positively associated with the water, lactic acid, sodium carbonate, and sucrose SRC values; a* and b* values; hardness; and color. However, the first principal component was negatively associated with the L value, WI, spread ratio, and interior and exterior appearance of the cookies. The b* value, flavor, texture, color, odor, and overall acceptability were positively associated with the second principal component. These results clearly demonstrated that the addition of SEDBP significantly affected the quality of cookies. Cookies with 0 % SEDBP addition had higher SRC, hardness, and a* values; cookies with 10–20 % SEDBP had higher b*, color, and odor values; cookies with 30 % SEDBP had higher overall acceptability, flavor, texture, and L values; and cookies with 40–50 % SEDBP had higher exterior and interior appearance scores, spread ratio, and WI.

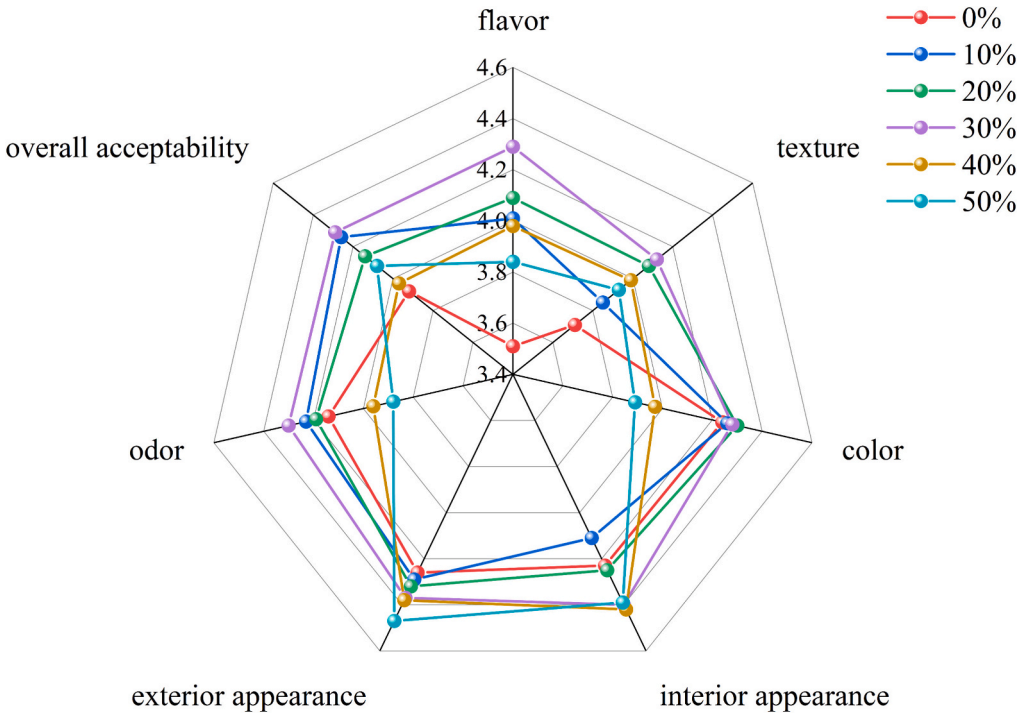

Fig. 5. Effect of steam-exploded donkey bone powder (SEDBP) addition at different concentrations on the sensory evaluation of cookies.

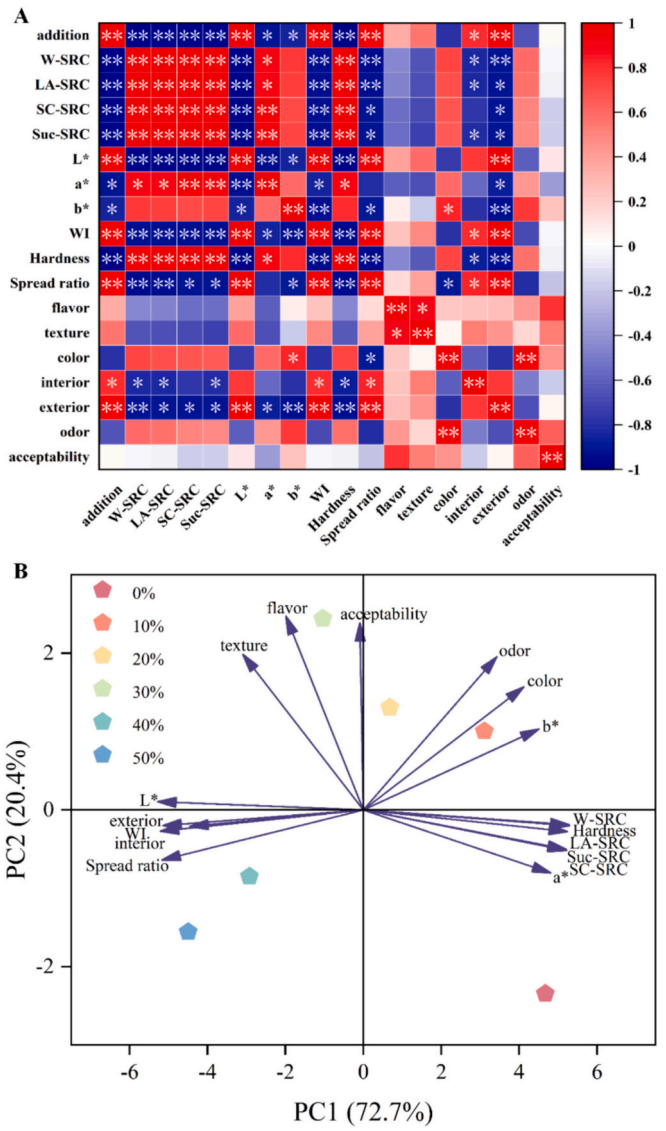

Fig. 6. Heat map of the correlations (A) and Principal component analysis (B) among various parameters of cookies; $^{*}p < 0.05$, $^{**}p < 0.01$. Addition, concentration of steam-exploded donkey bone added to the cookies; interior, appearance of the interior of cookies; exterior, appearance of the exterior of the cookies; acceptability, overall acceptability; W-SRC, LA-SRC, SC-SRC, and Suc-SRC, water, lactic acid, sodium carbonate, and sucrose solvent retention capacity, respectively; WI, whiteness index.

4. Conclusion

Steam explosion, autoclave and steam processing were applied for donkey bone powder preparation in this study. The results showed that steam explosion was an efficient treatment for donkey bone powder production with desirable functional property. Steam explosion treatment effectively reduced the particle size and improved the hydration properties and ABTS radical-scavenging rate of donkey bone powder. And steam explosion was conducive to increasing the protein digestibility, Ca^{2+} release, and the release of amino acids during digestion of donkey bone powder. The addition of SEDBP reduced the SRC of water, sucrose, sodium carbonate, and lactic acid of the mixed powder, thereby affecting the physicochemical properties and sensory evaluation of the corresponding cookies. Among them, cookies with 30 % SEDBP addition had better odor, texture, and lightness values, resulting in the highest overall acceptability scores. In conclusion, steam explosion enhanced the physicochemical properties and bioaccessibility of donkey bone powder, and the suitable addition of SEDBP was beneficial for improving whole wheat cookie quality. This work will help expand animal bone applications and develop nutrition-fortified foods.

CRediT authorship contribution statement

Yue Li: Writing – original draft, Investigation. **Xinru Qiu:** Investigation. **Yuanshuai Jiang:** Investigation. **Feng Kong:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Guiqin Liu:** Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101826.

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