



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Exploring *in vitro* digestibility of proteins in fresh pork and Chinese sausages: Effects of *Staphylococcus carnosus* RT6 on peptidomics and bioactivity

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ABSTRACT: This study investigated and compared the differences in gastrointestinal digestion among fresh pork, Chinese sausage, and Chinese sausage inoculated with *Staphylococcus* (S.) *carnosus* RT6. The results indicated that fermentation treatment improves gastric digestibility by inducing protein denaturation, transforming β -sheets into random coils and β -turns. The sausages inoculated with *S. carnosus* exhibited the best protein digestion characteristics, with the highest combined gastric/intestinal digestibility (77.38%). During *in vitro* gastrointestinal digestion, sausage inoculated with *S. carnosus* RT6 released more peptides (517) than traditional Chinese sausages (304) and fresh pork (134). The *S. carnosus* RT6 inoculate also yielded a higher proportion of peptides with molecular weights <1000 Da (36.21%) and more unique peptides. Furthermore, the digested peptides from the *S. carnosus* RT6 treatment were more widely distributed across the 3D structure of actin than those from the other treatments. The digested products of sausage inoculated with *S. carnosus* RT6 demonstrated superior radical scavenging activity, with DPPH, hydroxyl ($\cdot$ OH), and ABTS radical scavenging rates reaching 71.3%, 83.2%, and 80.5% respectively, and enhanced overall antioxidant capabilities. This study highlights the potential application of *S. carnosus* RT6 as a starter culture to enhance the nutritional quality of fermented sausage.

Keywords: Fresh pork; Chinese sausage; *In vitro* digestion; Protein digestibility; Peptidomic

1. Introduction

Pork is the most widely produced and consumed meat species globally, providing numerous essential nutrients such as proteins, minerals, and vitamins that constitute an integral part of daily diets^[1-2]. Among pork products, Chinese sausages are widely favored by consumers for their fine texture, desirable taste, and unique flavor^[3]. Technologically, Chinese sausages are produced by stuffing minced raw meat and fat, blended with specific spices, into casings, followed by drying and fermenting for several weeks. However, the traditional manufacturing process is characterized by long production cycles, inconsistent product quality, and potential safety risks that require further attention. To standardize production, inoculation with starter cultures has become the most prevalent method to reduce fermentation time, inhibit the growth of harmful bacteria, and

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Received 18 October 2025
Received in revised form 18 October 2025
Accepted 21 April 2026

enhance overall product quality^[4-5]. Li et al.^[6] demonstrated that dry fermented sausages inoculated with *Staphylococcus simulans* QB7 are a good source of bioactive peptides, whose stability against gastrointestinal digestion determines their bioaccessibility. Furthermore, Han et al.^[7] found that fermented sausages inoculated with *Lactiplantibacillus plantarum* and *Companilactobacillus alimentarius* exhibited superior color, texture, and flavor. These improvements were attributed to the metabolites of these beneficial microorganisms, which alter the physicochemical properties of meat proteins, thereby improving product quality. Traditionally, the quality of Chinese sausages is evaluated via physicochemical indices and sensory evaluation, such as color, texture, pH, taste, and flavor. Although these indicators effectively reflect eating quality, they offer limited insight into their nutritional value. Recently, the nutritional value of Chinese sausages has become increasingly crucial to consumer acceptance and for optimizing processing methods^[8-9].

Protein digestibility is a key indicator for measuring the nutritional value of meat products^[10]. Suboptimal digestibility can reduce the bioavailability of essential amino acids, potentially hindering growth and development while increasing the risk of intestinal diseases^[11-12]. Existing research indicates that various processing methods, including prolonged pickling, enzymatic tenderization, and emerging processing technologies, can induce protein oxidation, cross-linking, unfolding, and aggregation. These modifications alter protein structure and functional properties, thereby influencing their sensitivity to digestive enzymes and the subsequent release and bioavailability of amino acids and peptides during digestion. Zhu et al.^[13] studied the effect of NaCl on the structure and digestive properties of myofibrillar proteins. The study found that heating beef myofibrillar proteins at 85 °C and 0.4 M NaCl substantially improved its digestibility to 85.66%. This discrepancy could be due to NaCl could promote protein unfolding, therefore exposing the previously hidden cleavage sites to the action of digestive enzymes, increasing the MPs digestion. Furthermore, Zhao et al.^[14] demonstrated that treatment with proteinase K and bromelain significantly increased the degree of hydrolysis of beef protein by 4.5-17.3%. This improvement could be attributed to proteolysis by these enzymes acting as a pre-digestion step, thereby enhancing meat protein digestibility. Recent studies had also highlighted the impact of protein source and processing on nutrition and bioactive peptide release, as seen in insect protein hydrolysates^[15] and plant-based protein-enriched pasta^[16]. Fermentation-based biological systems are often viewed more favorably by consumers than purely physical processing systems. Recent investigations have demonstrated that fermentation treatment and the incorporation of starter cultures such as *S. carnosus* and *S. xylosus* can also induce significant degradation and oxidation of pork proteins^[17-18]. Therefore, protein modifications resulting from fermentation and the addition of starter culture may also substantially affect protein digestibility.

Beyond protein digestibility, the quantity and composition of hydrolysate peptides are critical determinants of nutritional quality^[19]. Peptidomics has emerged as a powerful tool for assessing protein digestion, enabling systematic characterization of digestive products and in silico prediction of bioactive functions through database mining. However, existing studies predominantly focus on within-product comparisons of processing effects (e.g., salt curing, heat treatment, ultrasound, or high pressure), leaving three

critical gaps: (i) limited comparative investigations into protein digestive characteristics between fresh pork and Chinese sausages prepared by different fermentation methods; (ii) poorly understood regulatory mechanisms of *S. carnosus* RT6 on the secondary structure of Chinese sausage proteins; and (iii) insufficient characterization of correlations between peptidomic profiles and bioactivity in fermented sausage digestion products. Therefore, this study systematically examined protein digestibility, microstructural evolution, and digestive product characteristics across three pork matrices: fresh pork, traditional Chinese sausage, and *S. carnosus* RT6-inoculated sausage. Peptidomics coupled with computational prediction was employed to characterize peptide composition and bioactivity, thereby elucidating the regulatory mechanisms underlying *S. carnosus* RT6-mediated fermentation of protein secondary structures and their correlations with digestion efficiency, peptidomic characteristics, and bioactivity. These findings provide novel insights into the nutritional distinctions among the three pork matrices.

2. Materials and methods

2.1. Materials

Fresh lean pork (from the hind leg) and back fat from the carcasses of six-month-old crossbred Huainan pigs (110 ± 5 kg) were purchased from the Pangdonglai supermarket in Xinxiang, China, within 24 hours of slaughter. Sodium dodecyl sulfate, Trichloroacetic acid (TCA), sodium dodecyl sulfate (SDS), Coomassie Brilliant Blue G-250, Nile Blue, and were acquired from Solarbio Technology Co., Ltd. (Beijing, China). Pepsin from porcine stomach (CAS: 901-75-6) and porcine pancreatic trypsin (CAS: 9002-07-7) were provided by Fuji Co., Ltd. (Osaka, Japan).

S. carnosus RT6 was previously isolated from Harbin dry sausage, identified by 16S rDNA sequencing, and stored at the School of Food Science, Henan Institute of Science and Technology.

2.2. Preparation of Chinese sausages

Fresh lean pork and back fat were minced through a meat grinder with a 5 mm orifice plate, respectively. The sausage formulation consisted of the following ingredients: 8,000 g of lean pork, 2,000 g of back fat, 300 g of sugar, 200 g of sodium chloride, 1 g of sodium nitrite, 5 g of sodium erythorbate, and Chinese liquor (200 g). *S. carnosus* RT6 was revitalized three times with 150 rpm oscillation in Mannitol Salt broth at 30 °C for 16 h to reach an OD₆₀₀ of 1.2-1.5 (pH 7.5). The total viable count (TVC) was determined using the bacterial culture method. 1 mL of bacterial solution was mixed with 9 mL 0.85 % sterile saline and homogenized using a stomacher for 4 min. 1 mL of a series of 10-fold dilutions was added and then mixed with 20 mL of plate count agar and incubated at 37 °C for 48 h, which was recorded as about 10⁸ CFU/mL. Centrifuge the remaining bacterial solution at 9,000 g for 10 min to collect the cell pellets, then wash twice with sterile water and resuspend in deionized water. The meat was thoroughly mixed and then divided into three groups, named S₀, S₉, and SC. Among them, the S₀ and S₉ groups did not add *S. carnosus* RT6, while the SC group did add *S. carnosus* RT6, which was inoculated with *S. carnosus* RT6 to a final concentration of 10⁷ CFU/g meat. The ingredients were well mixed and stuffed into natural pig casings (pig small intestine) with a diameter of 3.0

cm. Each sausage had a length of 15 cm. S₉ and SC groups were hung for 1 day at 25 ± 2 °C with a relative humidity of 30%–50%, and were placed in a fermentation chamber for 8 days at the same temperature, with a relative humidity of 75%–80%, while the S₀ group samples are not subjected to drying but are directly used in the following experiment. Fresh pork was the control sample (C).

2.3. Measurement of pork protein profiles

Total protein from sausage was extracted using the method of Sun et al.^[18]. The total protein concentration was determined by the Biuret method. After that, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to characterize the protein profiles before and after digestion, as described by Zhang et al.^[9].

2.4. Secondary structure of pork protein

The secondary structures of sausage protein were recorded on a Fourier transform infrared (FTIR) spectrophotometer (Nicolet 8210E, Thermo Fisher Scientific, Madison, WI, USA) according to the method of Zou et al.^[20]. The spectral information between the wavenumbers $1700\text{--}1600\text{ cm}^{-1}$ was used to calculate the content of various protein secondary structures.

2.5. Confocal laser scanning microscopy (CLSM)

The morphological characteristics of the proteins in the homogenized sample before and after *in vitro* digestion were observed using CLSM (Leica TCS SP5, Heidelberg, Germany). A 1.0 mL protein solution was evenly mixed with 20 μL of a 0.1% (w/v) Nile Blue solution to stain the protein phase. Then, 5 μL of the solution was coated on a microscope slide with coverslips to observe the distribution of meat protein using a $40 \times \text{HCPL APO}/20 \times \text{oil immersion objective}$ at a 633 nm helium-neon laser^[18].

2.6. Particle size measurements

To better understand the particle size of the homogenized sample before and after *in vitro* digestion, the mean particle size ($d_{4,3}$) of the sample solutions was monitored using a particle size analyzer (Mastersizer 3000, Malvern, Worcestershire, UK).

2.7. In vitro digestion

Four groups of samples were placed in a steamer drawer with a lid above boiling water until the center temperature of the samples reached 72 °C, then cooled to 25 °C for subsequent analysis—sample pretreatment following the method of Zhang et al.^[9] with slight modifications. In the ice bath, the sample equivalent to 300 mg of protein was added to 18 mL of simulated gastric fluid (SGF) and homogenized (9500 rpm, 2×30 s, with a cooling gap of 60 s). In this study, the INFOGEST static *in vitro* simulation of gastrointestinal food digestion of all samples was determined following the method of Brodkorb et al.^[21] with slight modifications. The sample solution was adjusted to pH 3.0 with 6 M hydrochloric acid solution, and pepsin was added to make the total volume of the gastric phase 20 mL (containing 6.9 mmol/L KCl, 0.9 mmol/L KH_2PO_4 , 25 mmol/L NaHCO_3 , 47.2 mmol/L NaCl, 0.12 mmol/L $\text{MgCl}_2(\text{H}_2\text{O})_6$, 0.5 mmol/L $(\text{NH}_4)_2\text{CO}_3$, 0.15 mmol/L CaCl_2 , 2,000 U/mL of final enzyme activity). The samples were incubated on a shaker (37 °C, 200 rpm) for 2 h, and the pH

value of the solution was then adjusted to 7.0 with 2 M NaOH to inhibit pepsin activity. During pepsin digestion, four groups of samples were labelled C-G, S₀-G, S₉-G, and SC-G.

After pepsin digestion, 10 mL of gastric digestive solution was withdrawn and mixed with 10 mL of simulated intestinal fluid, bile salts and pancreatin to simulate the environment of intestinal juice (containing 6.8 mmol/L KCl, 0.8 mmol/L KH₂PO₄, 85 mmol/L NaHCO₃, 38.4 mmol/L NaCl, 0.33 mmol/L MgCl₂(H₂O)₆, 0.60 mmol/L CaCl₂, 10 mmol/L Bile salt, 100 U/mL of final enzymatic activity) and was incubated on a shaker (37 °C, 200 rpm) for 2 h. After withdrawal, the solution was heat-treated at 95 °C for 5 min to terminate trypsin activity. During trypsin digestion, four groups of samples were labelled C-I, S₀-I, S₉-I, and SC-I.

2.8. Protein digestibility

According to Zou et al.^[20], protein digestibility was evaluated with minor modifications. Samples before and after *in vitro* digestion were mixed, respectively, with ethanol in a 1:3 (v/v) ratio and stored for 12 h at 4 °C, then centrifuged at 12,000 × *g* and 4 °C for 20 min to collect precipitate and supernatant, respectively. The protein content in the precipitates was detected using a BCA method with a commercial kit (Thermo Scientific, Rock-ford, IL). Protein digestibility was calculated as follows:

$$\text{Digestibility (\%)} = \frac{W_0 - W_1}{W_0} \times 100\% \quad (1)$$

W_0 and W_1 represent the protein content (g) in the precipitate before and after digestion, respectively.

2.9. Determination of the peptide content

A 15% TCA solution (w/v) was mixed with the digests in a 1:1 (v/v) ratio, stored at 25 °C for 20 min, and centrifuged at 5,000 × *g* for 10 min. Then, the peptide content in the collected supernatants was measured according to the Lowry method^[22] with casein phosphopeptide as a standard.

2.10. Free amino acid analysis (FAA)

All samples were freeze-dried at −60 °C after *in vitro* digestion. FAA assays in the digestion product were determined according to the method of Zou et al.^[20].

2.11. Determination of peptide sequences in the digestion product

This study applied peptidomics to identify the peptide sequences in the digestion product. The digestion product solution obtained in Section 2.7 was filtered through a 3 kDa ultrafiltration tube and then centrifuged at 12,000 × *g* for 30 min to collect the filtrate. The filtrate was desalted using a C18 column (Shimadzu, Kyoto, Japan), dried by vacuum centrifugation, and redissolved in ultrapure water to obtain the desalting solution.

The peptide sequences in the digestion product were identified by LC-MS/MS. The measurement procedure was conducted using the method described by Gao et al.^[23]. A 3 µL sample was analysed using an EASY-nLC 1000 equipped with a C18 column (Acclaim PepMap C18, 75 µm × 25 cm) and coupled to a Q Exactive™ Plus mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The flow rate was controlled at 300 nL/min, and the column temperature was 40 °C. The electrospray voltage was 2 kV. The binary mobile phase contained 0.1% aqueous formic acid (phase A) and 0.1% formic acid in acetonitrile (phase B). The chromatographic gradient was as follows: 0–3 min, 2–6% B; 3–42 min, 6–20% B; 42–47 min, 20–35% B; 47–48 min, 35–100% B; 48–60 min, 100% B. The mass spectrometer was run in the

data-dependent acquisition mode and automatically switched between MS and MS/MS modes. The parameters were set as follows: (1) MS: scan range (m/z) = 100–1500; resolution = 70,000; automatic gain control (AGC) target = $3e6$; maximum injection time = 50 ms; include charge states = 1–6; (2) HCD-MS/MS: resolution = 17,500; isolation window = 2.0; AGC target = $1e5$; maximum injection time = 45 ms; collision energy = 28. The peptides were searched using the UniProt database (<https://www.uniprot.org/peptidesearch/>). Mass tolerance was set as 0.1 Da for secondary ions and 20 ppm for primary ions, with a maximum of 2 allowed missed cleavage sites. No enzyme was selected to conduct enzymatic cleavage. The cut-off global false discovery rate (FDR) for peptide and protein identification was set to 1%.

2.12. Antioxidant activity assay

The digestion product solution obtained in Section 2.9 was filtered through a 3 kDa ultrafiltration tube and then centrifuged at $12,000 \times g$ for 30 min to collect the filtrate. The filtrate was the peptide sample solution (< 3 kDa) and was diluted to 1 mg/mL using 20 mM phosphate buffer at pH 7.0 and used within 24 h.

The radical scavenging activity of DPPH, hydroxyl, and ABTS of the peptide sample solution was determined following the method described by Zhu et al.^[24].

2.13. Screening of potential bioactive peptides

In this study, the bioactivity of peptides was scored with the PeptideRanker (<https://distilldeep.ucd.ie/PeptideRanker/>); peptides with scores higher than 0.5 were identified as potential bioactive peptides and further compared with the biopep (https://www.uwm.edu.pl/biochemia/biopep/start_biopep.Php)^[25].

2.14. Statistical analysis

Statistical analysis was performed using IBM SPSS software (SPSS Inc., Chicago, USA), and all data obtained were reported as mean value $\pm$ standard deviation (SD , $n = 3$ for all treatments). All experiments were carried out in triplicate. Data differences between different samples were analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple-range tests, and statistical significance was considered at $P < 0.05$.

3. Results and discussion

3.1. Protein profile

During ripening, the prolonged exposure of sausages to atmospheric oxygen induces the oxidative degradation of meat proteins. This primary biochemical reaction serves not only as a critical source of characteristic sausage flavor but also possesses unique nutritional significance^[26–28]. As shown in Fig. 1A, the meat protein profiles of the Chinese sausage samples exhibited significant modifications compared to the control. Specifically, the intensities of the bands corresponding to actin (45 kDa), troponin T (35 kDa), and myoglobin (17 kDa) decreased in the S₉ sample. This reduction is likely attributable to the activity of endogenous proteinases during drying, which promotes the degradation of these proteins. In comparison, the bands associated with myosin heavy chains (200–220 kDa) in the SC sample nearly disappeared, while bands

with molecular weights < 20 kDa increased. This pattern is similar to results reported for protein degradation in Harbin dry sausages inoculated with *S. xylosus*^[18]. The increase in low-molecular-weight bands may be attributed to the formation of low-molecular-weight peptides. Protein degradation during sausage processing is likely driven by the hydrolytic activity of endogenous enzymes combined with proteases produced by starter cultures such as *S. carnosus* RT6. Starters with strong protease activity enhance proteolysis by cleaving proteins into smaller peptides, thereby improving protein digestibility.

3.2. Secondary structure of pork protein

To further elucidate the differences in the secondary protein structures of fresh pork, traditional Chinese sausage, and Chinese sausage inoculated with *S. carnosus* RT6, FTIR spectra of meat protein were collected, as presented in Fig. 1B. The upper curve represents the original spectrum of dry sausage in the range of 1800–1500 cm^{-1} . Two characteristic absorption peaks were observed at 1656.79 cm^{-1} and 1546.85 cm^{-1} , corresponding to the amide I band (1600–1700 cm^{-1}), associated with C = O stretching, and the amide II band (1500–1600 cm^{-1}), attributed to N–H bending, respectively. These amide bands reflect the primary peptide backbone vibrations associated with protein secondary structure^[29]. After fermentation treatment, these absorption peaks exhibited varying degrees of reduced intensity and a blue shift (toward higher wavenumbers). This phenomenon is likely attributable to the fact that fermentation, particularly when accelerated by *S. carnosus* RT6, promotes the degradation of total sausage protein (Fig. 1B). This degradation facilitates protein unfolding, thereby exposing additional amino acid residues that serve as potential binding sites for digestive enzymes.

Generally, the 1600–1700 cm^{-1} region of the IR spectra provides the most detailed information on protein secondary structures and is commonly used to quantify the secondary structure distribution of meat proteins, as shown in Fig. 1C. Typically, α -helix, β -turn, and random coil structures are more flexible and disordered, whereas β -sheets are relatively stable and rigid^[30]. Compared with the control (fresh pork), the percentage of β -turns and random coils in the sausage proteins significantly increased ($P < 0.05$) following the nine-day fermentation period. In contrast, the β -sheet content significantly decreased ($P < 0.05$). This structural transition occurs because fermentation treatment disrupts intermolecular interactions, leading to the gradual unfolding of α -helices and the subsequent transformation of β -sheets into random coil structures. Given the significant contribution of β -sheet content to protein stability, it is hypothesized that these modifications enhance the sensitivity of proteins to digestive enzymes, thereby enhancing digestibility. Notably, the SC sausage exhibited the lowest percentage of β -sheet, which can be attributed to the proteolytic activity of *S. carnosus* breaking the intramolecular hydrogen bonds of the polypeptide chain, leading to protein and a looser myofibrillar protein structure^[17]. Furthermore, previous research indicated that the addition of *S. carnosus* RT6 decreased the pH of sausage, which may induce protein denaturation ($P < 0.05$). In conclusion, the protein hydrolysis and structural shifts induced by starters were mainly affected by proteolytic activity and pH fluctuations, which collectively enhance the susceptibility of the substrate to digestive enzymes^[31].

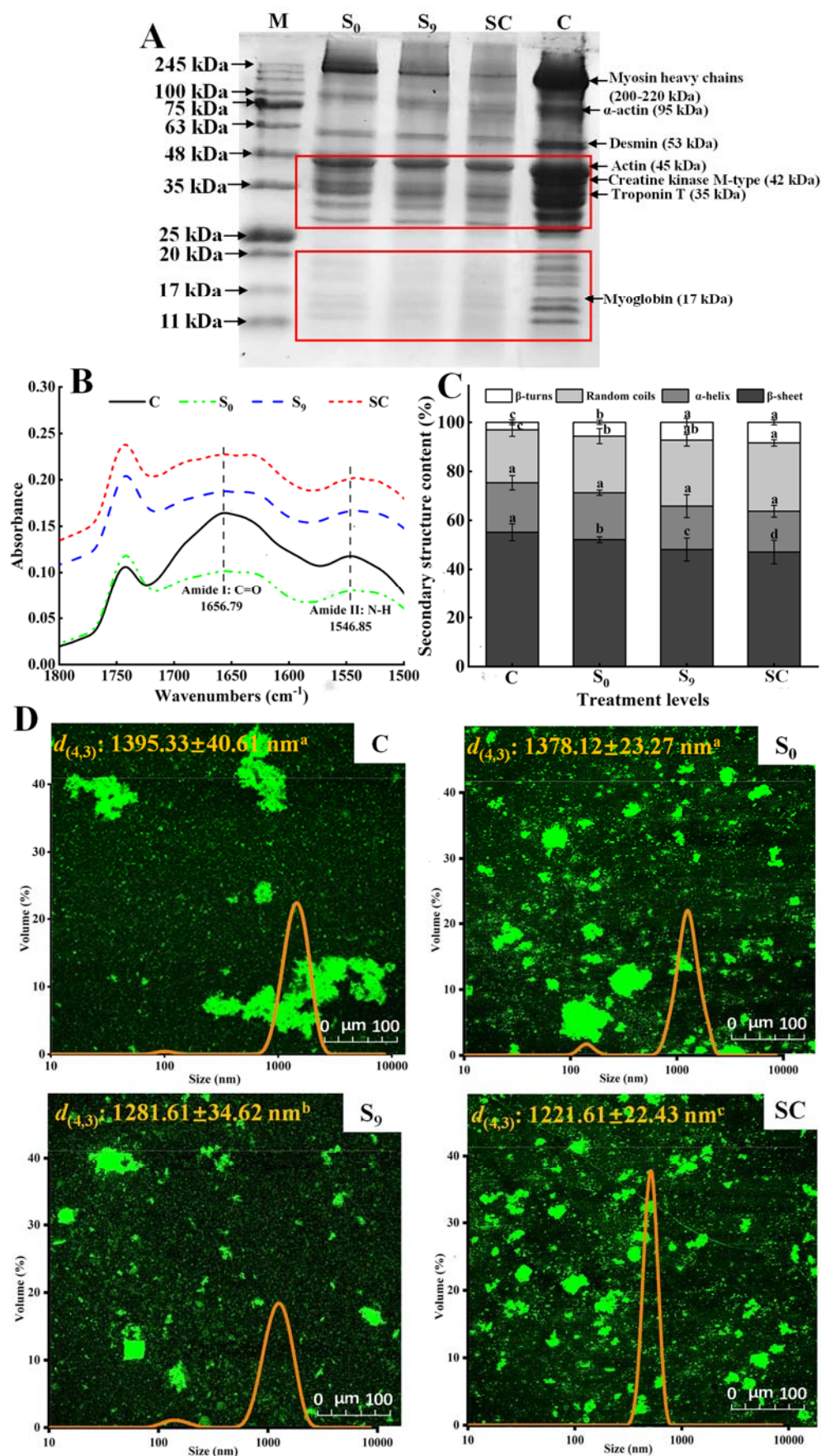


Fig. 1. SDS-PAGE (A), Fourier transform infrared spectra (B), secondary structure content (C), confocal laser scanning microscopy (CLSM) micrographs, and the mean volume particle size ($d_{(4,3)}$) (D) of the whole protein in four groups. C: fresh pork; S₀: Chinese sausage at 0 days of fermentation; S₉: Chinese sausage at 9 days of fermentation. SC: Chinese sausage inoculated with *S. carnosus* RT6 at 9 days of fermentation.

3.3. Microstructure and particle diameters of meat protein

Fig. 1D shows the CLSM images and average particle size ($d_{4,3}$) of the whole proteins from fresh pork and Chinese sausages during fermentation. Fresh pork (the control) consisted of protein structures with relatively large particles, and the distribution of meat protein was relatively concentrated in the solution, similar to a study by Liu et al.^[32], who investigated the stability of meat proteins in aqueous solution. Compared to the control, the aggregation ability of meat protein in Chinese sausage decreased gradually after 9 days of fermentation, and a relatively regular morphology was observed. Additionally, compared with other sausages, the distribution peak of protein particles in the SC sausages gradually shifted to the left, and the values of PDI and $d_{4,3}$ significantly decreased ($P < 0.05$), which is similar to the trend of CLSM images. This result was primarily due to the activity of extracellular protease, which breaks the chemical bonds between meat proteins, thereby inhibiting the aggregation of these proteins^[18,33]. We speculate that a lower degree of protein aggregation means a larger surface area exposed to digestive fluids, which may be beneficial for protein digestion.

3.4. Protein digestibility

As presented in Fig. 2A, after pepsin digestion, higher digestibility of proteins was observed in the S₉ and SC samples than in the control ($P < 0.05$). In particular, the protein digestibility of the SC sample showed a maximum increase of 24.52 % ($P < 0.05$). After further trypsin digestion, the proteins in the S₉ and SC samples were adequately digested, and their digestibility was still higher than that of the control. At the same time, there is no significant difference in protein digestibility between S₉ and SC samples, but SC still exhibited a slightly higher digestibility. This result could be attributed to two factors: first, the endogenous enzymes and enzymes secreted by microorganisms can directly degrade some proteins into small molecules such as peptides or amino acids during fermentation^[18,34]; second, the presence of beneficial microorganisms can not only reduce the size of protein particles and improve their dispersion uniformity (Fig. 1 D), but also promote protein unfolding, thereby increasing the contact area between proteins and digestive enzymes and exposing hidden cleavage sites within the proteins^[35]. Similarly, Zhang et al.^[9] reported that the high β -sheet and β -turn content of dry fermented sausage inoculated with *Lactobacillus plantarum* CD101 and *Staphylococcus simulans* NJ201 indicated the looser protein structure, resulting in high protein digestibility. This result suggests that the use of well-adapted starter cultures in fermented meat products increases the digestibility of pork protein.

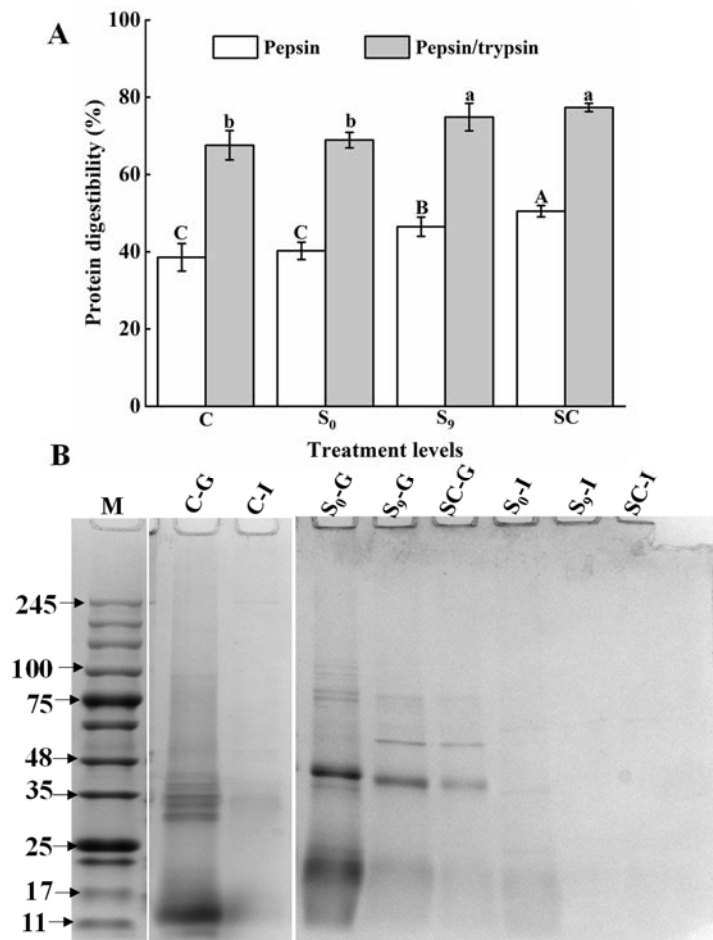


Fig. 2. Protein digestibility (A) and SDS-PAGE (B) of the whole protein in four groups after gastrointestinal digestion. M refers to the molecular weights of a protein standard. C: fresh pork; S₀: Chinese sausage at 0 days of fermentation; S₉: Chinese sausage at 9 days of fermentation; SC: Chinese sausage inoculated with *S. carnosus* RT6 at 9 days of fermentation.

3.5. Protein profile in the digestion product

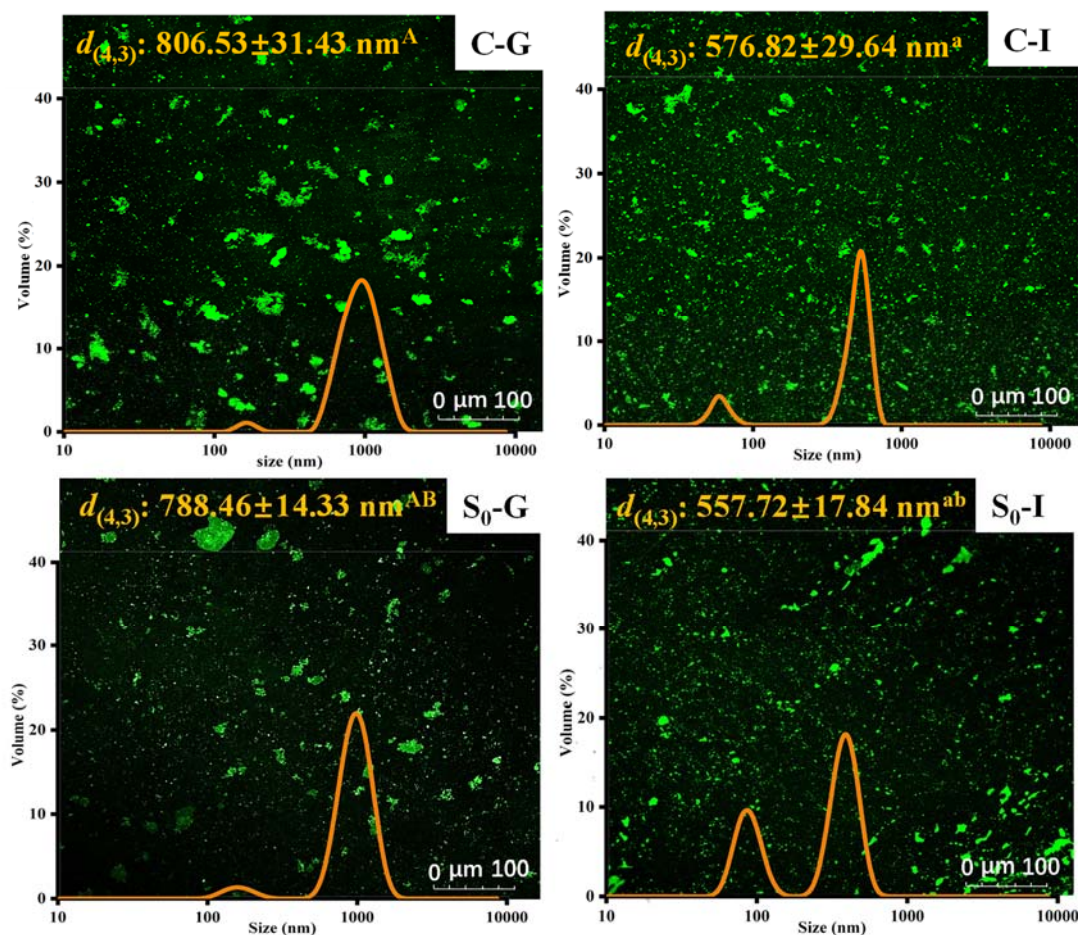
Fig.2B reflects the electrophoretic profiles of the total proteins in Chinese sausage during the simulated digestion. After pepsin digestion, pepsin cut high-molecular-weight proteins in all samples into smaller pieces. For the control, the proteins such as myosin (180-220 kDa) and actin (45 kDa) are degraded under the action of pepsin, and the optical density of the protein with a low molecular weight < 17 kDa was large, which was similar to that of pork products in the published article^[36]. For Chinese sausages, the molecular weight of the sample bands in the S₀ samples was mainly concentrated in the range of 100-63, 35, and < 17 kDa. Furthermore, only desmin (53 kDa) and actin (45 kDa) bands can be observed in the S₉ and SC samples, and a few smaller molecular weight fragments (< 20 kDa) could be observed at the bottom of the gels. After trypsin digestion, some bands can still be observed in the control, while almost no bands can be observed in the S₉ and SC samples. Zhang et al.^[9] observed similar results in dry fermented sausage inoculated with mixed starters during digestion. Fermentation treatment can denature proteins, making them more susceptible to enzymatic digestion. Therefore, after the two-step digestion with pepsin and trypsin, these proteins can be rapidly and efficiently hydrolysed into small fragments, usually with a molecular weight below about 5 kDa, which cannot be detected by SDS-PAGE, resulting in an 'almost no bands can be observed' outcome. This result indicated

that fermentation treatment and *S. carnosus* RT6 accelerated proteolysis, thus improving the hydrolysis pattern and digestive dynamics of protein in Chinese sausage.

3.6. Microstructure and particle diameters of protein in digestion product

The changes in protein particle size and microstructure cannot be ignored after digestion when trying to understand the differences in digestion characteristics between fresh pork and Chinese sausages. CLSM and average particle size ($d_{4,3}$) of meat protein after *in vitro* digestion are shown in Fig.3. All samples showed noticeable differences after gastrointestinal digestion. After pepsin digestion, the meat protein in all samples was degraded into fragments; these fragments are degraded into smaller fragments after further trypsin digestion. Meanwhile, the protein fragments of Chinese sausages are significantly smaller than those of fresh pork and are more evenly distributed in solution. This result may be because fermentation treatment can cause unfolding in the secondary structure of meat proteins, forming a conformation rich in random coils and β -turns, with more enzymatic cleavage sites exposed. This results in higher protein digestibility after gastrointestinal digestion, with fewer and smaller protein aggregates remaining in the solution^[37].

Additionally, the average particle size of meat protein exhibited trends similar to those of the CLSM images at the same digestion stage, showing a leftward shift in the distribution peak and a decrease in average particle size, respectively. Significantly, the SC samples showed the smallest mean particle size. This may be due to the fermentation treatment and the addition of *S. carnosus* RT6, which increases the contact area between meat protein and digestive enzymes, thereby improving the efficiency of enzyme reactions.



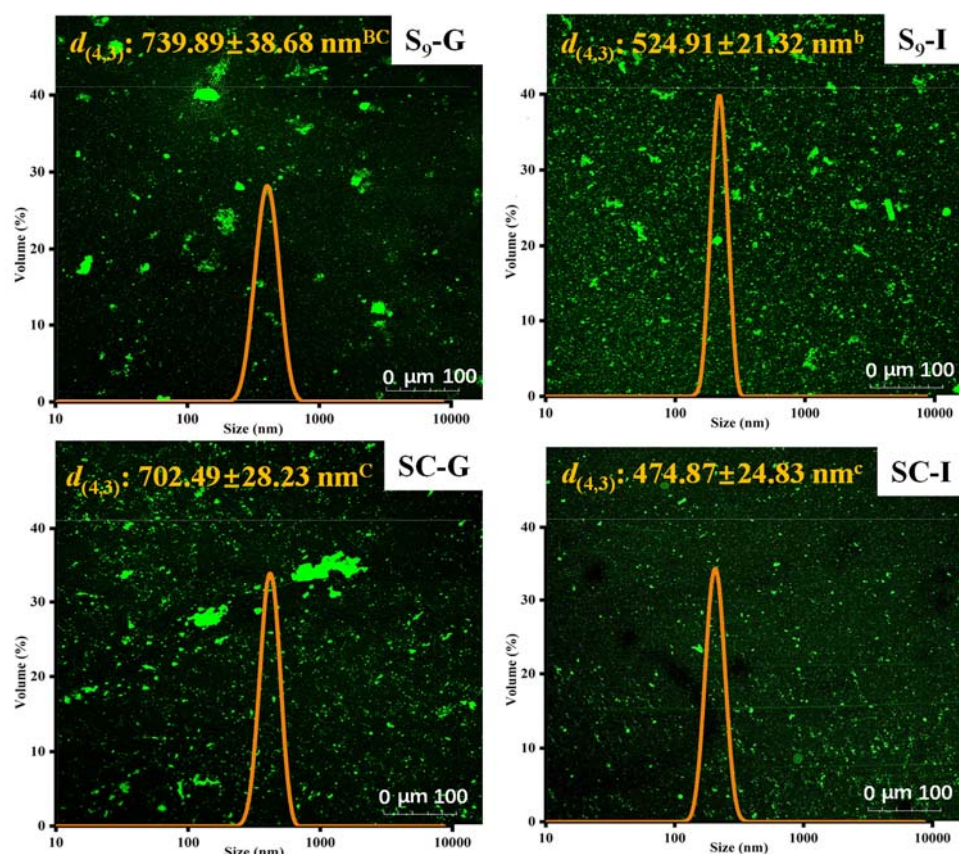


Fig. 3. Confocal laser scanning microscopy (CLSM) micrographs and the mean volume particle size ($d_{4,3}$) of the whole protein in four groups after gastrointestinal digestion. C-G: Gastric products of fresh pork. S₀-G: Gastric products of Chinese sausage at 0 days of fermentation. S₉-G: Gastric products of Chinese sausage at 9 days of fermentation. SC-G: Gastric products of Chinese sausage inoculated with *S. carnosus* RT6 at 9 days of fermentation. C-I: Intestinal products of fresh pork. S₀-I: Intestinal products of Chinese sausage at 0 days of fermentation. S₉-I: Intestinal products of Chinese sausage at 9 days of fermentation. SC-I: Intestinal products of Chinese sausage inoculated with *S. carnosus* RT6 at 9 days of fermentation.

3.7. Peptide content in digestion product

The peptide content (mg/mL) in fresh pork and Chinese sausage during the simulated digestion is shown in Fig. 4A. During the pepsin digestion process, the peptide content in S₉ and SC increased significantly ($P < 0.05$) by 19.6% and 24.1% compared to the control, which indicated that the fermentation treatment promoted the degradation of meat protein by pepsin, thus accelerating peptides production. However, the peptide content in S₉ and SC was significantly ($P < 0.05$) lower than that of the control during the trypsin digestion process, which may be due to the specific cut site of trypsin that can degrade some fragments of peptides to free amino acids, leading to a slower peptide production rate than its digestion rate^[31,38].

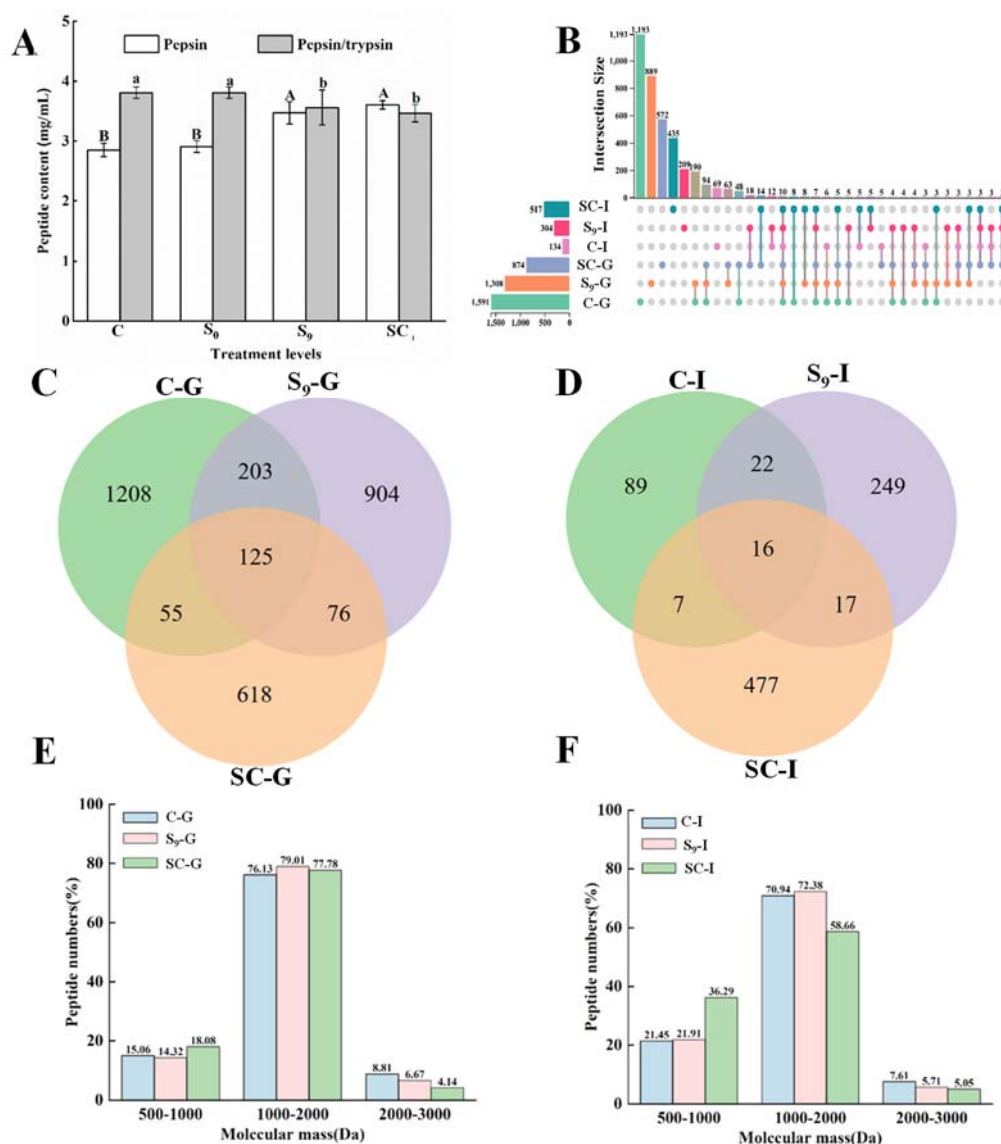


Fig. 4. Peptide content in four groups (A) after gastrointestinal digestion. Venn diagrams of peptide composition from the C, S₉, and SC groups after gastrointestinal digestion (B). Changes in peptide composition from the C, S₉, and SC samples in the gastric phase (C) and the intestinal phase (D). Peptide molecular mass distribution from the C, S₉, and SC samples in the gastric phase (E) and the intestinal phase (F). C-G: Gastric products of fresh pork. S₀-G: Gastric products of Chinese sausage at 0 days of fermentation. S₉-G: Gastric products of Chinese sausage at 9 days of fermentation. SC-G: Gastric products of Chinese sausage inoculated with *S. carnosus* RT6 at 9 days of fermentation. C-I: Intestinal products of fresh pork. S₀-I: Intestinal products of Chinese sausage at 0 days of fermentation. S₉-I: Intestinal products of Chinese sausage at 9 days of fermentation. SC-I: Intestinal products of Chinese sausage inoculated with *S. carnosus* RT6 at 9 days of fermentation.

3.8. FAA in digestion product

During protein digestion, FAAs are exposed by peptide bond cleavage and, therefore, can also be used to assess the degree of protein digestion^[38]. Table 1 shows the composition of FAA in the digestion product after digestion with pepsin/trypsin. The total FAA content was 1553.2, 1566.4, 1644.7, and 1711.8 µg/g for the control, S₀, S₉, and SC samples. The total FAA content in Chinese sausages was higher than in the control ($P < 0.05$), especially in the SC sample, which indicated that *S. carnosus* could promote sausage protein digestion, thus promoting amino acid release. It is speculated that after the pepsin digestion, the protein digestibility of fermented sausages is significantly higher than that of fresh meat, which initially promotes the

release of a large number of small-molecule peptides and free amino acids. These small peptides can be considered “preferred substrates” for the next stage of digestion^[39]. Due to their larger specific surface area, small peptides have more opportunities to interact with pancreatic enzymes in the intestine, making them ideal substrates for efficient and thorough hydrolysis into free amino acids.

Table 1 The free amino acids (FAAs) content (μg/g) from four groups after gastrointestinal digestion.

FAA	C-I	S ₀ -I	S ₉ -I	SC-I
Aspartic acid	26.9 ± 0.4 ^c	27.6 ± 0.9 ^c	39.1 ± 0.7 ^b	50.8 ± 1.1 ^a
Glutamic acid	23.7 ± 0.8 ^c	25.3 ± 0.5 ^c	38.6 ± 1.0 ^b	52.7 ± 0.8 ^a
Serine	12.1 ± 0.5 ^c	12.7 ± 0.7 ^{bc}	13.8 ± 0.5 ^b	15.1 ± 0.9 ^a
Glycine	14.9 ± 0.8 ^a	14.3 ± 0.5 ^a	8.8 ± 0.4 ^b	9.5 ± 0.5 ^b
Histidine	317.5 ± 8.3 ^a	312.5 ± 6.8 ^a	295.3 ± 6.9 ^a	289.6 ± 7.7 ^a
Threonine	58.9 ± 1.1 ^a	58.4 ± 0.8 ^a	57.9 ± 1.0 ^a	46.6 ± 1.2 ^b
Alanine	9.8 ± 0.5 ^b	10.6 ± 0.6 ^b	13.1 ± 0.9 ^a	14.2 ± 0.8 ^a
Arginine	260.9 ± 2.2 ^a	264.6 ± 3.2 ^a	242.6 ± 1.9 ^{ab}	217.5 ± 2.6 ^b
Proline	5.5 ± 0.3 ^b	5.9 ± 0.4 ^{ab}	6.3 ± 0.2 ^a	6.4 ± 0.5 ^a
Tyrosine	109.4 ± 1.9 ^c	110.2 ± 1.6 ^c	156.2 ± 3.7 ^b	189.9 ± 2.3 ^a
Valine	78.1 ± 1.1 ^a	76.3 ± 0.9 ^a	71.5 ± 0.5 ^{ab}	69.2 ± 0.8 ^b
Methionine	68.1 ± 1.0 ^b	69.8 ± 1.3 ^b	83.4 ± 1.4 ^a	85.6 ± 1.0 ^a
Cysteine	10.1 ± 0.4 ^b	10.8 ± 0.7 ^b	12.1 ± 0.3 ^{ab}	13.0 ± 0.2 ^a
Isoleucine	15.4 ± 0.6 ^{ab}	16.1 ± 0.6 ^{ab}	17.7 ± 0.7 ^a	17.3 ± 0.2 ^a
Leucine	211.7 ± 7.1 ^{ab}	214.5 ± 5.2 ^{ab}	222.6 ± 3.1 ^{ab}	237.1 ± 4.8 ^a
Phenylalanine	221.8 ± 3.9 ^b	226.3 ± 3.4 ^b	244.8 ± 4.2 ^{ab}	262.7 ± 5.1 ^a
Lysine	108.4 ± 2.9 ^b	110.5 ± 2.3 ^b	123.9 ± 1.8 ^{ab}	134.6 ± 1.1 ^a
Total EAA	1189.3 ± 13.6 ^b	1194.6 ± 16.4 ^b	1273.3 ± 15.8 ^{ab}	1332.6 ± 10.6 ^a
Total HAA	719.9 ± 6.3 ^c	729.7 ± 4.5 ^c	815.6 ± 6.6 ^b	882.4 ± 8.3 ^a
Total FAAs	1553.2 ± 19.2 ^b	1566.4 ± 23.8 ^b	1644.7 ± 13.5 ^{ab}	1711.8 ± 31.2 ^a

Different letters (a-d) within a row indicate significant differences ($P < 0.05$). C-I: Intestinal products of fresh pork. S₀-I: Intestinal products of Chinese sausage at 0 days of fermentation; S₉-I: Intestinal products of Chinese sausage at 9 days of fermentation. SC-I: Intestinal products of Chinese sausage inoculated with *S. carnosus* RT6 at 9 days of fermentation. HAA: Total hydrophobic amino acids; Total EAA: Total essential amino acids.

Furthermore, compared to the control, the digestion products of the S₉ sample showed a significant ($P < 0.05$) increase in the content of aspartic acid, glutamic acid, serine, alanine, tyrosine, and methionine. Furthermore, the SC sample also showed an increase in the content of aspartic acid, glutamic acid, serine, and tyrosine compared to the S₉ sample. Different profiles of FAA in the four groups suggest different degrees of protein digestion. This result was likely due to two factors: first, the endogenous enzymes and some microorganisms can directly degrade some proteins into amino acids during fermentation^[18,34]; second, the *S. carnosus* could have promoted the unfolding of meat protein and exposure of hidden cleavage sites previously buried in the protein^[9,35], promoting the binding of meat proteins to pepsin or trypsin, thereby accelerating the production of some amino acids. The free amino acids (FAAs) were classified into essential amino acids (EAA)—comprising methionine, valine, lysine, isoleucine, phenylalanine, leucine, tyrosine, threonine, and histidine—and hydrophobic amino acids (HAA), including leucine, alanine, valine, phenylalanine, methionine, proline, isoleucine, and tyrosine^[36]. The content of essential amino acids is an important factor determining the nutritional value of food. As shown in Table 1, the total EAA and HAA content in the SC sample is significantly higher than in the other groups. This indicates that the inoculation with *S. carnosus* RT6 can ferment sausages with higher nutritional value. Wang et al.^[17] found that when the active site of

protease from *S. epidermidis* bound to substrate actin, some residues wild fluctuated. The specific amino acids recognized by pepsin and trypsin are also susceptible to direct or indirect effects from mixed starter cultures, which may subsequently promote the hydrolysis kinetics of digestive enzymes, resulting in a significant increase in the content of EAA and HAA compared to the control group.

3.9. Peptide analysis in digests

3.9.1. Peptide sequences in digestion product

To characterize the changes of low-molecular-weight (< 3000 Da) water-soluble peptides in the digestive products of fresh pork and Chinese sausage at 9 days of ripening, the peptide composition was analyzed by nano-HPLC-MS/MS. The Venn diagrams of the peptides obtained from fresh pork and Chinese sausage are shown in Fig. 4B-D. Following pepsin digestion, the peptides were 1591, 1308, and 874 in the control, S₉, and SC samples, respectively. After trypsin digestion, the peptide numbers in these samples decreased to 134, 304, and 517 (Fig. 4B). Consistent with previous reports [37], the type of peptides is positively correlated with digestion efficiency during gastric digestion, while negatively correlated during intestinal digestion, indicating that the meat protein was digested more thoroughly in the intestine, which is consistent with the results of protein digestibility. Among the sequenced peptides, all samples shared 125 after pepsin digestion and 16 after trypsin digestion (Fig. 4C and D). As Escudero et al.^[40] observed in digested pork, these likely originated from the degradation of actin, myosin, and kinase. The fermentation treatment significantly influenced peptide profiles, with 1208, 904, and 618 unique peptides (newly released peptides) detected in the control, S₉, and SC samples after pepsin digestion, and correspondingly 89, 249, and 477 after trypsin digestion, which can be attributed to the metabolic characteristics of *S. carnosus* RT6^[41]. *S. carnosus* RT6 secretes extracellular proteases to perform 'pre-digestion' of muscle proteins during the fermentation phase. This process cleaves large molecular proteins into easily digestible low-molecular-weight peptides (< 3 kDa) and FAA, placing the initial substrates for subsequent *in vitro* gastrointestinal digestion in a structurally loosened, site-exposed, pre-activated state. This pre-degradation effect significantly reduces the hydrolysis barriers for pepsin, trypsin, and chymotrypsin, shortening the time required for complete enzymolysis and ultimately leading to a marked increase in the degree of hydrolysis and a rapid shift in the molecular weight distribution. At the same time, the protease's selective cleavage of the protein backbone disrupts the integrity of the protein's native conformation: tertiary and quaternary structures are dismantled, the stability of secondary structural elements decreases, and local conformational rearrangements occur around enzymatic cleavage sites, forming a more flexible substrate structure that is favorable for gastrointestinal enzyme activity, thereby promoting the production of low-molecular-weight peptides (< 3 kDa) and FAA.

3.9.2. Distribution of peptides in the digestion product

Based on the search range for the molecular weight of peptides in this study, the peptides were divided into three groups: 500 to 1000 Da, 1000 to 2000 Da, and 2000 to 3000 Da. As shown in Fig. 4E, the most significant percentage of peptides has molecular weights of 1000–2000 Da (> 50%), while the proportion of peptides in the 2000–3000 Da range is extremely low, indicating that pepsin tends to generate medium-length

peptides. After pepsin digestion, the proportion of peptides with a molecular weight of 2000–3000 Da in fresh pork was higher than that of Chinese sausages, while the proportion of peptides with molecular weights of 500–1000 Da was lower than that of the SC sample. With further digestion by trypsin (Fig. 4F), all samples showed a significant decrease in the proportion of medium-molecular-weight peptides (1000–2000 Da), accompanied by a concurrent sharp increase in small-molecular-weight peptides (500–1000 Da). This result indicates that the trypsin catalytic center cleaves larger molecular weight substrates more efficiently. The SC sample had the highest proportion of peptides in the 500–1000 Da range (36.29%) and the lowest proportion of peptides in the 1000–2000 Da range (58.66%), indicating that the meat protein in the SC samples was digested more thoroughly. This result may be attributed to the fact that the activity of *S. carnosus* RT6 in Chinese sausages promotes the unfolding of some meat proteins, exposing some of the buried binding sites, thereby accelerating the recognition of specific amino acids by pepsin and trypsin^[9], leading to more small peptide products (500–1000 Da) in Chinese sausages. Small peptides are easily absorbed by the human gut^[42].

3.9.3. The main peptides in the digestive product

After pepsin and pepsin/trypsin digestion, the top ten peptides with relatively high abundance in fresh pork and Chinese sausages are shown in Table 2. After digestion by pepsin, peptide 1 (AGDDAPRAVF), peptide 3 (VAPEEHPTL), peptide 5 (TVPPAVPGITF), peptide 13 (IKVPPPLPQF), and peptide 16 (VKEDQVFPMNPPKF) were found to be shared, while specific peptides were detected in different samples. Peptide 2 (FAGDDAPR), peptide 4 (FPPDVGGNVD), peptide 7 (FPPDVGGNVDYKN), peptide 9 (FPPDVGGNVDY), and peptide 12 (APPPPAEVHEVHE) were identified only in the control; peptide 15 (DIENDKQQL), peptide 19 (SGGQSEEEASINL), and peptide 20 (YELPDGQVIT) were identified only in the S₉ sample; peptide 11 (YALPHAIM), peptide 22 (AAFPPDVGGNVDYKN), and peptide 23 (TVIDQNRDGIIDKEDLRDTF) were identified only in the SC sample. Notably, the identified high-abundance peptides were mainly derived from myofibrillar proteins (e.g., myosin-4, actin, and Myosin light chain) and sarcoplasmic proteins (e.g., creatine kinase M-type, and aldolase) (Fig. 5A), which is attributed to the high proportion of myofibrillar proteins and sarcoplasmic proteins in meat protein. Additionally, there was a significant difference in the types of peptides produced by the same protein degradation in different treatment groups, which further confirms a change in enzyme cleavage sites. After digestion with pepsin/trypsin, the most abundant peptide in the control was derived from titin, accounting for 40% of the top ten peptides (Fig. 5B), followed by actin and troponin, which accounted for 20% and 20%, respectively. The most abundant peptides in the S₉ and SC samples were derived from troponin, accounting for 50% and 40% of the top ten peptides, respectively. Myosin light chain followed, accounting for 40% and 30%, respectively. These results can be interpreted as reflecting the digestion of proteins in Chinese sausage from a peptide perspective, further confirming that fermentation treatment and *S. carnosus* RT6 alter the digestion sites of meat proteins, leading to different peptide compositions in the digestion products.

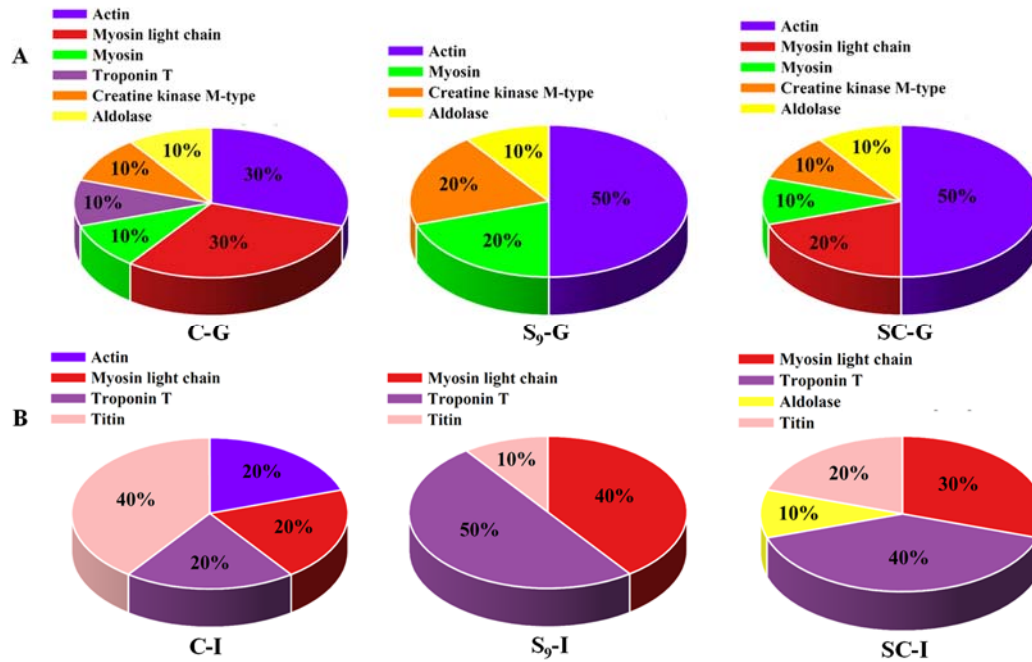


Fig. 5. Source of the top ten peptides from the C, S₉, and SC samples in the gastric phases (A) and the intestinal phases (B).

C-G: Gastric products of fresh pork. S₉-G: Gastric products of Chinese sausage at 9 days of fermentation. SC-G: Gastric products of Chinese sausage inoculated with *S. carnosus* RT6 at 9 days of fermentation. C-I: Intestinal products of fresh pork. S₉-I: Intestinal products of Chinese sausage at 9 days of fermentation. SC-I: Intestinal products of Chinese sausage inoculated with *S. carnosus* RT6 at 9 days of fermentation.

Table 2 The top ten peptides with relatively high abundance in fresh pork and Chinese sausage at 9 days of fermentation after gastrointestinal digestion.

No.	Peptide sequence	MW(Da)	Protein of origin	C-G	S ₉ -G	SC-G	C-I	S ₉ -I	SC-I
Peptide 1	AGDDAPRAVF	1018.49	Actin	√	√	√	√		
Peptide 2	FAGDDAPR	848.38	Actin	√					
Peptide 3	VAPEEHPTL	992.50	Actin	√	√	√			
Peptide 4	FPPDVGGNVD	1016.46	Myosin light chain	√					√
Peptide 5	TVPPAVPGITF	1098.61	Aldolase	√	√	√			
Peptide 6	DLAGRDLTDYL	1251.62	Actin		√	√			
Peptide 7	FPPDVGGNVDYKN	1421.66	Myosin light chain	√				√	
Peptide 8	DSGDGVTHNVPIYEG	1559.69	Actin		√	√			
Peptide 9	FPPDVGGNVDY	1179.53	Myosin light chain	√				√	
Peptide 10	FPPDVGGN	802.37	Myosin light chain					√	√
Peptide 11	YALPHAIM	915.47	Actin			√			
Peptide 12	APPPPAEVHEVHE	1408.68	Troponin T	√				√	
Peptide 13	IKVPPPLPQF	1135.68	Creatine kinase M-type	√	√	√			
Peptide 14	APPPPAEV	777.41	Troponin T						√
Peptide	DIENDKQQL	1102.53	Myosin		√				

15							
Peptide 16	VKEDQVFPMPNPPKF	1675.85	Myosin	√	√	√	
Peptide 17	AAFPPDVGGN	944.44	Myosin light chain				√
Peptide 18	RPVVPLIPPK	1212.78	Troponin T				√
Peptide 19	SGGQSEEEASINL	1320.59	Aldolase		√		
Peptide 20	YELPDGQVIT	1134.56	Actin		√		
Peptide 21	PVVPLIPPK	1056.68	Troponin T				√
Peptide 22	AAFPPDVGGNVDYKN	1563.74	Myosin light chain			√	
Peptide 23	TVIDQNRDGIIDKEDL RDTF	2363.18	Myosin light chain			√	
Peptide 24	SPIRLSP	769.45	Titin			√	
Peptide 25	PELLPDGDHD	1107.49	Aldolase				√
Peptide 26	FPPDVGGNVDYK	1307.62	Myosin light chain				√
Peptide 27	IPEGEKVDFDDIQKK	1760.90	Troponin T				√
Peptide 28	VPPKPEVPPP	1056.60	Titin				√
Peptide 29	APPPPAEVHEVHEEVH	1773.85	Troponin T			√	
Peptide 30	APPPPAEVHEVH	1279.64	Troponin T				√
Peptide 31	EEPEAPPPPAVPEEPK	1712.83	Titin				√
Peptide 32	PSRPVVPPLIPPK	1396.86	Troponin T				√
Peptide 33	IPEGEKVDFDDIQK	1632.81	Troponin T				√
Peptide 34	APPPPAEVHE	1043.51	Troponin T				√
Peptide 35	IKPPPVEPPPAPI	1351.79	Titin				√
Peptide 36	SEKERLEAQ	1089.55	Myosin			√	
Peptide 37	EAGPSIVHRK	1093.61	Actin			√	
Peptide 38	EPREPGHVEESHAQQT T	1931.88	Titin			√	
Peptide 39	EPREPGHVEESHAQQ	1729.78	Titin			√	
Peptide 40	EPREPGHVEESH	1402.63	Titin			√	
Peptide 41	APPPPAEVH	914.47	Troponin T			√	
Peptide 42	IGEDPKF	805.40	Myosin			√	

Note: C-G: Gastric products of fresh pork. S₉-G: Gastric products of Chinese sausage at 9 days of fermentation. SC-G: Gastric products of Chinese sausage inoculated with *S. carnosus* RT6 at 9 days of fermentation. C-I: Intestinal products of fresh pork. S₉-I: Intestinal products of Chinese sausage at 9 days of fermentation. SC-I: Intestinal products of Chinese sausage inoculated with *S. carnosus* RT6 at 9 days of fermentation.

Actin is an abundant protein in skeletal muscle, and its structure is easily influenced by the external environment during the fermentation process of meat products, thereby affecting its digestion and absorption. In this study, pepsin has been shown to hydrolyze actin (Table 2), to further clarify the locations of degraded peptides from fresh pork and sausage digests, the 3D structure of actin affected by spatial structure is shown in Fig. 6. Some new digestion regions were found in the actin of gastric digests from the S₉ (Fig. 6B) and SC (Fig. 6C) samples, indicating that fermentation treatment and *S. carnosus* increased the number of cleavage sites, thereby promoting digestion. During fermentation, the protein conformation was altered and rearranged, thereby breaking the steric hindrance of actin and increasing the accessibility of digestive enzymes to meat protein. This result suggests that the changes in the peptide profile were associated with the unfolding of the protein structure during fermentation.

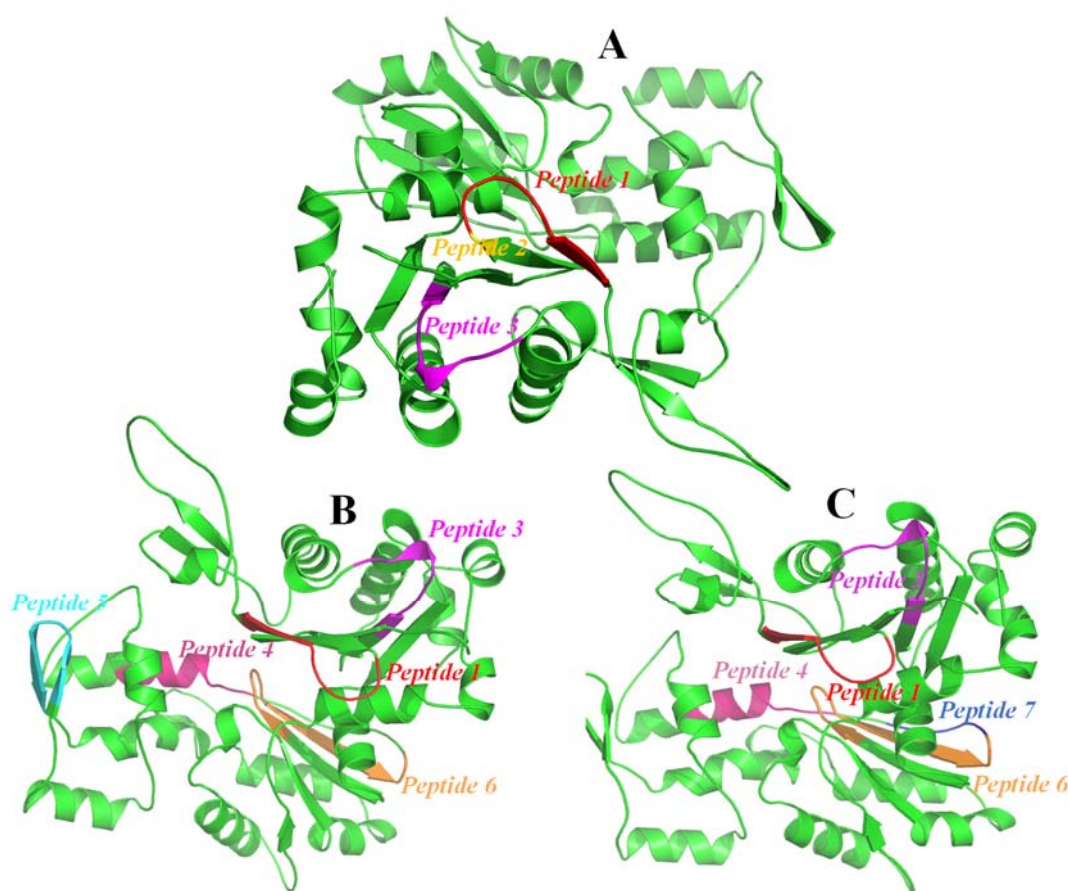


Fig. 6. Distribution of degraded peptides in the regions of actin from the C (A), S₉ (B), and SC samples (C). The positions of the new digested peptides of the sample's digests were highlighted by corresponding colors. C: fresh pork; S₉: Chinese sausage at 9 days of fermentation; SC: Chinese sausage inoculated with *S. carnosus* RT6 at 9 days of fermentation.

3.10. Antioxidation activities of digestion product in vitro

Bioactive peptides are short-chain molecules composed of approximately 2–20 amino acids, and their antioxidant activity is mainly evaluated by free radical scavenging capacity. The antioxidation activities of digestion products from the control and Chinese sausages were determined as shown in Fig. 7A–C. The radical scavenging activity of the peptide extracts from digestion products showed significant differences ($P < 0.05$) between the control (fresh pork) and Chinese sausages. Among them, the digestion product from the SC sample exhibited the highest radical scavenging activity of DPPH (Fig. 7A), hydroxyl (Fig. 7B), and ABTS

(Fig. 7C) ($P < 0.05$). The molecular weight distribution of peptides in digestive products is closely related to their antioxidant activity. Low molecular weight peptides (< 3 kDa, especially < 1 kDa) generally exhibit stronger DPPH/ABTS free radical scavenging ability and hydroxyl reducing power, mainly due to their higher membrane permeability—small peptides can more easily cross cell membranes or lipid bilayers to reach sites of reactive oxygen species generation. In addition, the active groups of small peptides (phenolic hydroxyl, imidazole, sulfhydryl, etc.) exhibit lower steric hindrance and greater reaction accessibility; meanwhile, their excellent water solubility also facilitates diffusion and activity in biological systems. After digestion with pepsin/trypsin, the digestion products of the SC sample had the highest number of peptides and the highest proportion of small peptides (< 1000 Da), suggesting that they may be the main contributors to antioxidant activity. Zou et al.^[20] also found that the more peptides with a molecular weight of < 1000 Da in the digestion products of pork sausages, the stronger the antioxidant activity. In summary, these results suggest that the digestion products of Chinese sausage inoculated with *S. carnosus* RT6 exhibit a superior antioxidation ability.

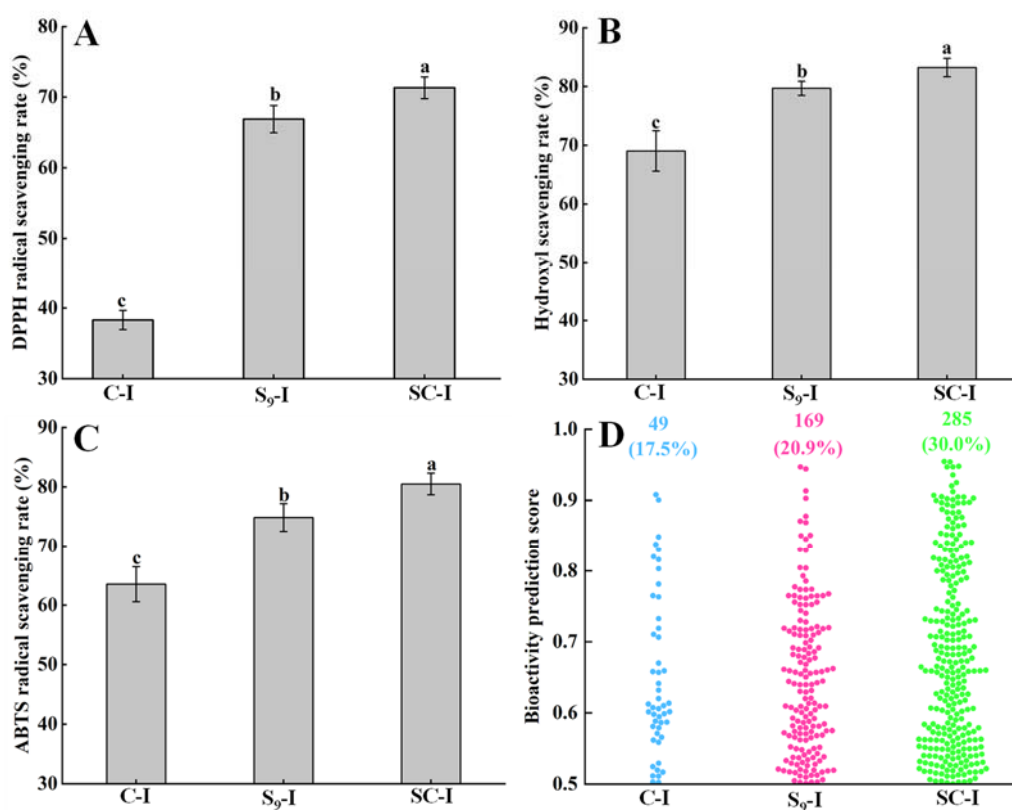


Fig. 7. Antioxidant activity of intestinal products of the C, S₉, and SC samples. DPPH radical scavenging rate (A), hydroxyl ($\cdot$ OH radical) scavenging rate (B), and ABTS radical scavenging rate (C). (D) The potentially bioactive peptides predicted by PeptideRanker. C-I: Intestinal products of fresh pork. S₉-I: Intestinal products of Chinese sausage at 9 days of fermentation. SC-I: Intestinal products of Chinese sausage inoculated with *S. carnosus* RT6 at 9 days of fermentation.

3.11. Screening of potential bioactive peptides

The functional activity of digestion products from the control and Chinese sausages was predicted using PeptideRanker. Typically, peptides with a threshold greater than 0.5 were considered potential bioactive peptides, and the higher the predicted score, the higher the probability of displaying biological activity. The

score and percentage of the potential bioactive peptides released are shown in Fig. 7D. It can be observed that the number and percentage of potential bioactive peptides in the digestive products from Chinese sausages are higher than those in fresh pork. To further elucidate the specific functions of each peptide, it was searched in the BIOPEP database. In this study, only the top 48 intensities of peptides with a score greater than 0.80 in digests of the sample were considered and are displayed in Table 3. After digestion with pepsin/trypsin, only RPPPGPPPP with DPP IV inhibitor and RPPPGPPPPGPPPPGP and APPGARPPPGPPPPPPGP with dipeptidyl carboxypeptidase inhibitor were derived from the control (fresh pork). In contrast, the other 42 peptides were all derived from Chinese sausage and had potential biological activity. This result suggests that the fermentation treatment can promote the digestion of meat protein, thereby producing many bioactive peptides. In addition, the SC sample also released unique peptides, such as GPGGPGGPGGPPG, PGSPGPRGLPG, GPGPVWPSGP, HPGSPGPPGNPGLPGVPG, PGSPGPPGSPG, GPSPGPGPSPG, GPADPPPTRGSGGP, and GPPGEMGPPG, which exhibit anti-amnesic and antithrombotic activities. These peptides were primarily derived from the degradation of collagen, actin, and G protein-coupled receptors, indicating that the increase in bioactive peptides is closely related to the degree of proteolysis.

Table 3 Identified (potential) bioactive peptides in the digestion products of fresh pork and Chinese sausage at 9 days of fermentation after gastrointestinal digestion. Only the peptides with a score > 0.8 in digests of all samples were considered.

No.	Identified sequence	Mass (Da)	Sample ID	Parent protein	BIOSE P sequences	Activity	Score
1	GPPPPRP	717.40	C-I, S ₉ -I, SC-I	Splicing factor 1	RP	ACE inhibitor/Neuropeptide/DPP IV inhibitor/Neurolysin inhibitor/Nepilysin inhibitor	0.95
2	GIGGAVIGMI GNVGAIAGP S	1824.00	C-I, S ₉ -I, SC-I	TS beta-glucoside transporter subunit EIIBCA	PS	DPP IV inhibitor	0.95
3	PGPPPPR	717.40	SC-I	Splicing factor 1	PR	ACE inhibitor/DPP III inhibitor	0.94
4	GPGPSGGPG GGGGG	967.42	SC-I	Serine and arginine rich splicing factor 11	GG	ACE inhibitor/DPP IV inhibitor/Nepilysin inhibitor	0.93
5	PGPSGGPGG GGGGG	967.42	C-I, S ₉ -I, SC-I	Serine and arginine rich splicing factor 11	GG	ACE inhibitor/DPP IV inhibitor/Nepilysin inhibitor	0.92
6	FPAGSRPF	878.45	SC-I	Alpha-amylase	PF	DPP IV inhibitor/DPP III inhibitor	0.92
7	GVGPPPPPP LP	1121.63	S ₉ -I, SC-I	Protein diaphanous homolog 1 isoform 1	PPPLP	Anti-amnesic	0.91
8	GPGGPGGPG GPPG	960.45	SC-I	Dynamin GTPase	PG	ACE inhibitor/Anti-amnesic/Antithrombotic/Regulating/DPP IV inhibitor/PAM inhibitor	0.91

9	RPPPGPPPP	911.509	C-I	Basic proline-rich protein	PPPP	DPP IV inhibitor	0.90
10	GSPPTFGGSP GFGGV	1320.62	SC-I	Nucleoporin 214	GGV	HMG-CoA reductase inhibitor	0.90
11	RPPPPPPAPP K	1150.67	SC-I	Titin	PAPPK	ACE inhibitor	0.90
12	PGSPGPRGLP G	991.53	SC-I	Collagen IV NC1 domain-containing protein	PG	ACE inhibitor/Antiamnestic/Antithrombotic/Regulating/DPP IV inhibitor/PAM inhibitor	0.90
13	SSGFGGGFV CAPGTGHGG A	1679.72	SC-I	IF rod domain-containing protein	GGA	Inhibitor of cytosol alanyl aminopeptidase	0.90
14	GPGGPGGPG GPGP	960.45	SC-I	Dynamin GTPase	GP	ACE inhibitor/Antiamnestic/Antithrombotic/Regulating/Antioxidative/Hypotensive/DPP IV inhibitor/Neprilysin inhibitor	0.90
15	KPRPPPPPP	982.58	C-I, S ₉ -I	Titin	PPPP	DPP IV inhibitor	0.90
16	SFSGWNGGG RLYGK	1485.72	SC-I	Heparin and heparin-sulfate lyase	GK	ACE inhibitor	0.90
17	DPIAPPGPPFP K	1232.66	SC-I	Titin	PK	Antioxidative/DPP IV inhibitor	0.90
18	RPPPGPPPPG PPPPGP	1510.81	C-I	Basic proline-rich protein	PPGP	Dipeptidyl carboxypeptidase inhibitor	0.90
19	GPGGPGGPG GAGGAV	1064.51	SC-I	RHS repeat-associated protein	AV	ACE inhibitor/DPP IV inhibitor	0.89
20	GPSGPPGPEG FP	1095.51	SC-I	Fibrillar collagen NC1 domain-containing protein	FP	ACE inhibitor/DPP IV inhibitor	0.89
21	GPGPVWPSG P	950.47	SC-I	G protein-coupled receptor 132	GP	ACE inhibitor/Antiamnestic/Antithrombotic/Regulating/Antioxidative/Hypotensive/DPP IV inhibitor/Neprilysin inhibitor	0.89
22	AGPAQPPPW PQPV	1341.69	SC-I	Synaptopodin 2	PV	DPP IV inhibitor	0.89
23	KPRPPPPP	885.53	SC-I	Titin	PPPP	DPP IV inhibitor	0.89
24	IGGAVIGMIG NVGAIAIGPS G	1824.00	SC-I	PTS β -glucoside transporter subunit	SG	ACE inhibitor	0.89
25	PISPPGPPIPR	1127.65	SC-I	EIIBCA	PR	ACE inhibitor/DPP III inhibitor	0.89
26	GHLDFFPNG GK	1188.57	SC-I	Titin	PR	ACE inhibitor/DPP III inhibitor	0.89
27	HPGSPGPPGN PGLPGVPG	1590.80	SC-I	Pancreatic triacylglycerol lipase	GK	ACE inhibitor	0.88
28	WFPAGSRP	917.46	SC-I	Collagen type XVI alpha 1 chain	PG	ACE inhibitor/Antiamnestic/Antithrombotic/Regulating/DPP IV inhibitor/PAM inhibitor	0.88
29	PGAGRPWP	837.43	SC-I	Alpha-amylase	RP	ACE inhibitor/Neuropeptide/DPP IV inhibitor/Neurolysin inhibitor/Neprilysin inhibitor	0.88
				Single stranded DNA binding protein 3	WP	ACE inhibitor/DPP IV inhibitor	0.88

30	KGPGSQHAP FLF	1285.66	S ₉ -I	YedE-related selenium metabolism membrane protein TBC1 domain family member 10C	LF	ACE inhibitor	0.87
31	GPAARFCAG	906.42	SC-I		AG	ACE inhibitor/DPP IV inhibitor	0.87
32	PGSPGPPGSP G	906.43	SC-I	Collagen type II alpha 1 chain	PG	ACE inhibitor/Antiamnestic/Antithrombotic/ Regulating/DPP IV inhibitor/PAM inhibitor	0.87
33	KPPPRPAPPP P	1150.67	S ₉ -I	Stoned B-like protein PDZ	PPPP	DPP IV inhibitor	0.87
34	GPGLGPRP	750.42	S ₉ -I	domain-contai ning protein	GPRP	Antithrombotic	0.86
35	GPSPGPGPSP G	906.43	SC-I	Actin	PG	ACE inhibitor/Antiamnestic/Antithrombotic/ Regulating/DPP IV inhibitor/PAM inhibitor	0.86
36	WRPPQPI	893.49	SC-I	Carbonic anhydrase	PI	DPP IV inhibitor	0.86
37	GPADPPPTRG SGGP	1262.61	SC-I	Ubiquitin E3 ligase ICP0	GP	ACE inhibitor/Antiamnestic/Antithrombotic/ Regulating/Antioxidative/Hypotensive/ DPP IV inhibitor/Neprilysin inhibitor	0.86
38	SPPGPPGTPR	962.50	SC-I	Titin	PR	ACE inhibitor/DPP III inhibitor	0.86
39	NWRPPQPL	1007.54	SC-I	Carbonic anhydrase	PL	ACE inhibitor/DPP IV inhibitor/Xaa-pro inhibitor/Lactocepin inhibitor	0.86
40	GLSPIFPGGG AGG	1086.55	SC-I	Elastin	GG	ACE inhibitor/DPP IV inhibitor/neprilysin inhibitor	0.85
41	GPPGEMGPP G	895.39	SC-I	Putative cuticular collagen	PG	ACE inhibitor/Antiamnestic/Antithrombotic/ Regulating/DPP IV inhibitor/PAM inhibitor	0.85
42	GPPGAAGAP GPQGFQGP	1462.70	SC-I	Collagen alpha-2(I) chain	QGP	ACE inhibitor	0.85
43	SAWDFNDGK	1039.44	SC-I	Alpha-amylase	GK	ACE inhibitor	0.85
44	RPPPPPPAPP KE	1619.89	S ₉ -I	Titin	KE	ACE inhibitor/DPP IV inhibitor	0.85
45	HDPSLLPWT	1065.53	S ₉ -I	Carbonic anhydrase	PWT	Antioxidative	0.84
46	APPGARPPPG PPPPPPGP	1652.89	C-I	Basic proline-rich protein	PPGP	Dipeptidyl carboxypeptidase inhibitor	0.84
47	KPRPPPPPPA PPK	1375.82	S ₉ -I, SC-I	Titin	PAPPK	ACE inhibitor	0.84
48	PAPAPAPPK	845.48	SC-I	EF-hand domain-contai ning protein	PAPPK	ACE inhibitor	0.84

Note: C-I: Intestinal products of fresh pork. S₉-I: Intestinal products of Chinese sausage at 9 days of fermentation. SC-I: Intestinal products of Chinese sausage inoculated with *S. carnosus* RT6 at 9 days of fermentation.

4. Conclusions

This study applied *S. carnosus* RT6 to the fermentation of Chinese sausages. By analyzing protein digestibility, protein profiles, and peptide composition, it first revealed the regulatory mechanism of *S.*

carnosus RT6 in the protein digestion process. It clarified the molecular pathway through which it promotes the release of bioactive peptides. Subsequent studies will conduct *in vitro* cell experiments to verify the physiological functions of bioactive peptides and optimize the inoculation process of the *S. carnosus* RT6 strain to develop Chinese sausage products with high digestibility and high bioactivity.

Declaration of interests

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.

References

- L. Yang, S. Chen, Z.P. Jiang, et al., Application of sodium alginate-pectin active intelligent film containing anthocyanins from *Aronia melanocarpa* and tea polyphenols in pork preservation and freshness monitoring, *Meat Sci.* 230 (2025) 109931. <https://doi.org/10.1016/j.meatsci.2025.109931>.
- S.Q. Zhu, Q. Li, M. Fu, et al., Study on the effect of tetrabutyl vitamin C ester on the freshness preservation of pork stored at a low temperature, *Food Chem. X.* 30 (2025) 103011. <https://doi.org/10.1016/j.fochx.2025.103011>.
- X.F. Shao, H.H. Wang, X.Y. Song, et al., Elucidating the pattern of flavor evolution during the steaming process of fermented sausages in two dimensions: Strain fermentation and steaming time, *Food Chem.* 480 (2025) 143945. <https://doi.org/10.1016/j.foodchem.2025.143945>.
- H. Wang, S.Q. Zhao, X.F. Xia, et al., Interaction of the extracellular protease from *Staphylococcus xylosus* with meat proteins elucidated via spectroscopic and molecular docking, *Food Chem. X.* 21 (2024) 101204. <https://doi.org/10.1016/j.fochx.2024.101204>.
- D.D. Wang, Z.Q. Xu, Y.Z. Wang, et al., Identification and characterization of novel antioxidant peptides from Yunnan dry-cured beef: A combined *in silico* and *in vitro* study, *Food Chem.* 477 (2025) 143485. <https://doi.org/10.1016/j.foodchem.2025.143485>.
- H.Y. Li, H.B. Fan, Z.H. Wang, et al., Effect of simulated gastrointestinal digestion on antioxidant, and anti-inflammatory activities of bioactive peptides generated in sausages fermented with *Staphylococcus simulans* QB7, *Food Sci. Hum. Wellness.* 13 (2024) 1662-1671. <https://doi.org/10.26599/FSHW.2022.9250145>.
- F.R. Han, Q.C. Wu, S. Li, et al., Quality enhancement and metabolic mechanism of fermented sausages with *Lactiplantibacillus plantarum* and *Companilactobacillus alimentarius*, *Food Biosci.* 71 (2025) 107287. <https://doi.org/10.1016/j.fbio.2025.107287>.
- H. Wang, C.J. Yu, Z. Wang, et al., Exploring *in vitro* protein digestion of dry fermented sausage under different thermal processing methods: Peptidomics and molecular simulation, *Food Chem. X.* 33 (2026) 103515. <https://doi.org/10.1016/j.fochx.2026.103515>.
- Y.W. Zhang, M.Q. Feng, J. Zhang, et al., Peptidomics insights into the interplay between the pre-digestion effect of mixed starters and the digestive pattern of sausage proteins, *Food Res. Int.* 162 (2022) 111963. <https://doi.org/10.1016/j.foodres.2022.111963>.
- M. Gawat, M. Boland, J. Chen, et al., Effects of microwave processing in comparison to sous vide cooking on meat quality, protein structural changes, and *in vitro* digestibility, *Food Chem.* 434 (2024) 137442. <https://doi.org/10.1016/j.foodchem.2023.137442>.
- S. Ribes, L. Aubry, M. Kristiawan, et al., Fava bean (*Vicia faba* L.) protein concentrate added to beef burgers improves the bioaccessibility of some free essential amino acids after *in vitro* oral and gastrointestinal digestion, *Food Res. Int.* 177 (2024) 113916. <https://doi.org/10.1016/j.foodres.2023.113916>.
- Z.H. Qiu, Y.Y. Shi, Y. Zheng, et al., Comparison of *in vitro* digestive characteristics of proteins from different sources in simulated elderly gastrointestinal conditions, *Food Chem.* 463 (2025) 141299. <https://doi.org/10.1016/j.foodchem.2024.141299>.

- C.Z. Zhu, M.J. Zhang, H. Chen, et al., Effect of NaCl on the structure and digestive properties of heat-treated myofibrillar proteins, Food Chem. 463 (2025) 141521. <https://doi.org/10.1016/j.foodchem.2024.141521>.
- D. Zhao, Y.J. Xu, T.Y. Gu, et al., Peptidomic investigation of the interplay between enzymatic tenderization and the digestibility of beef semimembranosus proteins, J. Agric. Food Chem. 68 (2020) 1136-1146. <https://doi.org/10.1021/acs.jafc.9b06618>.
- J.H. Park, S.I. Lee, W.S. Kwon, et al., Health benefits of co-supplementing mealworm protein hydrolysate and cranberry fruit extract, Ital. J. Food Sci. 35 (2023) 1-9. <https://doi.org/10.15586/ijfs.v35i1.2264>.
- H. Singh, R. Sharma, A. Gupta, et al., Characterization of jackfruit seed enriched pasta: product-functionality profile, secondary protein structures, bioactive composition and molecular morphology, Qual. Assur. Saf. Crops Foods. 15 (2023) 11-19. <https://doi.org/10.15586/qas.v15i2.1217>.
- H. Wang, J.H. Xu, Q. Liu, et al., Interaction between protease from *Staphylococcus epidermidis* and pork myofibrillar protein: Flavor and molecular simulation, Food Chem. 386 (2022) 132830. <https://doi.org/10.1016/j.foodchem.2022.132830>.
- F.D. Sun, H. Wang, Q. Liu, et al., Proteolysis and quality characteristics of Harbin Chinese sausages caused by the addition of *Staphylococcus xylosus* protease, Food Chem. 404 (2023) 134692. <https://doi.org/10.1016/j.foodchem.2022.134692>.
- B. Arslan, Y.L.L. Xiong, A. Soyer, Antioxidant potential and stability of bovine liver peptides in fermented sausage (sucuk): A pathway to value-added functional meat products, Food Biosci. 62 (2024) 105209. <https://doi.org/10.1016/j.fbio.2024.105209>.
- L.F. Zou, X. Yu, Y. Zhou, et al., *In vitro* digestibility of proteins, peptidomic analysis and antioxidant ability of sodium-reduced pork sausage with partial substitution of NaCl by KCl, Meat Sci. 197 (2023) 109049. <https://doi.org/10.1016/j.meatsci.2022.109049>.
- A. Brodkorb, L. Egger, M. Alminger, et al., INFOGEST static *in vitro* simulation of gastrointestinal food digestion, Nat Protoc. 14 (2019) 991-1014. <https://doi.org/10.1038/s41596-018-0119-1>.
- I.D. Nwachukwu, R.E. Aluko, A systematic evaluation of various methods for quantifying food protein hydrolysate peptides, Food Chem. 270 (2019) 25-31. <https://doi.org/10.1016/j.foodchem.2018.07.054>.
- B.H. Gao, X.B. Hu, H. Xue, et al., Isolation and screening of umami peptides from preserved egg yolk by nano-HPLC-MS/MS and molecular docking, Food Chem. 377 (2022) 131996. <https://doi.org/10.1016/j.foodchem.2021.131996>.
- S. Zhu, W. Yang, Y. Lin, et al., Antioxidant and anti-fatigue activities of selenium-enriched peptides isolated from *Cardamine violifolia* protein hydrolysate, J. Funct. Foods. 79 (2021) 104412. <https://doi.org/10.1016/j.jff.2021.104412>.
- Y. Gong, M.Q. Feng, J. Sun, Effect of different thermal processing methods and thermal core temperatures on the protein structure and *in vitro* digestive characteristics of beef, Food Chem. 464 (2025) 141751. <https://doi.org/10.1016/j.foodchem.2024.141751>.
- Y.Y. Hu, Q. Chen, R.X. Wen, et al., Quality characteristics and flavor profile of Harbin Chinese sausages inoculated with lactic acid bacteria and *Staphylococcus xylosus*, LWT-Food Sci. Technol. 114 (2019) 108392. <https://doi.org/10.1016/j.lwt.2019.108392>.
- L. Mei, D.M. Pan, T.T. Guo, et al., Role of *Lactobacillus plantarum* with antioxidation properties on Chinese sausages, LWT-Food Sci. Technol. 162 (2022) 113427. <https://doi.org/10.1016/j.lwt.2022.113427>.
- K. Zampouni, A. Soniadis, D. Dimakopoulou-Papazoglou, et al., Modified fermented sausages with olive oil oleogel and NaCl-KCl substitution for improved nutritional quality, LWT-Food Sci. Technol. 158 (2022) 113172. <https://doi.org/10.1016/j.lwt.2022.113172>.
- M.A. Pissia, A. Matsakidou, A. Paraskevopoulou, et al., Structural characteristics and physicochemical properties of freeze-dried snail meat, LWT-Food Sci. Technol. 155 (2022) 112980. <https://doi.org/10.1016/j.lwt.2021.112980>.
- H. Liu, Q. Li, S. Jiang, et al., Exploring the underlying mechanisms on NaCl-induced reduction in digestibility of myoglobin, Food Chem. 380 (2022) 132183. <https://doi.org/10.1016/j.foodchem.2022.132183>.
- Y.F. Liu, I. Oey, P. Bremer, et al., Proteolytic pattern, protein breakdown and peptide production of ovomucin-depleted egg white processed with heat or pulsed electric fields at different pH, Food Res. Int. 108 (2018) 465-474. <https://doi.org/10.1016/j.foodres.2018.03.075>.
- H.T. Liu, H. Zhang, Q. Liu, et al., Solubilization and stable dispersion of myofibrillar proteins in water through the destruction and inhibition of the assembly of filaments using high-intensity ultrasound, Ultrason. Sonochemistry. 67 (2020) 105160. <https://doi.org/10.1016/j.ultsonch.2020.105160>.

- H. Wang, J.M. Liu, Q. Chen, et al., Biochemical properties of extracellular protease from *Staphylococcus epidermidis* isolated from Harbin Chinese sausages and its hydrolysis of meat protein, *Food Biosci.* 42 (2021) 101130. <https://doi.org/10.1016/j.fbio.2021.101130>.
- D. Yu, M.Q. Feng, J. Sun, et al., Protein degradation and peptide formation with antioxidant activity in pork protein extracts inoculated with *Lactobacillus plantarum* and *Staphylococcus simulans*, *Meat Sci.* 160 (2020) 107958. <https://doi.org/10.1016/j.meatsci.2019.107958>.
- S. Jiang, M. Zhang, H. Liu, et al., Ultrasound treatment can increase digestibility of myofibrillar protein of pork with modified atmosphere packaging, *Food Chem.* 377 (2021) 131811. <https://doi.org/10.1016/j.foodchem.2021.131811>.
- L. Li, Y. Liu, X.Y. Zou, et al., *In vitro* protein digestibility of pork products is affected by the method of processing, *Food Res. Int.* 92 (2017) 88-94. <https://doi.org/10.1016/j.foodres.2016.12.024>.
- X. Bai, S. Shi, B.H. Kong, et al., Analysis of the influencing mechanism of the freeze-thawing cycles on *in vitro* chicken meat digestion based on protein structural changes, *Food Chem.* 399 (2023) 134020. <https://doi.org/10.1016/j.foodchem.2022.134020>.
- H. Chen, G.M. Zhao, X.L. Yu, et al., Exploring *in vitro* gastrointestinal digestion of myofibrillar proteins at different heating temperatures, *Food Chem.* 414 (2023) 135694. <https://doi.org/10.1016/j.foodchem.2023.135694>.
- Z.F. Bhat, J.D. Morton, A.E.A. Bekhit, et al., Emerging processing technologies for improved digestibility of muscle proteins, *Trends Food Sci. Technol.* 110 (2021) 226-239. <https://doi.org/10.1016/j.tifs.2021.02.010>.
- E. Escudero, M. Á. Sentandreu, F. Toldrá, Characterization of peptides released by *in vitro* digestion of pork meat, *J. Agric. Food Chem.* 58 (2010) 5160-5165. <https://doi.org/10.1021/jf904535m>.
- S.Q. Hu, G.H. Zhou, X.L. Xu, et al., Contribution of cathepsin B and L to endogenous proteolysis in the course of modern Jinhua ham processing, *Food Control.* 135 (2022) 108584. <https://doi.org/10.1016/j.foodcont.2021.108584>.
- H. Wang, J.H. Xu, Q. Liu, et al., Effect of the protease from *Staphylococcus carnosus* on the proteolysis, quality characteristics, and flavor development of Harbin Chinese sausage, *Meat Sci.* 189 (2022) 108827. <https://doi.org/10.1016/j.meatsci.2022.108827>.