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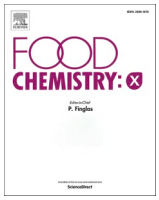

Cowhide gelatin peptide as a source of antioxidants for inhibiting the deterioration of pudding quality during storage

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ABSTRACT

To investigate the effect of gelatin peptide on the inhibition of quality deterioration in stored pudding, gelatin peptide with antioxidant properties was added to pudding products. For this purpose, a pudding recipe containing gelatin peptides was created. The gelatin peptides were characterized based on their antioxidant activity and protein structure. It was found that gelatin peptides had better antioxidant properties, lower thermal stability and crystallinity, higher hydrophobic amino acid content, and greater surface hydrogen bond exposure than commercially available peptides. Properties such as the pH, colony growth, and sensory characteristics of the pudding were characterized at 4 °C and 25 °C. The results showed that the addition of 0.5–1.0 % gelatin peptide to pudding was capable of significantly ($P < 0.05$) slowing down the decline in pH and sensory scores of the pudding and significantly inhibiting colony growth. It could prolong its storage life by five days at 4 °C and three days at 25 °C.

1. Introduction

Pudding products are made from a mixture of milk proteins and colloids (Lim & Narsimhan, 2006), where the latter enhance textural properties to generate the product's mushy consistency (D. Li, Zhang, Jiang, & He, 2021a). The main source of milk protein in pudding is milk powder, which contains 9.8 %–28.5 % milk fat, 15 %–24 % protein, and 32 %–67.5 % lactose (Y. H. Li, Wang, Guo, Shao, & Xu, 2019). Due to the pudding product's water content (up to 75 %), pH (approximately 6.5), cold storage conditions (4 ± 2 °C), and room temperature conditions (25 ± 5 °C) (Moufle, Jamet, & Karoui, 2017), the pudding is likely prone to microbial spoilage, protein oxidation, and lipid oxidation in pudding (Degirmenci & Erkurt, 2020). Therefore, it takes precautionary measures to address the deteriorating pudding quality caused by the above reactions.

Currently, natural antioxidants and antimicrobial agents are used to treat pudding products to extend their shelf life. For example, Dur-e-shahwar Sattar *et al.* (Sattar, Ali, & Hasnain, 2017), compared the effects of ungerminated and germinated pulses on the antioxidant, functional, and sensory properties of rice pudding; they found that germinated pulses enriched the antioxidant and phenolic content of the pudding and displayed better sensory, storage, and nutritional properties. Dengyun Li *et al.* (D. Li, Zhang, Jiang, & He, 2021b), applied almond extract with antibacterial and antioxidant properties to milk pudding and determined that this addition improved the storage life and texture properties of pudding products. Rubén Pérez Pulido *et al.* (Perez Pulido, Toledo del Arbol, Grande Burgos, & Galvez, 2012), successfully inhibited the growth of *Staphylococcus aureus* in rice pudding by applying a combination of high hydrostatic pressure and antimicrobial agents to the pudding. Oshima, S. *et al.* (Oshima, Hirano, Kamikado, Nishimura, Kawai, & Saito, 2014), added nisin A, an antibacterial agent, to milk pudding, and observed that it effectively inhibited the growth of bacteria in milk pudding while extending its storage period. However, though these methods are proven to effectively improve pudding quality and prolong its storage period, they are not economically efficient and require specific conditions in order to be effective. Therefore, there is an urgent need to explore effective approaches that effectively and conveniently extend pudding storage time.

The skin and bones of pigs, cattle, and other mammals contain collagen, which can be used as a source of gelatin (Dille, Haug, & Draget, 2021). Cow skin accounts for 29.4 % of total gelatin production (Tang, Zhou, Zhu, Zhang, Xie, Wang, et al., 2022), and the usage of cowhide is economically beneficial while supportive of environmental protection (Wei, Zhu, Li, Liu, Zhang, Kou, et al., 2022). It has been found that the

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production of cow skin gelatin through boiling, high-pressure treatment, ultrasonic treatment, enzymatic action, and acid or alkali treatment (He, Gao, Wang, Han, Yu, Shi, et al., 2021), enables the extraction of bioactive peptides through the breakage of intermolecular crosslinks and certain peptide bonds (Rather, Akhter, Ashraf, Mir, Makroo, Majid, et al., 2022). These gelatin peptides have been shown to lower blood sugar, boost immunity, increase immune factors, and enhance antioxidant and antibacterial properties (Zhang, Liu, Qi, Xv, Li, Guo, et al., 2023), thus highlighting their unique properties among bioactive products. Adding these ingredients to dairy products has been found to enhance their functional properties as well as influence their physical, chemical, microbiological, and sensory properties (Soutelino, Rocha, de Oliveira, Mársico, & Silva, 2023). For example, Barbara Duquenne *et al.* (Duquenne, Vergauwen, Capdepon, Boone, De Schryver, Van Hoorebeke, et al., 2016), applied pig skin gelatin peptides to frozen dairy mousse products and determined that the mousse with the gelatin peptides remained stable when frozen and maintained its hardness throughout storage. Samaneh Ayati *et al.* (Ayati, Eun, Atoub, & Mirzapour-Kouhdasht, 2021), applied fish gelatin active peptides to yogurt and determined that this improved the functional properties of the yogurt products, including antioxidants, ACE inhibitors, and DPP-IV inhibitors. Leon-Lopez *et al.* (León-López, Pérez-Marroquín, Campos-Lozada, Campos-Montiel, & Aguirre-Álvarez, 2020), identified a functional whey fermented beverage containing collagen peptides, which displayed high bioavailability, nutritional value, and antioxidant capacity, while also inhibiting the growth of pathogenic microorganisms in the beverage. Although the number of reports on the benefits of gelatin peptides pertinent to the food industry is relatively high, the number of scientific articles covering dairy applications is comparatively lower. This indicates the need for more data and further research, since aspects related to the storage period, and sensory, technical, functional, and nutritional changes caused by gelatin peptides in these products remain unknown (Soutelino, Rocha, de Oliveira, Mársico, & Silva, 2023).

Given the advantages of gelatin peptides in dairy storage, this study aims to assess the inhibitory effect of different amounts of gelatin peptide on the deterioration in pudding quality during storage, by applying gelatin peptide (homemade cowhide gelatin peptide) to pudding products. Compared with those of commercial peptides (commercially available cowhide collagen peptide), thereby providing an updated reference and theoretical basis for prolonging the storage period of pudding.

2. Materials and methods

2.1. Materials

Fresh cowhide was purchased from Lanzhou Xiaoxihu market, large pieces of fat removed, washed off the blood stains, cut into 0.5 m × 0.5 m frozen and preserved; Whole milk powder was purchased from Inner Mongolia Yili Industrial Group Co.; white sugar was purchased from Beijing Hualian Supermarket, Anning District, Lanzhou City; food-grade carrageenan, locust bean gum, are all purchased from Guangdong Xinyuan Biological limited company; commercially available peptides were purchased from Anyang Biotechnology.

Alkaline protease, trypsin, papain, neutral protease was purchased from Shanghai Solarbio; Sodium dodecyl sulfate (SDS) was purchased from Lanzhou Yichen Biological limited company; Glutathione (GSH) standards, 2-mercaptoethanol were purchased from Tianjin Guangfu Science and Technology Development limited company; O-Phthalaldehyde (OPA), sodium tetraborate, 1,1-Diphenyl-2-picrylhydrazyl radical 2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH), $FeSO_4$, Salicylic acid, H_2O_2, potassium ferricyanide, 2, 2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) purchased from Yuanye, Shanghai; Amino acid mixture standard purchased from Hikaratsu Pharmaceutical Industry Co., LTD. All other chemicals used were of analytical grade.

2.2. Preparation of gelatin peptides

The standard operating procedure is shown in Fig.S1. The cowhide was rinsed with water after thawing, following which mechanical hair removal and degreasing were conducted. The treated cowhide was then cut into 1 cm × 1 cm pieces, put into a beaker with 5 % sodium bicarbonate solution, and left to soak for six hours for further degreasing. After removing the cowhide and rinsing it with water, it was mixed in a material ratio of 1:4 (cowhide: distilled water), treated at 0.1 Mpa in a high-pressure steam sterilizer for 30 min, and boiled in water for five hours. After filtration, impurities were removed and the cowhide was freeze-dried, ground into a powder (cowhide gelatin powder), sealed, and stored at room temperature.

To determine the best performing enzyme, we did the following steps: The cowhide gelatin powder (6 %) was subsequently dissolved in water. Trypsin, papain, neutral protease, and alkaline protease were used to digest cowhide gelatin with the same enzyme activity (5000U/g) at their respective theoretical optimal pH values and temperatures (Table S1). The peptide extraction rate was used as an indicator to compare and screen for the best-performing enzymes (Figure S2-a). The results indicated that the alkaline protease peptides had the highest peptide extraction rates.

To determine the optimal conditions for alkaline protease hydrolysis and extraction, a single-factor test and response surface optimization were conducted (Table S2-5, Fig. S2-3). During the reaction, the amount of enzyme was 5200 U/g, the enzymolysis time was 3.5 h, the content of substrate was 6 %, the extraction rate of gelatin peptide was 45.85 %, and the degree of hydrolysis (DH) was 20.39 %. After the reaction, the enzymatic hydrolysate was inactivated in a water bath at 95 °C for 15 min and centrifuged at 3000 r/minute for 10 min. The supernatant was then freeze-dried and ground into powder, sealed, and stored at room temperature.

2.3. Gelatin peptide extraction rate assay

Based on the method by Frank *et al.* (Frank, Harold, David, & George, 1983), the concentration of the peptide solution was determined by the OPA method. The OPA mixture was prepared by dissolving 40 mg of OPA in 1 mL of methanol, and subsequently adding 25 mL of sodium tetraborate solution (100 nmol/L), 10 mL SDS (5 %), 0.1 mL of 2-mercaptoethanol, and 13.9 mL of water, under dark conditions. A 150 μL peptide sample was then mixed with 2 mL of the prepared OPA mixture. This mixture was incubated for two minutes at room temperature in the dark, to measure the absorbance value at a wavelength of 340 nm. Simultaneously, a series of GSH solution was prepared, with GSH contents of 0.25, 0.5, 0.75, 1.0, 1.25 mg/mL GSH. Approximately 0.25 mL each standard solution was then added to 10 mL OPA mixture. This mixture was shaken evenly and placed at room temperature for eight minutes, and the absorbance value of the mixture was measured at 340 nm. Origin 2020 was used to plot the standard curve and obtain the regression equation: $Y = 0.5208x\text{-}0.0146$, $R^2 = 0.9984$ (Fig. S4).

$$\text{Peptide extraction rate}\ (\%) = \frac{c \times v}{m} \times 100$$

where: c, peptide concentration, mg/mL; v, volume of crude peptide extraction, mL; m, weight of raw materials, mg.

2.4. Determination of degree of hydrolysis of gelatin peptide

According to the method described by Spellman *et al.* (Spellman, McEvoy, O'Cuinn, & FitzGerald, 2003) with slight modifications. The hydrolysate (60 μL) was mixed with 2.4 mL of freshly prepared OPA reagent for 10 min to ensure that the absorbance of the mixture at 340 nm was less than 1.0. A blank containing only 2.4 mL of OPA reagent was used. The DH was calculated according to the following formula:

$$DH(\%) = 100 \times n/N$$

$$n = (A_{340sample} - A340_{under\ sample}) \times Md/\varepsilon c.$$

where n represents the average number of peptide bonds hydrolyzed, N is the total number of peptide bonds per gelatin molecule (4287), M is the molar mass of gelatin (350.000 Da), and d is the dilution factor (41). ε and c are the molar extinction coefficients of OPA at 340 nm (6000 $M^{-1}cm^{-1}$) and protein concentrations (10 mg/mL), respectively.

2.5. Antioxidant properties

2.5.1. DPPH radical scavenging capacity

According to the method described by Tai, A. *et al.*, (Sakanaka, Tachibana, Ishihara, & Juneja, 2005) to determine the scavenging capacity of the DPPH radical. DPPH was dissolved with 95 % ethanol to prepare a 0.2 mmol/L DPPH solution. The 2 mL sample solution and 2 mL DPPH solution were mixed evenly and left at room temperature for 30 min away from light. The absorbance was determined at 517 nm. The formula for calculating DPPH is:

$$\text{DPPH radical scavenge} \Big/ \% = \left(1 - \left(\frac{A_i - A_j}{A_0}\right)\right) \times 100$$

Where: A_i is the absorbance of 2 mL sample solution + 2 mL DPPH solution; A_j is the absorbance of 2 mL of sample solution + 2 mL of 95 % ethanol solution, and A_0 is the absorbance of 2 mL DPPH solution + 2 mL of 95 % ethanol solution.

2.5.2. Hydroxyl free radical scavenging capacity

According to the method described by Jiang Haiping *et al.*, (Jiang, Tong, Sun, Xu, Zhao, & Liao, 2013) with slight modifications. 1 mL of 9 mmol/L $FeSO_4$ and 9 mmol/L salicylic acid–ethanol solution were used to create the reaction system. Approximately 1 mL of the sample solution was added to the reaction system and 1 mL of 8.8 mmol/L H_2O_2 was added to initiate the reaction. For the blank control solution, 1 mL of distilled water was used in place of the sample solution. Distilled water was used as the reference solution and the absorbance value was determined at 510 nm. The hydroxyl radical scavenging capacity was calculated according to the following formula:

$$\textit{Hydroxyl radical scavenging} \Big/ \% = \frac{A_0 - (A_x - A_{x0})}{A_0} \times 100$$

Formula: A_0 is the absorbance of the blank control solution; A_x is the absorbance after adding the sample solution; A_{x0} is the background absorbance of the enzymolysis solution.

2.5.3. Metal ion removal capacity

According to the method described by Gu Fenglin *et al* (Gu, Huang, Wu, & Zhu, 2018) with slight modifications. The mixture was prepared by mixing 1 mL of the sample, 2.5 mL phosphate buffer (0.2 mol/L, pH 6.6), and 2.5 mL of 1 % potassium ferricyanide solution. The mixture was placed in a water bath at 50 °C for 20 min and cooled rapidly. Subsequently, 2.5 mL of 10 % trichloroacetic acid solution was added, and the mixture was centrifuged from the core at 3000 r/minute for 10 min. Approximately 0.5 mL of 0.1 % $FeCl_3$ solution and 2.5 mL distilled water were added successively to 2.5 mL of the supernatant and left to stand at room temperature for 10 min. The absorbance was measured at 700 nm.

2.5.4. ABTS free radical scavenging capacity

According to the method described by Wiriyaphan *et al.*,(Wiriyaphan, Chitsomboon, & Yongsawadigul, 2011) with slight modifications. The ABTS stock solution was obtained by mixing 10 mL of ABTS solution at a concentration of 7 mmol/L with 176 uL of potassium persulfate solution at a concentration of 140 mmol/L, and placed in the dark at room temperature for 12–16 h. Prior to measurement of the peptide sample, the ABTS stock solution was diluted with sodium acetate buffer (0.02 mmol/L, pH4.5) to an absorbance of 0.70 ± 0.01 (734 nm). The ABTS radical scavenging capacity was calculated as follows:

$$\text{ABTS radical scavenging} \Big/ \% = \left(\frac{A_c - A_s}{A_c}\right) \times 100$$

Formula: Ac is the absorbance value of the ABTS solution; as is the absorbance value of the ABTS solution added to the sample.

2.6. Scanning electron microscope (SEM)

According to the method described by Gao *et al.* (Gao, Yang, Han, Yu, Song, Han, et al., 2019) with slight modifications. The lyophilized gelatin sample was ground into powder and placed on a metal plate in a vacuum drying chamber. A metal film (10 nm) was then sputtered on the surface of the gelatin and the structure was amplified by 150x, 500x, and 2,000x at a voltage of 15.0 kV.

2.7. Fourier infrared spectroscopy (FTIR)

Nicolet summit FTIR Analyzer (Thermo Fisher, Jiangsu, China) was used to measure the FTIR spectra of commercially available peptides and gelatin peptides from 4000 cm^{-1} to 400 cm^{-1} with a resolution of 4 cm^{-1}.

2.8. X-ray diffraction (XRD)

According to the method described by Ramachandran, T *et al.* (Ramachandran & Hamed, 2018) with slight modifications. The radiation source is Cu target Kα radiation, the voltage is 40kv, and the current is 40ma. The scanning angle is 10°-50°(2θ), and the scanning speed is 4°/min. The minimum repeating plane spacing d corresponding to the diffraction peaks is calculated as follows:

$$d(A) = \lambda/2\sin\theta$$

where λ is the X-ray wavelength and θ is the Bragg diffraction angle.

2.9. Differential scanning calorimeter (DSC)

According to the method described by Shela Gorinstein *et al.* (Gorinstein, Delgado-Licon, Pawelzik, Permady, Weisz, & Trakhtenberg, 2001) with slight modifications. The gelatinization thermal characteristics of the sample were tested using a differential calorimetry scanner (DZ-DSC300L, China). The sample was placed in an aluminum container, sealed and then placed in a heating dish, and heated to 20-100 °C at a temperature of 10 °C/min. The thermal curve was observed and the enthalpy change value was calculated.

2.10. Preparation of pudding products

The standard operating procedure is shown in Fig.S1. The pudding primitive ingredients are 7.5 g (4.5 %) milk powder,4.5 g (4.5 %) sugar,0.96 g (0.96 %) carrageenan, 0.64 g (0.64 %) locust bean gum, and 0.4 g (0.4 %) gelatin. To prepare the six groups of puddings products with different peptide addition, 0 %, 0.5 %, 1.5 % and 2.0 % of gelatin peptide and 1.0 % of commercially available peptide were added to the pudding raw materials. Water was added to supplement the sample to 100 g. The pudding solution with 1.0 % commercially available peptide was designated the control group (CK). To stir and dissolve evenly under water bath conditions to obtain pudding liquids. The pudding liquid was subsequently poured into a container, sealed for pasteurization, and heated to 80 °C. It was kept warm for 15 s, and then the temperature was quickly reduced to 4 °C. The six groups of pudding samples were placed at 4 °C and 25 °C, with 40 samples from each group. The sampling interval was two days and three pudding samples were randomly taken

from each group each time to measure sensory evaluation, texture, protein content, amino acid composition, pH, color and total number of colonies.

2.11. Sensory evaluation

Refer to GB/T 1983–2018 "*Jelly*" and GB25191-2010 "*National Food Safety Standard Modulated Milk*" to formulate scoring standards, and the score is a 100-point system. The appearance, taste and flavor of the above 12 groups (Different storage temperatures and concentrations) of pudding products were evaluated by 20 food major master students (10 males and 10 females, aged 24–28). All reviewers sign free and informed consent and agree to participate in the research (The study was carried out in accordance with the principles of the Declaration of Helsinki. This study was approved by the College of Food Science and Engineering of Gansu Agricultural University with the approval date of January 5, 2024). The sensory rating table is shown in Table 1.

2.12. Physicochemical propery

2.12.1 Texture.

According to the method described by Mihaylova D, *et al.*, (Mihaylova, Popova, Goranova, Petkova, Doykina, & Lante, 2021) with slight modifications. Measured using the TA.XTC-18 texture analyzer (Shanghai Xinyi Co., Ltd.). The specific measurement parameters are as follows: P/36R probe is used, the premeasurement rate is 2 mm/s, the test rate is 1 mm/min, the post-measurement rate is 1 mm/min, the compression degree is 50 %, the residence time is 5 s, the trigger value is 3 g, and the distance measurement is 10 cm.

2.12.2. Protein content

Refer to the Kjeldahl method in GB5009.5–2016 "*National Safety Standard-Determination of Protein in Food*". Measured by K9840 automatic Kjeldahl nitrogen determination instrument (Jinan Haineng Instrument Co., Ltd.).

2.12.3. Amino acid composition

Determination of amino acids in food according to *GB 5009.124–2016* "*Food safety national standard*". Weigh the appropriate amount of evenly mixed samples, add 10 mL 1:1 hydrochloric acid solution into the hydrolysis tube, mix well, place in 110 °C±1 °C electric blast incubator for hydrolysis for 22 h, and then cool to room temperature. Filter the hydrolysate into a 25 mL volumetric bottle and fill with water. Accurately absorb 0.5 mL filtrate and transfer it into 15 mL test tube, dry it with nitrogen, volume it with 0.02 mol/L hydrochloric acid solution to 10 mL, shake and mix it well, and then filter it through 0.22 μm microporous membrane. Amino acids were quantified using the A300 automatic amino acid analyzer (MembraPure, Bodenheim, Germany). The detection wavelengths were 570 nm and 440 nm, respectively. The chromatographic column was sulfonic acid cation exchange column with the sample size of 20 μL. The calculation formula of each amino acid content in the sample is as follows:

$$X = \frac{(C - C_0) \times V \times N}{m \times 10000}$$

In the formula: X represents the amino acid content in the sample, the unit is mg/L; C represents the concentration value of each amino acid in the sample calculated according to the concentration of the standard substance, the unit is mg/L; C_0 is the concentration value of each amino acid calculated according to the concentration of standard substance in the blank control, in mg/L; V is the constant volume, in mL; N is the dilution ratio; m is the weighing sample, in g.

Table 1
Sensory evaluation scale.

Sensory score	Evaluation criteria	Score value/ points
Appearance (30 points)	The pudding gel is complete, well formed, and soft and firm in texture.	21–30
	Pudding set completely, but too soft or too hard.	11–20
	The pudding is not set completely and has layers.	0–10
Taste (30 points)	Soft and smooth mouth, moderate elasticity, good taste.	21–30
	Medium taste, no obvious graininess, pudding elasticity is moderate.	11–20
	The taste is rough, and the pudding tissue is soft or hard, with obvious graininess.	0–10
Flavor (40 points)	Sweet and delicious, no odor, milk flavor.	28–40
	Sweet and almost palpable, with insufficient or overpowering milk flavor.	14–27
	The sweetness is too heavy or too light, with an abrupt gum or fishy taste.	0–13

2.12.4. pH

A HI99163 portable pH meter (Hanna Instruments, Italy) was used to detect the change in pH value with storage time. Random samples were taken and measured at 20 ± 1 °C (samples were discarded after measurement). Introduce the probe into the pudding and measure the pH.

2.12.5. Color

The color parameters (*L**, *a**, *b**) of the pudding surface were determined with a Konica CR-10 colorimeter (Konica Minolta Optoelectronics Co., Ltd). Three parallel samples per group, each pudding was measured 3 times.

2.12.1. Total number of colonies

The determination method of total number of colony count is determined by the plate colony counting method in GB4789.2–2016 "*National Standard for Food Safety: Microbiological Testing of Food Colonies*".

2.13. Statistical analysis

The Origin 2020 were used to perform basic processing and graphing of the experimental data, and the IMB SPSS Statistic 20.0 was used for multivariate ANOVA (Duncan method, $P < 0.05$). Pudding product trial data are expressed as mean ± standard deviation of three assays.

3. Results and discussion

3.1. Antioxidant properties of gelatin peptides

In order to investigate the antioxidant abilities of gelatin peptides in vitro, we conducted experiments to assess the free radical scavenging ability and Fe^{2+} reducing ability of DPPH, –OH, and ABTS. As shown in the Fig. 1, it was found that the free radical scavenging and Fe^{2+} reduction capacities of DPPH, –OH, and ABTS significantly increased ($P < 0.05$) with an increase in gelatin peptide concentration. These were also significantly higher than the commercially available peptides($P < 0.05$). This suggests that gelatin peptides have better antioxidant properties than commercially available peptides, which may be due to differences in the materials used to prepare the former. This may also due to the fact that homemade gelatin is made by partially hydrolyzing gelatin with high-pressure-assisted hot water. This is consistent with the findings from the study of L. Chen *et al.* (L. Chen, Ma, Zhou, Liu, & Zhang, 2014), who found that gelatinization occurs under the action of high pressure or high temperature, leading to the destruction of non-covalent bonds and covalent cross-linking that maintain and stabilize the triple helix structure. This breakage is conducive to the further enzymatic hydrolysis of small molecule peptides with stronger antioxidant properties.

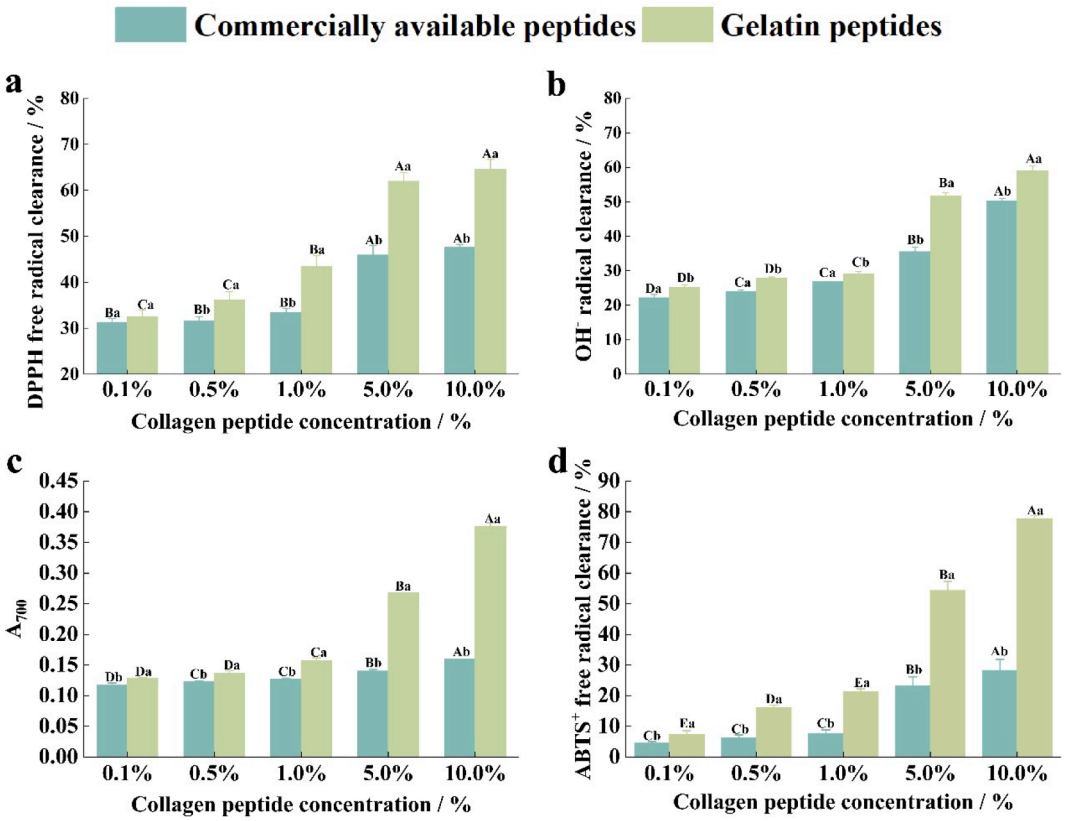

Fig. 1. a. Comparison of DPPH free radical clearance of peptides; b. Comparison of hydroxyl radical scavenging ability of peptides; c. Comparison of metal ion removal capacity of peptides; d. Comparison of ABTS radical scavenging ability of peptides. Note: Vertical lines in the figure represent error lines; Different capital letters in the figure represent the same peptide, and different concentrations are significantly different ($P < 0.05$); Lowercase letters indicate a significant difference between two peptides at the same concentration ($P < 0.05$).

In detail, DPPH free radical scavenging experiments are used to measure compounds' abilities to act as free radical scavengers or hydrogen donors, as well as to evaluate the antioxidant activity of food items (Gulcin, 2020). Unlike ABTS, DPPH free radical does not have to be produced before determination. Fig. 1-a shows that gelatin peptides demonstrate significant DPPH free radical scavenging ability ($P < 0.05$), which may be due to the presence of amino acids with a high capacity for hydrogen supply. This is consistent with a study by Jingbo Liu *et al.*, who found that the combination of Cys + Ala, Cys + Asp, and Cys + His displayed a stronger DPPH free radical scavenging ability than other amino acids (Shucheng He & Yinshi Zhu, 2018). This is consistent with the results of amino acid content. Moreover, the activity of the –OH free radical is considered to be very strong and harmful to organisms. Fig. 1-b shows that gelatin peptides demonstrate significant –OH radical scavenging ability ($P < 0.05$). This may be due to the relatively loose structure and higher number of enzyme cleavage sites in gelatin peptides, which occur as a result of their raw materials undergoing high-pressure treatment, thus improving the ability of –OH radical scavenging. (He, Han, Yu, Wang, Li, & Han, 2023). Furthermore, considering Fe^{2+} is the most potent pro-oxidant in the food system, chelation of iron ions may result in them losing their pro-oxidant properties. Fig. 1-c shows that that gelatin peptides display significant chelating ability for Fe^{2+} ($P < 0.05$), which may be due to amino acids in gelatin peptides having a strong ability for Fe^{2+} chelation. (Gulcin, 2020). In addition, ABTS·+ radicals are more reactive and have higher reaction rates than DPPH radicals, thus making it easy to observe reactions between preformed radicals and free radical scavengers (Gülçin, 2008). Fig. 1-d shows that gelatin peptides have more significant scavenging ability when it comes to ABTS free radicals ($P < 0.05$), thereby reducing ABTS·+ to ABTS. This may be due to the high content of basic amino acids in gelatin peptides. This is supported by research conducted by Shucheng He *et al.* (Shucheng He & Yinshi Zhu, 2018), who found that alkaline amino acids have high ABTS free radical scavenging capabilities. In summary, the antioxidant activity of gelatin peptides was found to be significantly better than that of commercially available peptides. This can be attributed to the improvement in proteolysis and release of bioactive peptides that occur under high-pressure conditions (Zhao, Huo, Qian, Ren, & Lu, 2017), which can effectively improve the antioxidant activity of cowhide gelatin enzymatic hydrolysis (He, Han, Yu, Wang, Li, & Han, 2024).

3.2. SEM

We used scanning electron microscopy to observe the microstructure of cowhide gelatin before and after enzymatic hydrolysis, and found significant differences, as shown in Fig. 2. On using the scanning electron microscope at magnification 150x, the gelatin (a) before enzyme digestion appeared broken with an irregular and flaky surface, while after enzyme digestion, the structure of the gelatin peptide (d) displayed a few changes and had a loose granular surface. At 500x magnification, the surface of the gelatin (b) before enzymatic digestion appeared loose and porous with an irregular macroporous spongy structure, most of which was still bonded together. However, the gelatin (e) after enzymatic digestion had disintegrated into small particles. At 2000x magnification, the gelatin (c) before enzymatic digestion displayed a dense lamellar body with irregular fragmentation on the surface, with some peptide chains still joined. However, the intact structure of gelatin (f) after enzymatic digestion was observed to have been destroyed. Therefore, based on electron microscopy, it was indicated that the gelatin molecules hydrolyzed from the overall lamellar form into smaller particles, which could expose key components. This is consistent with the findings of He and Nan *et al.*, who determined that high pressure and

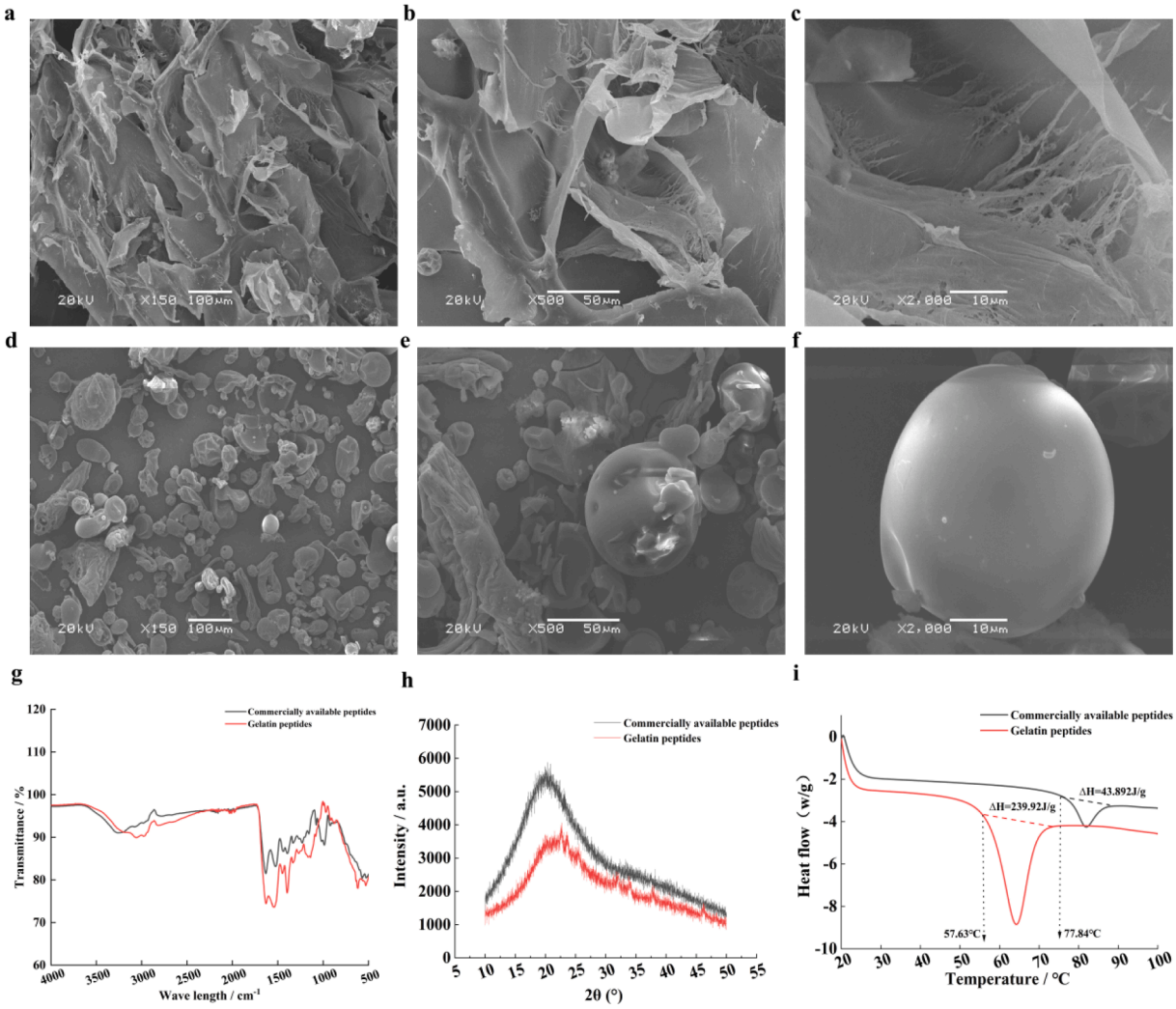

Fig. 2. (a-f) Scanning electron microscopy of gelatin and collagen peptide. (a-c) represent SEM images of cow skin gelatin at different multiples. (d-f) represent the SEM images of cow skin collagen peptide at different multiples, respectively. g. Effects of enzymatic hydrolysis on protein secondary structure. h. Comparison of X-ray diffraction images of collagen peptides. i. Differential calorimetry of collagen peptides.

enzymatic hydrolysis resulted in the destruction of inter-molecular hydrogen bonds and van der Waals forces present in gelatin. They found that this further led to a reduction in particle size, destruction of the gelatin structure, stretching of the gelatin molecules, and the exposure of more hydrophobic amino acids to the surface, thus improving the antioxidant capacity of enzymatic hydrolysis products (He, Han, Yu, Wang, Li, & Han, 2023; Nan, Zou, Wang, Xu, Zhang, Wei, et al., 2018).

3.3. FTIR

Infrared spectroscopy is often used to analyze the functional groups and secondary structures of proteins (Muyonga, Cole, & Duodu, 2004). According to Fig. 2-g, the infrared spectra of the two substances appeared to be similar, with four characteristic peaks representing amide A, amide I, amide II and amide III.

In detail, the amide A band is related to the vibration frequency of N—H, and the absorption band typically appears in the 3300–3500 region. When N—H groups form hydrogen bonds, the absorption summit moves to a lower frequency. The lower the intensity of the absorption peak, the stronger the ability to form hydrogen bonds (Matmaroh, Benjakul, Prodpran, Encarnacion, & Kishimura, 2011). As shown in Fig. 2-g, the absorption peak of the amide A band of the gelatin peptide moved to the lower wave number, indicating that more N—H bonds were formed in the peptide chain. This resulted in stronger hydrogen bond formation in the gelatin peptide, which is conducive to its structural stability. This is consistent with the results from a study conducted by Pace *et al.* (Pace, Fu, Lee Fryar, Landua, Trevino, Schell, et al., 2014), who found that protein stability increases with higher hydrogen bond content. In addition, the amide I and II bands of the two peptides were located at 1630 and 1540, respectively, but the gelatin peptide was found to have a redshift at these two positions, with a wide deformation of the absorption peak. This shows that the peptide bond was more prone to breakage during the process of gelatin peptide formation, while enzymatic hydrolysis of gelatin peptide was more thorough (Uriarte-Montoya, Santacruz-Ortega, Cinco-Moroyoqui, Rouzaud-Sández, Plascencia-Jatomea, & Ezquerra-Brauer, 2011). This finding is consistent with the results from Long He *et al.* (He, Han, Yu, Wang, Li, & Han, 2023), who determined that the secondary structure of gelatin undergoes severe damage, carbonyl content increases, lead to enzymatic hydrolysis was more thorough.

3.4. XRD

X-ray crystallography is the primary method used to determine the atomic structure of proteins, thus providing a visual representation of the differences across polypeptide crystal structures (Parker, 2003). In Fig. 2-h, the diffraction peak of the commercially available peptide at 2θ

was observed at 20.330°, while that of the gelatin peptide was seen at 22.690°. Both exhibited a relatively broad peak around 2θ = 21°. However, the intensity of the peak of the gelatin peptide was noticeably lower than that of the commercially available peptide, suggesting a decrease in crystallinity in the former after high-pressure treatment and the formation of more stable small molecule peptides. This observation aligns with the findings of He and L. Chen *et al.* (L. Chen, Ma, Zhou, Liu, & Zhang, 2014) (He, Han, Yu, Wang, Li, & Han, 2023), who proposed that high-pressure treatment promotes the development of the triple helix structure of gelatin molecules, leading to rearrangement and aggregation within the gelatin structure. Consequently, this enhances the enzymatic hydrolysis of gelatin, resulting in weakened crystallization of gelatin peptides and the production of more stable small molecule peptides. At the same time, the high-pressure and enzymatic hydrolysis treatments may also increase hydrophobic amino acid content in gelatin peptides, thereby improving their stability and antioxidant properties. Studies have also indicated that proteins with a high content of hydrophobic amino acids exhibit greater stability (Cristina Oliveira Neves, Aparecida Rodrigues, Teixeira Valentim, Cristina Freitas de Oliveira Meira, Henrique Silva, Ayra Alcântara Veríssimo, et al., 2020).

3.5. Thermal degeneration of gelatin peptides

Studies have shown that the thermal stability of proteins is determined by the partial hydrogen bond formed between the pyrrole ring and hydroxyproline developed from proline and hydroxyproline hydroxyl groups (Jongjareonrak, Benjakul, Visessanguan, Nagai, & Tanaka, 2005). Fig. 2-i illustrates that the thermal denaturation temperatures of the gelatin peptides and commercially available peptides, which are 57.63 °C and 77.84 °C, respectively. Additionally, the enthalpy change value was reflected by the DSC peak area (ΔH) (Schroepfer & Meyer, 2017). The enthalpy values of the gelatin peptides were found to be significantly higher ($P < 0.05$) than those of the commercially available peptides. Consequently, the gelatin was subjected to high pressure and enzymatic hydrolysis to produce gelatin peptides with lower thermal denaturation temperatures and higher enthalpy values. This led to a decrease in their thermal stability. This observation suggests that enzymatic hydrolysis of gelatin peptides is more thorough, resulting in greater exposure of the hydrogen bonds on their surfaces (He, Gao, Han, Yu, & Zang, 2021). This finding aligns with research conducted by He *et al.* (He, et al., 2021), who discovered that high-pressure treatment disrupts gelatin's structure, thereby reducing its thermal stability and allowing for more complete enzymatic hydrolysis. As a result, more hydrogen bonds on the surface are exposed, enhancing the antioxidative performance of the gelatin peptides.

3.6. Effect of gelatin peptides on pudding quality

3.6.1. Texture

Texture is a multi-parameter sensory property. It includes the rheological and structural properties of food which can be perceived by mechanical, visual, auditory and tactile sensors (Raheem, Carrascosa, Ramos, Saraiva, & Raposo, 2021), making it a key factor in pudding quality evaluations. The findings showed that the pudding with 0.5 % gelatin peptide added displayed no significant change in hardness, elasticity, and chewiness ($P > 0.05$) (Table 2). When 1.0–1.5 % gelatin peptide was added to the pudding, no significant change in hardness ($P > 0.05$) was observed, but a significant reduction in elasticity and chewiness was documented ($P < 0.05$). In addition, the pudding with 2.0 % gelatin peptide showed a significant reduction in hardness, elasticity, and chewiness ($P < 0.05$). This could be attributed to the high pH of the gelatin peptide prepared by alkaline protease, which deviates from the isoelectric point of the pudding gel system, resulting in the system's destruction. A similar study by H. Chen *et al.* (H. Chen, Wu, Huang, Feng, Ji, Zhao, et al., 2022; Lee & Choi, 2020), reported that carrageenan, locust bean gum, and gelatin in pudding are also influenced by pH, leading to smaller solution particles and an increase in weak zeta potential and particle repulsion, which decreases pudding firmness, elasticity, and chewiness.

Table 2
Effect of different amount of peptide on texture of pudding.

Peptide addition/%	Hardness/g	Elasticity	Masticatory
0	127.03 ± 6.99^{ab}	0.74 ± 0.02^{a}	50.84 ± 4.52^{a}
0.5	123.18 ± 2.08^{abc}	0.70 ± 0.04^{ab}	48.06 ± 1.80^{ab}
1	118.26 ± 4.09^{bc}	0.67 ± 0.02^{bc}	39.33 ± 1.97^{c}
1.5	117.75 ± 2.07^{bc}	0.64 ± 0.02^{cd}	33.76 ± 1.44^{d}
2	113.66 ± 5.94^{c}	0.60 ± 0.01^{d}	29.37 ± 0.85^{e}
CK	132.25 ± 6.51^{a}	0.62 ± 0.01^{d}	46.33 ± 0.91^{b}

Different lowercase letters indicate the significant difference in texture of pudding products under different peptide concentrations.

3.6.2. Protein content and amino acid composition

According to Fig. 3, the protein content of the puddings was observed to increase as gelatin peptide content increased. The protein content of the pudding with 2.0 % gelatin peptide was found to be significantly higher than that of the pudding with 0 % gelatin peptide ($P < 0.05$), indicating that the addition of gelatin peptides can increase the protein content of pudding and improve and improve its nutritional value.

The content of hydrophobic amino acids (0.652 g/100 g), acidic amino acids (0.31 g/100 g), and basic amino acids (0.211 g/100 g) in the pudding with 1.0 % gelatin peptide was found to be 13.99 %, 3.33 % and 18.54 % higher than those in the CK group, respectively (Table 3). The contents of Lys, Arg, Asp, and Ala in the pudding with 1.0 % gelatin peptide were also found to be significantly higher than those in the CK group. This indicated that the antioxidant activity of the pudding with 1.0 % gelatin peptide was stronger than that of the CK group, which is consistent with the findings of Jingbo *et al.* They discovered that alkaline amino acids such as Lys, His, and Arg exhibit antioxidant properties, as do the combinations of Cys + Phe, Cys + Asp, Cys + His, Cys + Ala, Cys + Thr, and His + Asp (Shucheng He & Yinshi Zhu, 2018). This may be due to the strong specificity of alkaline proteases for hydrophobic and alkaline amino acids, which is the reason they are used to prepare antioxidant peptides. Furthermore, the contents of hydrophobic amino acids, acidic amino acids, and basic amino acids in the pudding with 1.0 % gelatin peptide were 14.99 %, 8.77 %, and 42.57 % higher than those in the pudding with 0 % gelatin peptide, respectively. This is likely due to the specific amounts of gelatin peptide that were added to the pudding. Among these amino acids, the content of alkaline amino acids is higher, likely due to alkaline protease hydrolyzing the carboxyl terminal

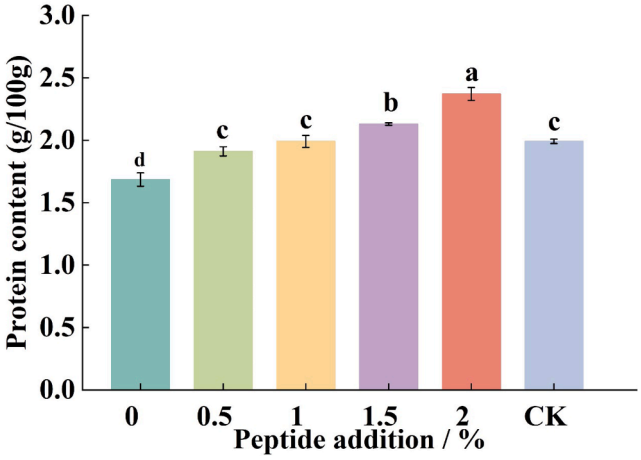

Fig. 3. Effect of different peptides addition on protein content. Lowercase letters indicate significant differences in protein content among different peptide addition ($P < 0.05$).

Table 3
Effect of different peptide additions on amino acid content.

Amino acid	0 % collagen peptide (g/100 g)	1 % collagen peptide (g/100 g)	1 % commercially available peptide (g/100 g)
Asp	0.085^{c}	0.11^{a}	0.10^{b}
Thr	0.042^{b}	0.051^{a}	0.047^{a}
Ser	0.046^{c}	0.062^{a}	0.055^{b}
Glu	0.20^{a}	0.20^{a}	0.20^{a}
Gly	0.033^{c}	0.15^{a}	0.10^{b}
Ala	0.070^{c}	0.12^{a}	0.088^{b}
Cys	0.0038^{a}	0.0033^{a}	0.0035^{a}
Val	0.078^{a}	0.076^{ab}	0.073^{ab}
Met	0.012^{a}	0^{b}	0^{b}
Ile	0.061^{a}	0.057^{ab}	0.055^{b}
Leu	0.11^{a}	0.11^{a}	0.10^{b}
Tyr	0.052^{a}	0.044^{b}	0.044^{b}
Phe	0.056^{a}	0.059^{a}	0.056^{a}
Lys	0.066^{b}	0.084^{a}	0.078^{b}
His	0.024^{a}	0.027^{a}	0.026^{a}
Arg	0.058^{b}	0.10^{a}	0.074^{b}
Pro	0.18^{b}	0.23^{a}	0.20^{b}

Different lowercase letters indicate significant differences in the same amino acid in pudding products with different peptide concentrations ($P < 0.05$).

of Lys to release more products with antioxidant activity (Xu, Shen, & Li, 2019). Notably, the content of hydrophobic amino acids in the pudding with 1.0 % gelatin peptide was significantly higher than that in the pudding with 0 % gelatin peptide and the CK group. This can be attributed to the presence of higher content of amino acids with free radical scavenging ability due to the high-pressure treatment. This finding aligns with the previous studies conducted by Tang and He *et al.*, which reported that high-pressure pretreatment facilitated the release of three peptides possessing potent antioxidant activity and augmented of hydrophobic amino acids (He, Han, Yu, Wang, Li, & Han, 2024) (Tang, et al., 2022).

3.6.3. Color

Color is an important visual parameter to consider when assessing consumer acceptability of a product (Yıldırım-Yalçın, Sadıkoğlu, & Şeker, 2021). As can be seen from Fig. 4 (Fig. S5), on day zero, the L^* and b^* values gradually decreased, while the a^* value gradually increased, with an increase in the addition of peptides. However, in the CK group, there was no significant difference observed for the L^* value and b^* value ($P > 0.05$), barring compared with the significant difference observed for a^* value and pudding with 0 % gelatin peptide ($P < 0.05$). This is possibly due to gelatin peptides having an effect on color, since the hydrolysis of alkaline protease makes the gelatin peptide darker.

Notably, with the extension of storage time, the L^* and b^* values showed a significant downward trend ($P < 0.05$), while a^* value showed a significant upward trend ($P < 0.05$). This may be due to the Maillard reaction between the amino compounds produced by the oxidative degradation of proteins (Chen, Leinisch, Greco, Zhang, Shu, Chuang, et al., 2019) and the carbonyl compounds produced by the decomposition of lactose. The colorful byproducts of this reaction may affect the color of the pudding. Simultaneously, during the manufacturing process, the pudding will produce more precursor material for the Maillard reaction due to the excessive heat load, thereby accelerating the reaction and contributing to the change in the color of the pudding (Tokusoglu, Akalin, & Unal, 2006). This aligns with the findings of Sunds *et al.*, who observed that the concentration of Arg (a marker produced in the early stages of the Maillard reaction) increased in UHT milk with an increase in storage time and temperatures ranging from 10 °C to 40 °C. This suggests that the Maillard reaction continues to occur in dairy products during storage (Sunds, Rauh, Sørensen, & Larsen, 2018). In addition, Martelli *et al.* (Martelli, Bancalari, Neviani, & Bottari, 2020), determined that the pink discoloration of Pecorino Toscano PDO cheese skin, which increases the a^* value of the cheese surface, is controlled by *Serratia acetifaciens*, indicating environmental contamination of the cheese skin. Therefore, microorganisms may also be responsible for pudding discoloration. However, the chroma of the pudding with 0.5 % gelatin peptide and the pudding with 1.0 % gelatin peptide changed slowly, possibly due to the antioxidant and antibacterial properties (López-García, Dublan-García, Arizmendi-Cotero, & Gómez Oliván, 2022) of gelatin peptides to inhibiting the reactions described above. Furthermore, on comparing color changes in the pudding at 25 °C and 4 °C, a delay was observed at 4 °C, which may be due to the low temperature and the antioxidant properties of gelatin peptides. The latter may have inhibited protein and fat oxidation in the pudding, as well as microbial growth and reproduction, thereby limiting the speed of Maillard reaction and delaying the color change of the pudding (Liang, 2000).

In summary, when the quantity of gelatin peptide added to the pudding is 0.5–1.0 %, inhibition of color change is clearly visible in pudding products.

3.6.4. pH

pH has an important effect on the gel system in pudding products and is a key factor in the evaluate of the product quality during storage (H. Chen, et al., 2022). Based on Fig. 5 (a-b) (Fig. S6 (a-b)), on day zero, it was observed that the addition of gelatin peptide (0.5 %, 1.0 %, 1.5 %, 2.0 %) significantly increased the pH of the pudding products ($P < 0.05$), while the CK group showed insignificant changes in pH ($P > 0.05$). This could be due to the alkaline protease used in gelatin peptide production, which would have alkalized the gelatin peptides and led to an increase in the pH. In addition, as the preservation time was extended, the pH of all pudding products is gradually reduced due to fat oxidation, causing rancidity (Sol Morales, Palmquist, & Weiss, 2000). This aligns with the findings of Frankel *et al.* (Frankel, 1993), who suggested that inhibiting fat oxidation (especially spontaneous oxidation) in milk and dairy products is a key factor in preserving quality and extending their storage life. Simultaneously, due to bacterial growth and reproduction in the product, food quality deteriorates and produces acid, which reduces the pH (Sibel Akalın, 2014). Further reduction in pH may also occur due to lactose degradation and acid production (Moufle, Jamet, & Karoui, 2017).

Notably, with the extension of storage time, at 25 °C and 4 °C, when 0.5–1.0 % gelatin peptide was added, as compared to the pH decline rate significantly lower than in the pudding with 0 % gelatin peptide ($P < 0.05$). This could be due to the gelatin peptides inhibiting fat oxidation and microbial reproduction. However, with the extension of storage time, 1.5–2.0 % gelatin peptide was added to the pudding pH decline rate significantly ($P < 0.05$) higher than the pudding with 0 % gelatin peptide. Both samples showed obvious rancidity and deterioration on the ninth day. It may be that excessive amounts of gelatin peptides destroyed the balance between pro-oxidants and antioxidants, thus affecting the stability of milk and accelerating oxidation (Sol Morales, Palmquist, & Weiss, 2000). On comparing the samples at different temperatures with the same storage time, the pH of the different pudding samples at 4 °C decreased slower than those at 25 °C. This may be due to low temperatures inhibiting microbial activity and oxidation reactions, thus slowing microbial growth and oxidative rancidity (Sibel Akalın, 2014).

In summary, when 0.5–1.0 % gelatin peptide was added to the pudding products, an alleviation in the declining trend of pH values was clearly observed.

3.6.5. Sensory evaluation

According to Fig. 5(c-d) (Fig. S6(c-d)), on day zero, the sensory score of the pudding gradually decreased with an increase in gelatin peptide concentration ($P < 0.05$). Compared with the pudding with 0 % gelatin peptide, the sensory evaluation significantly declined when 1.5–2.0 % gelatin peptide was added to the pudding ($P < 0.05$). This may be due to the unique flavor of the alkaline protease used in the production of gelatin polypeptides, which may not have been completely removed

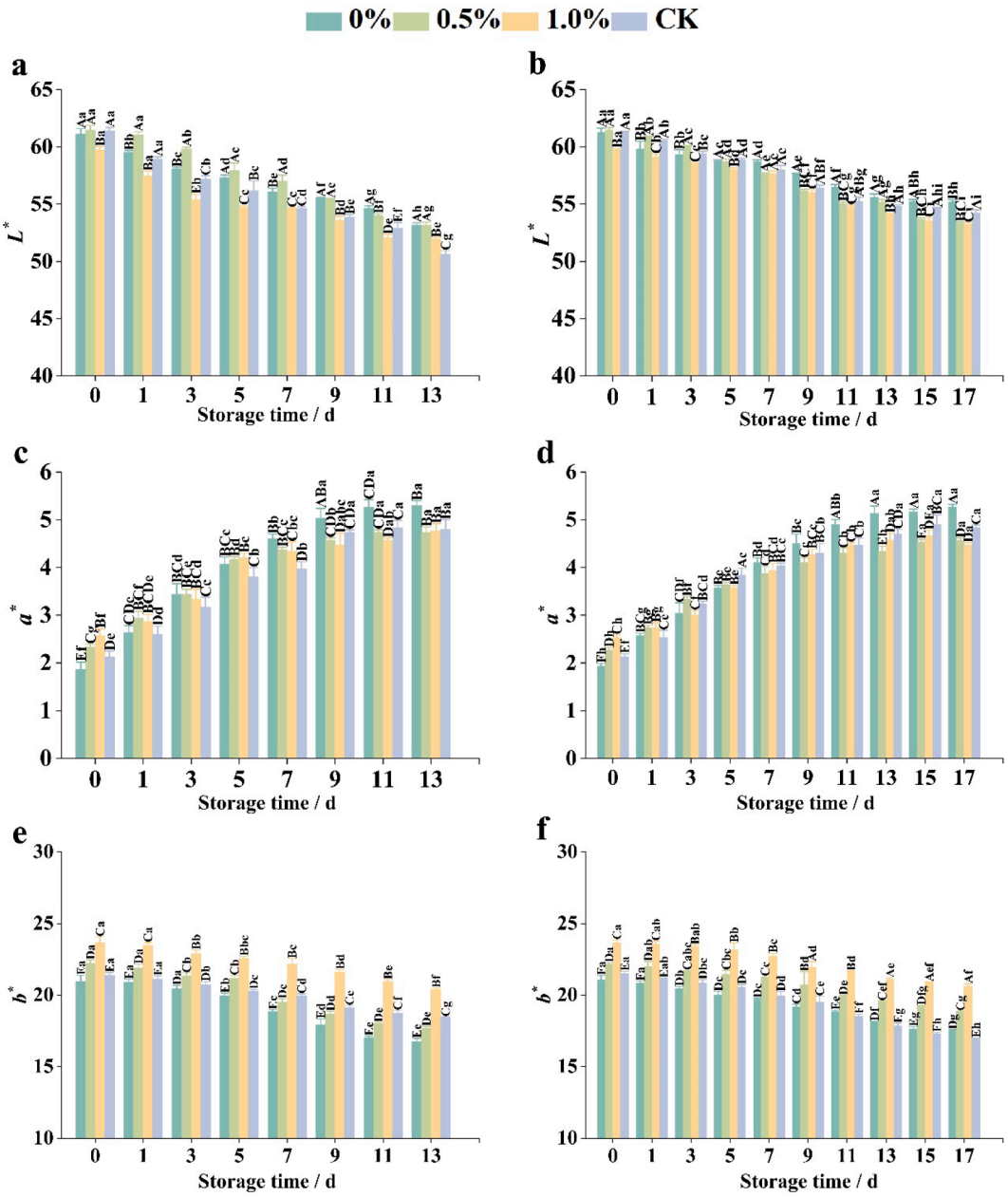

Fig. 4. Effect of peptide addition on chroma at different temperatures. Note: a, c, e represents the effect of different peptide addition amount on L^*, a^*, b^* value at 25 °C; b, d, f represents the effect of different peptide addition levels on L^*, a*, b* value at 4 °C. Vertical lines in the figure represent error lines; Different capital letters in the figure represent the same peptide, and different concentrations are significantly different ($P < 0.05$); Lowercase letters indicate a significant difference between two peptides at the same concentration ($P < 0.05$).

during late deodorization (W, X, X, G, Z, & biochemistry, 2019). Another cause may be the bitter substances in the gelatin peptide (Celestino, Iyer, & Roginski, 1997a). In order to minimize the sensory impact of gelatin peptide on pudding products, its content should be reduced as much as possible. The addition of low amounts of gelatin peptide (0.5–1.0 %) had the least effect on the pudding's sensory score.

Moreover, with prolonged storage time, sensory scores are observed to gradually decline, which may be due to lipid oxidation leading to oxidative odor production and protein pollution (Thomas, Scher, Desobry-Banon, & Desobry, 2004). This is similar to the results of Liang *et al.* (Liang, 2000), who found that an increase in storage time is associated with an increase in oxidation, which leads to poor pudding product quality. Another reason for the gradual decline in sensory scores and pudding quality could be due to protein degradation, which produces bitter peptides. This degradation occurs because proteases remain stable when pudding is stored, since they are endogenous or bacterial and can withstand high temperatures (Celestino, Iyer, & Roginski, 1997a). Notably, compared with the pudding with 0 % gelatin peptide, the changes in sensory scores were slower when 0.5–1.0 % gelatin peptide was added to the pudding, which may be due to the antioxidant nature of gelatin peptides reducing the oxidized odor substances produced by fat oxidation. However, it was observed that when 1.5–2.0 % gelatin peptide was added to the pudding, the sensory evaluators indicated significantly unpleasant rancidity beginning on day nine, which is consistent with the observed change in pH. In addition, the oxidation reaction accelerates 10 times with a 10 °C-temperature increase (Cluskey, Connolly, Devery, O'Brien, Kelly, Harrington, et al., 1997). Compared to the changes occurring at 25 °C, the sensory score was found to change slower at 4 °C, likely due to the inhibition of lipid oxidation and protease activity by low temperatures. This is consistent

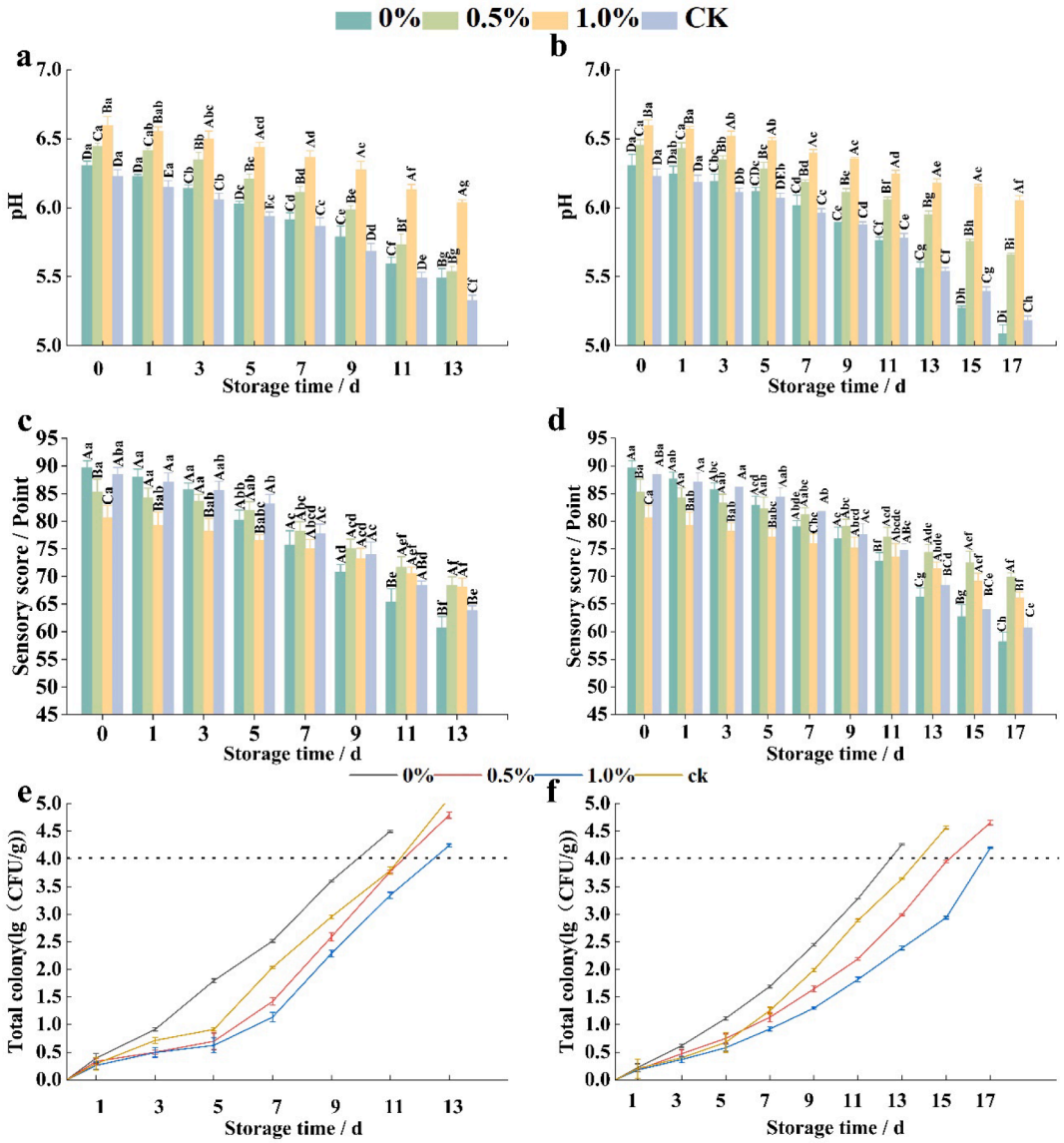

Fig. 5. (a-b) Effect of peptide addition amount on pH value at different temperatures. (c-d) Effect of peptide addition on sensory scores at different temperatures. (c-d) Effect of peptide addition on the total number of colonies at different temperatures. Note: a represents the change in pH at 25 °C, b represents the change in pH at 4 °C, c represents the change in sensory score at 25 °C, d represents the change in sensory score at 4 °C, e represents the change in the total number of colonies at 25 °C, and f represents the change in the total number of colonies at 4 °C. Note: Vertical lines in the figure represent error lines; Different capital letters in the figure represent the same peptide, and different concentrations are significantly different ($P < 0.05$); Lowercase letters indicate a significant difference between two peptides at the same concentration ($P < 0.05$).

with the results of Celestino *et al.*, who found that the non-protein nitrogen content of protein in whole milk powder during storage displayed an increasing trend, with the protease activity at 25 °C being higher than that at 3 °C (Celestino, Iyer, & Roginski, 1997b).

In summary, when 0.5–1.0 % gelatin peptide was added to the pudding, a clear alleviation in the decline of the sensory score of pudding products was observed.

3.6.6. *Total number of colonies*

The total number of colonies is an important indicator to evaluate whether a pudding product is edible. According to *GB/T 19883–2018*, when the total number of colonies in a pudding is 10^4 or greater, it is considered inedible. As observed in Fig. 5 (e-f) (Fig.S6 (e-f)), the total number of colonies increases with an increase in preservation time, which may be due to oxidation destroying the protein structure and accelerating degradation, thereby producing amino acids conducive for microbial growth in the pudding product (Wan, Feng, Wang, Du, Wang, Yu, et al., 2023). Notably, colony growth was observed to be the slowest when 0.5 %–1.0 % gelatin peptide was added to the pudding. This is possibly because gelatin peptides with antioxidant properties could inhibit protein oxidation and reduce free amino acid production. It may also be due to gelatin peptides having certain antibacterial properties (López-García, Dublan-García, Arizmendi-Cotero, & Gómez Oliván, 2022), which is consistent with the findings of Muhialdin *et al* (Muhialdin, Rani, & Hussin, 2020). They found that gelatin peptides have antioxidative and antibacterial effects that inhibit microbial growth and reproduction in pudding products. Moreover, the bacterial growth rate in the pudding with 2.0 % gelatin peptide was higher than that in the pudding with 0 % gelatin peptide. This may be due to the excessive addition of gelatin peptide, which makes the gel system difficult to maintain and accelerates bacterial growth. In addition, the storage period of pudding was found to be more effectively prolonged at 4 °C than at 25 °C, indicating that low temperatures combined with the antioxidant and bacteriostatic effects of gelatin peptide had obvious

effects on pudding quality during storage.

In summary, when 0.5–1.0 % gelatin peptide was added to the pudding, a clear inhibition in total colony growth of the pudding products was observed.

4. Conclusion

Although the production cost of gelatin peptides does not differ drastically from that of commercially available peptides, this study found that gelatin peptides are more economically beneficial due to their higher extraction rates, antioxidant properties, biological activity, degradation degrees, and hydrophobic amino acid content and purity. In summary, when 0.5–1.0 % gelatin peptide was added to the pudding it was found to significantly delay the color change, pH reduction, and sensory score reduction of pudding products. Moreover, due to the antioxidant properties of gelatin peptide, it was found that a small amount of gelatin peptide (0.5–1.0 %) extended the storage life of pudding by three days at 25 °C and five days at 4 °C. In addition, gelatin peptides not only affect the storage process of pudding, but also can further explore its effects on the human body after consumption.

Ethical approval

Ethics approval was not required for this research.

CRediT authorship contribution statement

Wanlin Wang: Methodology, Visualization, Writing – original draft. **Yining Zhao:** Investigation, Project administration, Software. **Long He:** Formal analysis, Resources. **Zhaoyang Song:** Writing – review & editing. **Chaoxue Shi:** Supervision. **Pei Jia:** Data curation. **Qunli Yu:** Conceptualization, Validation. **Lin Han:** 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.2024.101327.

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