Preparation and characterization of sesame peptide-calcium chelate with different molecular weight

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\textbf{ABSTRACT}
Sesame peptides could be used to treat underlying diseases and a suitable carrier of nutrients. In this study, sesame peptides were mainly prepared from SSM (sesame meal), and the chelating ability of different molecular weight sesame peptide components to calcium and the physical characterization of the chelates were investigated. After that, the molecular weights were verified by HPLC (high performance liquid chromatography), and the Zeta potential, PDI (polydispersity) of SSMP (sesame meal peptides) of different molecular weights were determined. In the SSMP-Ca chelates, the chelation rate of SSMP-2 (sesame meal peptide components 2) to calcium is 72.72 ± 0.17%. Fluorescence spectrum analysis found that the fluorescence intensity of chelates decreased, and the maximum absorption peak was red-shifted. FT-IR (Fourier Transform Infrared spectroscopy) research showed that the chelate vibration absorption wavelengths of the three characteristic peaks -NH\textsubscript{2}, COO\textsuperscript{-}, and C = O had change, indicating that the amino and carboxyl groups in sesame peptide participated in the chelation reaction. SEM (scanning electron microscope) analysis showed that SSMP and calcium could form a chelate with specific stability. Most of its microstructures were encapsulated and adhered to each other to create a “bridging effect” and appear as aggregates. These results provided a basis for using sesame peptides in the food or pharmaceutical industry.

\textbf{Introduction}
Calcium is an essential inorganic element in the human body, accounting for 1.5–2.2\% of the total weight of the human body.\textsuperscript{[1]} It has certain regulatory functions for intracellular metabolism, promoting bone growth and nerve conduction.\textsuperscript{[2,3]} The lack of calcium can cause more diseases, such as osteoporosis, rickets, and hypertension.\textsuperscript{[3,4]} From the perspective of functional food and pharmaceutical industries, to help overcome calcium deficiency, the most common calcium supplements are inorganic calcium, such as calcium carbonate and calcium lactate.\textsuperscript{[5]} Since the intestinal environment of the organism is a slightly alkaline environment, these inorganic calcium supplements will quickly produce different calcium salts after entering the organism, which reduces the bioavailability of calcium in the supplement.\textsuperscript{[6–8]} Therefore, it is a pressing task in the field of food or medicine to develop new calcium supplements to meet the broad needs of human health and sales market.

As a macromolecular substance composed of amino acids with different functional values from protein,\textsuperscript{[9]} peptides have excellent digestion and absorption in the gastrointestinal system, so polypeptides are often carriers for other substances.\textsuperscript{[10,11]} In recent years, there have been studies on the preparation of peptide-calcium chelates. Wang et al. prepared cucumber seed peptide-calcium chelate and characterized the physical structure of the obtained chelate.\textsuperscript{[12]} Huang et al. designed egg white
peptide-calcium chelates (EWP-Ca) using response surface methodology and received the best preparation process: the mass ratio of peptide to calcium is 4:1, the temperature is 53°C, the system pH is 8.2, and the time is 30 minutes. In addition, spectroscopy confirmed that the carboxyl oxygen and amino nitrogen atoms in the egg white peptide might combine with calcium to form a chelate compound during the chelation process.[13] Liu et al. chelated the hydrolyzate of wheat germ protein and CaCl₂ to obtain wheat germ peptide-calcium chelate.[14] Wu et al. used protease to decompose porcine collagen and then combined the enzymatic hydrolyzate with calcium to get porcine collagen peptide-calcium chelate.[15] According to reports, sesame peptide has shown a series of functional values such as antioxidant activity, blood pressure-lowering effect, cholesterol-lowering and thrombolysis.[16–18] Additionally, peptide-calcium chelates can enhance intestinal calcium absorption by preventing calcium precipitation in the gut, overcoming the negative effects of phosphate and phytate.[19] Therefore, peptide-calcium chelates are considered as a potential dietary calcium supplement due to their advantages in promoting calcium absorption.[20] Lu et al. explored the free radical scavenging ability of sesame peptides with different molecular weights.[21] They showed that the smaller the molecular weight, the stronger the anti-free revolutionary capacity of sesame peptides. However, the studies of SSMP-Ca chelation with different molecular weights have not been reported.

This study clarified the difference between different molecular weight sesame peptides and calcium chelate. The chelating ability of SSMP of various molecular weights to calcium was studied, and the chelates structure was characterized. These findings can facilitate the high-value and highly targeted use of protein peptides extracted from sesame meals as potential functional carriers with biological activity.

Materials and methods

Materials

Subcritical sesame meal, homemade in the laboratory. Alkaline protease (1.97 × 10⁵ U/g) and Gly-Gly-Tyr-Arg were purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China). Cytochrome c, Aprotinin, Bacitracin, and L-glutathione were purchased from Sigma Co., Ltd. (St. Louis, MO, USA). Calcium chloride (CaCl₂) and potassium bromide (KBr) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other reagents used are of analytical grade.

Preparation, purification and separation of SSMP

A 2% SSM (sesame meal) slurry was prepared accordingly and were adjusted to pH 10.9, and then 9700 U/g of alkaline protease substrate was added, and the reaction was kept at 51°C for 2 h. After the response, place it in a boiling water bath for 15 min, take it out and to 25°C. Then centrifuge at 8000 rpm/min and 4°C for 30 min, and place the supernatant at −50°C for vacuum freeze-drying to obtain SSMP.

The prepared SSMP was made into a 2% solution and filtered with a 0.45 µm water membrane. Using organic molecular weights of 1000, 3000, 5000, 8000, and 10000 Da the ultrafiltration membrane element adopts the principle of cross-flow filtration to perform ultrafiltration and retention of SSMP at 0.5 MPa, 25°C to achieve the effect of separating different components. After using organic ultrafiltration membrane elements with different molecular weights to permeate and intercept SSMP, obtained SSMP-1 (sesame meal peptide components 1, 8–10 kDa), SSMP-2 (sesame meal peptide components 2, 5–8 kDa), SSMP-3 (sesame meal peptide components 3, 3–5 kDa), SSMP-4 (sesame meal peptide components 4, 1–3 kDa), SSMP-5 (sesame meal peptide components 5, < 1 kDa).

Then use the 150 Da organic nanofiltration membrane element to carry out nanofiltration desalination treatment for each component, and can play a role in concentration. The SSMP-1, SSMP-2, SSMP-3, SSMP-4, and SSMP-5 obtained by the ultrafiltration mentioned above and interception are placed in the material tank. At a pressure of 3 MPa, nanofiltration desalination treatment is performed. After being freeze-dried at −50°C, store it for later use.
**Determination of ultrafiltration recovery and nanofiltration salt removal rate**

The recovery rate of peptide is calculated according to formula (1).

$$W = \frac{m_1}{m_2} \times 100\%$$  \hspace{1cm} (1)

In the formula: $W$ represents the recovery rate of the polypeptide; $m_1$ represents the mass of the permeate after ultrafiltration and the mass of the retentate after freeze-drying (Note: when using a 10000 Da ultrafiltration membrane, $m_1$ only counts the mass of the permeate after freeze-drying); $m_2$ indicates the mass of peptide used in the material tank each time. This study used the direct titration method to determine the chloride in each component before and after nanofiltration, as described in the Chinese standard GB 5009.44–2016. The salt removal rate is calculated according to the formula (2).

$$T = \frac{x_1}{x_2} \times 100\%$$  \hspace{1cm} (2)

In the formula: $T$ represents the salt removal rate; $x_1$ represents the chloride content in the component solution before nanofiltration; $x_2$ represents the chloride content in the component retentate after nanofiltration.

**Molecular weight, zeta potential and PDI coefficient**

Basis the method of Liu et al to verify the molecular weight of the peptide fraction after ultrafiltration.\(^{[22]}\) Agilent 1260 high performance liquid chromatography (Agilent 1260, Agilent Co., USA) and TSKgel G2000SWXL column (7.8 mm×30 cm, 5 μm) (G2000SWXL, TOSOH Co., JPN) were used. The standard products are cytochrome c (12384 Da), aprotinin (6511.63 Da), bacitracin (1422.69 Da), L-glutathione oxidized type (612.63 Da), Gly-Gly-Tyr-Arg (451.48 Da). The absorption peak of the target is detected at 220 nm, and the ratio of the peak area of the actual retention time is used as the distribution ratio of the molecular weight interval. The zeta potential and PDI value were determined according to Sun et al. method\(^{[23]}\) by using Nano-ZS90 Zetasizer (Nano-ZS90, Malvern, UK). Each target was accurately prepared into a sample solution and placed in the Zeta potentiometer to measure the obtained dispersion.

**Preparation of SSMP-Ca and determination of the chelation rate**

According to the method described by Zhang et al., the process for the chelation reaction of sesame peptide and calcium was determined with some modifications .\(^{[24]}\) Accurately weigh SSMP and its components and prepare a 2% peptide solution. Then add CaCl\(_2\) at a mass ratio of 15:1 (sesame peptide: additive), place it in a water bath, shake, and chelate at 30°C for 60 min. After the chelation is completed, centrifuge at 10000 rpm/min and 4°C for 20 min, and the supernatant obtained is the peptide-calcium chelate, denoted as SSMP-1-Ca\(^{2+}\), SSMP-2-Ca\(^{2+}\), SSMP-3-Ca\(^{2+}\), SSMP-4-Ca\(^{2+}\), SSMP-5-Ca\(^{2+}\). Finally, it was freeze-dried at −50°C and stored for later use. Using EDTA (EthyleneDiamineTetraaceticAcid) ligand complexometric titration method. Carry out the calculation of chelation rate according to formula (3).

$$C = \frac{V_1}{V} \times 100\%$$  \hspace{1cm} (3)

In the formula: $V_1$ represents the volume of EDTA consumed to titrate a certain chelated ion; $V$ represents the volume of EDTA consumed to titrate the total amount of a certain ion.
**Fluorescence spectrometry and FT-IR spectroscopy**

Regarding the method adopted by Liao et al., fluorescence spectroscopy was analyzed by the F-4700 fluorescence spectrophotometer (F-4700, HITACHI, JPN) with a slight modification.\(^{[25]}\) Each sample was prepared into a 10 mg/mL solution, and the fluorescence emission spectrum of the target object at 300–600 nm was measured at an excitation wavelength of 300 nm.

FT-IR spectroscopy was performed using a Nicolet710 Fourier Transform Infrared Spectrometer (Nicolet710, Nicolet Co., USA) according to the method of Wang et al.\(^{[26]}\) Put a small amount of the target sample into the grinding body, add potassium bromide, grind it evenly, and press the tablet. Potassium bromide powder was used as the blank background, the resolution was set to 4 cm\(^{-1}\), the number of scans was 16, and full-wavelength (400–4000 cm\(^{-1}\)) scans were performed.

**Scanning electron microscope of different components SSMP chelated calcium**

The microstructure of the sesame peptide and sesame peptide-calcium complex was analyzed by using SUPRA\(^{TM}\) 55 thermal field emission scanning electron microscope (SUPRA\(^{TM}\) 55, Zeiss, Germany). The powder samples were sprayed and sputtered with gold. The specimen is observed under an accelerating voltage of 5 kV.

**Results and discussion**

**Ultrafiltration recovery rate and membrane flux**

The membrane flux and the peptide recovery rate was determined, see Table 1a. 10 kDa ultrafiltration membrane is added to the sample the membrane flux before is 300 mL/min. After cleaning after use, the membrane flux is 170 mL/min. It can be seen that the membrane flux has been reduced by 43.33%. This is because sesame peptide is a crude product that contains macromolecular proteins and some impurities, which causes the clogging of the ultrafiltration membrane and reducing its reusability. The drug can be used later. Flushing restores its membrane flux.

Careful considerations, this study only collected the permeate and measured the recovery rate after freeze-drying. When using other membrane elements, to obtain different components, the permeate and the retentate were collected separately, and the membrane flux was restored to more than 80% of the original basis. The peptide recovery rate was more than 95%. Therefore, it shows that ultrafiltration has a good retention and separation effect on sesame peptides.

**Table 1.** Results of membrane flux and sample recovery before and after ultrafiltration membrane use, and the result of nanofiltration desalination treatment and the percentage of components in the original sample. A Membrane flux and sample recovery before and after ultrafiltration membrane use. B Nanofiltration desalination treatment and the percentage of components in the original sample.

| Ultrafiltration membrane | Membrane flux (mL/min) | Sample recovery rate |
|--------------------------|-------------------------|----------------------|
|                          | Before use | After use |                      |
| 10 KDa                   | 300        | 170       | 79.52%               |
| 8 KDa                    | 180        | 135       | 95.24%               |
| 5 KDa                    | 150        | 128       | 97.58%               |
| 3 KDa                    | 150        | 140       | 95.36%               |
| 1 KDa                    | 120        | 110       | 94.58%               |

| Category | SSMP-1 (8–10 KDa) | SSMP-2 (5–8 KDa) | SSMP-3 (3–5 KDa) | SSMP-4 (1–3 KDa) | SSMP-5 (<1 KDa) |
|----------|------------------|------------------|------------------|------------------|-----------------|
| Salt removal rate | 95.17% | 93.38% | 90.46% | 88.73% | 85.61% |
| Percentage    | 2.80%  | 7.76%  | 15.72% | 24.32% | 5.15% |
Nanofiltration salt removal rate and component mass percentage

The five components of SSMP-1, SSMP-2, SSMP-3, SSMP-4, and SSMP-5 were desalted using a 150 Da nanofiltration membrane, and the percentage results of each component are shown in Table 1b. It can be seen from the data in the table that after nanofiltration of these five components to remove salt, it is found that the chloride content in SSMP-1 to SSMP-5 gradually increases, and the effect of nanofiltration to remove salt gradually decreases. Still, the overall salt removal rate is above 85%. This is because of the impact of the order of the ingredients. With the progress of the nanofiltration operation, the membrane elements are clogged, or other pollutants are polluted, which leads to the increase of chloride in the salt component after the nanofiltration is performed.

It can also be clearly seen from the above table that the portion of sesame peptide more significant than 10 kDa is not considered, and the reasonable loss of polypeptide during the ultrafiltration process is ignored. Compared with the original sesame peptide, SSMP-4 has the highest proportion, SSMP-3 has the second largest proportion, and SSMP-1 has the smallest proportion. This may be because most of the peptides produced during the hydrolysis of SSM by alkaline protease are concentrated between 1000–8000 Da, and the amounts of short peptides produced are not much.

Molecular weight identification results of different components

After analyzing the correlation between the average retention time of the standard and Log Mw, the regression equation was established as \( y = -0.2173x + 6.8741, R^2 = 0.9621 \). After substituting the retention time of the chromatogram of each peptide component in Figure 1a into the standard curve, the molecular weight distribution percentage as shown in Table 2b can be obtained. It can be seen from Table 2b that the proportion of 8–10 kDa in SSMP-1 is 69.1345%; the proportion of 5–8 kDa in SSMP-2 is 76.0302%; the proportion of 3–5 kDa in SSMP-3 is 82.6353%; the proportion of 1–3 kDa in SSMP-4 is 82.1978%; the proportion of <1 kDa in SSMP-5 is 94.2705%. This result further proves the separation effect of the ultrafiltration separation technology on sesame protein peptides. Since the sesame protein peptides are separated and fractionated step by step using ultrafiltration membranes of different molecular weights, the corresponding molecular weights in the small molecular weight components are higher.

Zeta potential and PDI coefficient of different molecular weight peptide components

The Zeta potential and PDI measurement results of each peptide component are shown in Figure 1b and 1c. PDI (polydispersity index) is an important physical and chemical index for evaluating the dispersibility of molecular polymers. The lower the PDI value of the target, the higher the degree of dispersion in water. Figure 1b shows the PDI difference between the peptide components. Their PDI values from high to low are: SSMP-1 (0.50 ± 0.04) > SSMP-3 (0.44 ± 0.10) > SSMP-4 (0.44 ± 0.01) > SSMP-5 (0.38 ± 0.07) > SSMP-2 (0.26 ± 0.01). It can be seen that the best dispersion in an aqueous solution is SSMP-2, and the worst dispersion is SSMP-1.

It can be seen from Figure 1c that each peptide component shows a negative potential, indicating that its surface charge has more negative charges. Their potential values are: SSMP-1 (−18.13 ± 2.44 mV), SSMP-2 (−20.00 ± 0.87 mV), SSMP-3 (−18.20 ± 0.56 mV), SSMP-4 (−17.13 ± 1.70 mV), SSMP (−15.53 ± 1.65 mV). It can be seen that SSMP-2 has the largest number of negative charges on its surface compared to other components. This may be related to the changes in the number of exposed ionizable amino groups and carboxyl groups on the protein’s surface.

It was found that when the product produced by proteolysis has the characteristics of high Zeta potential, it can enhance intra- and intermolecular electrostatic repulsion, promote the degree of the unfolding of protein structure, destroy existing protein aggregates or prevent further aggregates formed, thereby improving the dispersibility of the hydrolysate.\(^{[27,28]}\) Therefore, the low dispersion coefficient of SSMP-2 may be related to the level of its potential.
Determination of the chelation rate of different peptide components with calcium

Measured by EDTA complexometric titration, the chelation rate of each peptide component to Ca$^{2+}$ is shown in Figure 2a. It can be seen from Figure 2a that under the same conditions, the separated parts of sesame peptide have a good chelating ability to Ca$^{2+}$, and the chelating rate is above 50%. Among them, SSMP-2 with a molecular weight of about 5–8 kDa has the highest chelation rate for Ca$^{2+}$, which is 72.72 ± 0.17%; SSMP-4 with a molecular weight of about 1–3 kDa has the lowest chelation rate for Ca$^{2+}$, which is 53.35 ± 0.30%, there is a significant difference in the chelation rate of each component to Ca$^{2+}$ (P < .05). This may be because the SSMP-2 peptide contains more groups that can bind to Ca$^{2+}$. In addition, the zeta negative potential of SSMP-2 is the highest, which proves that it has more negative surface charges and the lowest PDI coefficient, which can be better dispersed in an aqueous solution, thereby increasing the contact area with Ca$^{2+}$, resulting in a high chelation rate. In addition, for the smaller molecular weight SSMP-4 and SSMP-5, the chelation rate of Ca$^{2+}$ is weak, which may be due to the small molecular weight resulting in fewer residue sites that can bind to ions in the polypeptide molecule.
Fluorescence spectrometry of different peptide components chelated calcium

The results of the fluorescence spectra of the separated components and the products obtained by chelating each component with Ca$^{2+}$ are shown in Figure 2b. In protein molecules, aromatic amino acids are exceptional among them. They all have a benzene ring in structure, which mainly includes Trp (tryptophan), Tyr (tyrosine), and Phe (phenylalanine), especially the polarity of the
microenvironment of Trp residues. Because of the particularity of their chemical structure and the difference in the side chain chromophores, they can emit fluorescence and produce emission spectra. It can be clearly seen from Figure 2b that all the separated components of sesame peptide have fluorescence absorption, and the maximum absorption peak is around 353 nm, which is consistent with the results of the Zhao et al. study on the fluorescence emission spectra of whey protein peptides. However, the fluorescence intensity of each component is significantly different, and the fluorescence intensity is respectively: SSMP-5 (3371) > SSMP-4 (1759) > SSMP-3 (1280) > SSMP-2 (1055) > SSMP-1 (588.4). This is because after the protein is hydrolyzed, the spatial structure of the protein changes, causing the side chain groups of the aromatic amino acid residues to be exposed, and the system’s polarity increases. However, the fluorescence intensity is mainly affected by the energy transfer from Tyr to Trp (depending on the distance between the two residues) and the quenching group in adjacent amino acid residues. Since ultrafiltration membranes of different molecular weights separate each component, the length of the polypeptide chain in each component is different, and the distance between Tyr and Trp residues may also be different, which results in the fluorescence intensity of each component.

It can also be seen from the figure that after the separated components of sesame peptide are chelated with Ca$^{2+}$, the fluorescence spectrum curve of the obtained chelate is significantly different from the original component. After the chelation reaction of each component with Ca$^{2+}$, the maximum absorption wavelength of the product’s fluorescence was shifted to the long-wavelength direction at 350 nm, and the redshift effect occurred, which proved the production of a new substance. In addition, the fluorescence absorption intensity of the obtained chelate is greatly reduced. This may be because when the peptide forms a chelate with Ca$^{2+}$, the increase in Ca$^{2+}$ content leads to structural changes in the original molecule and increases the folding and curling of the peptide molecule. The distance between the chromogenic residues is reduced, which leads to fluorescence quenching and decreases the fluorescence intensity, which is very similar to the study of Zhao et al. However, the decrease in the fluorescence intensity of each component after chelation with calcium did not show a regular change.

**FT-IR spectroscopy of different components SSMP chelated calcium**

The FT-IR spectroscopy characteristic vibrational absorption peak wavelengths of the separated components and their products after chelating with Ca$^{2+}$ are shown in Table 2b and Figure 3. It can be clearly seen from the data in the table that each separated component has the characteristic vibration absorption peaks of -NH$_2$, COO$, and C = O, which can prove that it has a polypeptide structure. After each peptide component reacted with Ca$^{2+}$ to form a chelate, under the identification of FT-IR spectroscopy, the -NH$_2$ and COO$^-$ vibration absorption wavelengths all shifted to the long-wavelength direction, and the redshift effect occurred. Such results are consistent with the results of fluorescence spectroscopy.

Compared with the infrared spectrum curve of the original peptide component, the -NH$_2$ vibrational absorption wavelength of each peptide component and Ca$^{2+}$ chelate shows a regular change. It was shows that, except for SSMP-5, the red-shifted wavelength of -NH$_2$ in the infrared spectrum of the chelate decreases gradually with the decrease of the molecular weight of the components. However, there is no obvious rule for the absorption wavelength of COO$, C = O$ stretching vibration, which may be caused by the difference in the number of carboxylic acid residues in the side chain of the exposed group in the polypeptide chain. Interestingly, by observing the value of the redshift wavelength of -NH$_2$ vibration absorption, it is found that there may be a certain correlation between the redshift wavelength and the chelation rate, that is, the chelation rate of the component with a large redshift wavelength to Ca$^{2+}$ high, low redshift wavelength components have low chelation rate to Ca$^{2+}$. Therefore, the changes in the vibration absorption wavelength of the infrared characteristic peaks of -NH$_2$ can also reflect the changes in the chelation rate of each component.
Figure 3. FT-IR results of chelates formed by SSMP components of different molecular weights and calcium. A: FT-IR results of SSMP-1 and SSMP-1-Ca$^{2+}$; B: FT-IR results of SSMP-2 and SSMP-2-Ca$^{2+}$; C: FT-IR results of SSMP-3 and SSMP-3-Ca$^{2+}$; D: FT-IR results of SSMP-4 and SSMP-4-Ca$^{2+}$; E: FT-IR results of SSMP-5 and SSMP-5-Ca$^{2+}$.
Figure 4. SEM microstructures of SSMP components with different molecular weights, as well as comparison results of SEM microstructures of chelates formed with each component and calcium. A: SEM microstructures of SSMP-1 and SSMP-1-Ca$^{2+}$; B: SEM microstructures of SSMP-2 and SSMP-2-Ca$^{2+}$; C: SEM microstructures of SSMP-3 and SSMP-3-Ca$^{2+}$; D: SEM microstructures of SSMP-4 and SSMP-4-Ca$^{2+}$; E: SEM microstructures of SSMP-5 and SSMP-5-Ca$^{2+}$. 
In summary, each separated peptide component can chelate with Ca\(^{2+}\), and the vibration absorption wavelength of the characteristic peak of -NH\(_2\) changes regularly, indicating that the amino and carboxyl groups in the peptide are involved in the chelation process, so it has a particular reference value for actual production.

**Scanning electron microscope of different peptide components chelated calcium**

In this study, the microscopic morphology of each peptide component and the chelates of each peptide component and calcium were observed by SEM. The analysis results are shown below. As shown in Figure 4a and 4b, the structure of SSMP-1 and SSMP-2 is relatively broken, and both exhibit irregular sheet-like structures. Except for other small fragments, the surface has tiny protrusions. Under high magnification, the surface can have a porous structure, and SSMP-1 and SSMP-2 can basically be considered as porous planar structures. This may be because during the freeze-drying process, the moisture in the polypeptide vaporizes and the moisture escapes from the polypeptide to produce the porous structure. As shown in Figure 4c, 4d, and 4E, the microstructures of SSMP-3, SSMP-4, and SSMP-5 are all fragments, but their surface is flat and smooth, which is quite different from others. This may be because in the ultrafiltration process, pure small molecular weight peptides are more likely to aggregate, resulting in a denser structure.

It can be clearly seen from Figure 4a that SSMP-1- Ca\(^{2+}\) is obviously different from other chelates. It is found that the addition of Ca\(^{2+}\) changes the structure of SSMP-1 from smooth to coral, which may be due to its surface the porosity leads to; and in the same field of view, the amount of Ca\(^{2+}\) is less. As for the way that other components form chelate with Ca\(^{2+}\), most of them are in the state of being wrapped by peptide molecules, and a few are in the state of attachment. In addition, compared with SSMP-4, SSMP-4- Ca\(^{2+}\) has serious structural damage and more fragments, and the amount of Ca\(^{2+}\) in the field of view is less.

In summary, sesame peptide and Ca\(^{2+}\) can form a chelate with certain stability. The microstructure of chelates mostly shows that polypeptide molecules encapsulate metal ions, and the molecules adhere to each other, forming a “bridging effect” and appearing in aggregation. This may be because the sesame peptide solution presents a negative potential, and the surface of the molecule has more negative charges. When metal ions are added, the positively charged ions are wrapped by the involute, thereby forming a stable chelate. A small number of microstructures show that a small amount of metal ions gathered and attached to the surface of the polypeptide molecule. This may be because the metal ions are attached to the surface after binding to different residue sites. In addition, peptide molecules with a smaller molecular weight will cause significant changes in their structure when they form a chelate.

**Conclusion**

This study reported the chelating ability of different molecular weight sesame peptides to calcium, and the previous differences in the physical structure of different molecular weight sesame peptide-calcium chelate. In comparison, SSMP-2 has the highest chelation rate of calcium; SSMP-4 has the lowest chelation rate of calcium. This is due to the difference in molecular weight leading to the difference in the number of residue sites that bind to calcium in the polypeptide molecule. Both the fluorescence spectrum and FT-IR showed that sesame peptide can form a stable chelate with calcium. However, there are differences in the spectral characteristics of the chelates formed by sesame peptides of different molecular weights and calcium, and the change of the characteristic peak wavelength in FT-IR is also related to the level of calcium chelation rate of each component. SEM shows that each chelate has obvious differences with the original structure, and all include two chelating modes, “encapsulated state” and “attached state.” In addition, the small molecular weight sesame peptide forms a chelates with calcium, and its structure will change significantly, and the structure will be severely damaged, which will reduce the chelation rate. This study shows that sesame peptide may be used as a functional
food and health medicine, and the molecular weight level is defined to better promote the bioavailability of calcium.

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**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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