Current Research in Food Science 8 (2024) 100651

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Uncovering the rheological properties basis for freeze drying treatment-induced improvement in the solubility of myofibrillar proteins

Huijuan Yang [a], Zhizhao Chen [a], Haifeng Wang [b,*], Danping Jin [b], Xiaoqi Wang [d], Fan Wang [f], Xuejiang Cen [f], Jinsong Liu [g], Qing Shen [c,e,**]

[a] College of Standardization, China Jiliang University, Hangzhou, 310018, China
[b] Collaborative Innovation Center of Seafood Deep Processing, Institute of Seafood, Zhejiang Gongshang University, Hangzhou, 310012, China
[c] Department of Clinical Laboratory, The Quzhou Affiliated Hospital of Wenzhou Medical University, Quzhou People's Hospital, Quzhou, 324000, China
[d] Tufts University Friedman School of Nutrition Science and Policy, Boston, MA, 02111, USA
[e] Zhejiang Province Joint Key Laboratory of Aquatic Products Processing, Institute of Seafood, Zhejiang Gongshang University, Hangzhou, 310012, China
[f] Zhejiang Provincial People's Hospital (Affiliated People's Hospital, Hangzhou Medical College), Hangzhou, Zhejiang, 310014, China
[g] Zhejiang Huijia Biotechnology Co. Ltd., Anji, Zhejiang, 313307, PR China

ARTICLE INFO

Keywords:
Freeze-drying
Functional characteristics
Rheology
Multivariate statistical analysis

ABSTRACT

Myofibrillar proteins (MPs) are an important nutritional supplement and have great significance in sports training and rehabilitation therapy. Currently, MPs preservation is still disputed since they are vulnerable to degradation, polymerization, and denaturation. Freeze-drying is an emerging technology for protein preservation, its effects on the functionality of MPs from different sources have not yet been thoroughly studied. This study aims to evaluate the performance differences of freeze-drying in maintaining the functional characteristics of MPs from fish and mammalian sources, providing valuable insights for the processing and preservation of MPs, and providing nutritional support for nursing and rehabilitation. The results showed that freeze-drying was an efficient method for protein preservation, and the effects of freeze-drying on both fish and mammalian sources MPs were significant ($p < 0.05$) consistent. Specifically, whether before and after freeze-drying, the solubility of fish MPs (FMPs) was significant ($p < 0.05$) lower than that of mammalian MPs, while the foaming and emulsifying properties were significant ($p < 0.05$) higher than those of beef and sheep MPs (BMPs and SMPs, respectively). Furthermore, the most efficient protein concentration for freeze-drying was 10 mg/mL, and with this concentration, the gel strengths of BMPs and SMPs showed an insignificant difference ($p > 0.05$) after freeze-drying.

1. Introduction

Meat is one of the best sources of high-quality bio-accessible protein, and the muscle proteins contain more digestible lysine than plant proteins, which is regarded as a good protein supplement for the human body and have great significance in sports training and rehabilitation therapy (MacKenzie and Luyet, 1967). In the consumption structure of meat and its products in China, mammalian meat accounts for the highest proportion, especially pork, accounting for about 60% of the total meat consumption, followed by beef and sheep, accounting for about 20% in total (Wang et al., 2022; Yang et al., 2021). However, due to the high-fat contained, mammalian meat is unfriendly to the 'three highs' population, therefore the proportion of fish in the meat consumption has increased recently (de Oliveira, Neto, dos Santos, Ferreira and Rosenthal, 2017).

Myofibrillar proteins (MPs) are the main components in meat (Shuai et al., 2021). Its functional properties, such as solubility, foaming, emulsifying and gel properties, have an important impact on food processing and product quality (Shuai et al., 2021). However, because the spatial structures are sensitive to physical factors (such as pressure, and temperature) and chemical factors (such as acid and alkali), MPs are easy to deteriorate, and their functional properties were affected, which

* Corresponding author.
** Corresponding author. Department of Clinical Laboratory, The Quzhou Affiliated Hospital of Wenzhou Medical University, Quzhou People's Hospital, Quzhou, 324000, China.
E-mail addresses: whf4341514@163.com (H. Wang), leonqshen@163.com, sq@zjgsu.edu.cn (Q. Shen).

https://doi.org/10.1016/j.crfs.2023.100651
Received 19 September 2023; Received in revised form 1 December 2023; Accepted 2 December 2023
Available online 14 December 2023

further affects the quality of meat products (Zhang et al., 2022, 2023). Therefore, the preservation of functional proteins has received more and more attention. The traditional method for the preservation of proteins is liquid-state storage at 2–8 °C, under which the proteins are difficult to aggregate. The proteins stored by this method are difficult to accumulate and precipitate, which is particularly effective for maintaining the stability of proteins, but a slow chemical degradation process is still on-going. Freezing storage is another conventional method of liquid state storage. At low temperature, the degradation rate of protein decreases or even ceased, and the sample can be stored stably for several months. However, the denaturation of proteins still might be caused by low temperature (Helmick et al., 2021), solute concentration (Tan et al., 2022), the formation of the ice-liquid interface (Arsiccio et al., 2020), the drastic change of pH value (Tan, Ye, & Xie, 2021), and microbial formation (Yumiko and Matsuda, 1981).

Freeze-drying, also known as lyophilization, is a well-known technique for t preserving the color, fragrance, taste and shape of premium foods (Waghmare et al., 2021). After sealed packaging, it can be stored, transported and sold at room temperature for a long time. Freeze-drying generally includes three processes: pre-freezing, sublimation drying, and analytical drying (Wenzel and Gieseler, 2021). Among which sublimation is the main process of freeze-drying, and about 98–99% of the solvent in sample will be removed through direct sublimation from solid to gas (Adams, Cook, & Ward, 2015). Additionally, in the process of freeze-drying, the solid components are kept in place by ice in their positions. When the ice sublimates, it will leave gaps in the dried residual materials, thus preserves the integrity of the biological and chemical structure and activity of the product. Given its work mechanisms, it is a recommended method for drying foods containing compounds that are thermally sensitive and prone to denaturation (Bhatta et al., 2020).

In recent years, application of freeze-drying to plant-based foods, like potato flour and noodles (Bao et al., 2021), plant-based foods (Ma et al., 2021), such as ginger (An et al., 2016), pumpkin (Ciurzyńska, Lenart, & Greda, 2014), coffee (Fissore, Pisano, & Barresi, 2014) and animal-based food, which mainly focus on chicken breast (Babić, Cantalejo, & Arroqui, 2009), beef sirloin (Zhang, Yoo, & Farouk, 2021), and pork loin (Ma et al., 2018), etc. Other studies investigating the effects of freeze-drying technology on protein properties maintaining, such as ovalbumin (Liu et al., 2020), hempseed protein isolates (Dong, Woo, & Quek, 2023), egg white proteins (Liu et al., 2022), and pea globulin aggregates (Oliete et al., 2019). Yet, although the preservation of MPs or myosin proteins from fish (Chen et al., 2022) and sheep (Zhu et al., 2023) have also been studied by freeze-drying, systematic comparisons of MPs derived from mammalian and fish are uncommon.

The solubility of proteins is a fundamental ingredient that must be properly guaranteed to develop other functional characteristics and ensure the maximum food production (Wang et al., 2020). Moreover, the gel property of MPs in meat products is crucial for the development in food processing, which is the key to control the quality of meat products. Temperature sweeping in rheology can effectively simulate the variations in elasticity and viscosity of MPs with temperature changes, indirectly reflecting the performance changes of meat products during the process (Wang et al., 2022a,b). Herein, the novelty of this work lies in the revelation of the improvement of protein solubility after freeze-drying based on the rheological properties of MPs derived from fish and mammals. This work selects MPs extracted from fish and mammalian (beef, sheep, and pork) meat as the research objects, uncovering the rheological properties basis for freezing treatment-induced improvement in the solubility of myofibrillar proteins, aiming at providing a theoretical reference for the future research on meat proteins and related meat products processing and nutritional support for nursing and rehabilitation.

2. Materials and methods

2.1. Materials

Frozen surimi was purchased from Zhejiang Yufu Food Co. Ltd. (Hangzhou, China). The longissimus dorsi muscles of beef (cattle breeding with Australian Valley in Australia), sheep (Tan sheep in Yanchi County of Ningxia, Ningxia China), and pork (black pig in Taihu, Jiangsu, China) were purchased from a local market (Xinxiang Gaojin Food co. LTD, Zhejiang, China). The fish and animals were aged 180 ± 3 days and frozen after slaughtering for 24–48 h.

The Bradford protein kit and sodium dodecyl sulfate (SDS) were purchased from Shenggong Bioengineering Co., Ltd (Shanghai, China). All chemicals and reagents including potassium chloride, tris (hydroxymethyl) methyl amino-methane, maleic acid, and Biuret A and B were of analytical grade and purchased from Aladdin Co. Ltd (Shanghai, China). These chemicals and reagents were used without any further purification.

2.2. Preparation of MPs suspensions

The samples were prepared according to a previous method (Wang et al., 2022a,b), with slight modification. After the preparation of four meat pieces, 10 times the volume of buffer A (50 mM KCl-20 mM tris-maleate, pH 7.0) was added to obtain the suspension. The suspension was mixed and homogenized (90,000 rpm) at 4 °C using an Ultra-Turrax homogenizer (T-18, IKA, Germany). After standing for 15 min, the suspension was centrifuged at 8000×*g*, 4 °C for 5 min (Hettich Rotina 420R, Hettich, Germany) to obtain the precipitate. Further, 10 times the volume of buffer B (0.6 M KCl-20 mM Tris-maleate, pH 7.0) was added to the samples and the mixture was homogenized. The extraction was performed for 1 h before centrifugation (Hettich Rotina 420R, Hettich, Germany) to obtain the supernatant containing MPs (8000×*g*, 4 °C, 10 min). Finally, the supernatant was washed with four times the volume of precooled (4 °C) deionized water, and the MPs were isolated through centrifugation (6000×*g*, 10 min). The obtained MPs was dissolved in 0.6 M KCl solution and stirred at 4 °C for 24 h, with pH kept at 7.0 throughout this process. Finally, the concentration was determined using the Biuret method (Wang et al., 2020). Based on the protein determination results, the suspensions with protein concentrations of 2, 5, and 10 mg/mL were prepared.

2.3. Preparation of MPs powders

MPs suspensions without concentration adjustment in section 2.2 were completely frozen at −80 °C for 24 h. Then, MPs suspensions were spread on the metal trays and dehydrated with a freeze dryer (Model Lyobeta 25, Telstar Industrial, S.L., Barcelona, Spain). The experimental parameters of freeze-drying process were the same in all treatments. Briefly, slow freezing for 24 h, and 48 h, 5 Pa of drying at the temperature of −80 °C. Next, the freeze-dried proteins were crushed to powder at room temperature (25 °C), and then be screened through a 40-mesh sieve (Chen et al., 2022). Finally, MPs powders were weighted, individually packed, and stored at −30 °C.

2.4. Protein re-dissolution

The content of crude protein in the freeze-dried samples was determined by the Kjeldahl method (Jung et al., 2003). The MPs were re-dissolve with 0.6 M KCl with continuous stirring at 4 °C for about 24 h to obtain MPs suspensions at the concentrations of 2, 5, 10 mg/mL. During the whole process, 0.01 M NaOH and HCl were used to maintain the pH of the MPs suspensions constant at 7.0. The concentration verification was carried out using the biuret method described by Wang et al. (2022a,b). The absorbance value at 540 nm was measured using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The prepared

MPs suspensions was placed in a 4 °C refrigerator for 2 h to make them stable before analysis.

2.5. Determination of solubility of suspensions

The protein solubility (PS) of MPs suspensions before and after freeze-drying and re-dissolution were determined according to the method of (Zhang et al., 2020) with slight modifications. The suspensions (100 mg) were centrifuged at 10,000×*g* for 30 min at 4 °C. After centrifugation, the protein concentration of the supernatant and the original suspension samples were determined according to the instructions given in the BCA Protein Assay Kit, where Bovine Serum Albumin (BSA) was used as a standard protein to measure the absorbance at 562 nm. Each analysis was conducted in triplicates. PS was calculated using the following equation:

$$\mathrm{PS}(\%) = \frac{\text{Protein concentration in supernatant (mg/mL)}}{\text{Total protein concentration (10 mg/mL)}} \times 100\ \% \quad (1)$$

2.6. Determination of emulsifying property of suspensions

The emulsifying properties (emulsifying activity, EAI; emulsifying stability, ESI) of MPs suspensions before and after freeze-drying and re-dissolution were performed as previously described by (Gao et al., 2022). The mixtures of 80% (v/v) suspensions and 20% (v/v) soybean oil were homogenized for 2 min with a homogenizer to obtain fully emulsified emulsions. A portion of 0.05 mL emulsion was collected and diluted to 5 mL with SDS solution (1%, w/v). The turbidity of the diluted emulsion was determined immediately (0 min) or after 10 min at 500 nm using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The SDS solution was used as the blank. The EAI was expressed by the following the equation:

$$\mathrm{EAI}\left(m^2/g\right) = \frac{2.303 \times 2 \times A_0 \times N}{c \times \varnothing \times 10,000} \quad (2)$$

Where A_0 is the absorbance value of the diluted emulsion measured (immediately) at 0 min after emulsion formation, N is the dilution factor (500), c is the protein concentration ($g{\cdot}mL^{-1}$) of the solution, and Φ is soybean oil accounting for the volume fraction of the emulsion.

The ESI was expressed by the following equation:

$$ESI(\%) = \frac{A_0}{A_0 - A_{30}} \times 100\ \% \quad (3)$$

Where A_{30} is the absorbance value of the diluted emulsion after 30 min.

2.7. Determination of foaming property of suspensions

The foaming properties (foaming capacity, FC; foaming stability, FS) of MPs suspensions before and after freeze-drying and re-dissolution were determined according to the method of Zhang et al. (2023). The diluted MPs dispersion (12 mL, 0.5%, w/v) was whipped with a homogenizer at 8000 rpm for 1 min at room temperature (25 °C). Subsequently, the volume was measured using a cylinder and FC was defined as the percentage of increased volume. After storage for 20 min, the percentage of residual foam volume was expressed as FS. The specific calculation formula is as follows:

$$FC\ (\%) = \frac{V_0 - V}{V} \times 100\ \% \quad (4)$$

$$FS\ (\%) = \frac{V_O - V_{20}}{V_0 - V} \times 100\ \% \quad (5)$$

Where V was the initial volume, V_0 was the volume immediately after foaming and V_{20} was the volume of liquid remaining after 20 min at room temperature.

2.8. Determination of rheological characteristics of suspensions

The rheological characteristics of MPs before and after freeze-drying were investigated at the concentration of 10 mg/mL using a stress-controlled rheometer (MCR 302, Anton Paar, Graz, Austria) equipped with a parallel plate geometry (50 mm diameter, angle 0°, gap 0.5 mm) according to the method of (Wang et al., 2022a,b). MPs suspensions were transferred on the bottom plate of the rheometer, and the edges of samples were covered with liquid paraffin to prevent dehydration during measurements. The sol-gel transitions of samples were investigated using temperature sweep tests, where *G'* was monitored at a frequency of 1 Hz and strain amplitude of 1%, with temperature increasing from 10 to 85 °C at a heating rate of 1 °C/min. Before each test, the samples were stabilized at 10 °C for 3 min, and then the samples were subjected to the test in triplicates at least.

2.9. Statistical analysis

All the experiments were performed in triplicates with data represented as the mean ± SD. The SPSS 21.0 software (IBM Corporation, NY, USA) was conducted to analyze the results with a one-way ANOVA ($p < 0.05$). The difference between least-square means was performed using Duncan's multiple range test.

3. Results and discussion

3.1. Protein solubility

The characteristics of natural myosin itself determine the PS of natural MPs. The PS is the key functional characteristic of protein utilization, and also the prerequisite for other functional properties, such as forming property and emulsifying property. The changes in PS of fish and mammalian MPs treated with and without freeze-drying treatment were shown in Table 1.

The solubility of the four kinds of natural MPs increased along with the increasing of protein concentration (2–10 mg/mL). The solubility of pork MPs (PMPs) was the highest (94.13 ± 0.06%) with a concentration of 10 mg/mL, followed by sheep MPs (SMPs) (92.37 ± 0.12%) and beef MPs (BMPs) (91.23 ± 0.06%), while the solubility of fish MPs (FMPs)

Table 1
Changes of solubility of fish (A) and mammalian sources (beef-B, sheep-C, and pork-D) myofibrillar proteins treated with and without freeze-drying treatment.

Sample source	Freeze-drying treatment	Concentration (mg/mL)	Protein solubility (%)
Fish	Before	2	79.77 ± 2.64^{Cb}
		5	85.9 ± 2.66^{Ba}
		10	90.93 ± 3.13^{Aa}
	After	2	48.87 ± 0.06^{Ec}
		5	51.57 ± 0.06^{Eb}
		10	66.17 ± 0.23^{Da}
Beef	Before	2	81.23 ± 0.06^{Cc}
		5	86.57 ± 0.06^{Bb}
		10	91.23 ± 0.06^{Aa}
	After	2	56.43 ± 0.06^{Fc}
		5	71.23 ± 0.06^{Eb}
		10	74.27 ± 0.06^{Da}
Sheep	Before	2	79.3 ± 0.01^{Fc}
		5	86.13 ± 0.06^{Db}
		10	92.37 ± 0.12^{Ba}
	After	2	66.77 ± 0.06^{Ec}
		5	84.13 ± 0.06^{Cb}
		10	89.57 ± 0.06^{Aa}
Pork	Before	2	85.47 ± 0.06^{Fc}
		5	93.17 ± 0.06^{Eb}
		10	94.13 ± 0.06^{Ca}
	After	2	74.23 ± 0.06^{Dc}
		5	83.60 ± 0.01^{Bb}
		10	87.33 ± 0.15^{Aa}

was the lowest (90.93 ± 3.13%). Yang et al. (2022) evaluated the impact of heating and drying processes in pea protein extraction and found that after freeze drying treatment, the solubility of pea protein was higher than that under spray drying treatment, resulted in better foaming properties.

The solubility of the suspensions obtained by re-dissolution of MPs powders after freeze-drying treatment was generally lower than that of natural MPs suspensions, and the decreasing degree of fish and mammalian MPs were different. At the concentration of 2 mg/mL, the FMPs decreased by 38%, and for the mammalian MPs, the solubility of BMPs reduced the most significant by 31%, and that of PMPs only decreased by 13%. At the concentration of 10 mg/mL, the solubility of FMPs decreased by 28%, while that of PMPs only decreased by 7%. These results showed that the influence of freeze-drying treatment on the solubility of mammalian MPs is not significant ($p > 0.05$), especially for PMPs, which was similar to the study of (Bao et al., 2022).

3.2. Protein foaming property

MPs have typical amphiphilic (lipophilic and hydrophilic) structures and have good surface activity. During the stirring process, MPs tend to form bubbles at the water/air interface. Generally, the smaller the protein molecular weight, the stronger the foaming ability. The changes in the foaming properties of fish and mammalian MPs treated with and without freeze-drying treatment were shown in Fig. 1.

The foaming properties of natural fish and mammalian MPs were totally different. The FC and FS of FMPs were significantly ($p < 0.05$) higher than those of BMPs and SMPs, and similar to those of PMPs. When the concentration of 5 mg/mL was applied, the FC (221.62 ± 20.83%) and FS (164.93 ± 12.47%) of FMPs was higher than that of PMPs (FC: 184.76 ± 23.55% and FS: 148.42 ± 10.05%).

Nevertheless, following the freeze-drying process, the redissolved FMPs' foaming performance altered most, declining by 28% and 16% at the concentration level of 10 mg/mL, while the BMPs' foaming performance declined by 29% and 7% at the same concentration level. The FC and FS of freeze-dried PMPs were reduced by 15% and 9% after being redissolved. These results showed that freeze-drying treatment had an insignificant ($p > 0.05$) influence on the foaming property of mammalian MPs, especially for PMPs.

3.3. Protein emulsifying property

The lipophilic and hydrophilic properties of proteins endow them with surface active substances, which play the important role of emulsifiers in the formation of water oil two-phase (Kamon et al., 2016). The protein emulsifying property is affected by the distribution and concentration of hydrophilic and lipophilic groups on the molecular surface and the protein concentration (Nawaz et al., 2019). The changes in emulsifying properties of fish and mammalian MPs treated with and without freeze-drying treatment were shown in Fig. 2.

The EAI of FMPs was significantly ($p < 0.05$) higher than that of BMPs and SMPs, but significantly ($p < 0.05$) lower than that of PMPs. Interestingly, at the concentration of 2 mg/mL, the EAI of fish and mammalian MPs reached the highest. Along with the increase in concentration, the EAI of MPs decreased. For example, the EAI of FMPs reduced by 55% when its concentration condensed from 2 to 10 mg/mL, and that of PMPs was decreased by 68% under the same conditions. However, after freeze-drying, the EAI and ESI of MPs decreased significantly ($p < 0.05$). At the concentration of 10 mg/mL, the decreasing order of emulsifying activity of MPs after re-dissolution was PMPs (68%), BMPs (43%), FMPs (39%), and SMPs (32%). The results showed that freeze-drying treatment had an insignificant ($p > 0.05$) influence on the emulsifying properties of FMPs.

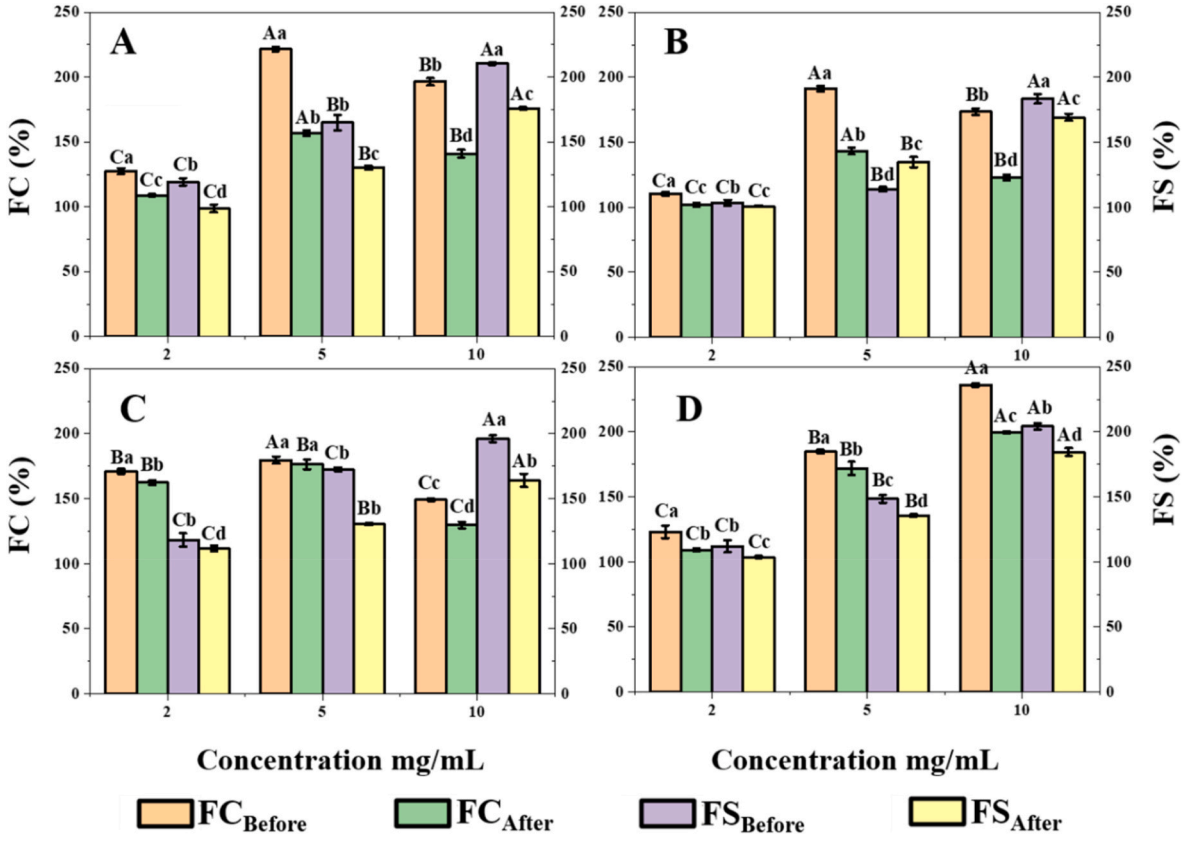

Fig. 1. Changes of foaming capacity (FC) and forming stability (FS) of fish (A) and mammalian sources (beef-B, sheep-C, and pork-D) myofibrillar proteins treated with and without freeze-drying treatment.

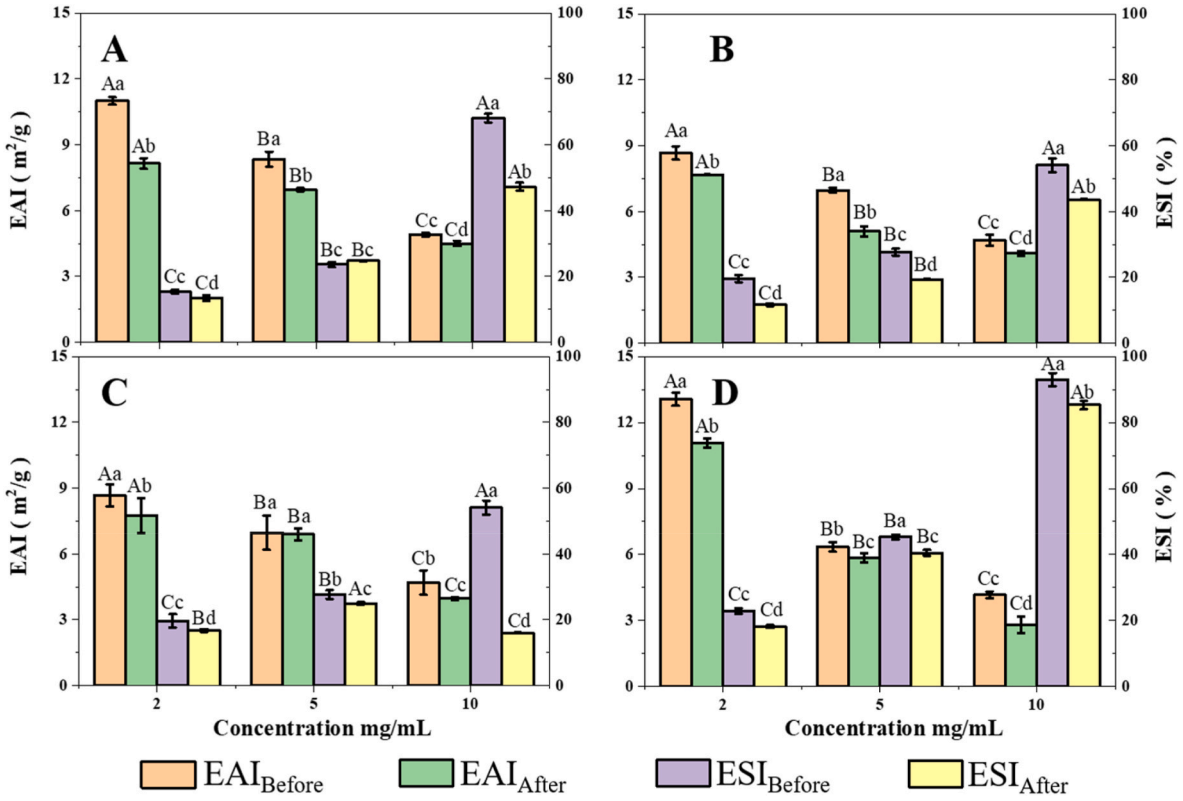

Fig. 2. Changes of emulsifying activity index (EAI) and emulsion stability index (ESI) of fish (A) and mammalian sources (beef-B, sheep-C, and pork-D) myofibrillar proteins treated with and without freeze-drying treatment.

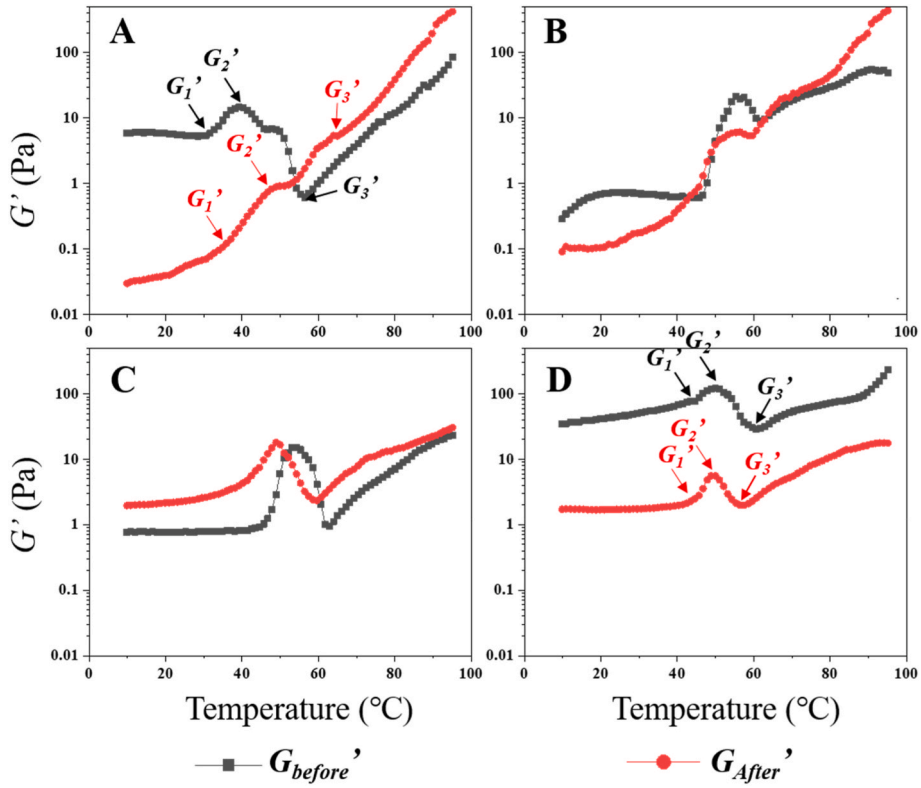

Fig. 3. Changes of storage modulus (*G'*) of fish (A) and mammalian (beef-B, sheep-C, and pork-D) sources myofibrillar proteins treated with and without freeze-drying treatment as increasing temperature according to dynamic rheological curves.

3.4. Rheological characteristic

Gelatinization reflects the ability of the protein to form a heat-induced gel, and is related to the texture, water and oil retention of meat products, which is an important functional characteristic of meat products (Miller et al., 2020). Dynamic rheology can monitor the denaturation, aggregation and spatial network formation of MPs in the process of gelation (Du et al., 2022). The variation in storage modulus (G') reflects the changes of protein structure.

As shown in Fig. 3, the variation of G' of all MPs with temperature could be divided into three stages: gel formation stage, gel deterioration stage, and gel strengthening stage (Xu et al., 2020). However, the gelling performance of fish and mammalian (beef, sheep, and pork) MPs was different. In the temperature range of 10–45 °C, the G' value of mammalian MPs increased, the protein molecules expanded slightly, and the interaction was weak (Baune et al., 2021). However, the G' value of FMPs decreased, which might be attributed to that the hydrogen bonds were broken with the temperature increased, which was the main force to maintain the protein structure. In 45–55 °C, the G' increased, meaning that proteins crosslinked. Subsequently, the G' decreased rapidly, which might be due to the deformation of myosin and/or the destruction of the structure of protein network (Ubeyitogullari and Ciftci, 2020). When the temperature rose further, a rigid protein network structure was finally formed. It was worth noting that the G' curves of FMPs had two peaks, while that of the mammalian MPs had only one peak, which was also reported by a study of Wang et al. (2022a, b). They suspected that the difference in the number of peaks in G' curves might be due to the difference in thermal stability between the head and tail of myosin in FMPs.

After freeze-drying treatment, the thermal gelation performance of fish and mammalian MPs changed. For example, the initial elastic modulus of FMPs treated with freeze-drying treatment was decreased. With the increase in temperature, the G' value basically showed a continuous upward trend. To accurately compare the influence made by freeze-drying treatment on MPs, the change point in dynamic rheological curves were shown in Table 2. In the temperature range of 55–62 °C, there were two right shift peaks for FMPs with freeze-drying treatment, while a higher value was discovered in the final gel strength of MPs with freeze-drying treatment. The trends of the G' values of BMPs and SMPs with freeze-drying were similar to those samples without freeze-drying treatment, while the final gel strength of BMPs after freeze-drying became higher. In addition, the G' value curves of PMPs generally declined after freeze-drying treatment, without shifting in the peak, and the gel strength finally decreased. This probably due to that during the freeze-drying process, the ice crystal position in the protein structure forms micropores similar to sponges. After the re-dissolution of 0.6 M KCl, the protein structure was restored by water absorption, but a certain amount of KCl in the protein suspension was not removed during freeze-drying. It might affect the performance of the head and tail of myosin during the thermal gelation process, resulting in the final gel strength and peak value of proteins from different sources after freeze-drying different from those in natural state.

3.5. Multivariate statistical analysis

To find out the difference in the functional properties indexes of different MPs (fish, beef, sheep, and pork) treated with and without freeze-drying, multivariate statistical analysis was applied which can collect scattered information for multidimensional variables, reduce the dimensions of multivariate data, and minimize the loss of original data (Song et al., 2021). From the score plot shown in Fig. 4A, the clusters of the four MPs with and without freeze-drying treatment had poor separation, and many overlapping parts could be observed, which were the correlated principal components (PCs), meaning that the freeze-drying treatment made insignificant ($p > 0.05$) change in the functional properties of MPs. The first two PCs of PCA explained 98.2 cum% of the total variance of the data set, which could well represent the dataset. The loading plot (Fig. 4B) was used to monitor the specific functional properties indexes for the separation of the samples. The discrete dots, such as FS, FC, solubility, EAI, and ESI were regarded as the major factors contributing to the score plot.

For the supervised analysis of functional properties indexes between these samples, the partial least squares discrimination analysis (PLS-DA) was applied (Chen et al., 2019). The variable importance in the projection (VIP) plot revealed the significance of the corresponding variables on the insignificant ($p > 0.05$) separation of these samples, in which the value of VIP >1 was considered as a criterion for significant ($p < 0.05$) variables (Chen et al., 2019). As shown in Fig. 4C, three indexes (solubility, ESI, and FC) were the dominant characteristic indicators to distinguish different samples. For example, the solubility of the SMPs was the highest whether treated with or without freeze-drying. However, the VIP scores of SMPs treated with and without freeze-drying were similar, which indicated that such difference could be attributed to the difference sources of MPs, while the effect of freeze-drying treatment was not significant. For further analysis of correlation coefficients of functional properties in different samples, the heatmap of correlation was constructed in Fig. 4D according to Pearson correlation analysis with several highly correlated ions depicted. For example, FC ($r = 0.94$, $p < 0.05$), and FS ($r = 0.94$, $p < 0.05$) showed a significant ($p < 0.05$) positive correlation to solubility. These results showed that the freeze-drying treatment well retained the functional characteristics of the MPs.

4. Conclusion

MPs determines the functional characteristics of meat products, but there is no effective preservation method. With the application of multivariate statistical analysis of the functional property indexes of MPs derived from fish and mammalian sources before and after the treatment, freeze-drying treatment was proved to be an efficient method for protein preservation in this study. The solubility of PMPs only decreased by 7% after freeze-drying treatment when the concentration was 10 mg/mL, while foaming activity and foaming stability were decreased by 15% and 9%, respectively. Especially for the impacts on emulsifying activity with a concentration of 10 mg/mL, freeze-drying treatment exhibited the least impact on FMPs, with only 8% decrease,

Table 2
Change point in dynamic rheological curves of fish (A) and mammalian sources (beef-B, sheep-C, and pork-D) myofibrillar proteins treated with and without freeze-drying treatment.

Sample source	Freeze-drying treatment	T_1 (°C)	G_1' (Pa)	T_2 (°C)	$G2'$ (Pa)	T_3 (°C)	$G3'$ (Pa)
Fish	Before	30.58	5.46	40.27	14.54	56.39	0.62
	After	37.04	12.944	47.79	6.83	64.99	2.13
Beef	Before	45.65	0.61	55.31	21.48	61.76	9.05
	After	45.65	0.89	55.31	6.04	61.76	8.05
Sheep	Before	44.58	0.93	53.17	15.26	62.84	0.96
	After	40.27	4.35	48.86	18.05	59.61	2.34
Pork	Before	44.58	77.61	49.94	121.43	60.69	28.84
	After	43.51	2.28	48.86	5.58	57.46	1.99

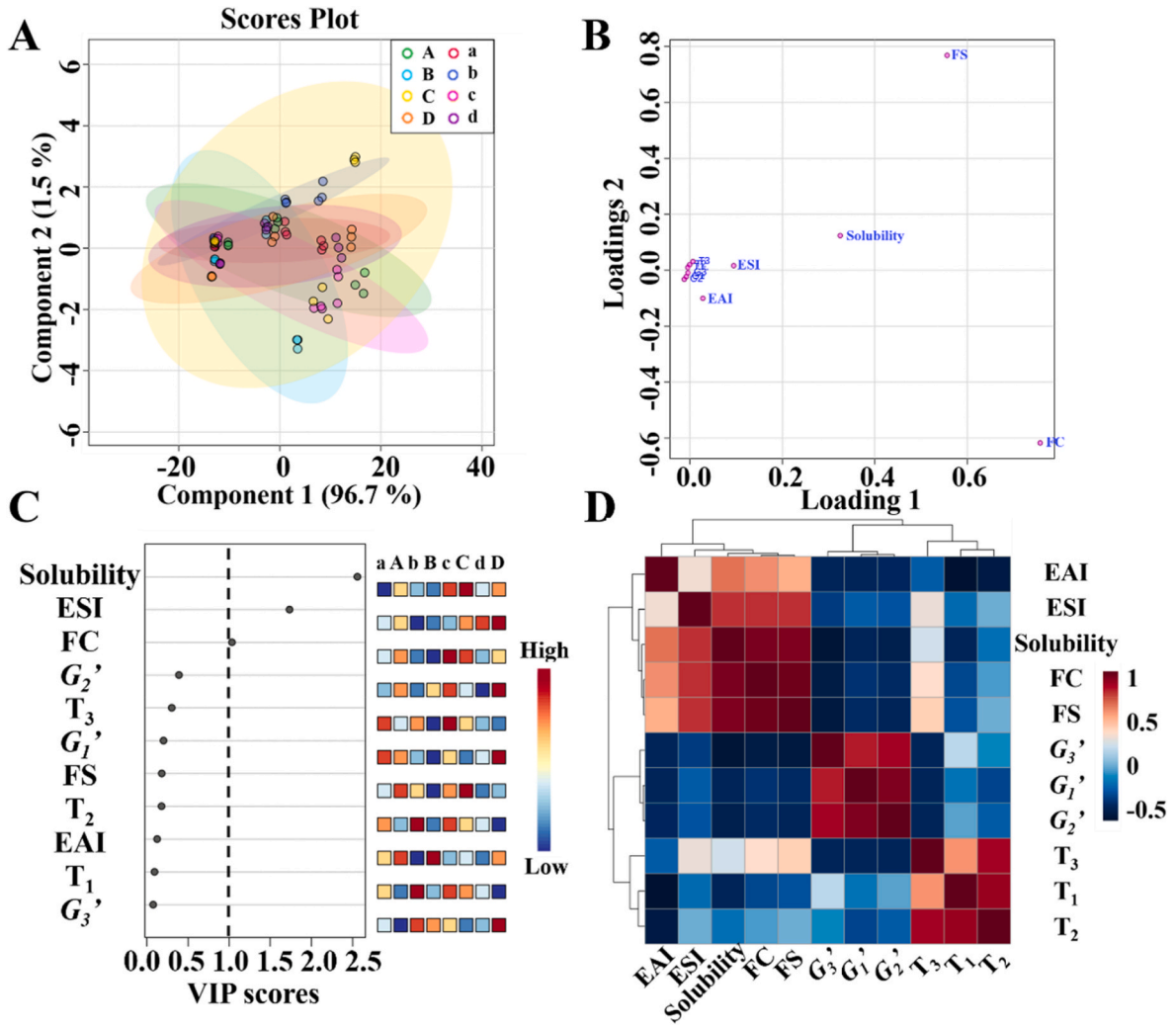

Fig. 4. Multivariate statistical analysis of functional properties data of fish (A/a) and mammalian (beef-B/a, sheep-C/c and pork–D/d) sources myofibrillar proteins treated with (capital letters) and without (lowercase letters) freeze-drying treatment: (A) scores plot, (B) loading plot, (C) VIP plot, and (D) correlation heatmap.

significantly ($p < 0.05$) lower than MPs derived from mammalian sources. Besides, the characterization of rheological properties showed that BMP and SMP gel strengths were unchanged after freeze-drying treatment, whereas FMP gel strengths increased under the concentration of 10 mg/mL. This study provides a reference for the meat related processing and the efficient preservation of MPs.

Ethics declarations

There are no ethical issues involved in this study.

CRediT authorship contribution statement

Huijuan Yang: conceived and designed the paper, collected and analyzed the literature and wrote the paper. **Zhizhao Chen:** conceived and designed the paper, collected and analyzed the literature and wrote the paper, edited the table and figures. **Haifeng Wang:** conceived and designed the paper, collected and analyzed the literature and wrote the paper, edited the table and figures. **Danping Jin:** conceived and designed the paper, edited the table and figures. **Xiaoqi Wang:** conceived and designed the paper, reviewed and edited the manuscript, All authors read and approved the manuscript. **Fan Wang:** conceived and designed the paper, reviewed and edited the manuscript, All authors read and approved the manuscript. **Xuejiang Cen:** conceived and designed the paper, reviewed and edited the manuscript, All authors read and approved the manuscript, and. **Qing Shen:** conceived and designed the paper, reviewed and edited the manuscript, All authors read and approved the manuscript, Xi Chen and reviewed and edited the manuscript. All authors read and approved the manuscript.

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.

Acknowledgements

We sincerely thank the National Natural Science Foundation of China Project (32301990), the Zhejiang Provincial Department of Education General Research Project (Y202044581) and Zhejiang Province TCM Science and Technology Project (2023ZL235).

Abbreviation

(MPs) Myofibrillar proteins

(FMPs) Fish myofibrillar proteins
(BMPs) beef myofibrillar proteins
(SMPs) sheep myofibrillar proteins
(PMPs) pork myofibrillar proteins
(SDS) sodium dodecyl sulfate
(PS) protein solubility
(EAI) emulsifying activity
(ESI) emulsifying stability
(FC) foaming capacity
(FS) foaming stability
(PCs) principal components
(PLS-DA) the partial least squares discrimination analysis
(VIP) The variable importance in the projection

References

Adams, G.D., Cook, I., Ward, K.R., 2015. The principles of freeze-drying. Cryopreservation and freeze-drying protocols 121–143.

An, K., Zhao, D., Wang, Z., Wu, J., Xu, Y., Xiao, G., 2016. Comparison of different drying methods on Chinese ginger (Zingiber officinale Roscoe): changes in volatiles, chemical profile, antioxidant properties, and microstructure. Food Chem. 197, 1292–1300.

Arsiccio, A., Mccarty, J., Pisano, R., Shea, J.E., 2020. Heightened cold-denaturation of proteins at the ice-water interface. J. Am. Chem. Soc. 142 (12), 5722–5730.

Babić, J., Cantalejo, M.J., Arroqui, C., 2009. The effects of freeze-drying process parameters on Broiler chicken breast meat. LWT–Food Sci. Technol. 42 (8), 1325–1334.

Bao, H., Zhou, J., Yu, J., Wang, S., 2021. Effect of drying methods on properties of potato flour and noodles made with potato flour. Foods 10 (5), 1115.

Bao, P., Chen, L., Hu, Y., Wang, Y., Zhou, C., 2022. l-Arginine and l-lysine retard aggregation and polar residue modifications of myofibrillar proteins: their roles in solubility of myofibrillar proteins in frozen porcine Longissimus lumborum. Food Chem. 393, 133347.

Baune, M.-C., Schroeder, S., Witte, F., Heinz, V., Bindrich, U., Weiss, J., Terjung, N., 2021. Analysis of protein-network formation of different vegetable proteins during emulsification to produce solid fat substitutes. J. Food Meas. Char. 15 (3), 2399–2416.

Bhatta, S., Stevanovic Janezic, T., Ratti, C., 2020. Freeze-drying of plant-based foods. Foods 9 (1), 87.

Chen, L., Wu, J.e., Li, Z., Liu, Q., Zhao, X., Yang, H., 2019. Metabolomic analysis of energy regulated germination and sprouting of organic mung bean (Vigna radiata) using NMR spectroscopy. Food Chem. 286, 87–97.

Chen, P., Qiu, Y., Chen, S., Zhao, Y., Wu, Y., Wang, Y., 2022. Insights into the effects of different drying methods on protein oxidation and degradation characteristics of golden pompano (Trachinotus ovatus). Front. Nutr. 9, 1063836.

Ciurzyńska, A., Lenart, A., Gręda, K.J., 2014. Effect of pre-treatment conditions on content and activity of water and colour of freeze-dried pumpkin. LWT–Food Sci. Technol. 59 (2), 1075–1081.

de Oliveira, F.A., Neto, O.C., dos Santos, L.M.R., Ferreira, E.H.R., Rosenthal, A., 2017. Effect of high pressure on fish meat quality–A review. Trends Food Sci. Technol. 66, 1–19.

Dong, X., Woo, M.W., Quek, S.Y., 2023. The physicochemical properties, functionality, and digestibility of hempseed protein isolate as impacted by spray drying and freeze drying. Food Chem. 433, 137310.

Du, J., Zhou, C., Xia, Q., Wang, Y., Geng, F., He, J., Cao, J., 2022. The effect of fibrin on rheological behavior, gelling properties and microstructure of myofibrillar proteins. Lebensm. Wiss. Technol. 153, 112457.

Fissore, D., Pisano, R., Barresi, A.A., 2014. Applying quality-by-design to develop a coffee freeze-drying process. J. Food Eng. 123, 179–187.

Gao, K., Rao, J., Chen, B., 2022. Unraveling the mechanism by which high intensity ultrasound improves the solubility of commercial pea protein isolates. Food Hydrocoll. 131, 107823.

Helmick, H., Turasan, H., Yildirim, M., Bhunia, A., Liceaga, A., Kokini, J.L., 2021. Cold denaturation of proteins: where bioinformatics meets thermodynamics to offer a mechanistic understanding: pea protein as a case study. J. Agric. Food Chem. 69 (22), 6339–6350.

Jung, S., Rickert, D., Deak, N., Aldin, E., Recknor, J., Johnson, L., Murphy, P., 2003. Comparison of Kjeldahl and Dumas methods for determining protein contents of soybean products. J. Am. Oil Chem. Soc. 80, 1169–1173.

Kamon, Y., Inoue, N., Mihara, E., Kitayama, Y., Ooya, T., Takeuchi, T., 2016. Hydrophilic crosslinked-polymeric surface capable of effective suppression of protein adsorption. Appl. Surf. Sci. 378, 467–472.

Liu, L., Dai, X., Kang, H., Xu, Y., Hao, W., 2020. Structural and functional properties of hydrolyzed/glycosylated ovalbumin under spray drying and microwave freeze drying - ScienceDirect. Food Sci. Hum. Wellness 9 (1), 80–87.

Liu, L., Shi, S., Zhang, Y., Li, Y., Guo, J., Zhang, M., 2022. Effect of microwave freeze drying on moisture migration and gel characteristics of egg white protein. J. Food Process. Preserv. 46 (9), e16899.

Ma, Q., Bi, J., Yi, J., Wu, X., Li, X., Zhao, Y., 2021. Stability of phenolic compounds and drying characteristics of apple peel as affected by three drying treatments. Food Sci. Hum. Wellness 10 (2), 174–182.

Ma, Y., Wu, X., Zhang, Q., Giovanni, V., Meng, X., 2018. Key composition optimization of meat processed protein source by vacuum freeze-drying technology. Saudi J. Biol. Sci. 25 (4), 724–732.

MacKenzie, A., Luyet, B., 1967. Freeze-drying and protein denaturation in muscle tissue; losses in protein solubility. Nature 215 (5096), 83–84.

Miller, D.K., Acevedo, N.C., Lonergan, S.M., Sebranek, J.G., Tarté, R., 2020. Rheological characteristics of mechanically separated chicken and chicken breast trim myofibril solutions during thermal gelation. Food Chem. 307, 125557.

Nawaz, A., Xiong, Z., Xiong, H., Irshad, S., Chen, L., Wang, P.k., Muhammad, Hafiz, Walayat, A.N., Qamar, S.H., 2019. The impact of hydrophilic emulsifiers on the physico-chemical properties, microstructure, water distribution and in vitro digestibility of proteins in fried snacks based on fish meat. Food Funct. 10 (10), 6927–6935.

Oliete, B., Yassine, S.A., Cases, E., Saurel, R., 2019. Drying method determines the structure and the solubility of microfluidized pea globulin aggregates. Food Res. Int. 119, 444–454.

Shuai, J.A., Miao, Z.A., Hui, L.A., Qian, L.A., Dx, A., Yn, A., Cl, A., 2021. Ultrasound treatment can increase digestibility of myofibrillar protein of pork with modified atmosphere packaging. Food Chem. 377, 131811.

Song, G., Wang, Q., Zhang, M., Yang, H., Xie, H., Zhao, Q., Wang, P., 2021. Real-time in situ screening of omega-7 phospholipids in marine biological resources using an iKnife-rapid-evaporative-ionization-mass-spectrometry-based lipidomics phenotype. J. Agric. Food Chem. 69 (32), 9004–9011.

Tan, M., Ding, Z., Xie, J., 2022. Freezing-induced myofibrillar protein denaturation: contributions of freeze-concentration and role of cellobiose. J. Food Eng. 329, 111076.

Tan, M., Ye, J., Xie, J., 2021. Freezing-induced myofibrillar protein denaturation: role of pH change and freezing rate. LWT–Food Sci. Technol. 152, 112381.

Ubeyitogullari, A., Ciftci, O.N., 2020. Fabrication of bioaerogels from camelina seed mucilage for food applications. Food Hydrocolloids 102, 105597.

Waghmare, R.B., Perumal, A.B., Moses, J.A., Anandharamakrishnan, C., 2021. Recent developments in freeze drying of foods - ScienceDirect. Innovative Food Processing Technologies 82–99.

Wang, C., Wang, J., Zhu, D., Hu, S., Kang, Z., Ma, H., 2020. Effect of dynamic ultra-high pressure homogenization on the structure and functional properties of whey protein. J. Food Sci. Technol. 57, 1301–1309.

Wang, H., Yang, H., Chen, X., Shen, Q., 2022a. Structural basis for high-intensity ultrasound treatment in the rheology of myofibrillar protein extracted from White Croaker in relation to their solubility. Lebensm. Wiss. Technol. 156, 112979.

Wang, H., Yang, Z., Yang, H., Xue, J., Li, Y., Wang, S., Zhang, M., 2022b. Comparative study on the rheological properties of myofibrillar proteins from different kinds of meat. Lebensm. Wiss. Technol. 153, 112458.

Wang, L., Xia, M., Zhou, Y., Wang, X., Ma, J., Xiong, G., Sun, W., 2020. Gel properties of grass carp myofibrillar protein modified by low-frequency magnetic field during two-stage water bath heating. Food Hydrocolloids 107, 105920.

Wenzel, T., Gieseler, H., 2021. Evaluation of packaging materials in freeze-drying: use of polymer caps and nested vials and their impact on process and product attributes. AAPS PharmSciTech 22, 1–10.

Xu, Y., Zhao, Y., Wei, Z., Zhang, H., Dong, M., Huang, M., Zhou, G., 2020. Modification of myofibrillar protein via glycation: physicochemical characterization, rheological behavior and solubility property. Food Hydrocolloids 105, 105852.

Yang, J., Mocking-Bode, H.C., van den Hoek, Theunissen, M., Voudouris, P., Meinders, M.B., Sagis, L.M., 2022. The impact of heating and freeze or spray drying on the interface and foam stabilising properties of pea protein extracts: Explained by aggregation and protein composition. Food Hydrocoll. 133, 107913.

Yang, H., Wang, H., Tao, F., Li, W., Cao, G., Yang, Y., Shen, Q., 2021. Structural basis for high-pressure improvement in depolymerization of interfacial protein from RFRS meat batters in relation to their solubility. Food Res. Int. 139, 109834.

Yumiko, Matsuda, 1981. Protein denaturation during freeze-drying of carp myofibrils. Nippon Suisan Gakk 47 (6), 813–815.

Zhang, T., Chen, T., Jiang, H., Liu, J., Wang, E., Zhang, M., Liu, X., 2023. pH-induced egg white protein foaming properties enhancement: Insight into protein structure and quantitative proteomic analysis at protein adsorption layer. Food Hydrocoll. 144, 109060.

Zhang, C., Li, X.-a., Wang, H., Xia, X., Kong, B., 2020. Ultrasound-assisted immersion freezing reduces the structure and gel property deterioration of myofibrillar protein from chicken breast. Ultrason. Sonochem. 67, 105137.

Zhang, R., Yoo, M.J., Farouk, M.M., 2021. Oxidative stability, proteolysis, and in vitro digestibility of fresh and long-term frozen stored in-bag dry-aged lean beef. Food Chem. 344, 128601.

Zhang, Y., Bai, G., Jin, G., Wang, Y., Wang, J., Puolanne, E., Cao, J., 2022. Role of low molecular additives in the myofibrillar protein gelation: underlying mechanisms and recent applications. Crit. Rev. Food Sci. Nutr. 1–19.

Zhang, Y., Bai, G., Wang, J., Wang, Y., Jin, G., Teng, W., Cao, J., 2023. Myofibrillar protein denaturation/oxidation in freezing-thawing impair the heat-induced gelation: mechanisms and control technologies. *Trends in Food Science & Technology*. Trends Food Sci. Technol. 138, 655–670.

Zhu, C., Wang, S., Bai, Y., Zhang, S., Zhang, X., Wu, Q., He, X., 2023. Effects of citrus fiber on the gel properties of mutton myofibrillar protein. Foods 12 (4), 741.