Ultrasonics Sonochemistry 93 (2023) 106299

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

Flavor mechanism of micro-nanoparticles and correlation analysis between flavor substances in thermoultrasonic treated fishbone soup

Wei He [1], Menglin Han [1], Ying Bu, Wenhui Zhu [*], Jianrong Li, Xuepeng Li [*]

College of Food Science and Engineering, Bohai University, Jinzhou, Liaoning 121013, China

ARTICLE INFO

Keywords:
Fishbone soup
Thermoultrasonic treatment
Micro-nano particles (MNPs)
Flavor
Correlation analysis

ABSTRACT

To study the physicochemical properties of micro-nanoparticles (MNPs) in thermoultrasonic treated fishbone soup, it was subjected to ultra-filtration with a 100 kDa ultrafiltration membrane to obtain large MNPs (LMNPs) and small MNPs (SMNPs). LMNPs and SMNPs were treated with force-breakers, and the interactions of the MNPs with five characteristic volatile compounds were investigated. LMNPs covered most proteins (222.66 mg/mL) and fatty acids (363.76 mg/g), while SMNPs was mostly soluble small molecules with taste substances like total free amino acids (85.26 mg/g), organic acids (2.55 mg/mL), and 5′-nucleotides (169.17 mg/100 mL). The stability of LMNPs is significantly higher than raw bone soup, and SMNPs can exist stably in the solution. Correlation analysis between flavor substance content and flavor suggested that the overall flavor profile of halibut bone soup was closely related to the content changes of 72 significant influence variables. The binding of LMNPs to characteristic flavor compounds was largely affected by hydrophobic interactions, hydrogen bonds, and ionic effects. While the binding of SMNPs to characteristic flavor compounds was largely determined by hydrophobic interaction and hydrogen bonding. This study explores the characteristics of MNPs and provides the possibility to clarify the interaction mechanism between MNPs and flavor.

1. Introduction

Flavor, an important quality that both producers and consumers focus on, is one of the most critical indicators of food quality. In addition to producing organic acids, amino acids, and nucleotides with taste, the main components of food systems, such as proteins and fats, can also interact with volatile flavor compounds to influence the perception of flavor in humans [1]. Meanwhile, chemical interactions between these components and flavor compounds are considered the main factors affecting the retention and release of food flavors, including electrostatic interactions, hydrophobic interactions, hydrogen bonding, van der Waals bonding, coordination interactions, π-π stacking, and so on [2]. Moreover, forces such as hydrogen bonding and hydrophobic forces can promote the formation of sodium caseinate or whey isolate-carboxymethylcellulose microcapsule network systems, thereby increasing the resistance of the system to the β-pinene trapping effect [3]. Proteins and alcohols, aldehydes, esters, and ketones can interact differently depending on the amino acid side chain structure, including hydrogen bonding, hydrophobic interactions, and ionic bonding [4].

Food-derived micro-nanoparticles (MNPs) are important nanomaterials produced during food processing and cooking [5]. MNPs are not formed by simple accumulation of units between nutrients. These particles often have layered and compartmentalized structures that can provide effective spatial and temporal control over the transport and release of nutrients or active substances [6]. Most of the nutrients aggregated in MNPs not only exist in the free state, but also participate in the formation of MNPs in the system, so these substances can greatly affect the taste of the system [2]. MNPs in apple juice can adsorb phenolic compounds, and then interact with proteins and polysaccharides, affecting the taste and volatile flavor of apple juice [7]. Zhou et al. [8] found that the aggregation state of MNPs in the tea soup was one of the crucial factors affecting the taste of tea soup, and particles larger than 200 nm had a noticeable bitter and astringent taste.

In our previous study, non-fried halibut bone soup was prepared by thermoultrasonic treatment based on the high-fat content of Greenland halibut (*Reinhardtius hippoglossoides)* [9]. MNPs have a significant optimizing effect on the halibut bone soup's stability and flavor. MNPs reduced the formation of undesirable flavor substances like acetic acid,

* Corresponding authors.
E-mail addresses: wenhuiby130@163.com (W. Zhu), xuepengli8234@163.com (X. Li).
[1] Wei He and Menglin Han should be considered as joint first author.

https://doi.org/10.1016/j.ultsonch.2023.106299
Received 16 December 2022; Received in revised form 2 January 2023; Accepted 10 January 2023
Available online 13 January 2023

butyric acid, 2-methylbutyric acid, etc., improved the homogeneity of the halibut bone soup, and enhanced its flavor binding capacity and quality. However, the effects of MNPs on taste components (such as 5′-nucleotides and organic acids) and volatile flavor substances have not been studied systematically. In this study, ultrafiltration technology was used to preliminarily separate MNPs in halibut bone soup; afterwards, nutrient content and colloidal characteristics of MNPs with different sizes were determined. A correlation model was established between the flavor substances content and the flavor of halibut bone soup. The main binding modes of MNPs with the five characteristic flavor substances alcohol, aldehyde, acid, ester, and ketone were explored by analyzing the adsorption rates of MNPs with the five flavor substances. Finally, the correlation model was established to explain the key mechanism of halibut bone soup binding flavor substances.

2. Materials and methods

2.1. Materials and reagents

The frozen halibut bones were purchased from Meijia Group Co., Ltd (Shandong, China) and stored in the refrigerator (−20 °C) for use. Organic acids and 5′-nucleotide standards were purchased from Aladdin Biochemical Technology Co., Ltd (Shanghai, China), and 17 kinds of free amino acid mixed standards were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). All other reagents were of analytical grade and were purchased from Sinopharm Group Reagent Co., Ltd (Shanghai, China).

2.2. Preparation of halibut bone soup

After washing, halibut fish bones were cut into small pieces of 3–5 cm. The food processor was used to grind bones into mud. Seal the fishbone mud (200 g) and deionized water in a retort bag at a ratio of 1:6 (w/v), and then heat them in an ultrasound-assisted treatment device (XO-120L-II, ATPIO, Nanjing, China) at 100 °C for 150 min, the thermoultrasonic parameter was 25 kHz, 400 W, and 10 min [9].

2.3. Ultrafiltration separation of MNPs

The halibut bone soup was filtered by 200 mesh gauze, then ultrafiltered with 100 kDa membrane. The filtered components were small micro nanoparticles (SMNPs), while the interception components were large micro nanoparticles (LMNPs) [10]. The two components were concentrated at 55 °C with a rotary evaporator (RE-52 AA, Yarong Biochemical Instrument Factory, Shanghai, China), then lyophilized and stored at −80 °C.

2.4. Determination of nutrient contents

The total sugars and water-soluble proteins content in halibut bone soup was determined by sulfuric acid-anthrone method and the biuret method, respectively [11,12]. The fatty acids content was determined by the method of Zhang et al. [13] using a gas chromatograph (7890A, Agilent Technologies Co Ltd, USA).

2.5. Formation, properties and microstructure of MNPs

2.5.1. Microstructure observation

The surface micro-morphology of halibut bone soup and MNPs were investigated by scanning electron microscope (SEM, S4800, Minolta Corporation, Tokyo, Japan). Referring to the method of Ballesteros et al. [14], MNPs powder was fixed on a special conductive tape, vacuum sprayed with gold (20–30 nm), and then photographed and analyzed at accelerating voltages of 5.0 kV and 10.0 kV, respectively.

The microstructures of the halibut bone soup and MNPs were analyzed by a Zeiss LSM710 confocal laser scanning microscope (CLSM, Oberkochen, Germany). Referring to the method of Liu et al. [15], Nile red and Nile blue A were prepared with absolute ethanol as a mixed fluorescent dye with a concentration of 1 mg/mL, which was used to observe the distribution of triglycerides and proteins in sample MNPs. The MNPs sample was prepared into a 20 mg/mL emulsion with ultrapure water. The CLSM was observed under a helium–neon laser (He/Ne) with excitation wavelengths of 633 nm (Nile blue A) and 488 nm (Nile red).

2.5.2. Particle size analysis

100 mg of the lyophilized sample was dissolved in deionized water (5 mL), and the particle size and particle dispersion index (PDI) of samples were measured using a Zeta potentiometer (NanoBrook 90 Plus Zeta, Brookhaven Instrument Co., Ltd., USA). The average particle diameter of the sample was recorded with the included Zetasizer software.

2.5.3. Interfacial tension analysis

The dynamic droplet analysis method was used to detect the change of surface tension (γ) with adsorption time (t) on the oil–water interface. The samples were dissolved in deionized water as emulsions with a concentration of 10 mg/mL, respectively. The droplet shape images were analyzed by Standard Contact Angle (SCA) software and the interfacial tension (γ) was calculated [16].

2.5.4. Kinetic stability

The separation index (SI) was measured referring to the method of Wang et al. [17] 10 mL of each sample (100 mg/mL) was taken and placed in a test tube, sealed, and stored at 4 °C for 24 h. The upper and lower separated phases were observed and measured continuously for 5 days. The initial total height of the sample before storage was denoted as H_T. The height of the upper phase of the sample stored for 24 h was measured and recorded as H_X. The separation index (SI, %) of the sample was calculated represented as:

$$SI(\%) = \frac{H_X}{H_T} \times 100 \quad (1)$$

2.5.5. Surface hydrophobicity

The surface hydrophobicity of halibut bone soup and MNPs was determined by the fluorescent probe method [18]. A concentration of 10 mmol/L 8-anilino-1-naphthalenesulfonic acid (ANS) was prepared as a fluorescent probe buffer using 10 mmol/L phosphate buffer (pH 7.0). The fluorescence intensity of the sample (arbitrary units, a.u) was measured using a spectrofluorometer (970CRT, Precision Scientific Instrument Co., ltd., Shanghai, China) to indicate the surface hydrophobicity of the halibut bone soup. The laser wavelength for fluorescence measurement was 390 nm and the emission wavelength was 430–510 nm.

2.5.6. Active sulfhydryl content

The active sulfhydryl group content in halibut bone soup and MNPs were determined following a procedure described previously [19]. 5,5-Dithio-bis-2-nitrobenzoic acid (DTNB) was prepared to 10 mmol/L with 20 mmol/L phosphate buffer (pH 8.0). The lyophilized powder sample was dissolved in deionized water to 2.5 mg/mL. The absorbance of the sample at 412 nm was measured by a UV spectrophotometer (UV-2550, Unico Shanghai Instruments Co., ltd., Shanghai, China). The unit of the sulfhydryl group is nmol/mg protein, that is, the amount of nanomolar sulfhydryl groups contained in every 1 mg protein, of which the molar extinction coefficient is 13600 M^{-1} cm^{-1}. The active sulfhydryl content (nmol/mg· pro) of samples was calculated represented as:

$$\text{Active sulfhydryl content(nmol/mg·protein)} = A_{412nm} \div (13600 \times \text{Protein concentration}) \times \text{dilution ratio} \times 10^6 \quad (2)$$

2.6. Flavor analysis

2.6.1. Headspace solid-phase microextractions-gas chromatography mass spectrometrometry (HS-SPME-GC–MS) analysis

The volatile flavor of halibut fish bone soup was analyzed using an Agilent 490 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA). As described previously, the lyophilized powder of each component was prepared into 20 mg/mL emulsion, and 5 mL of each was placed in a 20 mL headspace vial. The samples were stirred and equilibrated at 60 °C for 15 min. After equilibration, headspace adsorption was carried out with a 50/30 μm DVB/CAR/PDMS extraction needle for 30 min. The desorption time was 5 min [20].

The obtained GC–MS detection substances were processed by the computer NIST11 spectral library to determine the chemical composition. The substance with the highest content was taken as the standard, and the relative content of each chemical component was calculated represented as:

$$\text{Relative content}(\%) = \frac{A_i}{A_{\max}} \times 100 \quad (3)$$

Where, A_i is the peak area of each volatile substance, and A_{max} is the maximum peak area.

2.6.2. E-nose analysis

The odor profile of the samples was characterized by a portable e-nose system (PEN3, Win Muster Airsense Analytics Inc., Germany). 1 g lyophilized powder was dissolved in 5 mL deionized water and added to a 50 mL centrifuge tube. After wrapping with three layers of plastic wrap, the samples were equilibrated in a water bath at 37 °C for 30 min [21].

2.6.3. E-tongue analysis

As described previously [22], 0.1 g halibut bone soup and MNPs lyophilized powder were taken and dissolved in distilled water, respectively. Then the volume was fixed to 100 mL, and filtered with a 0.22 μm filter membrane. 70 mL sample solution was prepared and put into the test cup. The experiment was repeated 4 times, and the remaining 3 groups of stable data were retained for data analysis.

2.6.4. 5′-Nucleotide and organic acid analysis

1 g halibut bone soup and MNPs lyophilized powder were taken and dissolved in ultra-pure water, respectively. Then the volume was fixed to 100 mL. The 5′-nucleotides and organic acids content were analyzed by an HPLC system (Agilent1100, Agilent Technologies, Santa Clara, USA), as described earlier [23,24].

2.6.5. Free amino acids (FAAs)

According to the method of Akagündüz et al. [25], 5 mL of sample (100 mg/mL) was mixed with 15 mL TCA solution (15 %). After standing for 2 h, centrifugation was performed at 4 °C (8000 × *g*, 15 min). 5 mL supernatant was taken into a 50 mL centrifuge tube, and the pH was adjusted to 2.0 with 3 mol/L NaOH solution. Then the volume was adjusted to 10 mL, and filtered by a 0.22 μm filter membrane, then determined by an automatic amino acid analyzer (L-8900, Hitachi, Tokyo, Japan).

2.7. The interaction force between MNPs and volatile odor substances

0.1 g MNPs lyophilized powder was dissolved in 5 mL ultra-pure water. Based on the experimental results of previous studies [9], five characteristic flavor compounds, including 1-octene-3-alcohol, octanal, 2-methyl-butyric acid, ethyl acetate, and 4-methyl-2-pentanone were selected for flavor adsorption experiments. The five selected flavor compounds were dissolved in phosphate buffer (0.01 mol/L, pH 8.0) and sonicated in a water bath for 10 min to obtain a standard solution of flavor compounds at a concentration of 10 mg/L. The flavor compound standard solutions were stored in the shade to prevent decomposition in the light.

The effects of five force-breakers (urea, propylene glycol, guanidine hydrochloride, sodium sulphate, and dithiothreitol) were shown in Table 1 [26–28]. The reagents were dissolved with phosphate (0.01 mol/L, pH 8.0) to obtain standard solutions of urea (5 mol/L), propylene glycol (4 mol/L), guanidine hydrochloride (5 mol/L), sodium sulphate (1 mol/L) and DTT (0.1 mol/L) respectively. 2 mL of the interaction disruptor was mixed with an equal amount of the sample solution in a headspace flask and shaken for 30 min at room temperature to mix thoroughly. Another 2 mL of phosphate buffer solution was mixed with the same amount of sample solution to form a control group.

1 mL of the flavor substance standard was added to each sample mixture, immediately sealed and analyzed by HS-SPME-GC–MS, referring to **2.13**. The adsorption rate of the component to the volatile substance was calculated based on the difference between the peak area of the flavor substance and the control, represented as:

$$\text{Adsorption rate } (\%) = \frac{A_{Treatment}}{A_{Control}} \times 100 \quad (4)$$

Where, $A_{Treatment}$ and $A_{Control}$ denote the peak areas of the characteristic flavor substances obtained in the treatment and control groups, respectively.

2.8. Statistical analysis

Origin 9.0 and TBtools were used for plotting, and orthogonal partial least squares (OPLS) analysis was performed by SIMCA software. Data were statistically analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range test ($P < 0.05$) using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). All measurements were performed in at least three independent experiments and results were expressed as mean ± standard deviation (SD).

3. Results and discussion

3.1. Changes in nutrient contents in halibut bone soup and MNPs

Sugars, proteins, and lipids in food, as precursors to flavor formation, can have an important impact on food quality, both on their own and as derivatives [29]. Fig. 1A showed the changes in total sugars and water-soluble proteins content in halibut bone soup and MNPs. It can be seen that the LMNPs contained a large number of water-soluble proteins at 222.66 mg/mL, while the total sugars content was relatively low at 3.02 mg/mL. During the formation of MNPs, protein and lipids were thermally oxidized and degraded. Most of the water-soluble proteins migrating into the fishbone broth during the boiling process had a molecular weight greater than 100 kDa and thus was retained by the filter membrane during the ultrafiltration process. As for the sugars dissolved in the soup, some of the sugars have a small molecular weight and can pass through the membrane during ultrafiltration, while some of the polysaccharides may combine with proteins to form LMNPs. The majority of fatty acids in the halibut bone soup were present in the LMNPs and almost no fatty acids were present in the SMNPs (Fig. 1B&C). This may be due to the fact that fatty acids are mostly hydrophobic and readily bind to protein particles and are present on LMNPs, while LMNPs formed during boiling are more compact and less likely to be stripped under ultrafiltration shear.

Combining the changes in the content of water-soluble proteins, total sugars, and fatty acids, it can be concluded that the three types of substances interact with each other to make some large molecular weight compounds that cannot pass through the 100 kDa filter membrane, mostly present in the LMNPs.

Table 1
The five force-breakers and corresponding effects.

Reagent	Interaction			
	Hydrogen bond	Hydrophobic interaction	Ionic Effects	Disulfide bond
Urea	Weaken	Weaken	–	–
Propylene Glycol	Enhance	Weaken	Enhance	–
Guanidine Hydrochloride	Inhibition	Weaken	Inhibition	–
Sodium sulfate	–	Enhance	–	–
Dithiothreitol (DTT)	–	–	–	Decomposition

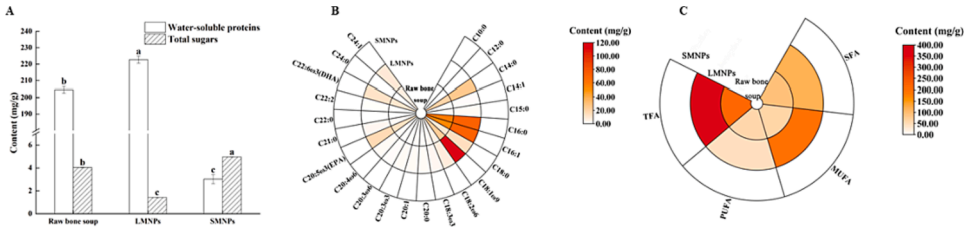

Fig. 1. Changes of nutrition content of halibut bone soup and MNPs. A: Total sugars and water-soluble proteins; B&C: Fatty acids.

3.2. Formation, properties, and microstructure of MNPs

3.2.1. Microstructure observation

To elucidate the morphological differences of MNPs in halibut bone soup, SEM and CLSM were used to observe the morphological characteristics of MNPs of different particle sizes. Fig. 2A is the SEM observation of halibut bone soup and MNPs. The raw bone soup had a complex structure, with each substance covering the other, possibly

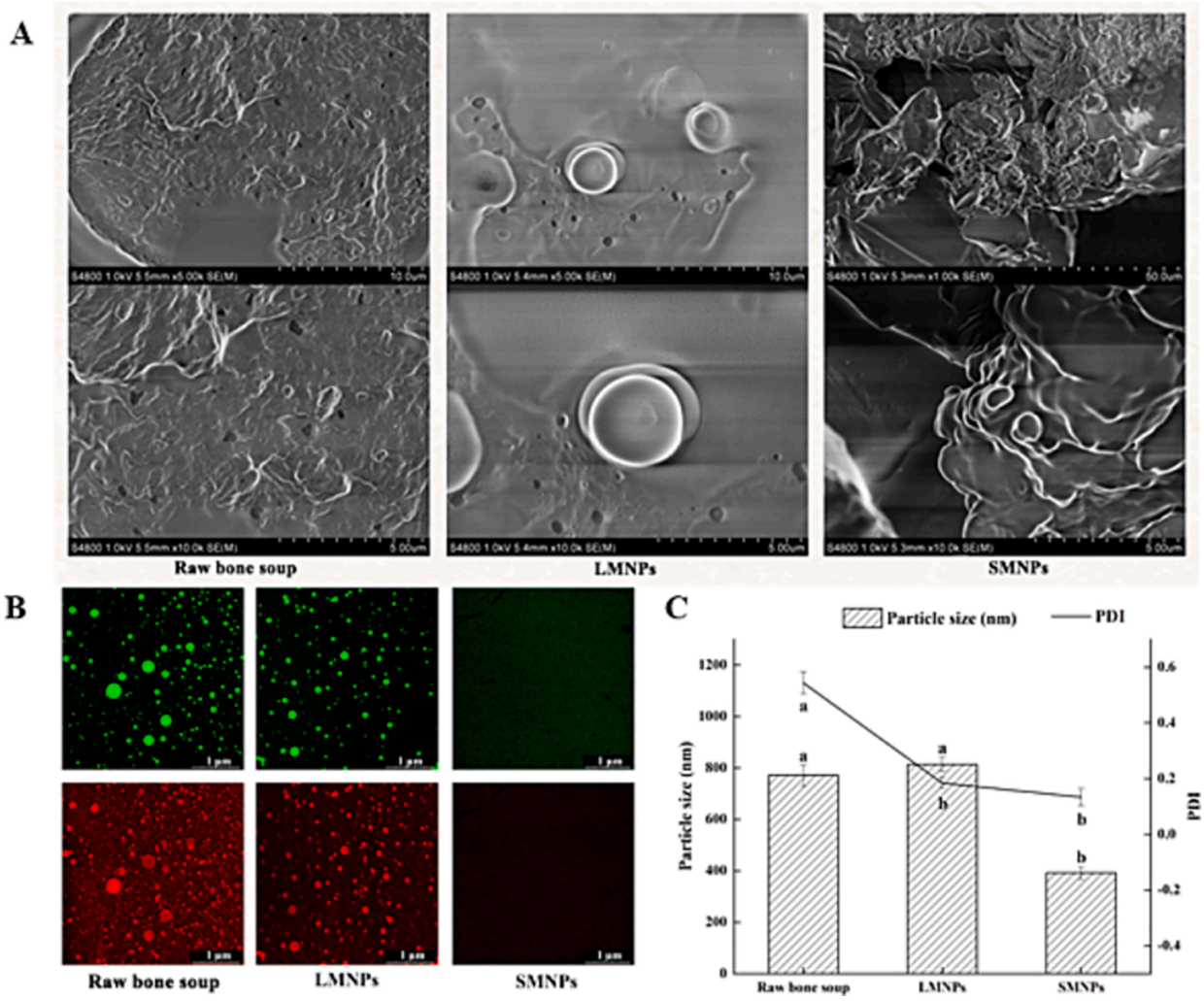

Fig. 2. SEM (A), CLSM (B) and particle size (C) observation.

with particles joining each other to form larger particles, in the form of clusters or dendrites. In LMNPs, the particles were spherical and could be clearly seen as evenly layered with inclusions, probably formed by fatty substances wrapped around globular proteins. LMNPs had larger particle sizes and more regular particle shapes, whereas SMNPs had irregular crystalline shapes and were probably composed of small molecular weight solutes.

Both proteins (green) and triglycerides (red) were distributed in spherical form in the soup sample through CLSM observation (Fig. 2B). The MNPs in the raw bone soup varied in size. After ultrafiltration, the LMNPs were more homogeneous in size. In comparison, no apparent forms of proteins and triglycerides were observed in the SMNPs. Notably, triglycerides mainly exist on the surface of LMNPs, wrapping around proteins to form lipid membranes. The lipid membranes on the surface of LMNPs are permeable, and some triglycerides may have entered the interior of the particles through the lipid membranes on the surface of LMNPs [30]. This result also confirmed that during the boiling process of halibut bone soup, lipids bind to the hydrophobic sites exposed by proteins to form tightly packed globular structures, and then form MNPs, which plays a role in stabilizing the fishbone soup system and increasing the soup flavor.

3.2.2. Particle size

Particle size, as the basic property of MNPs, can directly influence their surface properties and thus play a decisive role in the physicochemical and structural properties of MNPs [31]. As seen from Fig. 2C, the average particle size of MNPs in the raw bone soup was 770.97 nm, and the PDI was 0.54. After ultrafiltration, the average particle size of LMNPs does not increase significantly, reaching 813.80 nm, and PDI decreased significantly, reaching 0.18. While the average particle size of SMNPs decreased significantly, reaching 392.07 nm, and PDI was only 0.13. The sheer force of the ultrafiltration process reorganizes and grades the nutrients in MNPs, which makes the particle size more uniform and the size distribution more concentrated. This result is consistent with the results obtained from SEM and CLSM observations.

3.2.3. Interfacial tension

To investigate the aggregation behavior and stability of MNPs at the oil–water interface, the interfacial tension of MNPs emulsions at the oil–water interface was measured. After ultrafiltration, the interfacial tension of both LMNPs and SMNPs was lower than that of the raw bone soup (Fig. 3A), which means that the stability of both LMNPs and SMNPs was better than that of the raw bone soup. This may be due to the particle size fractionation of the components in the broth by ultrafiltration, and the reorganization of the particles by shear force, resulting in a more regular distribution of MNPs, which improves the stability of the system. In addition, the SMNPs had the lowest interfacial tension and the most stable system. This is because the LMNPs retain most of the nutrients in the soup, and after dissolution, the system appears as emulsion with large particles. SMNPs are mostly water-soluble small molecules with smaller particles in the system, and after dissolution appearing as clear and transparent solutions with the best stability.

3.2.4. Kinetic stability

Kinetic stability is an important indicator to see the differences more clearly in stability between different components. The stability of the raw bone soup and LMNPs gradually deteriorated as the increase of storage days (Fig. 3B). The stability of the concentrated lyophilized raw bone soup decreased rapidly after redissolution. The reason may be that the stable MNPs system was damaged by the concentrated lyophilization and redissolution, and the interaction among water-soluble proteins, lipids and sugars ruptured and reconnected many times, leading to the

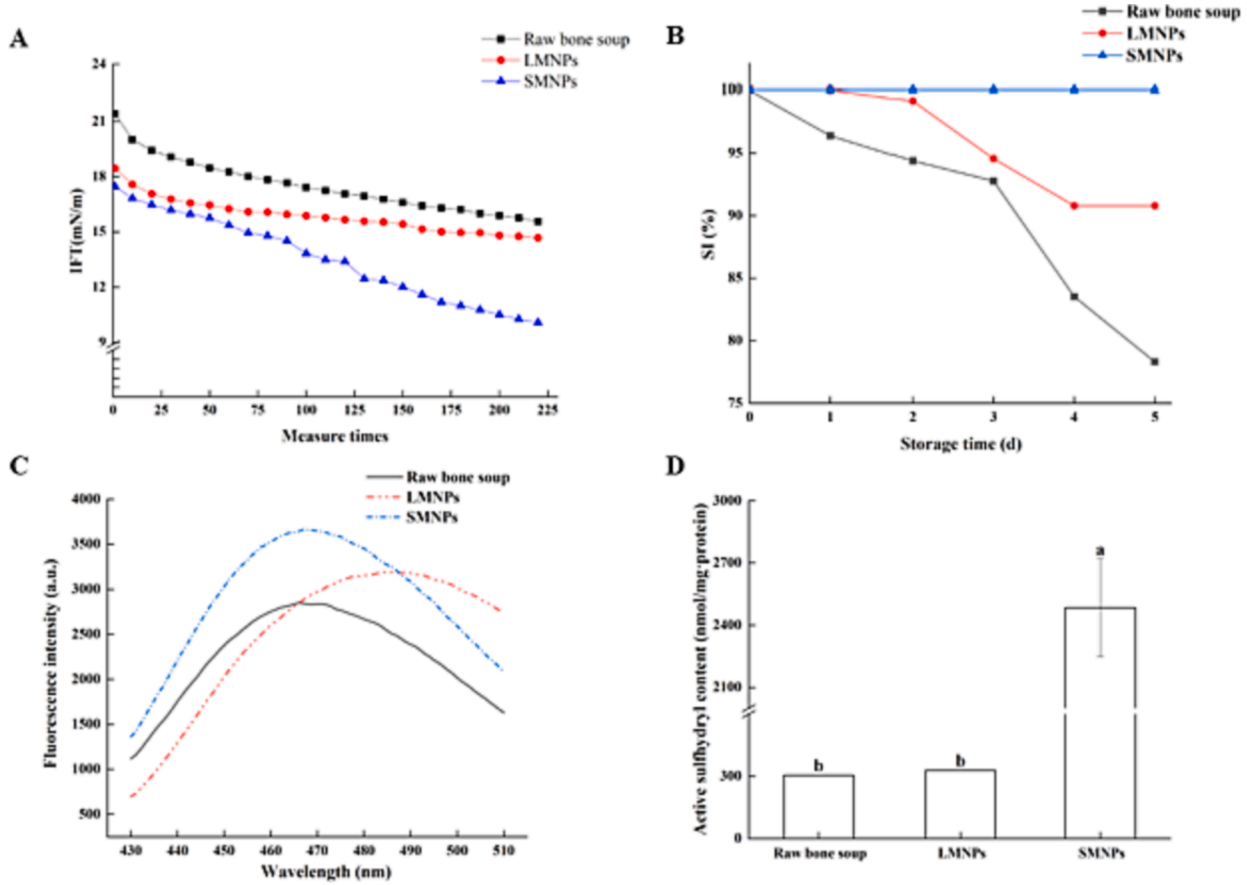

Fig. 3. Changes of interfacial tension (A), dynamic stability (B), surface hydrophobicity (C), and active sulfhydryl content (D) of halibut bone soup and MNPs.

loose structure and decreased stability of MNPs. The stability of LMNPs and SMNPs was improved by ultrafiltration classification. LMNPs can guarantee good stability during the first 2 days of storage, while SMNPs could be well dissolved in the solution system, maintaining for a long period of time, and no visible stratification was found within 5 days. As the increase of storage days, the water-soluble proteins, lipids, and sugars in the LMNPs were degraded by microorganisms, the stable emulsion system was disrupted, which led to delamination in the system, and the delamination volume gradually expanded.

3.2.5. Surface hydrophobicity

Surface hydrophobicity can estimate changes in protein exposure and aggregation [32]. The ultrafiltration separation significantly increased the surface hydrophobicity of LMNPs and SMNPs (Fig. 3C). The results indicate that ultrafiltration can expose hydrophobic groups on the surface of proteins in LMNPs and SMNPs, and the proteins in the system unfolded again. The contents of unaggregated and unfolded proteins increase, the exposed hydrophobic points increase, and the system's stability is improved. The higher surface hydrophobicity in LMNPs is beneficial for stabilizing fatty acids, thereby promoting the gelation of proteins and enhancing the degree of emulsification of broth. Although the protein content in SMNPs is low, the SMNPs have the highest surface hydrophobicity. This may be because the proteins in SMNPs are mostly peptides, with low protein folding and high exposure of hydrophobic groups, thus having a high surface hydrophobicity, which is conducive to maintaining hydrophobic components in the system. Meanwhile, the maximum fluorescence emission wavelengths of both LMNPs and SMNPs showed different degrees of redshift compared to raw bone soup, the exact cause of which needs to be further investigated, presumably due to the collision of MNPs in the fraction during ultrafiltration [33].

3.2.6. Reactive sulphhydryl content

The active sulfhydryl content indicates the degree of disulfide bond breakage and exposure of sulfhydryl groups in the protein [34]. As shown in Fig. 3D, the content of active sulfhydryl groups in the LMNPs didn't change significantly compared to raw bone soup ($P > 0.05$). It indicated that ultrafiltration did not cause significant exposure of disulfide bonds and sulfhydryl groups in the LMNPs proteins, and was able to maintain the LMNPs in a protein cross-linking condition relatively consistent with raw bone soup. In contrast, the content of active sulfhydryl groups in SMNPs was significantly higher than that of raw bone soup and LMNPs. Although the protein content in SMNPs was low, the protein molecular weight was small and unfolded to a high degree, most disulfide bonds were broken, and sulfhydryl groups were heavily exposed. Thus, cross-linking between proteins and binding between proteins and other substances differed from that of raw bone soup and LMNPs.

3.3. HS-SPME-GC–MS analysis

The composition and content of volatile compounds in samples determine the flavor quality to some extent. A total of 174 volatile compounds were detected in the halibut bone soup and MNPs by HS-SPME-GC–MS (Fig. 4A). All compounds can be classified into 11 categories, including alcohols, hydrocarbons, aldehydes, aromatic compounds, phenols, esters, ketones, acids, ethers, nitrogenous compounds, and sulphureous compounds (Fig. 4B).

Compared with raw bone soup, the contents and types of volatile compounds in LMNPs increased significantly, especially the main representative substances of aquatic product flavors, such as hydrocarbons, aldehydes, and alcohols, while the types and contents of volatile compounds in SMNPs decreased to some extent. Most of the volatile organic compounds were hydrophobic and had a strong affinity for lipid substances, and the hydrophobicity is positively correlated with the binding ability of lipids.

3.4. E-nose analysis

To study the odor differences of MNPs, their overall odor profile was described using an electronic nose. Radar fingerprints of the e-nose response values for halibut bone soup and MNPs are shown in Fig. 5A. The response values of e-nose to the W5S (nitrogen oxide sensitive), W1S (methyl compound sensitive), and W1W (sulfide sensitive) sensors were obvious. That is, each component contained more concentrated nitrogen oxides, methyl compounds, and sulfur compounds. The LMNPs had the highest odor response values among the three groups of samples because the LMNPs maintained the majority of MNPs, which allowed for the

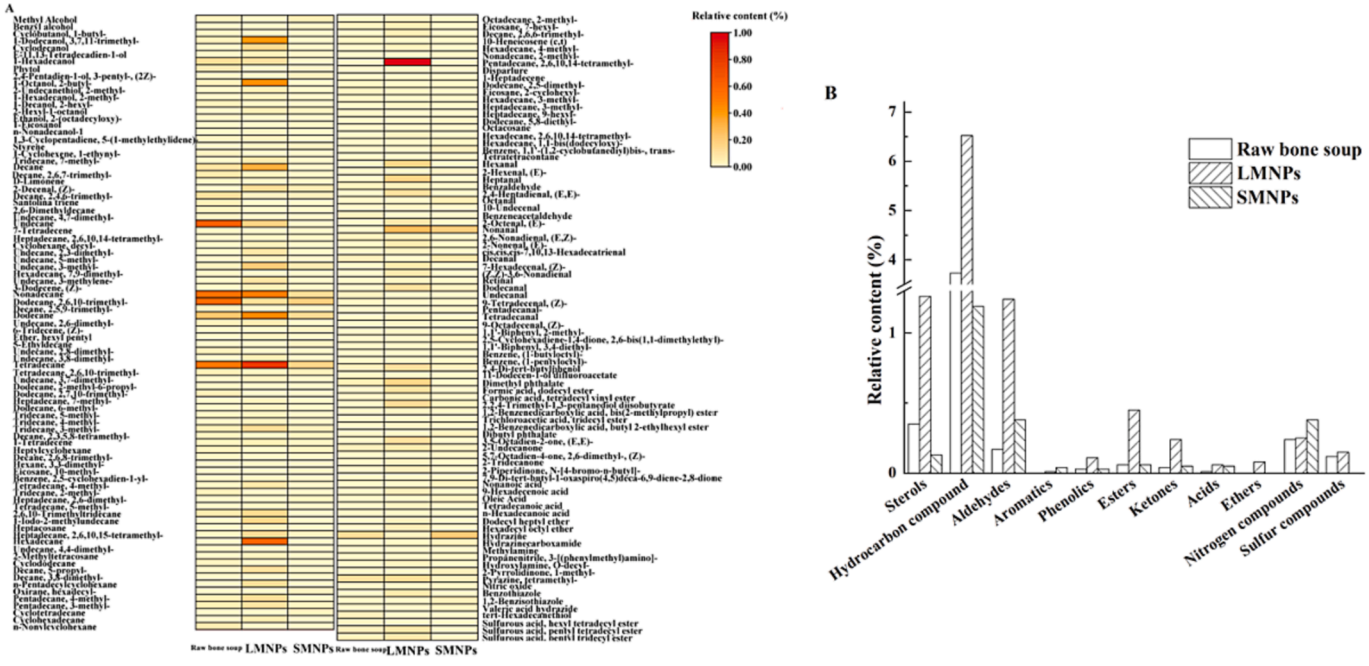

Fig. 4. HS-SPME-GC–MS analysis. A: Heatmap of volatile compounds content, B: Relative content of volatile compounds.

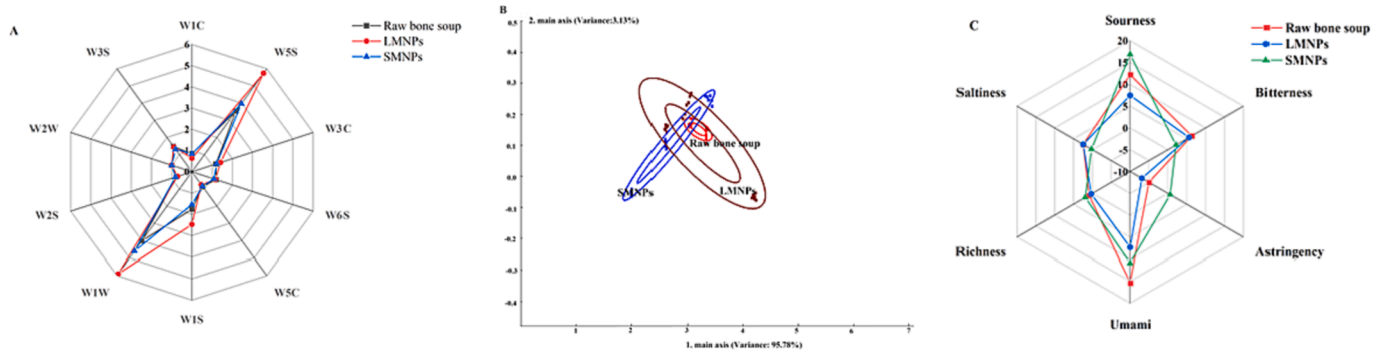

Fig. 5. Radar fingerprints (A), PCA analysis (B) of e-nose, and e-tongue (C) analysis of halibut bone soup and MNPs.

effective retention of volatile compounds in the fractions. Secondly, the increased surface hydrophobicity and reactive sulfhydryl content in LMNPs could promote the binding of hydrophobic volatile compounds to MNPs, resulting in higher response values. SMNPs had the relatively highest surface hydrophobicity and reactive sulfhydryl content and can absorb rich volatile compounds, so their odor characteristics were similar to those of raw bone soup and LMNPs.

PCA analysis was carried out to further analyze the differences in the odor characteristics of halibut bone soup and MNPs (Fig. 5B). The contribution of PC1 was 95.78 % and PC2 was 3.13 %, with a total contribution of 98.91 %, which exceeded 95 %, indicating that the main characteristics of the odor in the samples could be well represented [13]. The spatial distribution showed that the shapes of the three components overlap, leading to the conclusion that the smell of each component was similar, which is consistent with the outline presented in the radar map.

3.5. E-tongue analysis

E-tongue can analyze the sensory properties of samples through taste sensors [35]. Three fractions had high response values for umami, sourness, and bitterness (Fig. 5C), while the response values for saltiness, richness and astringency were all less than 1 and not perceptible to humans. The SMNPs had the highest sourness and the umami value was higher than LMNPs, which was consistent with the results of 5′-nucleotides and organic acids in the SMNPs. The sourness and umami of LMNPs were the lowest, but they had a bitter taste similar to raw bone soup, which may be related to the rich water-soluble proteins and fatty acids in LMNPs.

3.6. Changes in the content of 5′-nucleotides and organic acids

As taste active substances, 5′-nucleotides and organic acids can give food a special umami taste [36]. As shown in Table 2, the concentrations of 5′-AMP and 5′-IMP were low, and the TAV values of the two nucleotides were both less than 1, so the two nucleotides had no significant effect on the taste of fishbone soup and could be ignored. The contents of 5′-GMP in raw bone soup and SMNPs solution were 18.81 mg/100 mL and 160 mg/100 mL, respectively, and both of the TAV values were greater than 1, indicating that 5′-GMP contributed to the taste of raw soup and SMNPs. However, the content of 5′-GMP in LMNPs solution was only 4.40 mg/100 mL, and the TAV value was less than 1. Therefore, it is speculated that 5′-nucleotides have little effect on the taste of LMNPs. Succinic acid, the most abundant organic acid in the samples, were 2.48 mg/mL, 0.33 mg/mL and 1.73 mg/mL in the three components, respectively, and their TAV values were all greater than 1. It indicated that succinic acid contributed significantly to raw bone soup and MNPs. Lactic acid and citric acid are not significant contributors to the taste of raw bone soup and MNPs, as indicated by their contents and TAV values.

Overall, most of the taste substances of halibut bone soup were present in the SMNPs. This may be due to the small molecular weight, high polarity and water solubility of 5′-nucleotides and organic acids, which are difficult to bind to the proteins and lipids in LMNPs through hydrophobic interactions.

Table 2
Changes in 5′-nucleotides and organic acids content of halibut bone soup and MNPs.

Item	Threshold	Content		
		Raw bone soup (TAV)	LMNPs (TAV)	SMNPs (TAV)
5′-Nucleotides (mg/100 mL)				
5′-GMP	12.50	18.87 ± 0.51 (1.51)	4.40 ± 0.01 (0.35)	160.38 ± 1.87 (12.83)
5′-AMP	50.00	0.78 ± 0.04 (0.02)	0.64 ± 0.00 (0.01)	4.05 ± 0.15 (0.08)
5′-IMP	25.00	0.34 ± 0.03 (0.01)	0.52 ± 0.00 (0.02)	4.73 ± 0.08 (0.19)
Total		20.01	5.57	169.17
Organic acids (mg/mL)				
Succinic acid	0.11	2.48 ± 0.46 (22.51)	0.33 ± 0.01 (2.97)	1.73 ± 0.01 (15.68)
Lactic acid	1.26	0.44 ± 0.00 (0.35)	0.13 ± 0.00 (0.11)	0.72 ± 0.04 (0.57)
Citric acid	0.45	0.10 ± 0.01 (0.23)	0.08 ± 0.00 (0.18)	0.11 ± 0.00 (0.24)
Total		3.02	0.54	2.55

3.7. Changes in the content of FAAs

FAAs are not only important flavoring substances of food, but also the basis of characteristic flavor of food [37]. SMNPs had higher FAAs content than LMNPs (Fig. 6), probably due to the small molecular weight and mostly water-soluble nature of FAAs. Therefore, FAAs could pass smoothly through the ultrafiltration membrane into SMNPs. The results are consistent with those of 5′-nucleotides and organic acids. However, FAAs in LMNPs may be intercepted by proteins and lipids in MNPs and thus preserved.

Most of the differential volatiles belong to hydrocarbons, alcohols and aldehydes, which are consistent with the main flavor substances of aquatic products. The key difference substances mainly exist in LMNPs, and it is speculated that they combine well with volatile odorants and play an extremely important role in stabilizing the volatile flavor of fish bone soup. Moreover, SMNPs were rich in succinic acid, salty amino acids and sour amino acids, so it was speculated that SMNPs significantly contributed to the taste of fishbone soup.

3.8. Adsorption rates of volatile flavor substances by MNPs

The interaction between different types of characteristic volatile

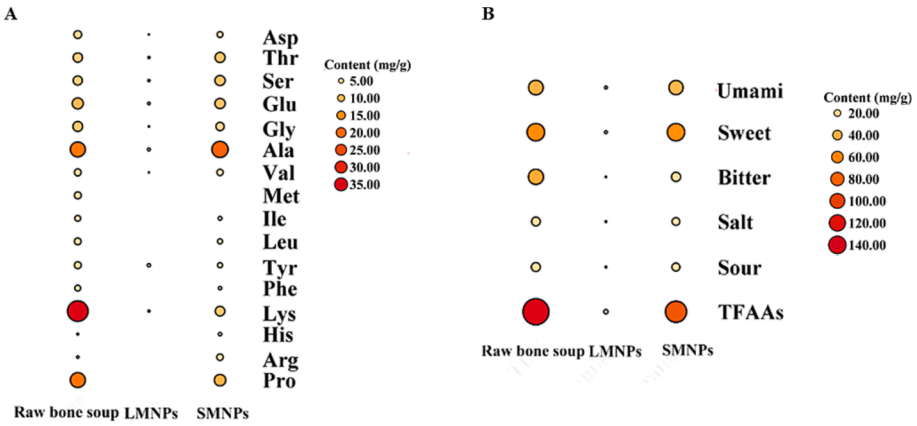

Fig. 6. Changes in FAAs content (A) and taste FAA content (B) of halibut bone soup and MNPs.

flavor substances and MNPs were further studied according to the adsorption rate of characteristic volatile flavor substances of MNPs under different force-breaker agents. As shown in Fig. 7, the adsorption rates of LMNPs were >0 for all four substances except 4-methyl-2-pentanone, indicating that LMNPs had adsorption capacity for 1-octen-3-ol, octanal, 2-methylbutyric acid and ethyl acetate, and no adsorption capacity for 4-methyl-2-pentanone. SMNPs had adsorption rates >0 for all five flavor substances, indicating the presence of adsorption for all the five substances. The addition of urea reduced the adsorption of octanal and 2-methylbutyric acid from 20.7 % and 51.95 % to −3.64 % and −84.99 %, respectively. The addition of guanidine hydrochloride reduced the adsorption of octanal and 2-methylbutyric acid to −16.8 % and −13.01 %, respectively. It can be inferred that the addition of urea and guanidine hydrochloride reduced the binding of octanal and 2-methylbutyric acid to the LMNPs. The changes in the adsorption rates of LMNPs with octanal and 2-methylbutyric acid under propylene glycol treatment were not as pronounced as the other treatments (the change in adsorption rate was only about 10 %), so it can be assumed that propylene glycol was able to maintain the binding of LMNPs with octanal and 2-methylbutyric acid better. Because urea can destroy hydrophobic interactions and hydrogen bonds in MNPs [38], guanidine hydrochloride has an inhibitory effect on hydrogen bonds and ionic effects, and also destroys the hydrophobic interactions in the system [26]. At the same time, propylene glycol can weaken hydrophobic interactions but enhance hydrogen bonding and ionic effects. Hydrophobic interactions, hydrogen bonding and ionic effects contribute significantly to the binding of LMNPs to octanal and 2-methylbutyric acid. In contrast, 1-octen-3-ol escaped in large amounts under DTT and the adsorption rate decreased from 10.69 to −119.31 %, so it can be presumed that it was absorbed by MNPs in the form of non-covalent and covalent bonds. In SMNPs, hydrophobic forces and other non-covalent interactions also play an important role in the adsorption of volatile flavor substances. Still, to a large extent, the weakening of non-covalent interactions promotes the combination of flavor and component. This may be because SMNPs themselves are in a highly stable state and the addition of the disruptor destabilizes the system, providing new opportunities for combining of flavor and components.

3.9. Correlation analysis between flavor substances and flavor of halibut bone soup and MNPs

OPLS is a multivariate statistical method that allows models to be easily interpreted by simplifying unrelated data variation and focusing categorical information on a few principal components [39]. The relative content of volatile flavor substances and the content of taste substances were used as X variables, and the W5S response value of e-nose and umami value of e-tongue were used as Y variables, respectively. The correlation model was established to explore the correlation between flavor profiles and flavor compounds of halibut bone soup and MNPs.

From the flavor OPLS score chart and the predicted and measured values of the OPLS model of halibut bone soup and MNPs in Fig. 8A&B (volatile flavor: Model 1, umami: Model 2). It can be seen that raw bone soup and MNPs can be well distinguished in terms of volatile flavor and taste. R^2X and R^2Y represent the interpretation rates for X and Y, and Q represents the predictive power of the model [40]. The R^2X, R^2Y and Q values of the two models satisfy $R^2Y(cum) > Q^2(cum) > 0.5$, and $R^2Y(cum) - Q^2(cum) < 0.3$, indicating that the two models have good explanatory and predictive capabilities. The data points for the three

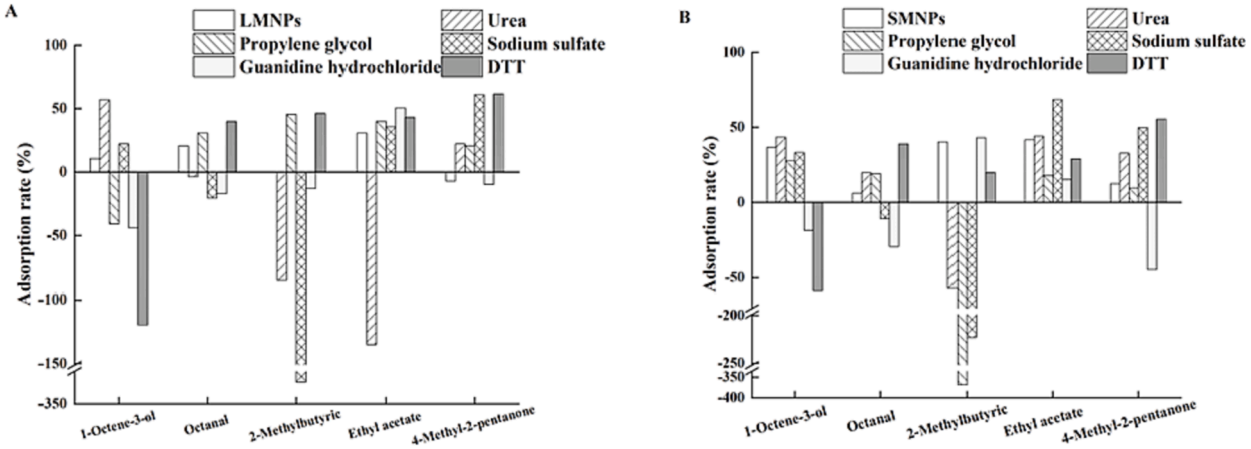

Fig. 7. The adsorption rate (A: LMNPs, B: SMNPs) of characteristic volatile flavor compounds with different reagents.

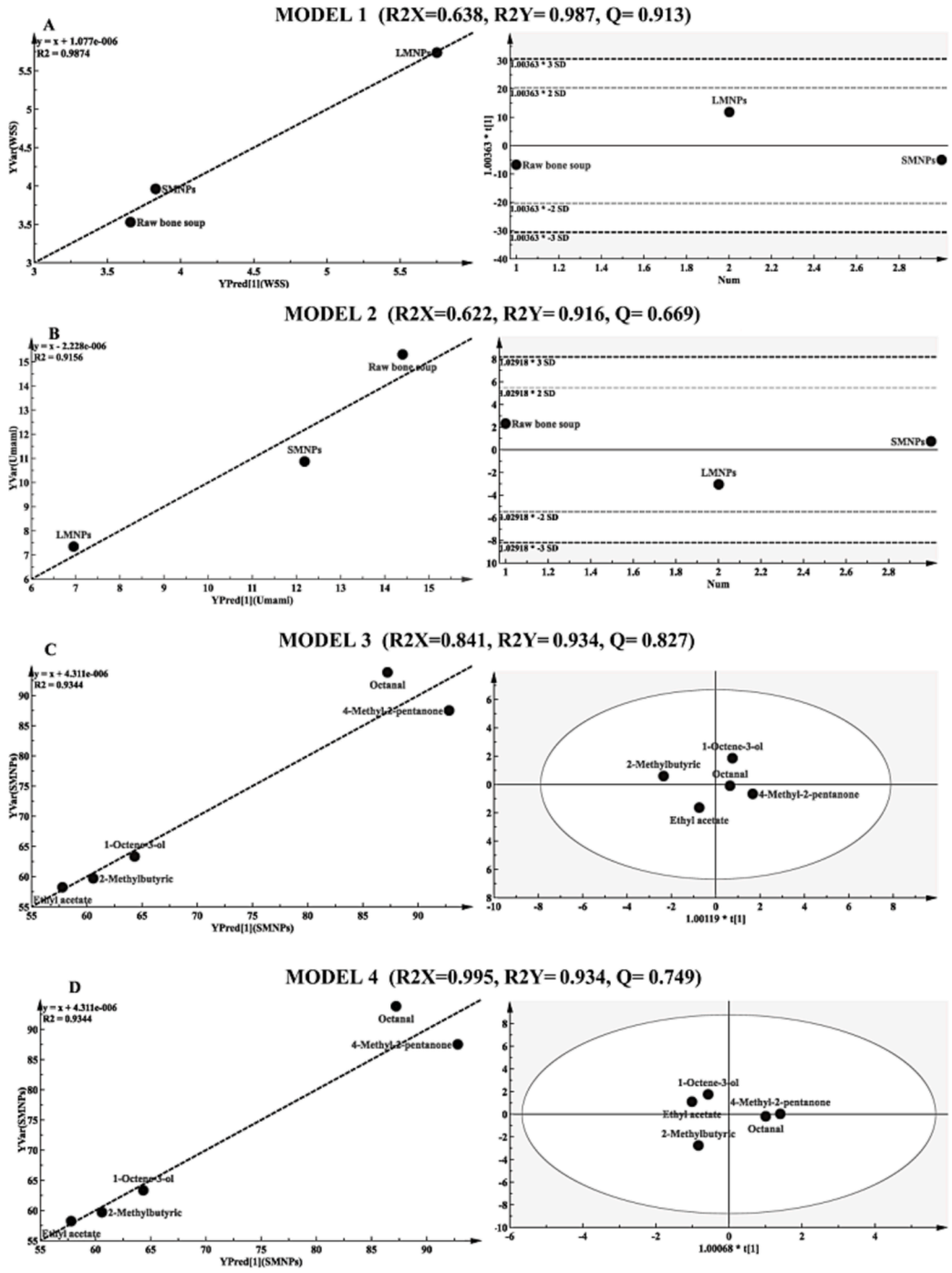

Fig. 8. The OPLS score and correlation between predicted values and measured values of OPLS model. A: Correlation between volatile flavor substances and odor characteristics; B: Correlation between taste substances and umami characteristics; C: Correlation between force-breakers and volatile flavor adsorption in LMNPs; D: Correlation between force-breakers and volatile flavor adsorption in SMNPs.

components were evenly distributed around the trend line and the corrected correlation coefficients for the volatile flavor and taste models were 0.9874 and 0.9156 respectively, indicating that the predicted and measured values of the OPLS model were a good match.

To judge the contribution of each volatile compound to the model, variable importance scores (VIP) analysis was performed on the OPLS model. The VIP values of flavor compounds are shown in Fig. 9A&B. The larger the VIP value is, the larger the contribution rate of X variable to the model is. VIP value >1 indicated that the compound was an important influence variable. Combined with the error line above 0, that is cross validation standard errors (cvSE) value less than 1, a total of 69 volatile flavor substances (Table S1) and the contents of succinic acid and salty amino acids and sour amino acids were identified as important influence variables of halibut bone soup and MNPs flavor [41].

In summary, most of the differential volatiles belong to hydrocarbons, alcohols and aldehydes, which are consistent with the main flavor substances of aquatic products. The key difference substances were mostly found in the LMNPs, which is presumed to bind well to volatile odor substances and play an extremely important role in stabilizing the volatile flavor of fish bone soup. The analysis also revealed that succinic acid, salty amino acids and sour amino acids were abundant in the SMNPs, so it is assumed that the SMNPs contribute significantly to the

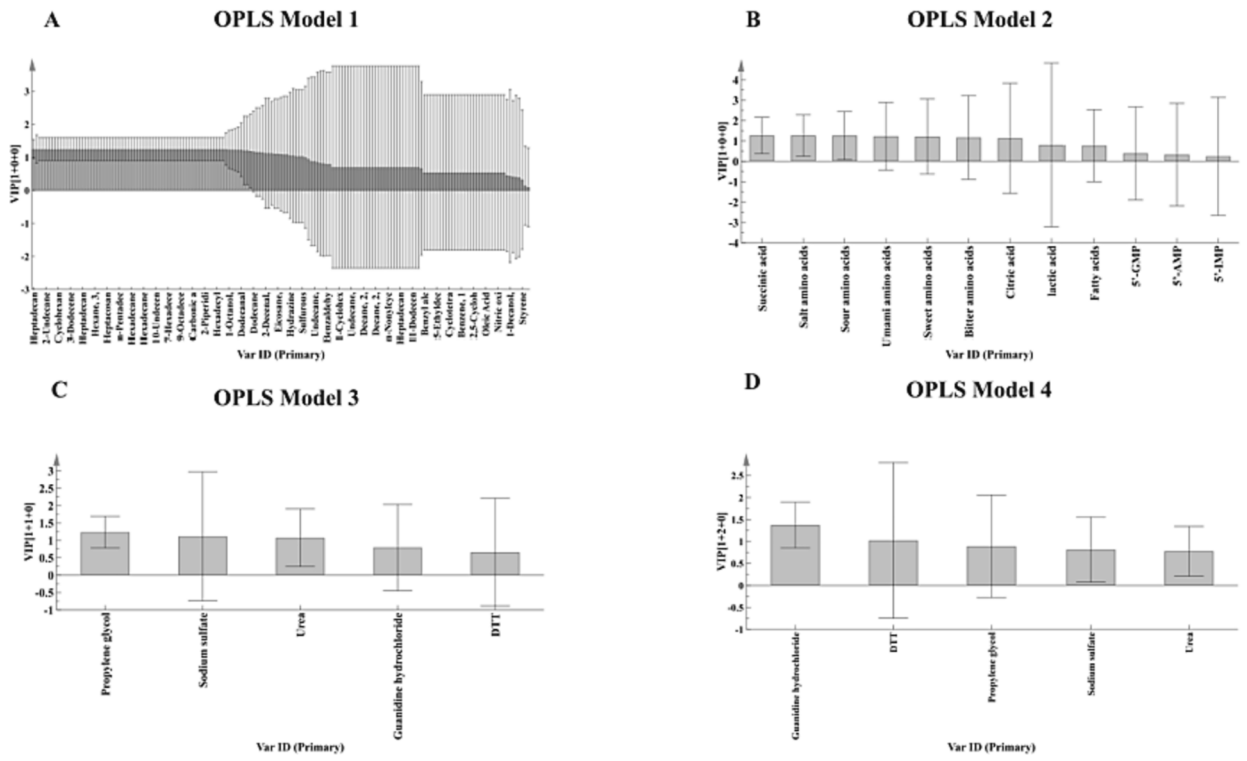

Fig. 9. The barplot of VIP value of OPLS models. A: Correlation between volatile flavor substances and odor characteristics; B: Correlation between taste substances and umami characteristics; C: Correlation between force-breakers and volatile flavor adsorption in LMNPs; D: Correlation between force-breakers and volatile flavor adsorption in SMNPs.

freshness of the fish bone soup.

The eigenvectors obtained by analyzing the loadings and eigenvalues of the principal components in the umami OPLS model (model 2), and hence the regression model was:

$$Y = 3.932 + 0.023X_1 + 0.015X_2 + 0.026X_3 + 0.070X_4 - 0.001X_5 + 0.074X_6 + 0.336X_7 + 0.024X_8 + 0.005X_9 + 0.0001X_{10} - 0.008X_{11} - 0.020X_{12}$$

In the formula, X_1 is umami amino acid, X_2 is sweet amino acid, X_3 is bitter amino acid, X_4 is sour amino acid, X_5 is fatty acid, X_6 is salty amino acid, X_7 is citric acid, X_8 is lactic acid, X_9 is succinic acid, X_{10} is 5′-GMP, X_{11} is 5′-AMP, and X_{12} is 5′-IMP.

3.10. Correlation analysis between adsorption rate and action force of MNPs on volatile flavor compounds

The OPLS model was used to explore the correlation between the adsorption of flavor substances of the components and effector disruptors. The sorption rates of MNPs for the five characteristic volatile flavor substances after treatment with urea, propylene glycol, guanidine hydrochloride, sodium sulphate and DTT, respectively, were used as X variables, and the adsorption rates of blank controls for the five characteristic flavor substances were used as Y variables.

From Fig. 8C&D, the OPLS model showed that the treatment groups in the OPLS model were well differentiated and had good explanatory and predictive power. The correlation between the predicted and measured values indicated that there is a correlation between the adsorption of flavor substances and the effector disruptors. The VIP values of the five chemical reagents are shown in Fig. 9C&D. VIP value > 1 indicates that the variable is an important influencing variable, and $0.5 <$ VIP value < 1 indicates that the variable is the main variable. Two important variables, propylene glycol and urea, that affect the binding of LMNPs to flavor substances were screened out. Guanidine hydrochloride was used as an important variable affecting the binding of SMNPs and flavor substances, and sodium sulfate and urea were the main variables affecting the binding of SMNPs and flavor substances. This showed that the binding of LMNPs to characteristic flavor substances is largely determined by hydrophobic interactions, hydrogen bonds and ionic effects, that is, non-covalent interactions. While the binding of SMNPs to characteristic flavor substances is largely determined by two non-covalent interactions, hydrophobic interactions and hydrogen bonding.

4. Conclusions

In this study, LMNPs and SMNPs were isolated and identified from non-fried halibut bone soup, and their physicochemical properties and flavor profiles were verified. It was demonstrated that the homogeneous morphology of the MNPs could improve their stability, which in turn played an important role in the stability of the halibut bone soup system. Regarding flavor, LMNPs are rich in protein and fatty acids, which can interact with a large number of volatile flavor substances and are the primary carriers of volatile flavor in fish bone soup. The SMNPs are mostly small soluble substances, which are stable in solution, and are rich in organic acids and 5′-nucleotides, which play a dominant role in the taste of the fish bone soup. The OPLS analysis showed that 72 flavor substances had a significant influence on the overall flavor of the fish bone soup, and a flavor model was constructed. The analysis of LMNPs and SMNPs treated with force-breakers showed that non-covalent interactions were the main role of MNPs in binding volatile flavor substances.

CRediT authorship contribution statement

Wei He: Writing – original draft, Methodology. **Menglin Han:** Writing – original draft. **Ying Bu:** Validation. **Wenhui Zhu:** Writing – review & editing. **Jianrong Li:** Project administration, Validation. **Xuepeng Li:** Data curation.

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.

Acknowledgment

This study was supported by scientific research project of Liaoning education department (LJKZ1017) and the Open Project Program of Beijing Key Laboratory of Flavor Chemistry (SPFW2022YB04).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ultsonch.2023.106299.

References

[1] J. Wang, M. Zhao, C. Qiu, W. Sun, Effect of malondialdehyde modification on the binding of aroma compounds to soy protein isolates, Food Res. Int. 105 (2017) 150–158, https://doi.org/10.1016/j.foodres.2017.11.001.
[2] M.L. Thanh, P. Thibeaudeau, M.A. Thibaut, A. Voilley, Interactions between volatile and non-volatile compounds in the presence of water, JAOCS 43 (1992) 129–135, https://doi.org/10.1016/0308-8146(92)90226-R.
[3] T. Koupantsis, E. Pavlidou, A. Paraskevopoulou, Glycerol and tannic acid as applied in the preparation of milk proteins-CMC complex coavervates for flavour encapsulation, Food Hydrocoll. 57 (2016) 62–71, https://doi.org/10.1016/j.foodhyd.2016.01.007.
[4] Y. Tan, K.J. Siebert, Modeling bovine serum albumin binding of flavor compounds (alcohols, aldehydes, esters, and ketones) as a function of molecular properties, J. Food Sci. 73 (2008) 56–63, https://doi.org/10.1111/j.1750-3841.2007.00591.x.
[5] G. Gao, H. Wang, J. Zhou, P. Rao, L. Ke, J. Lin, P. Sun, Y. Zhang, Q. Wang, Isolation and characterization of bioactive proteoglycan-lipid nanoparticles from freshwater clam (*Corbicula fluminea Muller*) soup, J. Agric. Food Chem. 69 (2021) 1610–1618, https://doi.org/10.1021/acs.jafc.0c02402.
[6] X. Chen, X. Yang, Characterization of orange oil powders and oleogels fabricated from emulsion templates stabilized solely by a natural triterpene saponin, J. Agric. Food Chem. 67 (2019) 2637–2646, https://doi.org/10.1021/acs.jafc.8b04588.
[7] X. Bai, T. Yue, Y. Yuan, H. Zhang, Optimization of microwave-assisted extraction of polyphenols from apple pomace using response surface methodology and HPLC analysis, J. Sep. Sci. 33 (2015) 3751–3758, https://doi.org/10.1002/jssc.201000430.
[8] J. Zhou, J. Liu, D. Lin, G. Gao, H. Wang, J. Guo, P. Rao, L. Ke, Boiling-induced nanoparticles and their constitutive proteins from *Isatis indigotica Fort.* root decoction: Purification and identification, J. Tradit. 7 (2017) 178–187, https://doi.org/10.1016/j.jtcme.2016.08.007.
[9] W. Zhu, W. He, W. Wang, Y. Bu, X. Li, J. Li, Y. Zhang, Effects of thermoultrasonic treatment on characteristics of micro-nano particles and flavor in Greenland halibut bone soup, Ultrason. Sonochem. 79 (2021), 105785, https://doi.org/10.1016/j.ultsonch.2021.105785.
[10] L. Ke, H. Wang, G. Gao, P. Rao, L. He, J. Zhou, Direct interaction of food derived colloidal micro/nano-particles with oral macrophages, NPJ Sci. Food 1 (2017) 3, https://doi.org/10.1038/s41538-017-0003-3.
[11] F. Leng, S. Sun, Y. Jing, F. Wang, Q. Wei, X. Wang, X. Zhu, A rapid and sensitive method for determination of trace amounts of glucose by anthrone-sulfuric acid method, Bulg. Chem. Commun. 48 (2016) 109–113.
[12] E. Subroto, E. Lembong, F. Filianty, R. Indiarto, S. Junar, The analysis techniques of amino acid and protein in food and agricultural products, Int. J. Sci. Technol. Res. 9 (2022) 29–36, https://doi.org/10.1016/S0167-4501(06)80096-8.
[13] J. Zhang, N. Tao, M. Wang, W. Shi, B. Ye, X. Wang, Q. Zhu, C. Hua, Characterization of phospholipids from Pacific saury (Cololabis saira) viscera and their neuroprotective activity, Food Biosci. 24 (2018) 120–126, https://doi.org/10.1016/j.fbio.2018.06.002.
[14] L. Ballesteros, M. Ramirez, C. Orrego, J. Teixeira, S. Mussatto, Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials, Food Chem. 237 (2017) 623–631, https://doi.org/10.1016/j.foodchem.2017.05.142.
[15] W. Liu, X. Li, B. Xu, B. Zhang, Self-assembled micellar nanoparticles by enzymatic hydrolysis of high-density lipoprotein for the formation and stability of high internal phase emulsions, J. Agric. Food Chem. 69 (2021) 11015–11025, https://doi.org/10.1021/acs.jafc.1c03070.
[16] W. He, W. Zhu, Y. Bu, W. Wang, X. Li, J. Li, Y. Zhang, Formation of colloidal micro-nano particles and flavor characteristics of Greenland halibut bone soup, J. Food Sci. 87 (2022) 216–230, https://doi.org/10.1111/1750-3841.15979.
[17] M. Wang, B. Huang, C. Fan, K. Zhao, H. Hu, X. Xu, S. Pan, F. Liu, Characterization and functional properties of mango peel pectin extracted by ultrasound assisted citric acid, Int. J. Biol. Macromol. 91 (2016) 794–803, https://doi.org/10.1016/j.ijbiomac.2016.06.011.
[18] A. Kato, S. Nakai, Hydrophobicity determination by a fluorescence probe method and its correlation with surface properties of proteins, BBA - Protein Struct. 624 (1980) 13–20, https://doi.org/10.1016/0005-2795(80)90220-2.
[19] X. Chen, X. Xu, M. Han, G. Zhou, C. Chen, P. Li, Conformational changes induced by high-pressure homogenization inhibit myosin filament formation in low ionic strength solutions, Food Res. Int. 85 (2016) 1–9, https://doi.org/10.1016/j.foodres.2016.04.011.
[20] W. Zhu, H. Luan, Y. Bu, X. Li, J. Li, G. Ji, Flavor characteristics of shrimp sauces with different fermentation and storage time, LWT-Food, Sci. Technol. 110 (2019) 142–151, https://doi.org/10.1016/j.lwt.2019.04.091.
[21] Y. Bu, W. He, L. Zhu, W. Zhu, J. Li, H. Liu, X. Li, Effects of different wall materials on stability and umami release of microcapsules of Maillard reaction products derived from *Aloididae aloidi*, Int. J. Food Sci. Technol. 56 (2021) 6484–6496, https://doi.org/10.1111/ijfs.15341.
[22] T.C.B. de Morais, D.R. Rodrigues, P.S.U.T. de Carvalho, S.G.A. Lemos, Simple voltammetric electronic tongue for the analysis of coffee adulterations, Food Chem. 273 (2021) 31–38, https://doi.org/10.1016/j.foodchem.2018.04.136.
[23] Y. Kong, X. Yang, Q. Ding, Y. Zhang, B. Sun, H. Chen, Y. Sun, Comparison of non-volatile umami components in chicken soup and chicken enzymatic hydrolysate, Food Res. Int. 102 (2017) 559–566, https://doi.org/10.1016/j.foodres.2017.09.038.
[24] X. Xu, M. You, H. Song, L. Gong, W. Pan, Investigation of umami and kokumi taste-active components in bovine bone marrow extract produced during enzymatic hydrolysis and Maillard reaction, Int. J. Food Sci. Technol. 53 (2018) 2465–2481, https://doi.org/10.1111/ijfs.13893.
[25] Y. Akagündüz, M. Mosquera, B. Giménez, A. Alemán, P. Montero, M. Gómez-Guillén, Sea bream bones and scales as a source of gelatin and ACE inhibitory peptides, LWT-Food Sci. Technol. 55 (2014) 579–585, https://doi.org/10.1016/j.lwt.2013.10.026.
[26] N. Yu, Y. Xu, Q. Jiang, W. Xia, Molecular forces involved in heat-induced freshwater surimi gel: Effects of various bond disrupting agents on the gel properties and protein conformation changes, Food Hydrocoll. 69 (2017) 193–201, https://doi.org/10.1016/j.foodhyd.2017.02.003.
[27] C. Riera, E. Gouezec, W. Matthey-Doret, F. Robert, I. Blank, The role of lipids in aroma/food matrix interactions in complex liquid model systems, Develop. Food Sci. 43 (2006) 409–412, https://doi.org/10.1016/S0167-4501(06)80096-8.
[28] T. Lv, Y. Wang, D. Pan, J. Cao, X. Zhang, Y. Sun, Y. Chen, Y. Liu, Effect of trypsin treatments on the structure and binding capacity of volatile compounds of myosin, Food Chem. 214 (2017) 710–716, https://doi.org/10.1016/j.foodchem.2016.07.115.
[29] M.A.J.S.V. Boekel, Formation of flavour compounds in the maillard reaction, Biotechnol. Adv. 24 (2006) 230–233, https://doi.org/10.1016/j.biotechadv.2005.11.004.
[30] X. Qian, X. Fan, H. Su, J. Zhang, N. Tao, J. Zhong, X. Wang, B. Han, Migration of lipid and other components and formation of micro/nano-sized colloidal structure in Tuna (*Thunnus obesus*) head soup, LWT-Food Sci. Technol. 111 (2019) 69–76, https://doi.org/10.1016/j.lwt.2019.04.088.
[31] K.A. Boora, K. Amarjeet, K.S. Kumar, M. Nitin, Characterization of heat-stable whey protein: impact of ultrasound on rheological, thermal, structural and morphological properties, Ultrason. Sonochem. 49 (2018) 333–342, https://doi.org/10.1016/j.ultsonch.2018.08.026.
[32] H. Liu, B. Wang, C.J. Barrow, B. Adhikari, Relating the variation of secondary structure of gelatin at fish oil-water interface to adsorption kinetics, dynamic interfacial tension and emulsion stability, Food Chem. 143 (2014) 484–491, https://doi.org/10.1016/j.foodchem.2013.07.130.
[33] H. Huang, T. Belwal, X. Lin, J. Limwachiranon, L. Zou, Z. Luo, Novel bind-then-release model based on fluorescence spectroscopy analysis with molecular docking simulation: New insights to zero-order release of arbutin and coumaric acid, Food Hydrocoll. 112 (2021), 106356, https://doi.org/10.1016/j.foodhyd.2020.106356.
[34] R. Liu, S. Zhao, B. Xie, S. Xiong, Contribution of protein conformation and intermolecular bonds to fish and pork gelation properties, Food Hydrocoll. 25 (2011) 898–906, https://doi.org/10.1016/j.foodhyd.2010.08.016.
[35] Y. Tahara, K. Toko, Electronic tongues–a review, IEEE Sens J. 13 (2013) 3001–3011, https://doi.org/10.1109/JSEN.2013.2263125.
[36] M. Zhang, X. Chen, K. Hayat, E. Duhoranimana, X. Zhang, S. Xia, J. Yu, F. Xing, Xing, Characterization of odor-active compounds of chicken broth and improved flavor by thermal modulation in electrical stewpots, Food Res. Int. 109 (2018) 72–81.
[37] A. Jurado, C. García, M.L. Timón, A.I. Carrapiso, Effect of ripening time and rearing system on amino acid-related flavour compounds of *iberian* ham, Meat Sci. 75 (2007) 585–594, https://doi.org/10.1016/j.meatsci.2006.09.006.
[38] F.O. Uruakpa, S.D. Arntfield, Network formation of canola protein–κ-carrageenan mixtures as affected by salts, urea and dithiothreitol, LWT-Food Sci. Technol. 39 (2006) 939–946, https://doi.org/10.1016/j.lwt.2005.06.007.
[39] Q. Meng, J. Zhou, D. Gao, E. Xu, M. Guo, D. Liu, Desorption of nutrients and flavor compounds formation during the cooking of bone soup, Food Control 132 (2022), 108408, https://doi.org/10.1016/j.foodcont.2021.108408.
[40] Y. Bu, Y. Liu, H. Luan, W. Zhu, X. Li, J. Li, Characterization and structure-activity relationship of novel umami peptides isolated from Thai fish sauce, Food Funct. 12 (2021) 5027–5037, https://doi.org/10.1039/d0fo03326j.
[41] Y. Qi, Z. Pi, S. Liu, F. Song, N. Lin, Z. Liu, A metabonomic study of adjuvant-induced arthritis in rats using ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry, Mol. Biosyst. 10 (2014) 2617–2625, https://doi.org/10.1039/c4mb00131a.