Physicochemical properties and microstructure of composite surimi gels: The effects of ultrasonic treatment and olive oil concentration

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ABSTRACT

This study was conducted to evaluate the effects of extra virgin olive (EVO) oil incorporation on the physicochemical properties and microstructure of surimi gels subjected to ultrasound-assisted water-bath heating. As the oil content was increased from 0 to 5 g/100 g, the breaking force and gel strength of the surimi gels significantly decreased, while the whiteness level exhibited the opposite tendency irrespective of the heating method. Compared with the traditional water-bath heating method, the ultrasonic heating promoted the unfolding of the α-helix structure and intensified the formation of β-sheet content and non-covalent bonds (ionic bonds, hydrogen bonds, and disulfide bonds), especially disulfide bonds, which contributed to the further crosslinking of the proteins and to gelation, thereby improving the gels’ strength. In addition, smaller cavities and compact microstructures were observed in the low-oil (<3 g/100 g) surimi gels under ultrasonic treatment, which effectively prevented water migration in the gel network and resulted in a high water holding capacity and uniform water distribution. However, the ultrasonic treatment barely remedied the poor microstructures of the high-oil (>3 g/100 g) surimi gels owing to oil coalescence, which weakened the protein-protein interaction. In conclusion, ultrasonic treatment combined with water-bath heating significantly improved the gelation properties of the low-oil surimi gels, although it did not remarkably improve those of the high-oil gels. The choice of a suitable oil concentration could be of great importance for the production and functioning of surimi products via ultrasound-assisted treatments.

Keywords:
Ultrasonic treatment
Surimi gel
Olive oil
Gelation properties

1. Introduction

As an important vehicle for the production of various surimi-based seafoods, surimi gels are widely favored by consumers owing to their unique sensory attributes and high nutritional value [1,2]. The “fish tofu,” crab legs, and fish sausages produced using surimi have long been popular in Thailand and other Asian countries [3,4]. During the rinsing process of frozen surimi manufacturing, large amounts of healthy lipids are removed from the surimi to improve the products’ storage stability during the freezing period [5]. However, lipid deficiency has a negative impact on the textural properties of surimi products, producing a rubber-like flavor, which will inevitably lead to a reduction in customers’ purchase intention [6].

Therefore, in place of lipids, exogenous additives, such as liquid oil (e.g., marine oils and vegetable oils) and solid fat (e.g., mutton tallow and lard), are normally added to surimi-based products to supplement the deficiencies in essential nutrients [7]. Furthermore, as the preference for a healthy diet continues to develop rapidly, consumers are excluding foods that are rich in saturated fatty acids, which have been linked to various diseases and are detrimental to human health [3,8]. In this regard, liquid oil is a more promising option than solid fat. Zhou et al. [2] reported that the presence of camellia tea oil could significantly improve the textural properties and lead to the formation of a firmer structure, especially with oil concentrations of 8 g/100 g. Elsewhere, Yan et al. [5] found that the conformation of myofibrillar proteins and the local chemical environment of surimi gels could be altered using fish-oil supplements, while Shi et al. [9] also observed that vegetable oils (e.g., soybean, peanut, corn, and rape oils) have different impacts on the quality of surimi-based products. Thus, the mechanism behind the effects of the oil type and concentration on the physicochemical changes of surimi gels remains unclear, and further research in this area is required.

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https://doi.org/10.1016/j.ultsonch.2022.106065
Received 24 April 2022; Received in revised form 4 June 2022; Accepted 12 June 2022
Available online 16 June 2022
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Olive oil, often known as liquid gold, has become largely synonymous with healthy eating. The oil is rich in unsaturated fatty acids and bioactive ingredients, which include polyphenols, tocopherols, phospholipids, and sterols [10], while the oil’s phenolic compounds can effectively prevent diabetes, cardiovascular disease, and cancer [11]. Furthermore, studies have demonstrated that olive oil containing low proportions of saturated fatty acids and high proportions of unsaturated fatty acids has the characteristics of stability, deformability, and good permeability, which could have an important effect on the physicochemical and sensory properties of surimi gels [9,12]. Based on the increase in the demand for functional seafoods, the preparation of composite surimi gels with olive oil could contribute to the development of functional surimi-based products.

In recent years, ultrasonic treatment has proven to have great potential in the food industry due to its advantages of being green, safe, and energy efficient [13,14,15]. In fact, it has been documented that ultrasonic treatment can alter conformational structures, which has a significant effect on improving the physicochemical properties of processed meat products [16,17]. Currently, ultrasonic processing is becoming increasingly common in the production of surimi-based products. Gao et al. [18] reported that ultrasonic pre-treatment facilitates the formation of compact microstructures and improves the puncture properties of surimi gels, while He et al. [19] found that ultrasonic treatment promotes the unfolding of the α-helix structure of proteins and enhances the hydrogen bonding and hydrophobic interactions between them, which significantly improves the gel properties irrespective of the sodium level. It has also been confirmed that the acoustic intensity of ultrasonic treatment prior to heating could result in secondary structural changes in proteins, thereby improving the gel’s strength [20]. Various studies have also focused on the use of ultrasonic treatment to prepare stabilized gel-like emulsions, with Cheng et al. [17] reporting that mono-frequency ultrasonic pre-treatment with a simultaneous dual frequency potentially improves the mechanical properties of whey emulsion gel. Li et al. [13] reported that high-intensity ultrasonic treatment induced structural changes and improved the emulsifying properties of chicken myofibrillar proteins. For certain, the use of high-intensity ultrasonic treatment can be expected to become a measured and targeted process for improving the quality of surimi gels and other food products.

The majority of studies have focused on emulsified protein gels and the pre-treatment of surimi via ultrasonic treatment, while there are few reports related to the influence of extra virgin olive (EVO) oil concentrations on the gelation properties of surimi gels induced via ultrasonic heating. Therefore, the present work aimed to investigate the effect of different concentrations of EVO oil on the physicochemical properties and microstructure of surimi gels treated via water-bath heating supplemented with ultrasonic treatment. This research provides a comprehensive understanding of the mechanism of composite surimi gels that are heat-induced via ultrasonic treatment as well as a theoretical basis for the development of new types of functional surimi-based products.

2. Materials and methods

2.1. Materials

Silver carp (Hypophthalmichthys molitrix) surimi (grade AAA) were obtained from Jingli Aquatic Food Co., Ltd. (Honghu, Hubei, China) and stored at −20 °C until utilization, while EVO oil was purchased from a local supermarket (Hefei, Anhui, China). All the chemical reagents used were of analytical grade and were supplied by Solarbio Science & Technology Co., Ltd. (Beijing, China) or Sigma–Aldrich Co. (St. Louis, Missouri, USA).

2.2. Preparation of composite surimi gels

Frozen surimi was thawed at 4 °C overnight before being sliced into cubes of around 1 mm³. The moisture content of thawed surimi was determined by moisture analyzer (MB27Z, Aohaoi Instrument Co., Ltd, China). EVO oil (0, 1, 2, 3, 4, and 5 g per 100 g of surimi) was added to the surimi, followed by salt (2.5 g per 100 g of surimi) for the solubilization of the myofibrillar proteins. Based on the above results of moisture measurement, the moisture content of the salted surimi was adjusted to 78 g/100 g by adding ice water. Following this, the mixture was chopped and emulsified for 1.5 min with a speed of 1,500 rpm using a commercial chopper (S2-5, Guangzhou Xuzhong Food Machinery Co., Ltd., China). To prevent the apparatus from being exposed to excessive heat, chopping was suspended for an equal period after running for 30 s. The mixed surimi pastes were then stuffed into polyvinylidene chloride (PVDC) casings with a diameter of 50 mm, which were sealed tightly at both ends until the subsequent heating process.

The emulsified sausage was sonicated using a KQ-300VDE ultrasound processor (Kunshan Ultrasonic Instruments Co., Ltd., China) before the surimi paste was subjected to water-bath heating combined with ultrasonic treatment; this was followed by further water-bath heating (denoted as US) and a separate treatment involving two-step water-bath heating (denoted as WB). The procedure for the US samples was as follows. First, sonication-assisted water-bath heating was conducted at a power of 270 W at 40 kHz and 40 °C for 50 min prior to water-bath heating at 90 °C for 30 min. The optimal settings were obtained via an additional optimization experiment, as described in the supplementary material. Meanwhile, the WB samples were treated via water-bath heating at 40 °C for 50 min followed by heating at 90 °C for 30 min. All the surimi gels were placed immediately in iced water for 20 min following heating and stored at 4 °C prior to further testing.

2.3. Gel properties

2.3.1. Texture properties

The surimi gels were equilibrated at room temperature (25 °C) for around 1 h before the experiment; then, they were cut into cylindrical shapes with a length of 20 mm. Following the procedure described by Gao et al. [21], textural tests were conducted on the gels using a TA-XT Plus texture analyzer (Stable Micro System, Surrey, UK) equipped with a P/5s spherical probe attachment. The test conditions were as follows: target pattern/auto force/pressing speed = 1.5 mm/s, testing speed = 1.0 mm/s, trigger force = 5 g, and pressing displacement = 15 mm. The breaking force (g) and deformation (cm) were recorded via the adjustment of the above parameters, while gel strength testing was performed using Eq. (1), with parallel tests repeated six times:

\[
\text{Gel strength (g × cm)} = \text{Breaking force (g)} \times \text{Deformation (cm)}
\]

2.3.2. Water holding capacity

A water holding capacity (WHC) assessment of the gel samples was carried out based on the method described by Liang et al. [22] with slight modifications. Briefly, approximately 4–5 g of sample slices (2 mm in thickness) were wrapped in three layers of filter paper and transferred to 50-mL centrifuge tubes for centrifugation (CT15RT centrifuge, Tianmei Scientific Instrument Co., Ltd., Shanghai, China) (6030×g for 15 min at room temperature). After removing the filter paper, the weights of the samples before and after centrifugation were recorded as \( m_1 \) and \( m_2 \), respectively. The WHC of the gels could be expressed in terms of Eq. (2):

\[
\text{WHC (％)} = \frac{(m_1 - m_2)}{m_1} \times 100
\]

2.3.3. Color

The surimi samples were cut into cylindrical shapes with a thickness of 1.5 cm prior to the color measurement, and the color characteristics of
the gel samples were measured using an SC-100 colorimeter (Beijing Kangguang Optical Instrument Co., Ltd., China). The values of lightness \((L^*)\), redness/greenness \((a^*)\), and yellowness/blueness \((b^*)\) were determined respectively in sextuplicate, while the level of whiteness was calculated using Eq. (3), as described in [7]:

\[
\text{Whiteness} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}
\]
2.4. Chemical interactions

The following procedure was described by Zhou et al. [2] and Yan et al. [5] and was performed with a minor modification. The chemical interactions of the composite surimi gels were distinguished according to the difference in the solubility of the chemical bonds among the five solutions (i.e., 0.05-M NaCl [SA], 0.6-M NaCl [SB], 0.6-M NaCl + 1.5-M urea [SC], 0.6-M NaCl + 8-M urea [SD], and 0.6-M NaCl + 8-M urea + 0.5-M 2-β-mercaptoethanol [SE]). Here 20 mL of the above chemical solutions was added separately to the chopped sample gels (4 g) and homogenized (PD500-TP Digital Homogenizer, Prima Technology Co., Ltd., Shanghai, China) for 1 min. The mixture was then stirred for 1 h at 4 °C followed by centrifugation at 8000 × g for 15 min. To prevent any interference from the mercaptoethanol, the Coomassie brilliant blue method was used to determine the protein concentration of the supernatant. The differences in protein concentration represent the existence of ionic bonds (SB-SA), hydrogen bonds (SC-SB), hydrophobic interactions (SD-SC), and disulfide bonds (SE-SD), respectively. Each measurement was performed in triplicate.

2.5. Total sulfhydryl content

Surimi gel samples (0.1 g) were mixed with 1 mL of the extracting solution and homogenized for 10 min at 8000 × g. The sulfhydryl groups content of the supernatant was determined using a micro-total mercapto-assay kit (Solarbio Science & Technology Co., Ltd., Beijing, China). The yellow compounds, with a maximum absorption peak at 412 nm, were used to determine the sulfhydryl groups content (SD) and the content of the supernatant. The differences in protein concentration represent the existence of ionic bonds (SB-SA), hydrogen bonds (SC-SB), hydrophobic interactions (SD-SC), and disulfide bonds (SE-SD), respectively. Each measurement was performed in triplicate.

2.6. Fourier-transform infrared spectroscopy analysis

The surimi samples were lyophilized under vacuum conditions to avoid moisture interference. The powders were pressed into thin slices before being measured using the attenuated total reflectance mode on a Fourier-transform infrared (FTIR) spectrometer (Nicolet 6700, Thermo-electric Group, USA). The spectra were scanned at a range of 500 and 4000 cm⁻¹ at a scanning frequency of 32 and a resolution of 4 cm⁻¹, while the spectrograms of the amide I fraction were analyzed using PeakFit software with self-deconvolution and multiple Gaussian curve fitting. The relative content of the protein’s secondary structure was ascertained by calculating the area of its corresponding peak, with each sample tested three times.

2.7. Scanning electron microscopy analysis

Based on the method described by Han et al. [23] and Liang et al. [24] with an appropriate modification, the surface morphology of the surimi gels was observed via a scanning electron microscopy (SEM) instrument (SU8010, Hitachi Equipment Systems Co., Ltd., Tokyo, Japan). The gel samples were sliced into cubes of around 2 mm³ and soaked in 0.1-M phosphate buffer (pH 7.2) for 30 min. Following this, the cubes were fixed overnight in 2.5% glutaraldehyde at 4 °C before being rinsed five times using the same buffer; the processed cubes were dehydrated immediately with a series of ethanol dilutions (30%, 50%, 60%, 70%, and 80% for 15 min, and 90% and 100% for 20 min.) followed by freeze drying for 24 h (FD-1B-50, Beijing Bo Medical Kang Co., Ltd., China). Finally, the samples were sputter coated with gold and examined using the SEM instrument.

2.8. Low-field nuclear magnetic resonance analysis

The relaxation measurements of the water distribution and composition of the surimi gels were carried out using a low-field nuclear magnetic resonance (LF-NMR) analyzer (NMIZ20-015V-1, Suzhou Niu-mag Analytical Instrument Corporation, China) following the approach described by Shi et al. [25] with some modifications. To avoid water loss and oil destruction, following the removal of the PDVC casing, 2-g samples were cut from the surimi gels and were placed immediately in cylindrical glass tubes with a diameter of 15 mm. The signal acquisition of the transverse relaxation time (T₂), with a resonant frequency of 18 MHz, was measured using a Carr–Purcell–Meiboom–Gill sequence. The operational key parameters were set as follows: SW = 250 kHz, TW = 3500 ms, P1 = 15 μs, P2 = 25 μs, NS = 8, TE = 0.5 ms, and NECH = 7000. The T₂ relaxation time data were subjected to an inversion operation, with each sample tested in triplicate.

2.9. Magnetic resonance imaging

Multi-layer spin-echo pulse sequence imaging was used to obtain coronal proton density images of the samples via magnetic resonance imaging (MRI) software. The T₂ weight images of the gel samples were determined with a TR of 1800 ms and a TE of 40 ms and were converted into color images via pseudo-color processing. The remaining parameters of all the images were set as follows: slice gap = 1.0 mm, slice width = 3.1 mm, and field of view = 100×100 mm.

2.10. Statistical analysis

The data obtained in this experiment were expressed as mean ± standard deviation. The statistical analysis (analysis of variance and an independent-sample t-test) was performed using IBM SPSS 23.0 software (SPSS Inc., Chicago, IL, USA), and any significant differences among the groups were compared using Duncan’s multiple range test (p < 0.05).

3. Results and discussion

3.1. Effect of ultrasonic treatment on the properties of surimi gels

3.1.1. Textural properties

The breaking force, deformation, and gel strength values of the composite surimi gels treated via the WB method or the US method, in which EVO oils with different contents (0–5 g/100 g) were added, are shown in Fig. 1A-C, respectively. With the increase in EVO oil content, the breaking force and gel strength of both groups (with added oil) decreased continuously compared with the control group (without oil); a slow decrease was obtained in the composite gels containing ≤ 3 g/100 g oil, while with an oil concentration beyond the range of 3 g/100 g, textural properties with lower values were obtained (p < 0.05) (Fig. 1A and C). The results were in accordance with those obtained by Shi et al. [9], who found that the addition of vegetable oils significantly reduced the breaking force, while a higher oil concentration tended to result in lower breaking force values. In addition, similar findings were reported in terms of fish oil [7] and virgin coconut oil (VCO) [3]. A possible explanation for this is that the increase in oil concentration reduced the protein concentration of surimi gel in the net structure, which interfered with the protein network arrangement [26] and weakened the compactness of the packing of the protein molecules [22], thus decreasing the breaking force as a result of lowering the gel strength. Furthermore, excess oil droplets could not uniformly fill the void of the protein gel matrix because of the leakage of oil located in the interstitial space of the gel network, which had a negative impact on the gel-forming capacity [27]. With the EVO oil fortification, the deformation underwent a slight change (p > 0.05) before a rapid decrease was observed when the oil concentration exceeded 3 g/100 g (p < 0.05) (Fig. 1B). This can be attributed to the tight structure formed after the surimi paste was stuffed, while the excess oil affected the degree of interaction between the protein molecules, resulting in a significant decrease in deformation [28].
The breaking force, deformation, and gel strength values of the samples undergoing US treatment were higher than those of the samples undergoing WB treatment at the same oil level. Notably, with the fortification of the EVO oil, the enhancement effect of the US treatment was more remarkable, especially in terms of gel strength ($p < 0.05$). Interestingly, the gel strength of the WB samples without oil and US samples with a 1 g/100 g oil content were 464.33 g × cm and 453.57 g × cm, respectively. These results further indicated that the use of ultrasonic heating in place of initial water-bath heating has great potential for improving the gel-forming capacity, albeit the addition of the EVO oil resulted in the deterioration of the gel properties.

Fan et al. [20] found that the gel strength of surimi was significantly strengthened with specific acoustic intensities of ultrasonic treatment (except for 0.35 W/cm²), while He et al. [19] also reported that ultrasonic treatment markedly improved the textural properties of silver carp surimi. Here, it was presumed that during ultrasonic-assisted heating at a low temperature, the physical and mechanical effects generated during the violent collapse of the cavitation bubbles promoted the dissolution of the myofibrillar protein and exposed more binding sites of endogenous transglutaminase, which is conducive to the increase in the covalent crosslinking intermolecular reaction in the heat-induced gelling process, leading to the enhancement of the puncture properties [29,30]. However, this result was in disagreement with that obtained by Liang et al. [22], who reported no marked changes in the breaking force and deformation of surimi-crabmeat mixed gels following ultrasonic treatment (210 W, 60 kHz). The reason for these different results is perhaps related to the diversity of the mixed gel systems and the ultrasound conditions and operation.

### 3.1.2. The WHC

The effects of WB and US treatment on the WHC of the surimi gels with various concentrations of EVO oil were determined, and the results are shown in Fig. 1D. With EVO oil fortification, the WHC of the composite gels was significantly enhanced (irrespective of the treatment conditions) compared with that of the control group (without oil), while an oil addition beyond the range of 3 g/100 g resulted in a rapid decrease. The increase in WHC was attributed to the fact that an appropriate oil concentration filled the protein matrix and acted as a migration barrier from the gel network, which effectively prevented the loss of water [3]. However, an excessive amount of oil weakened the protein–water interaction, which promoted the formation of the non-homogeneous structure and resulted in a lower WHC [26].

Furthermore, clearer effects of the WHC were observed in the same concentration with the US group compared with the WB group. The high shear energy and turbulence induced by the ultrasonic treatment resulted in the reduction of the particle size in the oil droplets and led to the dissolution of more of the myofibrillar molecules that were coated onto the oil droplet surfaces; this was followed by the formation of a homogenous and fine structure, thus restricting the flow of water and oil [31]. In addition, the ultrasonic-assisted heating contributed to the exposure of more hydrophobic residues, which likely promoted the combination with the oil droplets and increased the affinity of the hydropholic groups with the aqueous phase [32]. Both Zhang et al. [33] and Shi et al. [25] also demonstrated the feasibility of this, reporting that high-intensity ultrasound (600 W) improved the WHC of their gels.

### 3.1.3. Whiteness analysis

Color is one of the indispensable indicators for measuring the physical properties of foods and can determine whether the products are generally accepted by consumers. The effects of the different concentrations of EVO oil on the whiteness of the composite gels subjected to WB and US treatments are shown in Fig. 1E. Here, the whiteness in the US groups was slightly higher than that in the WB groups (except for the group with the addition of 2 g/100 g EVO oil), which suggested that the ultrasonic treatment had a mild effect on the improvement of the whiteness of the samples. The whiteness increased significantly with the addition of oil up to 2 g/100 g ($p < 0.05$) before undergoing a slight increase when the oil concentration exceeded 2 g/100 g under the two different heating treatments. Zhou et al. [27] found that the addition of lard/camellia seed oil clearly improved the whiteness of mixed gels, while significant increases in whiteness were observed elsewhere when the gels were supplemented with 5% virgin coconut oil (VCO) or a 5% VCO nano-emulsion [28]. In fact, our results were consistent with those obtained in previous studies [3,7,9]. There were two possible explanations for this phenomenon: i) The oil droplets in the gel matrix were attributed to a light-scattering effect, with the increase in oil content attributing to a light-scattering effect, with the increase in oil content at the expense of the hydration layer on the protein surface, thus weakening the binding ability of the protein and water [5]. Interestingly, it was found that the change trends of the hydrogen bonds were consistent with those related to the WHC (Fig. 1D), which suggested that hydrogen bonds could be crucial to the stabilization of the water. This conclusion was also in accordance with...
those reported by Yan et al. [5]. Zhou et al. [27] and He et al. [19] found that the hydrophobic interactions were closely related to the gel strength. The reduction in hydrophobic interactions could result in a low gel strength, as was the case in the current experiment. This change in bonds can be attributed to the fact that a part of the hydrophobic groups of the myofibrillar molecules coated onto the oil droplets strongly contacted with the oil particles, which affected the proximity of the hydrophobic groups to each other [31]. Compared with the gel samples without oil, the disulfide bonds presented a significant increasing trend with the addition of the EVO oil (p < 0.05). Similar results were obtained by Zhou et al. [35], who illustrated that fortification with fat promotes the formation of disulfide bonds in composite gels. One possible explanation for the increase in the value of disulfide bonds is that the myosin head–head and tail–tail interactions were enhanced as the oil concentration was increased during the heat-induced gelation.

The results also indicated that the contents of different chemical bonds in the US group were significantly higher than those in the WB group, especially in terms of the disulfide bonds (p < 0.05), suggesting that ultrasonic treatment would potentially alter the physicochemical properties and protein–protein interactions of specific gels. A remarkable reduction in the total sulfhydryl groups was observed in the US group, which was in accordance with the change in disulfide bonds (Table 1, and Fig. 2). In short, the ultrasonic treatment promoted the exposure and oxidation of more sulfhydryl groups in the protein, with the disulfides subsequently forming disulfide bonds followed by the generation of disulfide crosslinking [36]. This was likely because the cavitation induced by the ultrasonic treatment generated highly reactive free radicals (OH- and H·), which can cause the formation of hydrogen peroxide through chemical reactions. It has been proposed that this hydrogen peroxide could oxidize some sulfhydryl groups [15], which was in line with our results. However, further research is required to clarify the underlying mechanism behind the effect of ultrasonic treatment on the change in total sulfhydryl content [33].

The higher value of ionic bonds demonstrated that the electrostatic interaction between the proteins was enhanced by the ultrasonic treatment, with ultrasound-assisted water heating leading to the partial denaturation of the protein molecules and the exposure of more amino acid residues with positive and negative charges, thereby altering the charge distribution of the proteins [2]; this potentially explains why the ionic bonds changed.

### 3.3. Secondary structure

The protein secondary structure, which includes α-helix (1651–1660 cm⁻¹), β-sheet (1600–1639 cm⁻¹), β-turn (1661–1700 cm⁻¹), and random coil (1640–1650 cm⁻¹) components, can be accurately identified through the amide I band [24]. The contents of the secondary structures of the gels were calculated, with the results presented in Fig. 3. Compared with the control samples (without oil), the proportion of the α-helix content slightly increased (from 18.66% to 19.23%) with the addition of oil up to 3 g/100 g in the WB groups. A significant increase (from 19.23% to 24.87%) in the α-helix content was observed when the oil concentration exceeded 3 g/100 g, which indicated that a high EVO oil content could change the structure of surimi gels. This could be due to the excessive oil binding with the protein, which weakened the protein–protein interactions and subsequently hindered protein unfolding. A previous study found that the gel quality had an adverse correlation with the α-helix content [20] and could be the cause of the low gel strength and poor microstructure at higher oil concentrations. Conversely, Yan et al. [5] reported that the α-helix content tended to decrease when fish oil was added. The differences between the oil type and concentration and the gel manufacturing process could be the reason for these disparate results.

As shown in Fig. 3, a reduction of roughly 3%–4% of the proportion of α-helix content in the samples with the incorporation of EVO oil was observed in the US group, while the β-sheet content exhibited an opposite tendency. In addition, the secondary structure of the gels treated with ultrasound at lower oil concentrations changed more significantly, thus indicating the presence of synergistic effects between the ultrasonic treatment and the low-oil content. Here, compared with the conventional heat treatment (WB), the ultrasound-assisted heating treatment could have induced the denaturation of the proteins, which led to the suitable unfolding behavior of the protein and the exposure of the sulfhydryl groups, thereby facilitating the formation of a crosslinked network structure [19]. Furthermore, smaller particle size oil droplets produced via the ultrasonic treatment could have been uniformly inserted into the surimi paste, thus enhancing the oil–protein interactions [2] and ultimately changing the protein conformation. This could be the reason why the α-helix content decreased and the β-sheet...
indicated that ultrasonic treatment could promote the conversion of the α-helix to a β-sheet content, thus forming a more compact and uniform gel network [37]. Several previous studies have indicated that ultrasonic treatment could promote the conversion of the α-helix to a β-sheet content, thus forming a more compact and uniform gel network [17,20].

3.4. Microstructure of the surimi gels

The SEM micrographs of the composite gels containing different concentrations of EVO oil are shown in Fig. 4. Regardless of the heating method used, the EVO oil acted as a filler to influence the formation of a network structure in the composite gels. As shown in Fig. 4A–A5 and B0–B3, a notable improvement of the microstructures in the composite gels was observed, with the gels presenting a compact structure with a small pore diameter when the EVO oil content was increased to up to 3 g/100 g, especially in the groups with the highest concentration of oil (3 g/100 g) (Fig. 4A and B3). Conversely, as shown in Fig. 4A4–A5 and B4–B5, non-uniform structures with large holes were observed at higher concentrations of oil (>3 g/100 g).

Myofibrillar protein as the major protein in fish muscle, has a mostly rod-like structure and composed of a series of sarcomeres, which plays a key role in the gelation process of surimi [38]. After heating treatment, myofibrillar protein underwent denaturation and random aggregation to form three-dimensional network structure with large pores. When a small amount of oil was added into the surimi, the surface of oil was adsorbed by myofibrillar protein, and the adjacent oil droplets were tightly packed into the gel matrix, which facilitated the formation of denser gel structures [39]. Based on the above results, this may be the reason why an appropriate amount of EVO oil markedly decreased the holes in the gel structure. While the higher oil concentrations had a clear adverse effect on protein–protein interactions by increasing the intermolecular distances between the protein chains, leading to larger pores and a coarse microstructure [5]. This is in agreement with the findings of Zhou et al. [31], who reported that the ultrastructure of the low protein/fat ratio mixed gels (1:5, 1:10, and 1:15) presented a coarser and more irregular three-dimensional network with larger pores compared with that of higher protein/fat ratio mixed gels (15:1, 10:1, and 5:1). In addition, the poor gel structures with the addition of >3 g/100 g oil were in line with the lower puncture properties of the composite gels in the present study (Fig. 1). The likely reason for this is that coalescence caused by excessive amount of oil greatly disturbed or disrupted the organized arrangement of myofibrillar protein, resulting in the disordered arrangement of gel proteins, which had a negative impact on puncture properties [3]. This phenomenon was not observed when the oil concentration was ≤3 g/100 g. It was presumed that less oil decreased the puncture properties of gels, but significantly improved the water holding capacity and avoided the moisture exudation and migration, which was beneficial to forming a homogenous and compact gel structure [33].

As shown in Fig. 4, there was a clear promotion in the microstructures of the composite gels heated via ultrasonic treatment. This was probably because ultrasonic-assisted heating treatment was conducive to better unfolding and dissolution of myofibrillar protein [19], and the high-intensity shock waves generated by the implosion of the cavitation gas bubbles in the US group reduced the size of the oil droplets, which were sufficiently coated and entrapped by the dissolved protein [40], resulting in the formation of a more homogeneous and smoother gel composition. Both Li et al. [13] and Zhao et al. [41] also demonstrated that the oil droplets following ultrasonic treatment exhibited a small particle size and uniform dispersion. According to previous studies, the rod-like structure of myofibrillar protein after heat treatment might be converted into matrices/aggregates and spherical structures, and spherical structural proteins with small width and height and the organized protein aggregates made the gel network compact and stable [42]. At 40 °C, the myosin molecules (the major component of myofibrillar protein) began to stretch and gradually aggregated to form protein clusters, and then myosin clusters were crosslinked to establish a preliminary protein network. Similarly, one could speculate that the shear stress induced by ultrasound, in promoting the exposure of more active groups of myosin, enhanced the intermolecular chemical interactions in protein aggregates [43], and reduced the particle size of myosin aggregation [15,44], which might lead to the smaller aggregates.
and ordered aggregation. This created favorable conditions for the rapid formation of stable agglomeration of myosin under water bath heating in the second step (90 °C). Furthermore, ultrasound-assisted heating treatments could also compel the proteins to expose more intramolecular sulfhydryl groups, which can be oxidized into disulfide bonds to promote crosslinking and aggregation among the proteins, thereby resulting in a satisfactory structure [37]. However, the ultrasonic treatment barely improved the structures of the composite gels supplemented with oil concentrations of >3 g/100 g.

3.5. Low-field nuclear magnetic resonance results

To further investigate the mobility and distribution of water molecules in the composite gels, the $T_2$ relaxation time with different heating regimes was assessed via an LF-NMR analysis. As shown in Fig. 5A and B, three distinct peaks ($T_{2b}$, $T_{22}$, and $T_{23}$) were observed in the relaxation spectra. The $T_{2b}$ component in the range of 0.1–1 ms corresponded to the water that was closely associated with the macromolecules, and the $T_{22}$ component in the range of 20–400 ms corresponded to the immovable water entrapped within the myofibrils. The $T_{23}$ component in the range of 400–2,000 ms was attributed to the free water outside the myofibril lattice [19].

The proportion of the above $T_2$ distributions are presented in Fig. 5C. Here, the corresponding fraction ($PT_{2b}$) of the immobilized water ($T_{2b}$) at the highest point accounted for over 95%, indicating that $PT_{2b}$ was the major water species in the gel samples. However, no notable changes ($p < 0.05$) were observed in terms of the $T_{22}$ relaxation time in the WB and US groups (Fig. 5A and B), which revealed that water-bath heating combined with ultrasonic treatment had little effect on the immobilized water mobility and avoided excessive loss of immobilized water. Furthermore, following gelation, the low water mobility in the gel network indicated that the water molecules interacted only weakly with the surrounding chemical environment in the presence of the EVO oil [7]. With the increase in EVO oil content, both the $T_{2b}$ relaxation time and the peak area $PT_{2b}$ in the WB groups decreased significantly (from 0.65 to 0.48 ms and from 1.97% to 0.19%, respectively; $p < 0.05$) as a linear function (Fig. 5A and C). This phenomenon demonstrated that the bound water was more closely linked with the protein and that the relaxation rate of the water was restricted after the expelled water was removed [33,45]. The $T_{2b}$ and $PT_{2b}$ values of the samples with oil concentrations of ≤3 g/100 g increased gently in the US groups (Fig. 5B and C), while a decrease in $PT_{22}$ was observed (Fig. 5C), which indicated that part of the immobilized water was converted into bound water. These results were contrary to those obtained with the WB groups. This may have been because the cavities of the gel matrix structures were filled with oil droplets [6] and the water molecules were trapped in gel pores with a small size (Fig. 4) following the US treatment [37], thereby resulting in the acquisition of water, especially with the addition of 3 g/100 g oil. In addition, with the samples undergoing US treatment, the $PT_{2b}$ value decreased sharply (from 1.92% to 0.77%; $p < 0.05$), and the $PT_{22}$ underwent a significant change ($p < 0.05$) from 95.41% to 96.58% when the oil content exceeded 3 g/100 g (Fig. 5C). These results indicated that the ultrasonic treatment combined with heating promoted the unfolding of the protein structures [19], leading to the partial denaturation of the myofibrils; this was beneficial to the exudation of the salt-soluble proteins and the agglutination of gel network; with more moisture retained in the fiber structure ultimately becoming immobilized [25]. For surimi gel (heating at 40 °C and 90 °C), the ratio of $PT_{2b}$ was
The PT$_{23}$ value of the samples treated with ultrasound was slightly lower than traditional water bath heating, which indicates that ultrasonic treatment restrained the increase of free water existed outside the myofibrillar lattice, and the immobilized water being trapped in the myofibrillar protein network was better preserved and less transformed into free water. Previous studies have reported that mobilized free water lied outside the myofibrillar lattice represented the potential drip loss of meat structures [47]. Therefore, the decrease of PT$_{23}$ was beneficial to the enhancement of water holding capacity of surimi gel. Overall, ultrasonic treatment exhibited great ability on inhibiting the accumulation of free water.

Notably, shorter T$_{2b}$ relaxation times of the surimi gels treated with the WB and US methods were observed at higher oil contents (>3 g/100 g), suggesting that the gels with >3 g/100 g oil addition tended to have more restriction of bound water after heating treatment, which also implied that the bound water tightly bound to proteins inhibited the migration of immobilized form. Han et al. [23] have pointed out that the degeneration and aggregation of myosin in heat-induced gelation system might be relate to the change in immobilized water. Aside from this, high content of EVO oil was surrounded by more protein membranes, leading to the insufficient myofibrillar proteins participating in the gel structure [48], which was detrimental to the formation of high-degree crosslinking in the gel network. It is presumed that the formation of incomplete network structure of the gels with oil concentrations of >3 g/100 g might due to the restriction of more bound water transforming into immobilized water. This was consistent with the findings pertaining to the microstructural observations (Fig. 4). It seems that the ultra-structure of gels was closely related to the restriction of water mobility in the heat-induced myosin gel.

3.6. MRI results

As a rapid, non-destructive method, MRI can be used to determine the distribution of water in foods and to visualize internal structural changes [49]. The T$_2$ weighted images of the surimi gels containing various EVO oil concentrations and subjected to WB and US treatment are shown in Fig. 6. Here, the red color corresponds to a high proton signal density, and the blue corresponds to a low proton signal density [50]. On comparing the MRI images, it was clear that the pseudo-color images of the samples with the addition of EVO oil in the WB and US groups presented more red shading and less yellow than those without oil, which suggested that the surimi gels with the oil retained more moisture. Oil droplets might act as water migration barrier of gel network to retain water molecules more firmly, which was likely a major reason for this phenomenon [3]. With the increase in oil content (<3 g/100 g), the red-colored region increased significantly in the pseudo-color images, and the water distribution of the gels became more homogeneous (Fig. 6A0–A3 and B0–B3). This can be interpreted by the fact that oil globules occupied the void spaces in protein matrix and participated in the formation of a firmer structure, thereby preventing the loss of water [2]. However, the size of the external brighter regions underwent a continuous decrease in the samples with higher EVO oil concentrations (>3 g/100 g) (Fig. 6A4–A5 and B4–B5), indicating that the presence of high-content oil could not effectively maintain the moisture content of gels. As a comparison, the gel samples with US treatment displayed higher quantitative intensity than those with WB treatment, indicating that ultrasonic treatment was more beneficial in maintaining the water holding capacity of surimi gels. Moreover, the samples with 3 g/100 g oil content in US group showed the reddest and brightest pseudo-colour image (Fig. 6B3), which suggested that surimi gels with the addition of 3 g/100 g oil under ultrasonic treatment had palpable effect on the water maintenance. The MRI result was in accordance with that observed in terms of the T$_2$ relaxation.

![Figure 6](image-url)

Fig. 6. The T$_2$ weighted magnetic resonance images of the gel samples containing different EVO oil contents heat-induced via the WB (A0 – A5) and US (B0 – B5) methods. A0 – A5: the gels subjected to WB treatment with oil contents of 0, 1, 2, 3, 4, and 5 g/100 g; B0-B5: the gels subjected to US treatment with oil contents of 0, 1, 2, 3, 4, and 5 g/100 g. WB: traditional two-step water-bath heating; US: water-bath heating combined with ultrasonic treatment followed by further water-bath heating.
4. Conclusions

This study was aimed at revealing the effects of ultrasound treatment and the addition of EVO oil to the physicochemical characteristics and microstructure of surimi gel products. The incorporation of EVO oil in the surimi gels decreased the breaking force and gel strength but improved the whiteness, while the effect of the ultrasound treatment on the gelation properties was closely related to the oil content. With lower oil concentrations (0–3 g/100 g), the ultrasound treatment promoted the formation of hydrogen bonds and disulfide bonds and the conversion from α-helix to β-sheet content, thereby significantly enhancing the gel strength. Meanwhile, compact and homogeneous microstructures with smaller hole diameters were observed in the samples undergoing ultrasonic treatment; this hindered the flow of water molecules and resulted in a higher WHC and uniform water distribution, especially with the addition of oil concentrations of 3 g/100 g (these findings are supported by the LF-NMR and MRI analyses). However, with high-oil concentrations (>3 g/100 g), the ultrasound-assisted heating barely compensated for the poor gelation properties of the high-oil surimi gels, which presented non-uniform microstructures with large pores. Therefore, to maximize the nutritional value, the optimal oil concentration for ultrasonic processing should not exceed 3 g/100 g. This study provides a theoretical basis for the rational application of EVO oil in ultrasound-based processing to produce functional surimi-based seafoods with improved gel properties.

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.

Acknowledgments

This work was supported by the earmarked fund for the Anhui Provincial Modern Agri-industry Technology Research System (AHCYJSTX-08) and the China Agriculture Research System of MOP and MARA (CARS-48).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2022.106065.

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