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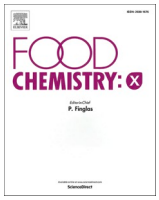

Effect of different orthogonal double frequency ultrasonic assisted freezing on the quality of sea bass

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ABSTRACT

The ice crystals formed in the body of the fish after freezing will cause irreversible damage to the fish's tissues, resulting in a decline in the fish quality. Therefore, based on the single frequency and double frequency ultrasonic freezing technology, the influence of orthogonal ultrasonic on the sea bass quality was studied. The results showed that the orthogonal ultrasonic wave could effectively improve the utilization rate of ultrasonic. In addition, SEM images showed that the muscle tissue in the dual frequency orthogonal ultrasonic assisted freezing group (DOUAF-40 (H) 20 (V)) was more uniform and dense. DOUAF-40 (H) 20 (V) group did not cause excessive oxidation of myofibrin on the one hand, and on the other hand reduced the duration of lipid oxidation in fish. The results showed that the orthogonal ultrasonic freezing technology inhibited the impact on fish quality during the freezing process, which provided a reference for the food freezing industry to improve aquatic products.

Introduction

Sea bass (*Lateolabrax maculatus*) has the characteristics of delicious meat, high nutritional and low fat value, mainly distributed in the Mediterranean Sea and surrounding waters (Munekata et al., 2020). The simultaneous action of microorganisms and enzymes on sea bass resulted in decreased water retention ability, protein degradation and lipid oxidation, thus reducing the quality of sea bass at room temperature. (Lan et al., 2021). Freezing is a normal method to keep fresh aquatic products. Therefore, during the freezing process of sea bass, it is necessary to reduce the damage of ice crystals (Tan et al., 2021). The freezing curve can be defined as three different stages:(1) precooling stage: 5 ~ −2℃; (ii) Phase transition: −2 ~ −5℃; Subcooling stage: −5 ~ −18℃. The most critical phase is the phase transition, when most of the free water forms ice crystals. (Xu et al., 2015). Common traditional freezing methods usually freeze more slowly, such as air freezing (Isfahan et al., 2017), immersion freezing (Liang et al., 2015) and so on. Ultrasonic assisted freezing (UAF) is a new green and safe freezing technology developed in recent years. It does not need additives in the process and relies on cavitation effect produced by ultrasonic to act on aquatic products (Ma et al., 2021b). In the study of Chen et al. (2022), UAF can shorten the formation process of large ice crystals in fish, promote the secondary nucleation of ice crystals, and form small and smooth ice crystals inside and outside the cells, thus boosting the aquatic products quality (Sun et al., 2021). On this basis, Ma et al. (2021a) found that contrasted with single frequency ultrasound, multi-frequency ultrasound could better reduce the ice crystals size, reduce the ice crystals damage to fish cells, and better maintain the large yellow croaker quality. Tian et al. (2020a,b) showed that contrasted with single frequency ultrasonic freezing, orthogonal frequency ultrasonic freezing would make potato freezing faster, with less drip loss and more conducive to potato preservation. Orthogonal ultrasonic can effectively solve the non-uniformity and cavitation shielding of single frequency ultrasonic and improve the utilization rate of ultrasonic wave (Zhai et al., 2017). Sun et al. (2021) showed that in the ultrasonic freezing process of different powers, the power of 175 W had the least influence on the common carp quality (*Cyprinus carpio*). Based on previous studies, it is found that the ultrasonic field intensity generated by orthogonal single frequency ultrasound is lower than that of orthogonal double frequency ultrasound. Tian et al. (2020a,b) showed in their research that

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compared with orthogonal single frequency ultrasound, the freezing rate of potatoes was significantly increased and the juice loss was significantly decreased under the freezing treatment of orthogonal double frequency ultrasound. However, the application of orthogonal dual-frequency ultrasound in fish aquatic products is less. This study explored the influence of orthogonal dual-frequency ultrasound on the sea bass quality change.

Based on the above research, this paper adopts the ultrasonic power of 175 W, optimal horizontal single-frequency ultrasonic, vertical single-frequency ultrasonic, horizontal dual-frequency ultrasonic, and orthogonal dual-frequency ultrasonic were selected to analyze the effects on the frozen sea bass quality, mainly from the size of ice crystal, freshness, protein structure changes.

2. Materials and methods

2.1. Materials and equipment

They bought fresh bass from a local market (Luchao Port Market in Shanghai, China) weighing 475–525 g, beat the head of the fish to death, washed it with water, and packed it in polyethylene bags in turn. on the basis of the way of Ma et al. (2021), the sea bass samples were divided randomly into six different treatments, such as immersed freezing (IF), single frequency horizontal ultrasound assisted freezing (SHUAF) at 40 kHz, single frequency vertical ultrasound assisted freezing (SVUAF) at 20 kHz, dual frequency horizontal ultrasound assisted freezing (DHUAF) at 20 and 40 kHz, dual frequency orthogonal ultrasonic assisted freezing (DOUAF), the vertical frequency is 20 kHz, the horizontal frequency is 20 kHz, dual frequency orthogonal ultrasonic assisted freezing (DOUAF), the vertical frequency is 20 kHz, the horizontal frequency is 40 kHz. Our research group drew and designed multi-orthogonal frequency auxiliary ultrasonic refrigeration equipment, and then submitted the drawings to Xicheng Ultrasonic Equipment Co., Ltd. (Jining, Shandong, China) for manufacturing, as shown in Fig. 1. The ultrasonic system makes up of four ultrasound transducers and a hexahedral freezing tank (side length: 60 cm). A 29.9% calcium chloride (w/v) solution is used as a secondary refrigerant in the ultrasound assisted freezing (UAF) and the secondary refrigerant temperature was controlled in the range of −30 ± 0.5 ℃. The ultrasonic time was set 30 s on, 30 s off. Fluke 2640A was used to record the temperature of bass samples in real time. A *T*-type thermocouple is inserted into the back muscle of the sea bass at the same distance from the head and tail. When the core temperature of sea bass is detected to reach −18 ℃, it is transferred to a freezer at −40 ℃ for five days. Then put it into a sink of 0.6 m*0.45 m, and thaw it with running water. The temperature of tap water is about 15 ℃ and the flow rate is 0.5 m/s.

2.2. Freezing curve

The freezing curve was decided by the correction method of Tan et al. (2021). The multi-point thermometer (Fluke 2640A, USA) was used by placing its probe on the back of sea bass samples to monitor the changes in the central temperature of sea bass caused by six different freezing methods (Fig. 1). Five temperature test points were set up for each frozen fish sample and the temperature curve of the back muscle center of sea bass is selected to plot the figure.

2.3. Water retention

2.3.1. Thawing loss

Thawing loss was decided by Bian et al. (2022). The sea bass were thawed in tap water to a central temperature of 4 ℃. Blot the skin of the sea bass with filter paper. The formula of thawing loss is shown:

$$\text{Thawing loss}\,(\%) = \frac{W_2}{W_1} \times 100\%$$

W_0 (g) is shown as the sea bass samples before freezing
W_1 (g) is shown as the sea bass samples after freezing
W_2 (g) $= W_0 - W_1$

2.3.2. Cooking loss

Cooking loss was dicided by a modified method according to Cheng et al. (2022). Weigh 2 g (1 cm*1 cm*2 cm) seabass from the back muscle of the seabass sample, wrap it with filter paper, and then put it in a polyethylene bag in turn. Finally, the polyethylene bag was boiled in

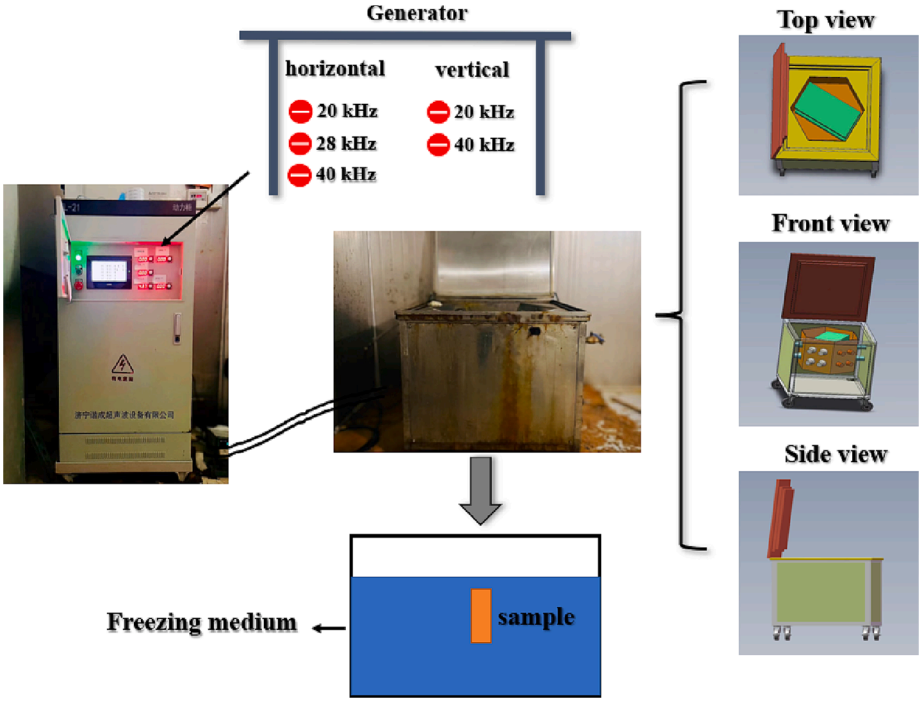

Fig. 1. Schematic diagrams of the multi-orthogonal frequency assisted ultrasound freezing system.

85℃ water for 10 min. The formula of thawing loss is shown:

$$\text{Cooking loss}\,(\%) = \frac{W_2}{W_1} \times 100\%$$

W_0 (g) is shown as the sea bass samples before cooking
W_1 (g) is shown as the sea bass samples after cooking
W_2 (g) = $W_0 - W_1$

2.3.3. Water holding capacity (WHC)

The WHC method was determined by Merlo et al. (2019). The sea bass sample was cut into small pieces (about 2 g) and then placed on filter paper to dry water during centrifugation. The sea bass samples were centrifuged (5000 × g) for 10 min. The formula of thawing loss is shown:

$$\text{Centrifugal loss}\,(\%) = \frac{W_2}{W_1} \times 100\%$$

W_0 (g) is shown as the sea bass samples before centrifugation
W_1 (g) is shown as the sea bass samples after centrifugation
W_2 (g) = $W_0 - W_1$

2.4. Magnetic resonance imaging (MRI) and Low-field nuclear magnetic resonance (LF-NMR)

MRI and LF-NMR were surveyed water distribution and migration of sea bass samples by a modified method according to Tan et al. (2021). LF-NMR was measured by LF-NMR analyzer (MesoMR23-060H). I, Niu Mark Company, Shanghai, China), magnetic field intensity of 0.5 T, proton resonance frequency of 20 mHz. The sea bass sample was cut into 2.0 cm × 2.0 cm × 1.0 cm piece (5 g), removed moisture and wrapped in plastic bag. The packaged samples were examined in a cylindrical probe with a diameter of 70 mm at an operating temperature of 32℃. Water distribution images of sea bass samples were made using MRI imaging software and MSE spin sequence equipment.

2.5. Total volatile basic nitrogen (TVB-N)

TVB-N of sea bass samples were decided by Cheng et al. (2022). 5 g of fish flesh was taken from the back muscle of the sea bass sample for measurement. TVB-N was decided by microtitration and mg N/100 g was used to represent the muscle value of sea bass.

2.6. Texture profile analysis (TPA)

After thawing the sea bass, take the back muscle and cut it into TA 2.0 cm × 1.5 cm × 1.5 cm. XT Plus Texture measuring Analyzer (Stable Micro Systems, Ltd, Godalming, Surrey, UK), equipped with P/5 probe, probe size 50 cm*50 cm, which is compressed from the longitudinal muscle of cut fish block, Other parameters include test speed of 1 mm /s, compression interval of 5 s, compression percentage of 50%, and relaxation time of 5 s (Li et al., 2021). Six groups of sea bass cut after different treatments were placed in the center of the probe and measured along the longitudinal muscle direction of the fish.

2.7. Color value

Color was determined by a modified method using a colorimeter (CR-400, Konica Minolta, Tokyo, Japan) according to Cartagena et al. (2021). L value indicates the lightness, a value indicates the red–green degree, b value indicates the yellow–blue degree. The total color change of sea bass samples is denoted by △E, the formula of is △E shown:

$$\Delta E = \sqrt{(L_0 - L_1)^2 + (a_0 - a_1)^2 + (b_0 - b_1)^2}$$

L_0 value is shown as the initial lightness of sea bass samples.
a_0 and b_0 values are shown as the initial chromaticity of sea bass samples.
L_1 value is shown as the lightness of sea bass samples after treatment.
a_0 and b_0 values are shown as the chromaticity of sea bass samples after treatment.

2.8. pH value

pH value was decided by a modified method on the basis of Lv et al. (2021). The sea bass samples were minced into 2 g and dispersed in 20 mL normal saline (0.9%, m/v) and centrifuged at 4℃ (1000 × g) for 5 min. pH values of sea bass samples were tested using a pH meter (Mettler Toledo, Greifensee, Switzerland).

2.9. Scanning electron microscopy (SEM)

The SEM method was decided by a modified method on the basis of Cheng et al. (2022). The samples of frozen sea bass were placed in the cold storage at −18℃, cut into 5 mm × 5 mm × 3 mm sides with Leica blades, and freezer-dried in a freezer-dryer (model FDU-2110, rated power supply AC220V, 50 Hz, 2.4KVA, maximum working power 11A). The longitudinal and transverse sections were observed under 5 kV scanning electron microscope (SU5000, HITACHI, Japan).

2.10. Extraction of myofibrillar protein

The myofibrillar solution was decided by a modified method on the basis of Li et al. (2021), and modified a little. Homogenize 2 g fish and 20 mL TRIS-Buffer A (0.05 M sodium chloride) under the condition of 1160 × g for 15 min at a temperature of 4 ℃. At the end of the procedure, pour out the supernatant and repeat the procedure. Finally, the sediment was collected and stirred with 20 mL TRIS-Buffer B (0.6 M sodium chloride) pre-cooled. It was used to measure the changes of protein structure of sea bass after different freezing treatments.

2.11. Measurements of carbonyl content and total sulfhydryl content

The contents of sulfhydryl (SH) and carbonyl were decided by a modified method in the kit, and the unit was μmol/g. SH test kit and carbonyl test kit are purchased from Solarbio. (Solarbio Technology Co., Ltd, Beijing, China). The purpose of this study was to detect the changes of sulfhydryl and carbonyl contents in proteins of sea bass samples after different freezing treatments.

2.12. Secondary structures of myofibrillar protein

The secondary structure of myofibrin was decided by Fourier infrared spectrometer (Nicolet IS50, Thermo Scientific Inc., Waltham, MA, USA). After freeze-drying, the sample was ground with potassium bromide powder. Using Gaussian curve fitting in Origin 2021 (Origin-Lab, Northampton, MA, USA) de-unwinding the spectrum in the 1600 and 1700 cm^{-1} range representing the amide I region. The peak area divided by the amide I area is used to detect different types of secondary structures.

2.13. Tertiary structure of myofibrillar protein

The tertiary structure of myofibrin was determined by fluorescence spectrophotometer (F-7100, Hitachi, Tokyo, Japan). The extracted protein solution was put into a fluorescence spectrophotometer for detection. The imaging parameters are as follows: the scanning speed is 1200 nm/min, the emission wavelength is 300 ~ 400 nm, the excitation wavelength is 295 nm and the slit width is 5 nm.

2.14. Statistical analysis

All experimental samples were independently repeated three times. SPSS 22.0 software was used to compare the groups, one-way analysis of variance (ANOVA) was used, and the laboratory results were recorded as mean ± standard deviation.

3. Results and discussion

3.1. Freezing rate

The freezing rate depends on how fast the temperature decreases over time. The faster the temperature decreases, the higher the freezing rate will be; otherwise, the slower the freezing rate will be. With the increase of freezing rate, small and uniform ice crystal formation can reduce tissue damage and drip loss, and improve frozen food quality (Nowak et al., 2019). The center temperatures of sea bass by different freezing methods are shown in Fig. 2 (A). The freezing rate according to the core temperature of the IF, SHUAF-40, SVUAF-20, DHUAF-20/40, DOUAF-20 (H) 20 (V), and DOUAF-40 (H) 20 (V) sea bass samples were 58, 51, 46, 39, 36 and 33 min, respectively. Sea bass samples treated with DOUAF-40 (H) 20 (V) ultrasound froze the fastest and formed small, smooth ice crystals inside and outside the cells (Qiu et al., 2020).

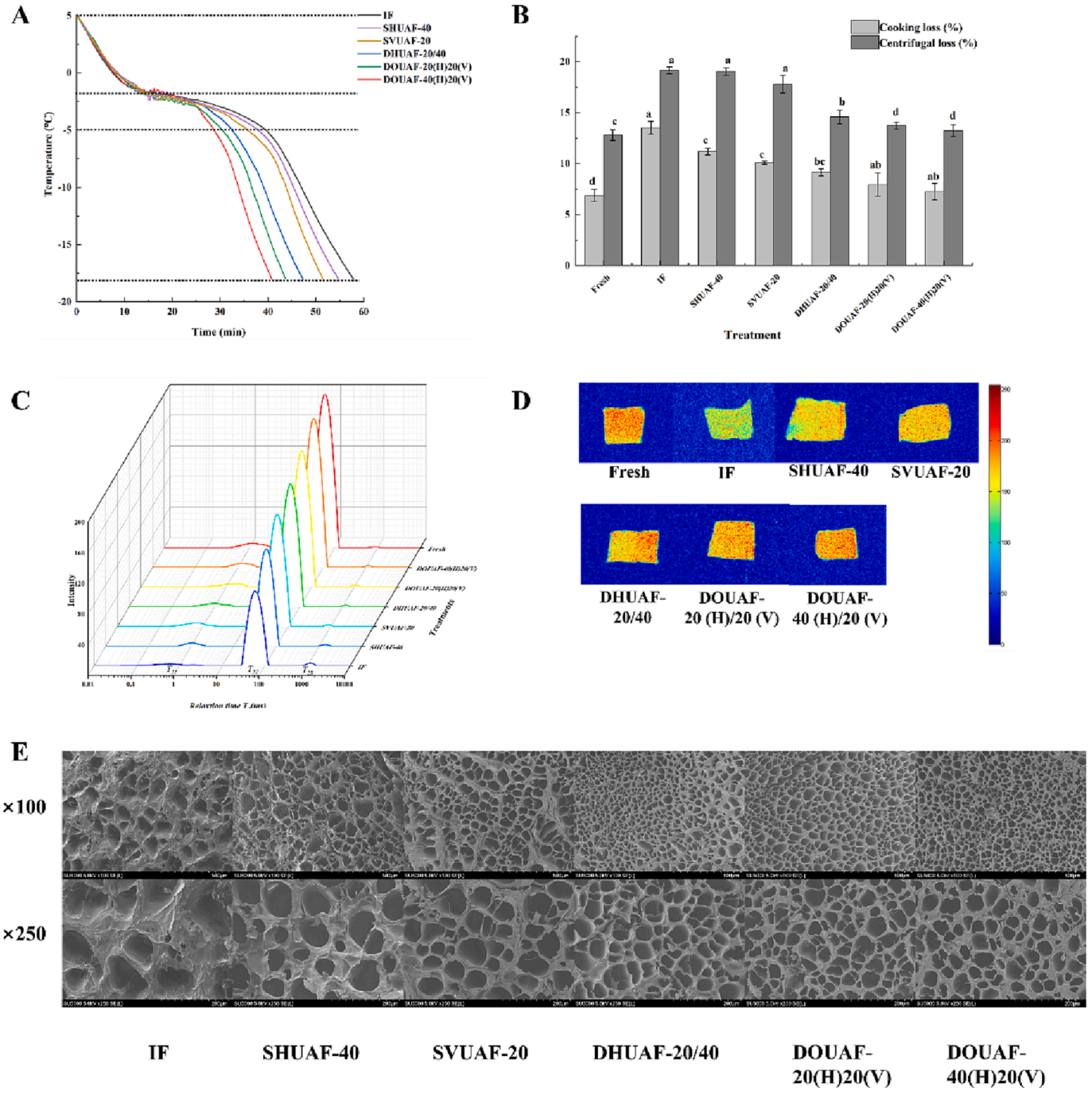

Fig. 2. Effect of different UAF treatments on the sea bass samples. A: freezing curves; B: thawing methods on the centrifugal loss and cooking loss; C: relative peak area percentages of different water states during T2 relaxation time, D: a magnetic resonance images; E: effect of different UAF treatments on scanning electron microscope view of transverse section microstructure of sea bass.

According to studies (Ma et al., 2021a), ultrasonic freezing technology can enhance the formation rate of ice crystal nuclei and improve the freezing rate through cavitation and mechanical action, so as to form small and uniform ice crystals in cells and reduce the damage to cells during freezing process. Dual/Orthogonal-frequency ultrasonic wave can effectively solve the single frequency ultrasonic inhomogeneity (Chen et al., 2020) and cavitation shielding in the propagation process.

In conclusion, in the freezing process of sea bass, the small and smooth ice crystals formed inside and outside the cells can reduce the damage, that is, under a certain intensity of ultrasonic conditions, the freezing speed is faster, and the impact on the quality of sea bass is smaller. The experiment shows that the freezing rate of DOUAF-40 (H) 20 (V) group is the fastest. The ultrasonic field intensity generated by orthogonal ultrasonic in this group is higher than that in IF group, and the single frequency (SHUAF-40, SVUAF-20) ultrasonic group, so the freezing rate of this group is faster. The difference between this group and the DOUAF-20 (H) 20 (V) group lies in the different ultrasonic frequencies in the horizontal direction. The reason is that the ultrasonic intensity (40 kHz) in the horizontal direction is greater than that of 20 kHz. Therefore, the ultrasonic intensity after orthogonal is stronger in the DOUAF-40 (H) 20 (V) group, that is, its freezing rate is faster.

3.2. Effect of different UAF treatments on thawing loss, cooking loss and WHC

Thawing loss, cooking loss and water holding capacity (WHC) of sea bass samples indicate intracellular water distribution and stability of residual water, which are important indicators of meat quality (Li et al.). The thawing loss of each group ranged from 0.59 to 0.92%, among which DOUAF-40 (H) 20 (V) ultrasonic treatment had the lowest water loss than other ultrasonic frequencies (Fig. 2 (B)). The thawing loss of each group ranged from 0.59 to 0.92%, among which, the IF group was the lowest, because the field intensity generated by DOUAF-40 (H) 20 (V) group was higher than that of the other groups, thus improving the cavitation effect of ultrasonic waves, resulting in more stable water retention rate and less water loss of sea bass. As the Fig. 2. shows, cooking loss and water holding capacity (WHC) of the sea bass samples. The decrease in water content of muscle cells is due to cell destruction, which is caused by the formation of ice crystals in muscle cells during freezing (Gao et al., 2019). Bian et al. (2022) showed that different combinations of ultrasonic frequencies caused different effects on WHC, because after the ice crystals formation, the original protein structure was destroyed and more active groups, such as sulfhydryl and carbonyl groups, were produced. The ice crystals produced by different ultrasonic combinations of single frequency, double frequency and orthogonal had different effects on the myosin structure of sea bass, so selecting the appropriate ultrasonic frequency combination was the key. Ma et al. (2021a) studied that multi-frequency ultrasound can effectively reduce water loss compared to other frequency ultrasound combinations, similar to this study. The reason why DOUAF-40 (H) 20 (V) group was closer to fresh samples and different from other groups was that the ultrasonic intensity generated by orthogonal ultrasound was greater than that of other groups, so the resulting ice crystals were smaller than those of the other groups, which reduced the cell damage caused by the influence of ice crystals, thus reducing the cooking loss and centrifugal loss.

3.3. Effect of different UAF treatments on water distribution and water state

The distribution of water and the state of water are often explained in terms of hydrogen proton relaxation time, thus affecting the WHC and frozen food quality. The stability of water molecules in the system library depends on the mobility of water molecules, that is, the mobility of hydrogen protons. In the last few years, MRI has been widespreadly used in food quality. According to it, the distribution of hydrogen proton density and water can be observed more accurately, efficiently and intuitively (Wang & Xie, 2019). Bian et al. (2022) thawed frozen large yellow croaker and visualized the water distribution in the fish using NMR technology. The hydrogen proton signal density in the sample is represented by red and blue, with red areas representing high water content and blue areas representing low water content. The results show that the reduction of relaxation time leads to the decrease of mobility of hydrogen protons. As shown in Fig. 2(C), the characteristic peaks, T_{21} (0.1–10 ms), T_{22} (10–100 ms), and T_{23} (over 100 ms) depend on the following two groups of factors: (1) sarcoplasmic protein, myofibrillar protein and other macromolecules; (2) water inside and outside the cell, namely bound water and free water (Qiu et al., 2022). The fixed peak water area of Fresh group, IF group, SHUAF-40 group, SVUAF-20 group, DOUAF-20 (H) 20 (V) group, DOUAF-40 (H) 20 (V) group and fresh sample group showed an increasing trend, while the peak water area of free water showed a decreasing trend ($P < 0.05$). There were no significant differences among different treatment groups ($P > 0.05$). The experimental results showed that different ultrasonic treatments could shorten the migration time of fixed water, and the DOUAF-40 (H) 20 (V) group had the best effect. Li et al. (2023) also found that UAF shortened the time of fixed water and free water loss in sea bass muscles during freezing, which is similar to this study. UAF affects the ice crystals growth and distribution in freezing time, thus making fish muscle cells more intact. The reason is that ultrasound makes the ice crystals smaller and smoother, which is less damaging to muscle tissue. The DOUAF-40 (H) 20 (V) group was different from the other groups because the ultrasonic intensity generated by orthogonal ultrasound was greater than that of the other groups, and the orthogonal ultrasound group caused smaller ice crystals to form inside and outside the cells than the other groups, which reduced the damage of the ice crystals to the cells and thus reduced the precipitation of free and fixed water in the cells.

As shown in Fig. 2 (D), the hydrogen proton density increased in all groups with the highest hydrogen proton density in DOUAF-40 (H) 20 (V) group. The results showed that orthogonal UAF could improve the water retention capacity of fish muscle more than single frequency UAF and horizontal UAF. The hydrogen content of DOUAF-40 (H) 20 (V) group was higher than that of other groups because the ultrasonic wave intensity generated by DOUAF-40 (H) 20 (V) group was stronger and the ice crystals generated were smaller, which reduced the cell destruction rate and the loss of hydrogen atoms. Under orthogonal ultrasonic conditions, the molecular rearrangement and hydrogen proton distribution in the sample will be regular, which enhances the hydrogen bond between water molecules, thus reducing the migration and loss of water (Tian et al., 2020a,b). Orthogonal UAF decreased moisture migration of another important reason is that the sample in the process of freezing, vertical and horizontal ultrasonic field has increased the intensity of ultrasonic, and the ice crystal formation and growth of small and uniform, reduce the content of free water, cause cells to solute concentration increased, including lipid, protein, carbohydrate, etc. (Tian et al., 2020a,b).

3.4. Effect of different UAF treatments on physicochemical quality

The effects of different UAF treatments on physicochemical sea bass quality were represented by the following tables, including color parameters, TVB-N and texture profile analysis (TPA) (Table 1).

The color and pH value of fish is the first criterion for consumers to choose, which is an important criterion for judging freshness (Kono et al., 2017). L* and b* values are related to fat oxidation, and a* values are related to myoglobin content (Sun et al., 2017). The overall color change of sample fish depends on the water content of muscle tissue, myoglobin oxidation and lipid oxidation (Shi et al., 2022). The L* (lightness) values of the DOUAF-40 (H) 20 (V) ultrasonic treatment is closest to the value of fresh group. The L* values of each group decreased in the order of Fresh, DOUAF-40 (H) 20 (V), DOUAF-20 (H) 20 (V), DHUAF-20/40, SVUAF-20, SHUAF-40, and IF. The study showed that

Table 1
Effect of different UAF treatments on color parameters, TVB-N of sea bass.

Treatment	Color			△E	pH	TVB-N
	L*	a*	b*			
Fresh	53.67 ± 1.36^{a}	−2.12 ± 0.02^{a}	0.48 ± 0.97^{d}	–	6.91 ± 0.06^{a}	9.44 ± 0.17^{a}
IF	44.37 ± 0.72^{d}	−2.83 ± 0.14^{d}	3.75 ± 2.91^{a}	11.95 ± 1.79^{a}	6.38 ± 0.03^{d}	11.59 ± 0.08^{f}
SHUAF-40	47.73 ± 0.46^{c}	−2.50 ± 0.08^{c}	2.68 ± 0.21^{b}	8.38 ± 1.09^{b}	6.59 ± 0.02^{c}	11.25 ± 0.18^{e}
SVUAF-20	49.50 ± 0.27^{c}	−2.42 ± 0.06^{bc}	2.57 ± 0.24^{b}	7.03 ± 0.58^{bc}	6.66 ± 0.03^{c}	10.17 ± 0.06^{d}
DHUAF-20/40	50.81 ± 1.14^{b}	−2.36 ± 0.02^{bc}	1.78 ± 0.29^{bc}	5.95 ± 1.25^{bc}	6.80 ± 0.02^{b}	10.83 ± 0.25^{c}
DOUAF-20 (H) 20 (V)	51.42 ± 0.84^{ab}	−2.31 ± 0.05^{abc}	1.29 ± 0.16 ^{cd}	4.65 ± 0.73^{c}	6.82 ± 0.02^{ab}	10.47 ± 0.15^{b}
DOUAF-40 (H) 20 (V)	52.03 ± 0.28^{ab}	−2.23 ± 0.04^{ab}	1.06 ± 0.16 ^{cd}	4.12 ± 0.69^{c}	6.87 ± 0.03^{ab}	10.07 ± 0.15^{b}

Values are expressed as mean ± standard deviation. "a-d" represents significant differences among groups ($p < 0.05$).

the L value of SOUAF-40 (H) 20 (V) group had the best effect because of the strong ultrasonic field generated by orthogonal ultrasonic wave, and the small ice crystals formed by DOUAF-40 (H) 20 (V) could reduce the damage to cells, thus effectively reducing lipid oxidation. Based on this theory, dual-frequency/orthogonal frequency ultrasonic reduces cavitation shielding, thus improving ultrasonic utilization rate, and orthogonal frequency ultrasonic can better increase cavitation effect (Tian et al., 2020a,b). Monteschio et al. (2017) showed that the destruction of protein structure would lead to the divergence of light, resulting in the change of L* value. There was no obvious difference in a* and b* values between the fresh sample and the SOUAF-40 (H) 20 (V) ultrasonic treatment ($p > 0.05$).

The pH of the treated sea bass samples began to change due to the attraction of polar groups in the frozen protein molecules (Mei et al., 2021). However, the difference under different ultrasonic treatments were not significant. Compared with the fresh group, the pH value of the different ultrasonic treatment group was lessened, and the DOUAF-40 (H) 20 (V) ultrasonic treatment group was closest to the fresh group. The reason for the decrease of pH value is the inorganic phosphorus and lactic acid accumulation, and the degradation of ATP in the freeze–thaw process (Tolstorebrov et al., 2014).

TVB-N usually indicates the degradation capacity of proteins and amines (Bekhit et al., 2021). Enzymatic deterioration during freezing causes the breakdown of proteins and other nitrogenous substances, resulting in an increase in the value of compounds that accumulate organic amines, such as TVB-N (Zhou & Xie, 2021). The TVB-N of the sea bass samples were shown in Table 1. TVB-N of fresh, IF, SHUAF-40, SVUAF-20, DHUAF-20/40, DOUAF-20 (H) 20 (V) and DOUAF-40 (H) 20 (V) samples were slightly changed compared with that of fresh perch samples, but the differences between samples were not significant.The texture profile analysis of sea bass were shown in Table 2.

Texture analysis (TPA), namely hardness, elasticity, resilience and chewability, is an important index to evaluate the freshness of fish (Ntzimani et al., 2021). The Table 1 shows that ultrasonic treatment with different frequency combinations had a significant effect on the hardness of sea bass samples. The DOUAF-40 (H) 20 (V) ultrasound treatment values were closest to those of fresh samples, hinging on the cavitation influence of ultrasound during freezing, reducing ice crystal damage to muscle cells, resulting in protein molecule aggregation and water loss, which reduced hardness, elastic resilience and chewability values (Wu et al., 2021). The reason for the difference among different groups was that the ultrasonic intensity generated by the DOUAF-40 (H) 20 (V) group was greater than that of the other groups, and the small and smooth ice crystals produced were less destructive to the cells, thus

Table 2
Effect of different UAF treatments on texture profile analysis of sea bass.

Treatment	Hardness	Springiness	Resilience	Chewiness
Fresh	4165.98 ± 32.11^{a}	0.52 ± 0.003^{a}	0.152 ± 0.002^{a}	407.52 ± 2.29^{a}
IF	3493.51 ± 46.85^{e}	0.42 ± 0.004^{e}	0.110 ± 0.006^{a}	348.97 ± 2.90^{f}
SHUAF-40	3626.69 ± 38.65^{d}	0.44 ± 0.007^{de}	0.108 ± 0.005^{c}	360.93 ± 4.49^{e}
SVUAF-20	3711.59 ± 28.93 ^{cd}	0.46 ± 0.006^{d}	0.116 ± 0.009^{c}	370.55 ± 2.07^{d}
DHUAF-20/40	3820.18 ± 38.89^{c}	0.48 ± 0.008^{c}	0.132 ± 0.004^{b}	380.24 ± 2.12^{c}
DOUAF-20 (H) 20 (V)	3980.03 ± 32.04^{b}	0.50 ± 0.003^{bc}	0.138 ± 0.003^{c}	388.30 ± 2.77^{bc}
DOUAF-40 (H) 20 (V)	4047.36 ± 49.74^{b}	0.50 ± 0.007^{a}	0.148 ± 0.002^{b}	393.81 ± 3.89^{b}

Values are expressed as mean ± standard deviation. "a-f" represents significant differences among groups ($p < 0.05$).

reducing the loss of proteins, and thus reducing the hardness, springiness resilience and chewiness value.

3.5. Effect of different UAF treatments on the muscle tissue and ice crystal morphology

The irregularly shaped ice crystals that form in the muscles of sea bass destroy the muscle tissue during freezing.

As shown in Fig. 2 (E)., SEM images of the back muscles of sea bass underwent different ultrasonic treatments (the size of the hollow cavity in this image reflects the formation of ice crystals), and the area, length and width of ice crystals produced under different ultrasonic treatments are shown in the Table 3. In all different ultrasonic treatment groups, the DOUAF-40 (H) 20 (V) group could better preserve all the tissue structure of fish, because the ultrasonic field intensity generated by orthogonal ultrasound was greater than that of single frequency and horizontal ultrasound, thus reducing the ice crystals size and effectively reducing the ice crystals harm to the tissue structure of fish (Tian et al., 2020a,b). The research showed that the ice crystals formed in the cells of sea bass were obviously smaller and uniform during the freezing process of different ultrasonic methods, which reduced the damage to the cells.

3.6. Influence of different UAF treatments on the muscle protein and lipid oxidation

3.6.1. Sulfhydryl and carbonyl content (The primary structure of proteins)

Because sea bass belongs to the high protein, low fat sea fish. Therefore, the deterioration degree of protein quality is of great significance for the study of maintaining the quality of fish. The level of protein denaturation and oxidation of fish muscle was determined by the content of sulfhydryl and carbonyl As shown in Fig. 3 (A), by SHUAF-40, SVUAF-20, DHUAF-20/40, DOUAF-20 (H) 20 (V) and DOUAF-40 (H) 20 (V) treatment, the total mercapto content of myogen in each group were reduced by 71.7 %, 52.1 %, 35.6 %, 28.1 %, 11.7 % and 9.1 %, respectively, compared with that in the fresh group. Contrasted with the

Table 3
Effect of different UAF treatments on size of ice crystal of sea bass.

Treatment	Area (μm^2)	Length (μm)	Width (μm)
IF	9714.93 ± 64.04^{a}	143.72 ± 6.36^{a}	99.65 ± 4.04^{a}
SHUAF-40	5324.92 ± 74.74^{b}	106.22 ± 6.66^{b}	66.47 ± 0.67^{b}
SVUAF-20	3040.98 ± 80.07^{c}	72.82 ± 3.23^{c}	53.45 ± 1.30^{c}
DHUAF-20/40	1742.35 ± 80.23^{d}	59.30 ± 0.99^{d}	39.47 ± 0.82^{d}
DOUAF-20 (H) 20 (V)	1656.32 ± 44.48^{d}	58.89 ± 0.39^{d}	37.96 ± 0.07^{d}
DOUAF-40 (H) 20 (V)	1631.62 ± 45.24^{d}	56.41 ± 0.86^{d}	37.49 ± 0.17^{d}

Values are expressed as mean ± standard deviation. "a-d" represents significant differences among groups ($p < 0.05$).

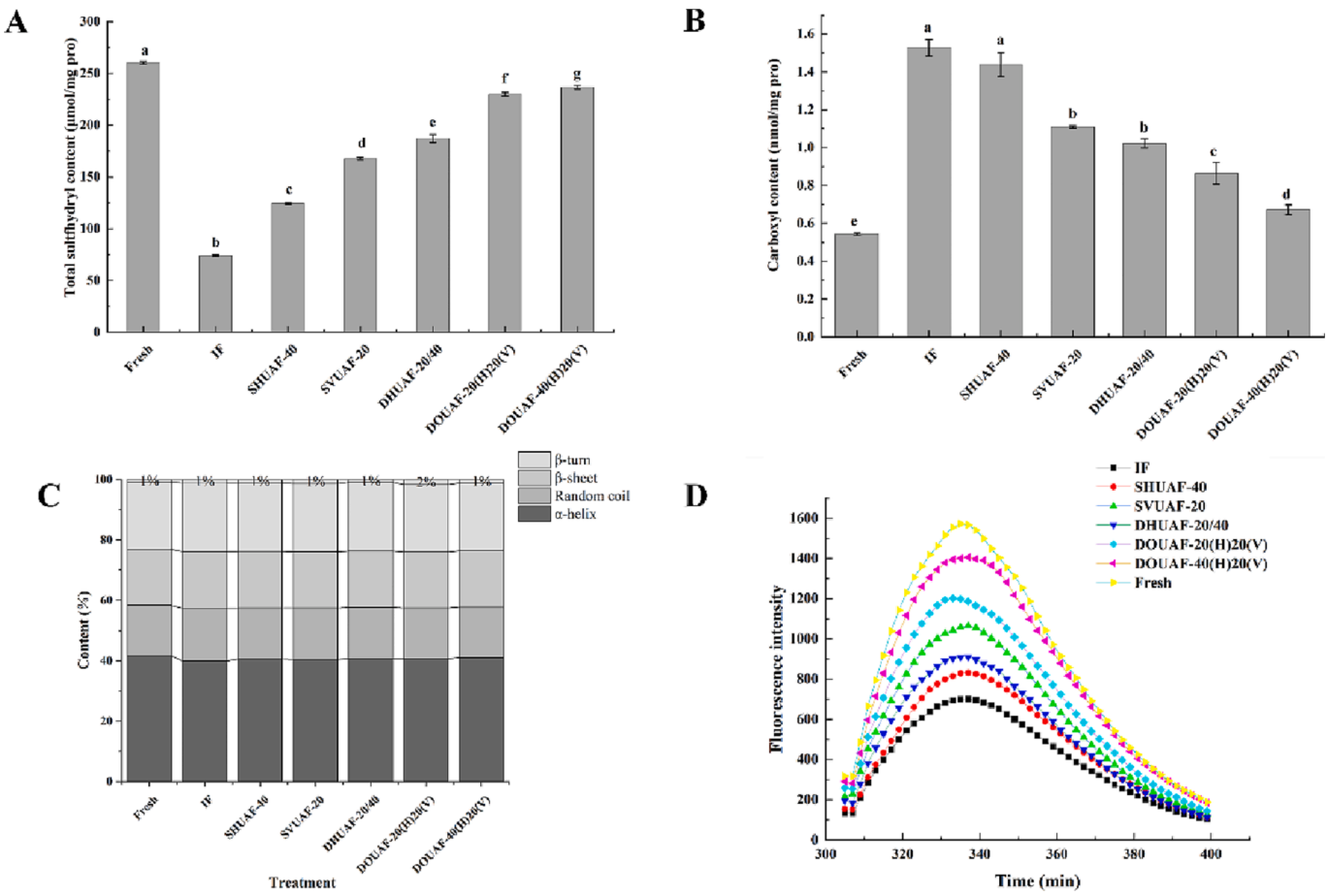

Fig. 3. Effect of different UAF treatments on sea bass. A: total sulfhydryl content; B: carbonyl content; C: secondary structure; D: tertiary structure.

fresh group, the total myogenic carbonyl content in IF group, SHUAF-40 group, SVUAF-20 group, DHUAF-20/40 group, DOUAF-20 (H) 20 (V) group and DOUAF-40 (H) 20 (V) group was increased by 64.5%, 62.3 %, 51.1 %, 49.2 %, 37.2 % and 19.2 %, respectively. The DOUAF-40 (H) 20 (V) group was the closest to the fresh sample group. It was found that UAF was an effective method to shorten protein oxidation time, and the stability of protein can be improved by effectively enhancing ultrasonic intensity in a certain range (Ma et al., 2021a). The ultrasonic intensity of orthogonal ultrasound is higher than that of horizontal ultrasound, which can better maintain the integrity of muscle cells because of it can form smaller ice crystals that damage cells less. (Yu et al., 2022). Excessive ultrasonic power will lead to the destruction of amino acid sequence of protein, resulting in local instantaneous high temperature and pressure, which will produce H+, OH–, H2O2 with strong oxidation capacity, so that the protein oxidation rate increases (Zhang et al., 2019). In this study, different UAF treatments with lower power (175 W) were used to prevent excessive oxidation of myogens in UAF, in line with previous studies (Ma et al., 2021a).

3.6.2. *Secondary structure of proteins*

The secondary structure of MPs in different ultrasonic treatment groups is basically formed in α-helix mode. Contrasted with Fresh samples, the α-helix contents of MPs treated with IF group, SHUAF-40 group, SVUAF-20 group, DHUAF-20/40 group, DOUAF-20 (H) 20 (V) group and DOUAF-40 (H) 20 (V) group decreased by 4.83 %, 2.65 %, 2.43 %, 2.23 %, 1.67 % and 2.91 %, while the contents of β-sheets increased by 1.41 %, 1.13 %, 0.71 %, 0.55 %, 0.48 % and 0.26 % (Fig. 3 (B)). Among them, the reason for the change of secondary structure is that the protein molecular structure is changed under the action of UAF in space, losing its structure and making it soluble. The intensity of orthogonal ultrasound is higher than that of single and double horizontal frequency ultrasound, which makes the protein more easily denatured. (Huang et al., 2019).

3.6.3. *Tertiary structure of protein*

Trends in the tertiary structure of proteins are usually represented by fluorescence spectra (Fig. 3 (D)). The change of protein fluorescence spectrum usually reflects the content of tryptophan, phenylalanine and tyrosine in the protein, mainly through the change of tryptophan content reaction. (Wang et al., 2021). Based on different ultrasonic freezing treatments, protein fluorescence intensity of sea bass samples was decreased by DOUAF-40 (H) 20 (V) group, DOUAF-20 (H) 20 (V) group, DHUAF-20/40 group, SUOAF-20 group, SHUAF-40 group and IF group. The reason was that the tryptophan in the protein was oxidized after freezing, which resulted in protein degradation (Chen et al., 2020). The best effect of the DOUAF-40 (H) 20 (V) group was because the ultrasonic field intensity generated by the DOUAF-40 (H) 20 (V) group was greater than that of the other groups, and the small ice crystals produced would reduce the damage of proteins, thus reducing the loss of tryptophan content.

4. Conclusion

Based on the research shows that, contrasted with the single frequency and horizontal multi-frequency ultrasonic assisted technology, orthogonal ultrasonic can increase the ultrasonic field intensity in the horizontal and vertical directions, so as to reduce the large ice crystals formation and reduce the loss of protein. Compared with IF, orthogonal ultrasound-assisted freezing can reduce thawing loss, centrifugal loss and water migration, and increase WHC of frozen sea bass. Meanwhile,

the color and texture of sea bass after orthogonal ultrasonic freezing were better than those of IF treatment group. SEM images showed the microstructure of sea bass, among which the muscle tissue of DOUAF-40 (H) 20 (V) group was more uniform and compact than that of other groups, and the size of ice crystals produced by DOUAF-40 (H) 20 (V) group was smaller than that of other groups. In summary, this study shows that orthogonal ultrasound-assisted freezing can effectively shorten the damage of sea bass muscle tissue caused by freezing process, thus improving the frozen fish quality. These findings indicate a way for industry to effectively improve the frozen aquatic products quality and contribute to the provision of high frozen aquatic products quality in the era of epidemic.

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CRediT authorship contribution statement

Huan Yu: Conceptualization, Methodology, Investigation, Writing – review & editing, Formal analysis, Writing – original draft. **Jing Xie:** Methodology, Validation, Writing – review & editing, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Further reading

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