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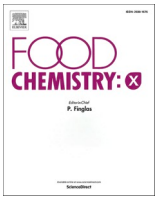

Effects of different power multi-frequency ultrasound-assisted thawing on the quality characteristics and protein stability of large yellow croaker (*Larimichthys crocea*)

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ABSTRACT

This study investigated the impact of multi-frequency ultrasound-assisted (20/28/40 kHz) thawing (MUAT) at different power levels (195, 220, 245, and 270 W, respectively) on the flesh quality and protein stability of large yellow croakers. Compared with flowing water thawing (FWT) and the other MUAT sample, flesh quality results indicated that the MUAT-220 W significantly reduced ($p < 0.05$) thawing loss, total volatile base nitrogen (TVB-N), total free amino acids (FAAs) and thiobarbituric acid reactive substances (TBARS). Low-field nuclear magnetic resonance (LF-NMR) spectroscopy indicated that MUAT-220 W samples had higher immobilized water content and lower free water content. In addition, the MUAT-220 W sample contained higher sulfhydryl and lower carbonyl contents compared to the FWT sample. Secondary and tertiary structural results of myofibrillar proteins (MPs) showed that MUAT-220 W significantly reduced thawing damage to MPs. Therefore, MUAT-220 W improved the quality and protein stability of the large yellow croaker during the defrosting process.

1. Introduction

Large yellow croaker is a nutritious fish with a high protein content (Wu et al., 2024). Fresh large yellow croakers become perishable, and the alterations occurring in the flesh of the fish significantly impact both its quality and consumer preference (Xiang et al., 2024). Therefore, the fish can be frozen to extend the shelf life and avoid seasonal shortages. Thawing is an essential step before secondary processing or consumption of frozen goods, and it significantly affects the frozen goods' ultimate quality. Inappropriate thawing methods can lead to damage quality, increase in juice loss, protein denaturation, and lipid oxidation (Leygonie, Britz, & Hoffman, 2012). Thus, it is especially crucial to discover a quick, eco-friendly way to thaw fish that also enhances its quality.

Ultrasound-assisted thawing (UAT) is a green and rapid thawing technology. According to a study by Sun et al. (Sun, Kong, Liu, Zheng, & Zhang, 2021), UAT speeds up the process of thawing food and preserves its quality better than traditional thawing methods. The UAT makes the internal and external of the food absorb the attenuated heat at the same time, avoiding the phenomenon of uneven thawing caused by the local high temperature inside the food. On the other hand, the microjet formed by ultrasonic Kissam et al. (Kissam, Nelson, Ngao, & Hunter, 1982) and Li et al. (Li et al., 2020) indicated that thawing speed of UAT was more efficient than that of air thawing and water thawing, and could ensure the quality after thawing. Fig. 1 illustrates the process of ultrasonic thawing. The multi-frequency ultrasonic equipment can generate a higher level of mechanical interference and cavitation yield than single-frequency ultrasonic equipment (Hu et al., 2015). In addition, the use of dual-frequency ultrasound enables a broader range of energy dissipation (Ma, Huang, Peng, Wang, & Yang, 2015).

As far as we know, there are few studies on the application of multi-frequency ultrasound in food materials. Many studies using ultrasound to freeze aquatic products are still in their infancy, and most studies still use mono-frequency ultrasound for thawing. The effects of mono-, dual- and tri-frequency ultrasound-assisted thawing (UAT) on the physico-chemical quality, water-holding capacity, moisture migration and

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distribution and myofibrillary structure of frozen large yellow croaker were detected on previous laboratory studies (Bian, Cheng, Yu, Mei, & Xie, 2022). The results showed that multi-frequency UAT treatment significantly improved the thawing speed, maintained the stability of myogenic fibers and reduced lipid oxidation. The multi-frequency UAT samples had better water retention capacity and physicochemical quality. Sun et al. (Sun et al., 2021) investigated the effects of ultrasonic-assisted thawing at different power levels on carp (*Cyprinus carpio*) and showed that ultrasonic-assisted thawing at 300 W accelerated the thawing process and improved the quality of the thawed fish. Higher power ultrasound can cause damage to frozen food tissues and protein structure (Wang et al., 2021). With this background in mind, this study aims to disseminate solutions based on multi-frequency ultrasonic thawing technology, an innovative emerging non-hot food engineering technology, to build sustainable and resilient food systems, improve the nutritional status of food, and enhance food and nutrition security. Based on the previous studies, we explored the effects of MUAT at different power levels (195, 220, 245 and 270 W) on the meat quality and protein stability of large yellow croaker.

2. Materials and methods

2.1. Materials preparation

The specimens (475 ± 25 g) were obtained from a port town (Pudong New Area, Shanghai). The newly obtained samples were disemboweled and washed with deionized water. The samples that had been washed and dried were placed into polyethylene bags to flash freezing. Fish samples undergo different treatments.

2.2. Thawing process

The large yellow croakers were thawed in a random manner using five different methods: water thawing (FWT), and MUAT at 195 W (MUAT-195), 220 W (MUAT-220), 245 W (MUAT-245), and 270 W (MUAT-270), respectively. The MUAF instrument was designed by ourselves (Fig. 2). The samples were placed in the ultrasound device (20/28/40 kHz) at a temperature of 20 ± 1 °C. The temperature in the center of the samples was continuously monitored and recorded in real-time using a T-type thermocouple during the thawing process. The thawing process is completed when the temperature in the center of the sample has risen to 4 ± 1 °C.

2.3. Thawing loss determination

The concept of thawing loss was derived from the description provided by Xu et al. (Xu, Zhao, Yang, Mei, & Xie, 2024). The formula was as follows:

$$\textit{Thawing loss}\ (\%) = \frac{(W_1 - W_2)}{W_1} \times 100\%$$

W_1(g): the samples' weight before freezing; W_2(g): the samples' weight after thawing.

2.4. Water distribution and migration

A sample block measuring 2.0 × 1.5 × 1.5 cm was removed from the dorsal muscle portion of the large yellow croakers. It was wrapped in polythene cling film to prevent loss of surface moisture. The samples were placed in an NMR tube (70 mm in diameter). Transverse relaxation (T2) was measured on an LF-NMR analyzer at a proton resonance frequency of 20 MHz. The key parameters were set as follows: SW = 100 kHz, RFD = 0.08, NS = 4, P1 = 19 μs, P2 = 37 μs, RG1 = 20 db, DRG1 = 6 db, PRG = 1, and TW = 2000 ms. MRI studies were performed to generate pseudo-color pictures of the materials, which were weighted based on proton density. Image acquisition parameters were as follows: slice width = 1.4 mm, repetition time = 500, echo time = 20 ms. Three images were acquired for each set of samples.

2.5. Total volatile basic nitrogen (TVB-N)

Representative 5 g samples of the large yellow croaker dorsal muscle from each of the six experimental groups were taken for determination of TVB-N values using a Kjeltec analyzer (FOSS 8400, Hilleroed, Denmark). The results were expressed as mg N/100 g.

2.6. Thiobarbituric acid reactive substances (TBARS)

The TBARS assay was conducted using the methodology outlined by Kubra et al. (Kubra, Telat, & Gonca, 2024) with minor modifications. A representative 5 g sample of the large yellow croaker dorsal muscle from six different groups was mixed and homogenized with 20 mL of 20% (mass concentration) trichloroacetic acid (TCA). The mixed samples were allowed to stand at room temperature for 60 min before being centrifuged at 8000 ×*g* for 15 min at 4 °C. A total of 5 mL of the supernatant and TBA solution (2 mmol/L) were added to a test tube and

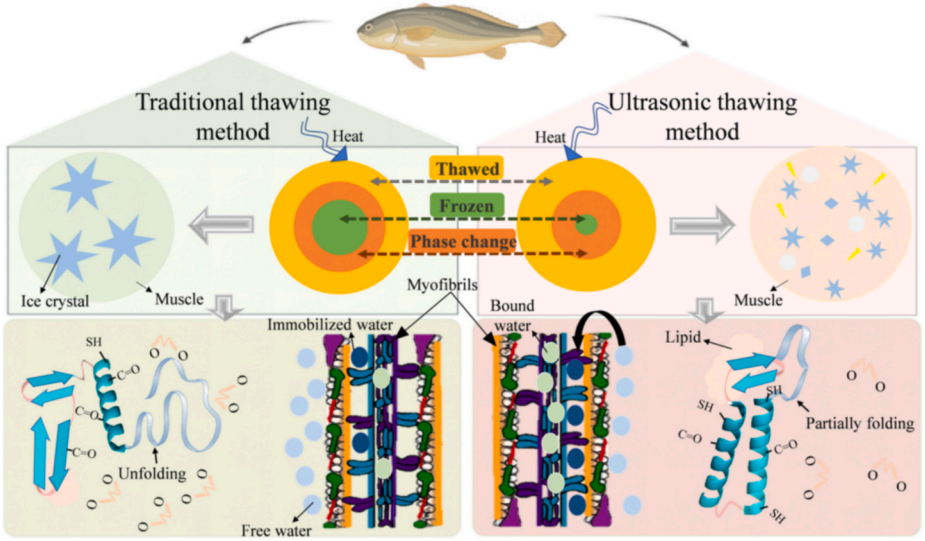

Fig. 1. The mechanism of ultrasonic thawing.

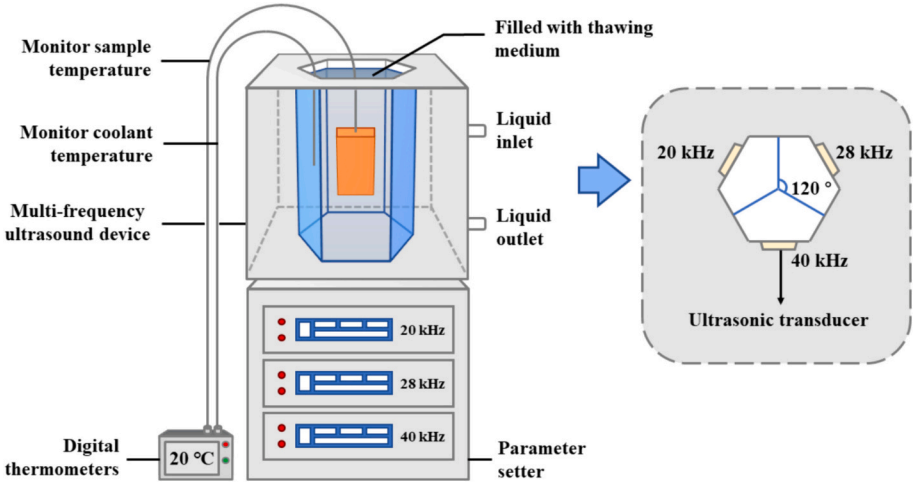

Fig. 2. Schematic diagram of multi-frequency ultrasound-assisted thawing (UAT) system.

heated at 100 °C for 40 min, until cooled at room temperature to be tested. MDA forms a pink compound when it reacts with thiobarbituric acid, and the absorbance of this compound peaks at 538 nm. By utilizing this unique property, the MDA content was quantified in the samples.

2.7. Free amino acids (FAAs)

The quantification of FAAs was conducted using the methodology outlined by Dong et al. (Dong et al., 2023) with modifications. A representative 2 g sample of the large yellow croaker dorsal muscle from six different groups was added to 10 mL of 5% TCA to mix the homogenate. The supernatant was centrifuged at 10,000 ×*g* for 10 min. Repeat the above steps. All supernatants were mixed and diluted to 25 mL. FAAs were filtered through a 0.22 μm filter to remove impurities. Afterward, the filtered FAAs were examined using an ultra-high-speed automatic amino acid analyzer to determine their composition and concentration.

2.8. Extraction of MPs

MPs were extracted according to the methodology described by Huang et al. (Huang et al., 2022). 2 g flesh and 20 mL Tris-buffer A (0.05 M) were mixed and centrifuged 15 min at 4 °C (11, 960 ×*g*). The sediment continues to follow the above steps. After the second separation, the sediment was combined with 20 mL of Tris-buffer B (0.6 M) and put it for 3 h at a temperature of 4 °C. The combination was thereafter subjected to centrifugation at a speed of 11,960 ×g for 15 min. The supernatant was MPs.

2.9. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The MP composition analysis was conducted using the methodology described by Tan et al. (Tan, Ye, Chu, & Xie, 2021). SDS-PAGE was conducted sequentially on a pre-cast 12% Hepes-tris gel. Following electrophoresis, protein molecular weight was determined to use protein standards purchased from EZ Biolab. Electrophoresis was performed at room temperature, 150 V, for 40–50 min. After electrophoresis was completed, the protein bands were stained with Coumarin Brilliant Blue Rapid Staining Solution, and then decoloured with Coumarin Brilliant Blue Rapid Decolouring Solution. Finally, the degradation of myofibrillar proteins was analysed by using gel image-specific analysis soft.

2.10. Carbonyl content determination

The carbonyl content was determined by 2,4-dinitrophenylhydrazine derivatization. The results were given as μmol/mg protein.

2.11. Total sulfhydryl content determination

The total sulfhydryl content was measured based on the method described by Yang et al. (Yang, Fang, Xie, Mei, & Xie, 2024). The total sulfhydryl contents in μmol/g of protein were determined using the extinction coefficient of 2-nitro-5-thiobenzoate (NTB).

2.12. Secondary structures of the MP

Fourier transform infrared (FTIR) spectroscopy is a method employed to analyze the secondary structure of MPs. Potassium bromide powder and dried myofibril samples were ground and mixed well. The mixture was pressed into thin sheets. The following are the test parameters: the scan wavelength range is 500 to 4000 cm^{-1}, and the resolution is 4 cm^{-1}.

2.13. The tertiary structure of the MP

The intrinsic fluorescence of MPs was measured using a fluorescence spectrophotometer (F-7100) in the wavelength range of 300–400 nm. The excitation wavelength was 295 nm. The width of the slit was 5 nm. The scanning speed was 1200 nm/min.

2.14. Statistical analysis

The findings were presented using the mean ± standard deviation after performing all analyses in triplicate. To conduct multiple comparisons, we utilized one-way analysis of variance (ANOVA) with the assistance of SPSS 26.0 software. Additionally, the generation of charts was conducted using Origin software.

3. Results and discussion

3.1. Analysis of thawing loss

The variations in thawing loss indicate the water-holding capacity of fish (Yao, Jin, Zhang, Yang, & Xu, 2023). In the thawed fish, the thawing loss was caused by the large and irregular holes left by the ice crystals between MPs. In addition to ice crystal damage, protein denaturation also contributed significantly to the increase in thawing loss (Tan et al., 2021). Samples thawed with FWT had a thawing loss of 2.86%, however, the thawing losses of the MUAT-195, MUAT-220, MUAT-245 and

MUAT-270 groups were 2.63%, 2.49%, 2.52% and 2.49%, respectively (Table 1). All the flesh structure of the fish after being frozen had been damaged. The highest thawing loss of FWT samples was observed during the thawing process. It has been demonstrated that MUAT treatments improve the structural properties of myosin and reduce the dense aggregation of muscle proteins, which is expected to enhance water retention (Sun, Sun, Xia, Xu, & Kong, 2019). The destruction of flesh fibers reduces the water retention by capillary force decreased during the thawing process, making it unlikely to be reabsorbed into the cell interior (Guo et al., 2021). Wang et al. (Wang, Yan, Ding, & Ma, 2022) also reported that MUAT had less damage to the samples compared with FWT in the experiment of detecting the quality after thawing. Among all the treatments, it was observed that MUAT-220 displayed the lowest level of thawing loss with increasing ultrasound power. This phenomenon can be explained by the inhibition of microscale gas nuclei formation in fish tissues as a result of mechanical harm produced by ultrasonic waves over a specific threshold. According to Guo et al. (Guo et al., 2021), the use of suitable ultrasonic power resulted in a decrease in thawing loss in white yak meat that had been thawed.

3.2. Analysis of water distribution and migration

LF-NMR is a useful method for assessing fish freshness (Guo et al., 2024). Three distinct kinds of water can be distinguished from one another based on the LF-NMR results (Huang et al., 2023). As shown in Table 1, the LF-NMR curve of all thawing methods exhibited the presence of these three water groups. The peaks of samples shifted with different thawing ways and MUAT power, suggesting that the thawing methods have an impact on the water distribution in fish flesh. It was found that there was no significant difference in pT_{21} across all of the samples ($p > 0.05$), which suggests that the tight binding of the bound water to the proteins in the flesh is unaffected by any mechanical stress or changes in the microstructure (Cai, Zhang, Cao, Cao, & Li, 2019). The content of pT_{23} in the fresh sample was not significantly different from MUAT-220, MUAT-245, and MUAT-270. The thawed samples in the FWT had significantly lower levels of pT_{22} than other samples, and the level of pT_{23} was the highest after thawing. Chu et al. (Chu, Tan, Bian, & Xie, 2022) also found that the UAT samples led to better water retention than those not subjected to ultrasonic treatment. According to previous studies (Sun et al., 2021), it is probable that the integrity of the muscle fibers in the FWT samples was damaged, which led to an increase in the fluidity of the water that was immobilized in the intercellular space. It was found that the pT_{22} of the MUAT-220 exhibited the highest value. This could be attributed to the relaxation of the gap between flesh fibers by the appropriate power treatment. Meanwhile, the MUAT-220 also improved the sarcomere shortening and reduced water loss caused during thawing. Compared with MUAT-220, the lower pT22 of MUAT-245 and MUAT-270 can be attributed to the destructive effects of high-power ultrasonic. This ultrasound can induce mechanical vibration, causing damage to both the perimysium and endomysium, ultimately disrupting the structure of muscle fibers (Wu et al., 2022).

Table 1
The thawing loss, water distribution, TVB-N, and TBARS results of frozen large yellow croaker under different thawing methods.

Treatment	Thawing loss (%)	pT_{21}/%	pT_{22}/%	pT_{23}/%	TVB-N (mg N/100 g)	TBARS (10^{-2} mg/MDA kg)
FS	–	2.28 ± 0.32^{a}	96.57 ± 0.13^{a}	1.14 ± 0.21^{c}	9.52 ± 0.07^{d}	11.71 ± 0.04^{e}
FWT	2.86 ± 0.05^{a}	2.40 ± 0.88^{a}	93.44 ± 0.76^{d}	4.16 ± 0.38^{a}	10.26 ± 0.07^{a}	13.59 ± 0.21^{a}
195 W	2.63 ± 0.04^{b}	2.71 ± 1.07^{a}	94.67 ± 0.39^{c}	2.62 ± 1.20^{b}	9.95 ± 0.07^{b}	12.77 ± 0.22^{bc}
220 W	2.49 ± 0.01^{b}	2.47 ± 0.32^{a}	96.00 ± 0.46^{ab}	1.54 ± 0.18^{bc}	9.76 ± 0.02^{c}	12.26 ± 0.15^{d}
245 W	2.52 ± 0.04^{b}	3.24 ± 0.31^{a}	95.35 ± 0.05^{bc}	1.40 ± 0.33^{bc}	9.78 ± 0.06^{c}	12.57 ± 0.17^{c}
270 W	2.49 ± 0.11^{b}	2.82 ± 0.83^{a}	94.87 ± 0.27^{c}	2.31 ± 0.86^{bc}	9.93 ± 0.04^{b}	12.97 ± 0.13^{b}

The methods include fresh sample (FS), flowing water thawing (FWT), multi-frequency UAT at 195 W, 220 W, 245 W, and 270 W. The letter from "a" to "e" are used to describe the significance of differences between the samples ($p < 0.05$).

In Fig. 3 (a), the colors red and blue colors represent high and low proton concentrations, respectively. The proton densities of the MRI images varied among the different thawing samples, with MUAT-220 appearing as the highest and FWT the lowest proton densities. During the thawing process, this change in densities can be attributed to the destruction of cell membranes that occurs. The MPs were difficult to absorb extracellular water, leading to a partial loss of fixed water as free water because of this damage.

3.3. Analysis of TVB-N

TVB-N values are dependent on the level of microbial and enzymatic activities causing the spoilage. Therefore, TVB-N is widely acknowledged as a reliable predictor of the freshness and safety of food (Chen, Mei, & Xie, 2024). The TVB-N value of the fresh fish group (FS) was 9.52 mg N/100 g and increased by 7.78, 4.52, 2.52, 2.73, and 4.31% for the FWT, MUAT-195, MUAT-220, MUAT-245, and MUAT-270, respectively (Table 1). Many basic volatile compounds are formed during the decomposition of proteinaceous and nonproteinaceous nitrogenous compounds. It is possible that the longer thawing time and longer enzyme activity on proteins caused by FWT treatment resulted resulted in greater protein breakdown and higher TVB-N values (Chu et al., 2022). This might be the reason why the TVB-N values of FWT-treated samples were substantially higher than those of MUAT.

Significant differences in the TVB-N value were observed in MUAT samples at different thawing powers, as presented in Table 1. The findings indicated that TNB-N values were lower in UAT-220 and UAT-245. These results indicate that the appropriate ultrasonic power has a passivating effect on enzymes and a killing effect on microorganisms, thus slowing down the decomposition of nitrogenous substances.

3.4. Analysis of TBARS

The large yellow croaker is rich in lipids, which is prone to oxidative rancidity during storage, reducing its freshness and quality (Abdel-Naeem, Sallam, & Malak, 2021). Notably, the thawed samples contained significantly more TBARS than the FS samples ($p < 0.05$). This result may be due to the formation of ice crystals during freezing, leading to cellular damage and the release of oxidation precursors, thereby promoting lipid oxidation (Li et al., 2020). The MUAT-treated samples significantly reduced TBARS content compared to FWT samples ($p < 0.05$). Wu et al. (Wu et al., 2022) also reported that MUAT had less damage to the ground pork compared with FWT in the experiment of detecting the quality of the prepared ground pork after thawing. The TBARS content of MUAT-220 was significantly lower than that of other MUAT treatments ($p < 0.05$). The rapid thawing speed of the MUAT treatments could shorten the time for fat oxidation. Furthermore, the impact of UAT on flesh fiber tissue was minimal, thereby restricting the release of oxidation factors and inhibiting lipid oxidation (Guo et al., 2021).

The TBARS content of MUAT-270 after thawing is higher than that of other samples, which was due to the increase in ultrasonic power and the high temperature generated by the cavitation effect, resulting in more obvious lipid oxidation. Jayasooriya et al. (Jayasooriya, Torley,

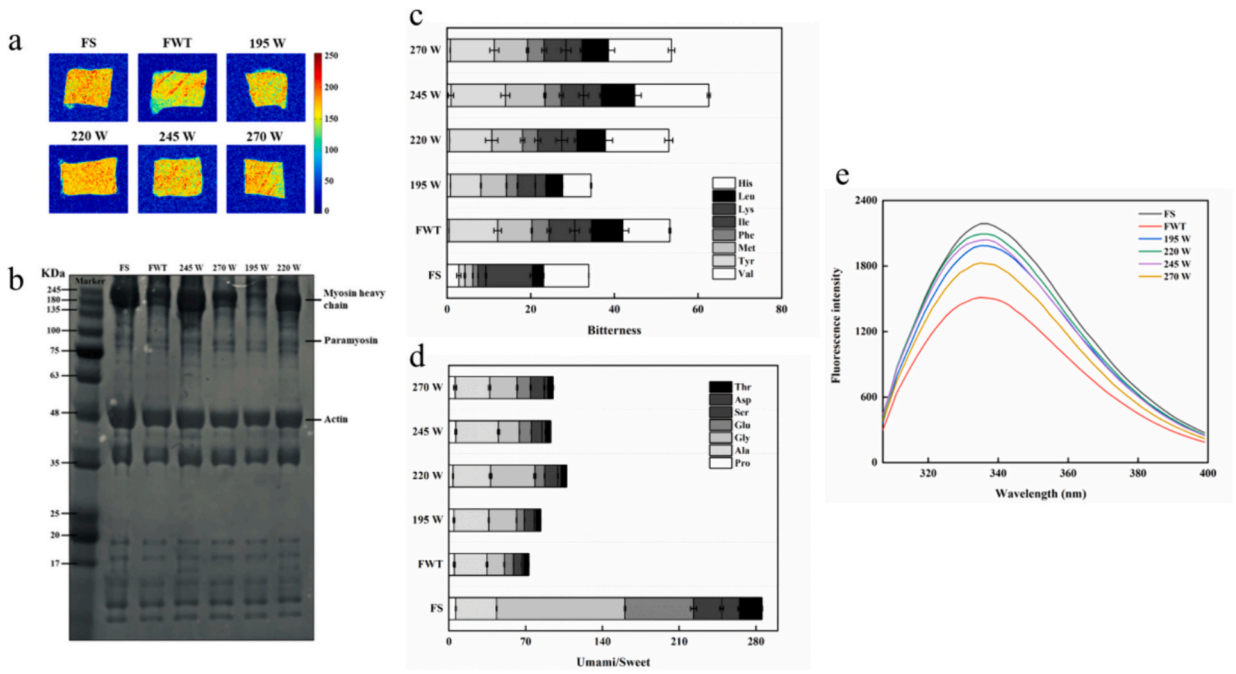

Fig. 3. MRI (a), MP SDS-PAGE (b), amino acids related to bitterness (c), amino acids related to umami and sweet (d), protein tertiary structure (e) of frozen large yellow croaker with different thawing treatments which include fresh fish group (FS), flowing water thawing (FWT), multi-frequency UAT at 195 W, 220 W, 245 W, and 270 W. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

D'Arcy, & Bhandari, 2007) also pointed out that the appropriate ultrasonic power could avoid surface overheating during thawing.

3.5. Analysis of FAAs

FAAs are fresh flavor components and nutrient elements in foods that are involved in protein synthesis and play a crucial role in the flavor and nutrition of foods (Guo et al., 2021). Changes in the content of FAAs are shown in Table 2. Fresh sweetness is a complex and comprehensive taste. Currently, it is generally believed that the content of five kinds of amino acids has a great influence on fresh sweetness, which are glutamic acid, alanine, glycine, and aspartic acid (Özden, 2005). All thawed samples contained lower levels of glutamate, alanine, glycine, and aspartic acid than fresh fish, but MUAT-treated samples contained higher levels than FWT-treated samples (Fig. 3 (d)). In particular, the MUAT-220 showed the smallest decrease in total FAAs contents compared to other samples. Kang et al. (Kang, Gao, Ge, Zhou, & Zhang, 2017) demonstrated the generation of free radicals through cavitation induced by ultrasonic waves in water. This phenomenon was found to increase the degradation level of proteins during thawing. This might account for the higher ultrasound-induced FAAs levels in this study. In addition, the total FAA contents of MUAT-220, MUAT-245, and MUAT-270 after thawing were not significant, but the total FAA contents of MUAT-270 were the lowest. The results show that high-intensity ultrasound caused the water molecules to produce hydroxyl groups. The presence of H^+ ions in water can potentially bind with negatively charged FAAs, leading to the loss of negatively charged polar FAAs with water (Zou et al., 2018). The bitter amino acid concentration did not change significantly between the MUAT-195 and FS samples, indicating that the MUAT treatment partially preserved the fresh sweetness and reduced the bitterness of the samples.

Table 2
The FAAs results of frozen large yellow croaker under different thawing methods.

Treatment	FAAs							
	Asp	Thr	Ser	Glu	Gly	Ala	Val	Met
FS	16.22 ± 1.35^{a}	20.44 ± 0.80^{a}	25.59 ± 1.75^{a}	62.69 ± 2.61^{a}	117.07 ± 0.50^{a}	37.25 ± 0.34^{b}	2.83 ± 0.53^{a}	1.89 ± 0.08^{e}
FWT	1.77 ± 0.05^{c}	4.62 ± 0.13^{c}	7.64 ± 0.18^{d}	8.35 ± 0.20^{c}	15.77 ± 0.34^{e}	29.87 ± 0.68^{e}	0.47 ± 0.05^{b}	8.15 ± 0.17^{b}
195 W	1.57 ± 0.07^{c}	4.52 ± 0.07^{c}	9.15 ± 0.15^{c}	6.97 ± 0.16^{d}	25.40 ± 0.26^{c}	31.74 ± 0.40^{d}	0.80 ± 0.03^{b}	6.11 ± 0.08^{d}
220 W	2.82 ± 0.12^{b}	5.20 ± 0.08^{b}	12.20 ± 0.33^{b}	8.86 ± 0.17^{c}	40.37 ± 0.93^{b}	34.35 ± 1.01^{c}	0.59 ± 0.02^{b}	7.38 ± 0.52^{c}
245 W	3.18 ± 0.11^{b}	4.93 ± 0.03^{bc}	9.66 ± 0.03^{c}	10.97 ± 0.13^{b}	18.93 ± 0.14^{d}	38.86 ± 0.98^{a}	0.92 ± 0.63^{b}	9.42 ± 0.26^{a}
270 W	2.90 ± 0.11^{b}	5.16 ± 0.21^{b}	12.60 ± 0.41^{b}	12.28 ± 0.48^{b}	25.12 ± 0.67^{c}	31.48 ± 1.02^{d}	0.74 ± 0.04^{b}	7.94 ± 0.28^{b}

Treatment	FAAs							
	Ile	Leu	Tyr	Phe	Lys	His	Pro	Total
FS	1.89 ± 0.15^{b}	2.67 ± 0.11^{c}	1.43 ± 1.16^{d}	1.27 ± 0.06^{c}	11.12 ± 0.55^{a}	10.78 ± 0.04^{c}	6.23 ± 0.28^{ab}	319.34 ± 4.26^{a}
FWT	6.16 ± 1.12^{a}	7.49 ± 1.42^{a}	11.64 ± 0.91^{ab}	4.08 ± 0.45^{a}	4.03 ± 0.36^{b}	11.24 ± 0.22^{c}	4.93 ± 0.86^{abc}	126.20 ± 3.25^{c}
195 W	4.26 ± 0.07^{a}	4.01 ± 0.06^{bc}	7.28 ± 0.12^{c}	2.64 ± 0.10^{b}	2.52 ± 0.06^{c}	6.81 ± 0.15^{d}	4.62 ± 0.72^{bc}	118.42 ± 1.01^{c}
220 W	5.76 ± 1.38^{a}	6.78 ± 1.78^{a}	10.08 ± 1.47^{b}	3.62 ± 0.74^{a}	3.63 ± 0.58^{b}	15.18 ± 0.97^{b}	3.68 ± 0.49^{bc}	160.48 ± 9.95^{b}
245 W	5.36 ± 1.16^{a}	7.96 ± 1.56^{a}	13.02 ± 1.09^{a}	3.93 ± 0.51^{a}	4.24 ± 0.39^{b}	17.73 ± 0.36^{a}	6.40 ± 0.82^{a}	155.51 ± 5.48^{b}
270 W	5.24 ± 1.19^{a}	6.30 ± 1.53^{ab}	10.55 ± 1.09^{b}	3.97 ± 0.57^{a}	3.83 ± 0.44^{b}	15.08 ± 0.78^{b}	5.66 ± 1.30^{a}	148.84 ± 7.48^{b}

The methods include fresh sample (FS), flowing water thawing (FWT), multi-frequency UAT at 195 W, 220 W, 245 W, and 270 W. The letter from "a" to "e" are used to describe the significance of differences between the samples ($p < 0.05$).

3.6. Analysis of SDS-PAGE

The electrophoresis pattern of SDS-PAGE can reveal the aggregation, degradation, and cross-linking of proteins. β-Mercaptoethanol, serving as a reducing agent, can disrupt the disulfide bond within SDS-PAGE electrophoresis (Liao et al., 2022). Fig. 3 (b) demonstrated that the gel electrophoresis patterns exhibited myosin heavy chains (MHC) and actin chains (AC) as the predominant bands, which varied depending on the thawing procedures. Among them, MHC was the most important protein related to the analysis of the physicochemical properties of fish flesh. Tokur et al. (Tokur & Korkmaz, 2007) noted that the myosin-heavy chain was more prone to proteolysis than other proteins. The FS samples had the darkest and broadest MHC, and the thawed-treated bands were lighter in color. This might be due to the oxidation of MHC during the thawing process and the formation of disulfide bonds and carboxyl groups leading to protein degradation. In the study on the effect of high pressure freezing on natural actomyosin degeneration, Cheng et al. (Cheng, Sun, Zhu, & Zhang, 2017) pointed out that the natural structure of proteins is unstable at low temperatures. The electrophoretic bands of MUAT-220 were closest to FS, probably because the faster thawing speed inhibited the decrease of MHC concentration and reduced protein degradation, which better maintained the original physicochemical properties of myogenic fibronectin.

3.7. Changes in sulfhydryl and carbonyl contents

It is possible to determine the oxidation of proteins during fish preservation by analyzing the levels of sulfhydryl and carbon groups. The sulfhydryl groups are among the most dynamic functional groups of the protein. They are easily oxidized to disulfide bonds and other oxidation products as storage time increases. It is the usual practice to take into account variations from the sulfhydryl groups when determining the oxidation levels of proteins found in marine commodities. Table 3 indicates that there were statistically significant changes ($p < 0.05$) in the levels of carbonyl and sulfhydryl in the thawed samples compared to the FS samples ($p < 0.05$). The transformation of amino acid side chain groups could potentially account for the observed increase in carbonyl contents. MUAT-220 demonstrated the lowest carbonyl contents and the highest sulfhydryl contents ($p < 0.05$). Furthermore, the process of dehydrogenation readily converted protein sulfhydryl groups into disulfide bonds (Wang et al., 2020). Comparatively, the MUAT-treated samples showed lesser changes in sulfhydryl and carbonyl contents compared to the FWT samples. The reason for this phenomenon might be that the short thawing time reduced the reaction of enzymes and microorganisms in the samples, thus inhibiting the occurrence of oxidation reaction (Zhang, Sun, Chen, Liu, & Kong, 2021). The highest total sulfhydryl contents and the lowest carbonyl contents were found in the MUAT-220 among all samples. It is worth noting that excessive ultrasonic radiation leads to cavitation and microfluidic phenomena, resulting in the decomposition of water and the production of highly reactive free radicals, which could easily oxidize proteins (Ashokkumar et al., 2010).

3.8. Analysis of secondary structures determination

The secondary structure of MPs mainly includes α-helix, β-sheet, β-turn, and random coil, which is a structure composed of some peptide segments' backbones in the peptide chain (Wang et al., 2016). α-helix is a typical and important three-dimensional structure in protein secondary structure. The increase in β-turn and random coil contents usually reflects the increased looseness of the MP structure (Li et al., 2019). The decrease in α-helix contents is mainly related to the irreversible denaturation of proteins (Table 3) (Cai et al., 2019). MUAT-treated samples showed significantly higher α-helix contents in comparison to FWT ($p < 0.05$). The α-helix levels initially increased and then decreased as ultrasonic power increased. The stabilization of the α-helix structure primarily relied on hydrogen bonds, specifically between amino hydrogen (NH-) and carbonyl oxygen (C = O) within the polypeptide chain (Cao & Xiong, 2015). The longer thawing time likely disrupted these hydrogen bonds. The content of α-helix in MUAT-220 samples was significantly higher than that in other samples. Ultrasound-induced microjets enhanced both heat and mass transfer, thereby accelerating the thawing rate and reducing protein denaturation. Conversely, MUAT-270 showed the lowest α-helix contents due to the mechanical oscillation, elevated temperatures, and hot spots generated by excessive ultrasonic power, which led to protein denaturation or interruption of hydrogen bond interactions.

3.9. Analysis of the tertiary structure of the protein

Endogenous fluorescence spectroscopy is a technique used to track alterations in the tertiary structure of proteins by quantifying the tryptophan residues (Zhang et al., 2012). Thus, alterations in the tertiary structure may be evaluated by the use of intrinsic emission spectroscopy and measures of fluorescence intensity. The intrinsic fluorescence intensity (IFI) of MPs is shown in Fig. 3 (e). The fluorescence intensity observed in MUAT-220 was notably greater than that detected in the remaining samples, indicating a denser protein structure for MUAT-220 in which tryptophan residues were deeply buried in the protein core. The appropriate ultrasonic power was more easily attenuated in frozen fish compared to unfrozen tissue, ultimately preventing localized overheating and effectively reducing the thawing time (Shore, Woods, & Miles, 1986). Zhang et al. (Zhang, Sun, Chen, Kong, & Diao, 2020) found that higher ultrasonic power caused protein aggregation, which altered the polar environment. The fluorescence intensity of FWT was the lowest compared to the other samples, mostly because of the chromophores' exposure coming from protein unfolding, leading to a reduction in fluorescence intensity.

4. Conclusion

Frozen preservation is a widely used method for preserving aquatic products, which can effectively inhibit the activity of microorganisms and the reaction process of endogenous enzymes, extend the shelf life of aquatic products, and better maintain the nutritional value and flavor of

Table 3
The carbonyl content, total sulfhydryl content, and secondary structure results of frozen large yellow croaker under different thawing methods.

Treatment	Carbonyl content	Total sulfhydryl content	Secondary structure (%)			
	(μmol/ g protein)	(μmol/ g protein)	α-helix	β-sheet	β-turn	random coil
FS	1.60 ± 0.16^{e}	71.35 ± 1.13^{a}	45.89 ± 1.97^{a}	21.80 ± 1.13^{d}	17.12 ± 0.95^{a}	15.21 ± 2.51^{b}
FWT	2.48 ± 0.10^{a}	58.42 ± 0.58^{e}	32.13 ± 0.49^{d}	34.05 ± 0.35^{a}	16.88 ± 0.16^{a}	16.95 ± 0.02^{b}
195 W	2.25 ± 0.13^{b}	59.98 ± 0.41^{de}	34.73 ± 0.10^{c}	30.95 ± 1.55^{ab}	17.14 ± 2.40^{a}	17.18 ± 0.76^{ab}
220 W	1.86 ± 0.05^{d}	64.66 ± 0.50^{b}	39.51 ± 0.83^{b}	25.95 ± 2.15^{cd}	14.45 ± 4.59^{a}	20.30 ± 1.61^{a}
245 W	1.97 ± 0.05^{cd}	62.41 ± 0.13^{c}	35.58 ± 0.04^{cd}	27.78 ± 0.32^{bc}	19.67 ± 0.83^{a}	16.99 ± 0.56^{b}
270 W	2.12 ± 0.02^{bc}	61.47 ± 1.89^{cd}	34.19 ± 1.61^{cd}	31.53 ± 0.54^{ab}	17.32 ± 1.26^{a}	16.97 ± 0.21^{b}

The methods include fresh sample (FS), flowing water thawing (FWT), multi-frequency UAT at 195 W, 220 W, 245 W, and 270 W. The letter from “a” to “e” are used to describe the significance of differences between the samples ($p < 0.05$).

food. Ultrasonic assisted thawing is gradually applied in the field of food preservation. Multi-frequency ultrasonic assisted thawing is an effective method to accelerate the thawing process and improve the quality characteristics of frozen large yellow croaker.

This study sought to examine the effects of varying power levels of MUAT technology on the quality characteristics and stability of MP in the large yellow croaker. MUAT significantly reduced the damage to quality during thawing. Specifically, an optimal ultrasonic power of 220 W exhibited the ability to effectively minimize the thawing loss in the large yellow croaker, preventing the increase of TBARS and TVB-N. Meanwhile， the total loss of FAAs in MUAT-220 was lower than that in other samples. In addition, the UAT treatment inhibited the migration and loss of fixed and free water caused by thawing. Notably, MUAT-220 demonstrated a lower degree of MP structure destruction, as evident from protein oxidation, secondary structure, and tertiary structure analysis. Therefore, the application of MUAT-220 treatment could minimize the adverse effects of thawing on the quality and MP stability of the large yellow croaker. Therefore, MUAT-220 can improve the quality of thawed large yellow croaker, especially at specific power and frequency, and can obtain better quality, which can be further studied in the future. In this paper, multi-frequency ultrasound is applied in the process of assisted thawing to study its effect on the quality of large yellow croaker in the process of thawing, so as to promote the application of multi-frequency ultrasound in the food freezing industry. The application of multi-frequency ultrasonic wave in the thawing process of large yellow croaker provides some theoretical and practical references.

Ultrasonic-assisted thawing is gradually applied in the field of food preservation. Multi-frequency ultrasonic-assisted thawing is an effective method to accelerate the thawing process and improve the quality characteristics of the frozen large yellow croaker. Multi-frequency ultrasonic assisted thawing at 220 W minimizes deterioration of quality and protein stability of the large yellow croaker caused by freezing and thawing. Therefore, MUAT-220 can improve the quality of thawed large yellow croaker, especially at specific power and frequency, and can obtain better quality, which can be further studied in the future.

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CRediT authorship contribution statement

Xinrui Yang: Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Chuhan Bian:** Validation, Software, Methodology, Formal analysis, Data curation. **Yixuan Dong:** Methodology, Investigation, Formal analysis, Data curation. **Jing Xie:** Validation, Supervision, Methodology, Funding acquisition. **Jun Mei:** Writing – review & editing, Software, Project administration, Conceptualization.

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.

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Data availability

The authors do not have permission to share data.

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