Impact of Ultrasound-assisted Saline Thawing on the Technological Properties of mirror carp (Cyprinus carpio L.)

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ABSTRACT
The aim of the study was to evaluate the positive effect of ultrasound-assisted saline thawing (UST) on the technological properties (water mobility, water holding capacity, colour, pH, shear force, TVB-N, oxidation reaction and microstructure) of mirror carp (Cyprinus carpio L.). The results present in the study showed that different thawing methods had negative impacts on the quality of mirror carp to varying degrees. Among them, UST samples had significant lower thawing loss, centrifugal loss and cooking loss than ultrasound thawing (UT) and air thawing (AT) samples (P < 0.05). The analysis result of low-field nuclear magnetic resonance illustrated that UST inhibited the mobility and distribution of water effectively. Decrease in shear force and TVBN values were observed in all thawing samples, and the UST samples maintained the significant better texture property and freshness than UT and AT samples did (P < 0.05). In addition, the treatment of UST obtained 1% salt concentration inhibited the oxidation reactions effectively. Investigation of the microstructure of samples demonstrated that the treatment of UST kept the relatively complete structure of tissue than other thawing methods. Therefore, UST can be an alternative strategy to the traditional thawing of meat.

1. Introduction

Mirror carp (Cyprinus carpio L.), is a kind of important commercial freshwater fish, which has lower cholesterol levels and higher polyunsaturated fatty acids [1]. Fish becomes more and more popular and the per capita fish consumption in the world is increasing yearly because of the health property [2]. Due to the chemical composition and the easily microbial contamination of fish, it is easy for fish to get spoil and loss the freshness at room temperature. Therefore, as one of the most common and effective methods for food preservation, freezing is used to ensure the quality and extend the shelf-life of fish for ease of transportation [3]. At the same time, thawing is an essential process during transport [4]. During the traditional thawing processes, the microbial growth and chemical spoilage may lead to the uncontrollable quality deterioration, such as liquid losses, discoloration, protein denaturation and unpleasant flavor and so on [5–7]. Therefore, it is important to select the proper thawing method that can cause the minimal damage on fish quality.

Ultrasound-assisted is recognized as a green and innovative technology applied in meat processes [8]. The appropriate ultrasonic frequency can convert the vibration energy into heat energy and increase the internal medium temperature [9]. So as to effectively prevent the local overheating of meat and reduced the damage on muscle cell. The study of Zhang, Zhang, Zhou, Wang, and Zhang [10] proved that ultrasound-assisted treatment can effectively facilitate the extraction of meat protein and improve the meat quality. Recently, ultrasonic is also commonly used for thawing to improve the meat quality, which has the characteristics of the high thawing speed and thawing uniformly [11–12]. Li, Zhao, Muhammad, Song, & Liu [13] reported that ultrasound-assisted thawing better maintained the quality and inhibited the lipid oxidation of bighead carp fillets. Recently, ultrasound has been widely used as an auxiliary method in the thawing process of meat products.

New technologies are required in the meat cooling processes to improve the sustainability and efficiency of the meat industry. Saline thawing is an effective thawing method for reducing thawing time and inhibiting bacterial growth, which is new fast thawing method used in the food processing industry to control food quality. Thawing rate has the great influence on the food quality. The traditional thawing methods with lower thawing rate, such as water immersion thawing and air thawing, might cause the deterioration of meat products quality.

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2. Materials and methods

2.1. Sample and material preparation

According to the procedures approved by the Malmö-Lund Ethical Committee, fresh mirror carps were purchased from commercial abattoir (Harbin, Heilongjiang, China) and individually euthanized in pH-buffered tricaine methanesulfonate (250 mg/L). The fish was cut into pieces (25 ± 5 g). The fresh mirror carp piece was defined as the FS group. The fish samples were individually packaged in moisture-impermeable, poly-ethylene bags and frozen at -18 °C for 7 days. The frozen samples were thawed by air thawing (AT), ultrasonic thawing (UT), ultrasound-assisted saline thawing (UST), and ultrasound-assisted saline thawing (USTT).

AT samples were thawed at room temperature until the center temperature of the sample reached 4 °C. The treatment of UT was performed with ultrasonic power at 200 W (10°C) with ultrasound assisted freezing machine (Nanjing Xianou Co., Ltd., China). For UST, the packed samples were fully immersed in the coolant with salt concentration of 0.05% (UST1), 0.10% (UST2) and 0.20% (UST3) under the ultrasound powers of 200 W until the center temperature of the sample reached 4 °C.

2.2. Water holding capacity (WHC)

The WHC of fish was measured by thawing loss, centrifugal loss and cooking loss, which were determined by weighing the fish samples by different treatments under the following equations:

\[
\text{Thawing loss (\%)) = \frac{W_3 - W_1}{W_1} \times 100
\]

\[
\text{Centrifugal loss (\%)) = \frac{W_3 - W_2}{W_1} \times 100
\]

\[
\text{Cooking loss (\%)) = \frac{W_1 - W_3}{W_1} \times 100
\]

where \(W_1, W_2, W_3\) and \(W_4\) were the weight of the frozen samples, thawed samples (after removing the surface water), samples after centrifugation (10,000 × g, 15 min, 4 °C) and samples after cooking (75 °C, 25 min), respectively.

2.3. Water mobility and the distribution

The moisture migration of fish samples was measured by the method of Shi et al. [17] through a Minispec mq 20 1H low-field nuclear magnetic resonance (LF-NMR) analyser (Bruker Optik GmbH, Germany).

2.4. Colour and pH

The colour property of fish samples was determined by \(L^*\)-value (lightness), \(a^*\)-value (rightness) and \(b^*\)-value (yellowness) by Color Analyzer (ZE-2000, Juki Corp, Tokyo, Japan).

About 5 g minced fish sample was homogenized with 9 times the volume of distilled water and stood for 30 min at 4 °C. Then the pH value of samples was measured by a pH meter.

2.5. Total Volatile Binding Nitrogen (TVBN)

5 g minced fish sample and 50 mL of distilled water were stirred for 30 min. Adding 10 mL boric acid and 5 mL mixed indicator liquid into the supernatant and then distilled for 10 minutes. The mixture was then titrated with HCl (0.01 mol/L) until it turned blue-purple.

\[
TVBN (mg/100g) = \frac{V_1 - V_2}{100} \times 2800
\]

where \(V_1\) and \(V_2\) was the the titration volume of HCl in sample and blank, respectively.

2.6. Lipid oxidation

The degree of lipid oxidation was reflect through the thiobarbituric acid-reactive substances (TBARS) of the fish, which was determined due to the method of Li et al. [18].

2.7. Protein oxidation

The degree of protein oxidation was detected by carbonyl groups according to the method of Li et al. [18].

2.8. Scanning electron microscopy (SEM) observation

The microstructures of fish muscle thawed with different methods were observed through scanning electron microscope (SEM) (Bal-Tec Co., Manchester, NH, USA). The image of microstructure was showed with SEM at the accelerating voltage of 5 kV and 1,000 × magnification.

2.9. Statistical analysis

The experiments were performed in triplicate and all data were shown as mean values ± standard deviation (SD). The data was analyzed by Statistic 8.1 software package (Analytical Software, St. Paul, MN), and the means were considered significant at \(P < 0.05\).

3. Result and analysis

3.1. Water holding capacity (WHC)

Water holding capacity (WHC) is a key indicator to measure the moisture changes of fish after different thawing treatments, which is closely related to the colour, texture and sensory qualities of fish [19]. In the present study, the WHC of samples was reflected by thawing loss, centrifugal loss and cooking loss.

Thawing loss and centrifugal loss are intuitive expression of the moisture changes of the sample after thawing. As illustrated in Fig. 1, the thawing loss and centrifugal loss of AT samples were significant higher than those of other samples (\(P < 0.05\)). Specifically, UST2 samples had the lowest thawing loss and centrifugal loss at 5.52% and 17.17%, respectively. In addition, there were no significant differences in fish WHC between UST2 and UST3 (\(P > 0.05\)). The forming of ice crystals from water during freezing process destroyed the muscle cell and caused the water redistribution [20]. The subsequent slow thawing process hindered the reabsorption process of water, thereby promoting the water loss. In addition, the longer thawing time could result in the microorganisms growth and oxidation reaction happening [19]. Thus aggravating the water flowing outside the muscle tissue. On the contrary, the increase in ionic strength of muscle tissue treated by salt might cause the...
swelling of myofibillar matrix, which could improve the binding ability between protein and water [21]. In addition, ultrasound-assisted thawing, as a quick defrosting method, can reduce the water loss by shortening the thawing time. Moreover, gas nucleus informed due to the cavitation effect of ultrasound could reduce water loss by reducing the mechanical damage to fish tissues. This could explain the minimal changes in thawing loss of fish under UST treatment. Wang, Yan, Rashid, Ding, and Ma [22] reported that the slower thawing rates resulted the higher thawing loss of small yellow croaker.

To further evaluate the differences in WHC from samples with different thawing methods, the cooking loss of fish was also conducted. Cooking loss is the symbol of the total loss water and soluble substances after heating of muscle [23]. As shown in Fig. 1, samples treated with UST2 had the minimum cooking loss at 11.03%, which were significant (P < 0.05) lower than those of samples treated with AT and UT by 9.22% and 4.91%, respectively. The results were in agreement with the thawing loss and centrifugal loss, which indicated that ultrasonic-assisted salt water thawing was the most effective method to inhibit water loss. During the thawing processes, the huge changes in hydrophobic and hydrophilic groups of myofibril induced by slowly thawing led to the increase in cooking loss. However, the combination of appropriate ultrasonic power and salting conditions were helpful to increase the thawing rate and maintain the complete muscle microstructure [24]. Thus contributing to the lower water loss of fish.

3.2. Water mobility and the distribution

The redistribution of water is one of the most important indexes to explain the changes in the water mobility in muscle, which is also considered to be related to the water holding capacity of fish during thawing processes closely [25]. As shown in Fig. 2, the normalized treatment of water peak areas and the types of water state were observed in the LF-NMR spectrum, which represented the different degrees of the tightness between water and muscle and protein [26]. There are three types of water observed in the figure, which are called bound water ($T_{2b}$, 0-10 ms), immobilized water ($T_{21}$, 10-100 ms), and free water ($T_{22}$, 100-1000 ms), respectively. The peaks of all thawed-samples shifted rightward (increasing in relaxation time) to varying degrees compared to the fresh samples, which showed that the thawing processes led to the water mobility increasing and redistribution. As for the changes in $T_{2b}$, no significant differences were observed in samples with different thawing methods (P > 0.05), which might be due to the tighter binding between water and macromolecules [27]. While, the changes in $T_{21}$ of samples subjected to AT were significant higher than those of samples subjected to UT, UST1, UST2 and UST3 (P < 0.05). The peak of $T_{21}$ was the dominant water population in meat systems, and was associated with the meat quality [28]. The prolongation of $T_{21}$ reflected that thawing processes resulted in the increase in the water mobility, which might be resulted from the weakened water-protein interaction after thawing. Water in muscle cells migrated intracellular to extracellular space after freezing, while it was difficult to go back into intracellular space after thawing [29], especially the slowly thawing (AT). It is worth mentioning that the treatment of UST2 exhibited the minimum increase in $T_{21}$. On the one hand, proper salt ions might be helpful to maintain the osmotic pressure in muscle cells. Thus decreasing the water loss. On the other hand, ultrasonic-assisted salt water thawing accelerated the thawing speed and reduced the degree of protein

![Fig. 1. Effect of different thawing methods on the thawing loss, centrifugal loss and cooking loss of mirror carp (Cyprinus carpio L.). A-D indicate the significant differences (P < 0.05) of different thawing treatments.](image1)

![Fig. 2. Effect of different thawing methods on the the $T_2$ relaxation times of mirror carp (Cyprinus carpio L.).](image2)
degradation, which was contributed to reduce the muscle juice loss and inhibit water migration. By contrast, the salt concentration was too high to cause protein denaturation easily, which might reduce the ability to bind water [30].

3.3. Colour and pH

Colour, as the direct indicator to evaluate the visual appearances of the samples, is important for the quality and acceptability of food [31]. As depicted in Table 1, AT samples had obviously higher \( L^* \)-value and \( b^\ast \)-value \( (P < 0.05) \) than fresh fish samples, followed by UT, UST1, UST3 and UST2. While the \( a^\ast \)-value of samples showed the opposite trend. Samples treated by UST (3.42) had the minimal differences from fresh samples (3.67). During the air thawing processes, the water lost form muscle resulted in the water attaching to the muscle surface, which caused the increase in the \( L^* \)-value [32]. In addition, part of the pigment taken away as the loss of water during the thawing processes might be a factor that lead to the decrease in \( a^\ast \)-value and the increase in \( b^\ast \)-value. Also, the oxidation denaturation of myoglobin molecule at the stage of freezing and thawing caused the loss of optimum colour presentation [5]. By contrast, the thawing method of ultrasonic and salting reduced the time needed in thawing processes [33], which decreased the water loss in the extracellular space of muscle tissue. Thus inhibiting the increase in \( L^* \)-value. Moreover, appropriate addition of salt ions effectively protected spatial and structural integrity and delayed the oxidation reaction. So as to suppress the color deterioration of fish. However, the increased oxidation degree with incremental salting concentration might lead to fish discoloration [34]. This is the reason of the changes in the the colour when the salt ion concentration increased to 0.20%.

pH value, as one of the important indicator of raw fish quality, is related to the freshness of fish. The changes in pH of samples treated by different thawing methods were demonstrated in Table 1. Compared to the fresh samples, all thawing samples had lower pH values than the fresh sample. Among them, the pH value of AT samples was the lowest one. The reduction in pH values was because of the accumulation of lactic acid and inorganic phosphoric acid during thawing processes [35]. While there were no significant differences among UT and UST samples. The decrease in pH led to the denaturation of myofibrillar and sarcoplasmic proteins, which might result in the decline in water holding capacity of fish. The results was corresponded to the result of WHC (Fig. 1).

3.4. Texture

Tenderness is an important quality attribute of fish, which is commonly measured by the shear force [36]. The influence of different thawing methods on the shear force of fish samples were showed in Fig. 3. The shear force of fresh sample was 14.33 N, followed by 12.51 N, 13.17 N, 13.56 N, 13.66 N and 13.33 N for AT, UT, UST1, UST2 and UST3, respectively. The results indicated that thawing processes cause the significant decrease in shear force of fish \( (P < 0.05) \). During the freezing and thawing processes, the destruction and disintegration of muscle fiber structure induced by the formation of ice crystals, as well as the enzymatic action resulted in the reduction in shear force [5]. Samples thawed by UT and UST had significant higher shear force than that of AT samples \( (P < 0.05) \). The ultrasonic treatment can change the structure of muscle tissue and maintain the texture property of fish to a certain degree. The impact of salt on the meat quality is concentration-dependent. The appropriate salt content could cause the expansion of myofilament lattice and increase the texture property of fish during thawing processes. As for the combination of ultrasound and salting, it could increase the thawing speed during the thawing processes, which was helpful to control the reduction in shear force of fish (Fig. 1). While the study of Jiang, Nakazawa, Hu, Osako, and Okazaki [21] reported that the excessively high salt concentration increased the solubility and the unfold degree of protein, so as to cause the softening of the samples.

3.5. Total Volatile Binding Nitrogen (TVBN)

The changes in TVB-N values are direct reflection to evaluate the corruption degree of fish. As presented in Fig. 3, the TVB-N values of samples treated by AT, UT, UNT1, UNT2 and UNT3 were significant higher than that of FS sample by 17.35%, 9.43%, 6.04%, 3.58% and 3.02%, respectively. It can be concluded from the result that thawing processes promoted the formation of volatile nitrogen and reduced the freshness of fish or led to fish corruption, while the combination of ultrasound and salting effectively bridled the increase in TVB-N values to the lowest range. The increase in the TVB-N values was resulted from the degredation of protein and non-protein nitrogen compounds, which might be due to the growth of microbial during the freezing and thawing processes [37]. In addition, the higher TVB-N values was likely to cause unpleasant flavors in fish. Especially for air thawing processing. By contrast, the treatment of ultrasonics thawing can effectively increase the thawing speed by avoiding the local high temperature inside the samples. In addition, the exist of salt can inhibit the growth of microbial [21] and reduce the formation of protein aggregations [38]. Moreover, the ultrasinics treatment can promote the entry of salt ions into muscle cells. Therefore the combination of ultrasonics and salting treatments reduced the formation of TVB-N, so as to maintain the quality and extend the shelf-life of fish.

3.6. Lipid oxidation

Lipid oxidation causes the deterioration of freshness, quality and nutritive values of fish products. The estimation of TBARS is the most important parameter for the determination of the degree of lipid oxidation [22].

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**Table 1**

| Treatment | Colour | pH |
|-----------|--------|----|
|           | \( L^* \)-value | \( a^\ast \)-value | \( b^\ast \)-value | \( P \)-value |
| FS        | 50.07±0.32\(^\text{A}\) | 3.67±0.04\(^\text{A}\) | 6.57±0.05\(^\text{A}\) | 6.91±0.01\(^\text{A}\) |
| AT        | 54.12±0.31\(^\text{A}\) | 2.17±0.07\(^\text{B}\) | 8.55±0.04\(^\text{B}\) | 6.61±0.02\(^\text{C}\) |
| UT        | 53.06±0.25\(^\text{B}\) | 3.05±0.03\(^\text{C}\) | 7.50±0.03\(^\text{C}\) | 6.71±0.01\(^\text{D}\) |
| UST1      | 52.90±0.20\(^\text{C}\) | 3.15±0.02\(^\text{D}\) | 7.46±0.02\(^\text{D}\) | 6.73±0.03\(^\text{D}\) |
| UST2      | 51.61±0.31\(^\text{C}\) | 3.42±0.03\(^\text{D}\) | 7.17±0.05\(^\text{D}\) | 6.73±0.02\(^\text{E}\) |
| UST3      | 52.43±0.24\(^\text{B}\) | 3.25±0.05\(^\text{C}\) | 7.43±0.04\(^\text{D}\) | 6.72±0.01\(^\text{E}\) |

\(^\text{A-D}\)means the significant differences \( (P < 0.05) \) of different thawing treatments.
The microstructure of muscle is the direct reflection of the changes in the structural integrity of muscle [43], which is important to important oxidation. The influence of different thawing methods on the TBARS of fish was shown in Fig. 4. All thawing samples had higher TBARS values than fresh samples. During the freezing processes, the formation and development of ice crystals destroyed the muscle cells, which lead to the release of pro-oxidant substances and promoted the oxidation reaction. Especially for the slow thawing methods (AT), the thawing processes increased the chance of contact between oxidant substances and air and aggravated the occurrence of oxidation reaction. Therefore, AT samples had the highest TBARS value. The treatment of UT and UST on the fish suppressed the oxidation reaction to a certain extent. The ultrasonic waves could induce the formation of ice nucleation by moving through liquid medium quickly. Thus improving heat transmission efficiency and thawing rate. At the same time, the addition of salt in thawing process can increase the heat transfer, which also accelerated thawing [6]. In addition, the combination of ultrasound and salt can effectively increase the thawing rate and inhibit the lipid oxidation. However, the improper salt concentration might promote the lipid oxidation [39]. That the reason of the increase in TBARS value from UST3 group.

3.7. Protein oxidation

The carbonyl contents of meat were determined to analyze the protein oxidation of fish under different thawing methods. As observed in Fig. 4, the carbonyl content of fresh sample was 1.47 nmol/mg. The content of carbonyl increased significantly (AT: 1.84 nmol/mg, UT: 1.72 nmol/mg, UST1: 1.63 nmol/mg, UST2: 1.60 nmol/mg, UST3: 1.62 nmol/mg) after thawing (P < 0.05). The results reflected that thawing processes resulted in the occurrence of autoxidation. As for thawing samples, the freezing processes caused the damage on cell and changed the ultrastructure of the muscle, which led to the release of intracellular haem iron and enzymes. Thus leading to the exposure of ROS and the ultrastructure of the muscle, which led to the release of intracellular damage induced by freezing. As a result, the sarcosome structure is kept relatively intact. Moreover, the presence of a low concentration of salt could increase the solubilization/extraction of proteins and lead to the swell of filament lattice [44]. As for the treatment of UST, the combination of ultrasonic and saline has achieved excellent results in shortening the thawing time. Thus reducing the damage on the muscle and keeping the myofibers intact relatively. The visual observation of microstructures indicated that improper thawing methods caused the cell rupture and muscle structure damage [45]. Leygonie et al. [5] reported that the structural modifications is closely related to the quality attributes of meat. The deterioration of microstructural characteristics were associated with the decrease in water holding capacity and shear force (Fig. 3).

3.8. Scanning electron microscopy (SEM) observation

The microstructure of muscle is the direct reflection of the changes in the structural integrity of muscle [43], which is important to important to analyse the physical properties of meat. The influences of different thawing methods on fish microstructure were observed in Fig. 5 (transversely and longitudinally). The muscle bundle of fresh samples had complete structure and arranged tightly. In addition, the periphery of the myofiber kept almost intact and there was little gap between adjacent muscle bundle. As for AT samples, distorted myofibers with a rough surface were observed from the longitudinal figure. And the fascicular structure of muscle was obviously loosened compared to the fresh sample. By contrast, the microstructures of fish samples thawed by UT and UST were more complete than those of AT samples, but the space interfascicular was slightly larger than that of the unthawed samples. On the one hand, ultrasonic treatment attenuated faster and suppressed the occurrence of local overheating, which reduced the deterioration on the muscle microstructure [19]. In addition, salinization can offset the intracellular damage induced by freezing. As a result, the sarcosome structure is kept relatively intact. Moreover, the presence of a low concentration of salt could increase the solubilization/extraction of proteins and lead to the swell of filament lattice [44]. As for the treatment of UST, the combination of ultrasonic and saline has achieved excellent results in shortening the thawing time. Thus reducing the damage on the muscle and keeping the myofibers intact relatively. The visual observation of microstructures indicated that improper thawing methods caused the cell rupture and muscle structure damage [45]. Leygonie et al. [5] reported that the structural modifications is closely related to the quality attributes of meat. The deterioration of microstructural characteristics were associated with the decrease in water holding capacity and shear force (Fig. 3).

4. Conclusion

The positive effect of UST on the technological properties of mirror carp comparing with the UT and AT was evaluated in the study. The results showed that the various thawing processes reduce the quality of the fish to varying degrees. Based on the analysis of water mobility and water holding capacity, UST samples had the slightest changes in water migration and lower water loss. Likewise, samples thawed under UST maintained the significant better texture characteristics and microstructure, especially the salt concentration in UST was 1%. Moreover, the combination of ultrasound and saline thawing effectively reduce the degree of lipid and protein oxidation. In conclusion, UST was the best thawing method to maintain the quality of fish. The findings of the present study are of great theoretical and applicable value for the fish preservation. The further study we would reveal the influence of UST on the properties of myofibrillar protein.

Fig. 4. Effect of different thawing methods on the lipid oxidation and protein oxidation of mirror carp (Cyprinus carpio L.). A-D indicate the significant differences (P < 0.05) of different thawing treatments.
CRediT authorship contribution statement

Fangfei Li: Methodology, Software, Validation, Formal analysis, Investigation, Funding acquisition, Writing – original draft. Bo Wang: Software, Investigation. Baohua Kong: Investigation. XiuFang Xia: Conceptualization, Supervision, Funding acquisition, Writing – original draft. Yihong Bao: Conceptualization, Resources.

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.

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