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Original Research Article

Development of reduced-salt gel of silver carp meat batter using low frequency ultrasound: Effect on color, texture, cooking loss and microstructure

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Abstract

Purpose: *To develop reduced-salt silver carp meat gels using low frequency ultrasound.*
Methods: *Silver carp meat batters were prepared with 0.5, 1 (reduced-salt) and 2 % (regular salt), and sonicated (20 KHz, 500 W) for 30 and 40 min, or unsonicated (control). Changes in gel properties were evaluated in terms of color, texture, cooking loss and microstructure using color measurement, puncture test, cooking loss and scanning electron microscopic (SEM) analysis, respectively.*
Results: *Ultrasound and salt exposure led to marked effects on color, texture and cooking loss in fish meat gels ($p < 0.05$). Reduction in salt content increased the lightness (L*) and cooking loss; and also decreased the sample values of greenness (-a*), breaking force, rupture distance and gel strength. Scanning electron microscopy (SEM) on regular-salt level samples showed that ultrasonic exposure decreased dense aggregates and increased the number and distribution of small cavity samples. Reduced-salt samples (1 % salt) subjected to 30 min sonication had better color (lighter) than control (0 min sonication), better texture (higher gel strength) and cooking loss comparable to that of regular-salt level sample subjected to 30 min sonication, and similar to microstructures from normal salt samples without ultrasound exposure.*
Conclusion: *Low frequency ultrasound is suitable for preparing reduced-salt fish meat gels under suitable ultrasonic conditions.*

Keywords: *Silver carp, Fish meat batter, Gel, Reduced salt, Low frequency ultrasound*

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INTRODUCTION

Silver carp (*Hypophthalmichthys molitrix*) is one of the major inexpensive freshwater fish species harvested commercially in China [1]. In recent years, food products such as fermented sausages [2], surimi [3-5] and restructured fish [1] have been developed from silver carp.

Common salt or sodium chloride is a vital additive in the processing of many foods. However, excessive sodium intake is associated with hypertension [6]. Aligned with that, there is a great interest in reduced-salt products. However, reduction in salt content could influence the characteristics of meat products. Reduced salt content impairs the complete solubilization or dissociation of myofibrillar proteins, thereby contributing to coarse product structure [4]. Moreover, it has been shown that salt concentrations below 2 % negatively affect the mechanical and functional attributes of fish products [7].

Ultrasound is a novel, harmless and eco-compatible technique employed in food industries for production of food products. It involves the use of mechanical waves with frequencies beyond the auditory threshold of humans. In the food processing industries, ultrasound waves of low frequency are employed for altering the physical and chemical properties of foods [8], while those of high frequencies are useful for assessing the physicochemical attributes foods. Some studies have reported the enhancing effect of low frequency ultrasound on the properties of meat protein [8-11]. According to Li *et al* [12], ultrasound waves are capable of improving the properties of low-salt gels. In addition, it has been suggested that ultrasound parameters such as the type of equipment, frequency, and time and power of sonication should be considered in research studies [8,13].

The aim of the present study was to develop reduced-salt gels of silver carp meat batters using low frequency ultrasound. Available literature shows that there is limited information on this area of research.

EXPERIMENTAL

Materials

Fresh live silver carp fish (mean weight = 2.5 ± 0.2 kg) were purchased from a local supermarket (Wuxi, Jiangsu, China). Trained store personnel sacrificed the fish using percussive stunning. Then the fish were scaled, eviscerated, decapitated and washed in running tap water. Thereafter, they were covered with crushed ice, and transported to Food Processing Technology Laboratory within 20 min. Then, the fish were manually skinned and filleted. Only the white muscles were used: the red muscles were manually removed with a knife. The muscles were passed through a grinder using a 7-mm plate (MGJ-090, Foshan Shunde Mingjian, Guangdong, China). Samples were placed in polyethylene bags and kept at 4 ^{o}C prior to preparation of meat batter. All of the chemicals and reagents used in this study were of analytical grade (Sinopharm Chemical Reagent, Shanghai, China).

Meat batter preparation

The meat batter samples were prepared with three levels of salt (NaCl) namely 0.5, 1 % (reduced-salt concentrations) and 2 % (regular-salt concentration); and three levels of ultrasound treatment, namely 0 min (unsonicated sample used as control), 30 min and 40 min. The meat batters were prepared in line with a modified version of the procedure described earlier [14]. The preparation procedure was as follows: ground meat was homogenized in a food processor (JYL-D025, Joyoung, Shandong, China) with ice-cold water for 30 s, and salt was added at different concentrations as indicated in Table 1, followed with chopping for 30 sec. Approximately 60 g of each meat batter was placed in a plastic bag, spread out to a thickness of about 2 mm, and vacuum-sealed.

Table 1: Composition of the fish meat batters

Ingredient	Salt levels (%)		
	0.5	*1*	*2*
Fish meat	80.0	80.0	80.0
Salt	0.5	1.0	2.0
Ice water	19.5	19.0	18.0
Total	100.0	100.0	100.0

Ultrasound treatment and meat gel preparation

This was performed using a probe system (Intelligent Ultrasonicator Generator USC201L, 500 W and 20 kHz, Hangzhou Biao, Hangzhou, China) equipped with horn of 10 mm diameter.

The sample was immersed in 1500 mL iced-cold water in a 2000-mL glass beaker. The beaker was placed in ice bath. During the ultrasound application, the temperature of the iced-cold water was maintained below 15 ^{o}C. The distance between sample and tip of the probe was established at 2 cm. the meat batters were sonicated for periods of 30 and 40 min. Measurements were taken in triplicate. Meat batter not subjected to sonication served as control at the same temperature (15 ^{o}C). Samples were stored at 4 ^{o}C prior to analysis.

Meat gel was prepared as follows: after the ultrasound treatment, meat batter portions (about 35 g) were placed in plastic centrifuge tubes of inner diameter 2.5 cm, and height 10 cm. The tubes were hermetically sealed. The sealed

tubes were centrifuged at 2500 g at 3 ^{o}C for 15 min using Sigma Laboratory Centrifuges 4K15, Germany. Thereafter, the tubes were heated in a water bath at 40 ^{o}C for 1 h, and at 90 ^{o}C for 15 min. Then, the meat gels were immediately cooled by immersing the tubes in ice-cold water at 4 ^{o}C for 30 min. The meat gels were stored in a refrigerator at 4 ^{o}C prior to further analysis.

Color measurement

The meat gels were sliced into cylinder-shaped patterns of diameter 2.5 cm and length 2.0 cm. The color of the sample surfaces was determined with a color spectrophotometer (UltraScan, USA), after prior calibration using a white and black standard plate. Color was measured at four different points on a sample to get the magnitudes of lightness (*L**), redness (+*a**), greenness (-*a**), yellowness (+*b**) or blueness (-*b**) of the gels. The measurements were carried out in triplicate.

Puncture test

Puncture test was performed in line with a modified version of the procedure reported earlier [12]. The test was done on cylinder-shaped slices of meat gels (same dimensions as in color measurement) with TA.XT Plus texture analyzer bearing a 5-mm diameter plunger operating at a velocity of 1 mm/sec. The parameters measured were distance to rupture (cm) and breaking force (g). Gel strength, expressed in the unit of g.cm, was obtained from the product of breaking force and distance to rupture. The test was repeated six times for each sample at room temperature.

Cooking loss analysis

This was determined using a modified version of the procedure outlined previously [15]. Portions of each meat batter (approximately 10 g) were processed to meat gel using meat gel preparation as described above. The tubes were kept in inverted position for 1 h, and filter paper (Whatman no. 1 with diameter 90 mm) was put on the open tubes to absorb separated fluid. Cooking loss was estimated in terms of post-cooking weight decrease (%) relative to original weight. Measurements were done in triplicate.

SEM of meat gels

The ultra-structures of the 4 meat gel samples (sample containing 1 % salt with 30 min ultrasound, and samples containing 2 % salt subjected to 0, 30 and 40 min ultrasound) were examined under SEM (SU1510, Hitachi, Tokyo, Japan) which was set at the operating voltage of 5 kV and at a magnification of ×3k. Prior to SEM, the 4 samples were processed with a modified version of an earlier method [12]. The samples were subjected to fixation for 48 h using phosphate buffered solution of 2.5 % glutaraldehyde, pH 7.0. Following fixation, the samples were rinsed thrice with 0.1 M phosphate buffer at the same pH, and then dehydrated through increasing ethanol concentrations (30 – 100 %). Finally, the dehydrated samples were dried in a freeze drier dryer (Alpha 1-2 LDplus, Germany).

Statistical analysis

Data were subjected to statistical analysis using ANOVA. Groups were compared with Duncan's multiple range tests. All analyses were carried out with SPSS 20.0 software. Statistical significance of difference was assumed at $p < 0.05$.

RESULTS

Color

The values of *L**, *a** and *b** for fish meat gels are shown in Figure 1. The *L** value varied from 78.31 to 81.27; *a** value varied from -1.07 to -1.83, while *b** value varied from 7.20 to 9.74.

There were marked increases in *L** values of fish meat gels subjected to ultrasound for 30 min and 40 min, with decreasing NaCl content ($p < 0.05$). In addition, the *L** values of regular-salt fish meat gels were reduced by ultrasound exposure. However, the -*a** values at decreasing NaCl levels were reduced by ultrasound exposure for the two periods of time. Furthermore, at normal NaCl level, there were increases in -*a** value as a result of ultrasound exposure.

With decreased NaCl content, the *b** level was enhanced by the two ultrasound exposure periods, while in samples with normal NaCl content, *b** was reduced as a function of duration of ultrasound exposure. However, in samples containing 1 % NaCl, ultrasound exposure had no marked effect on *b** value.

Puncture test

The breaking force values of fish meat gels varied from 186.55 to 291.66 g. The distance to rupture values varied from 0.56 to 0.86 cm, while the gel strength varied from 107.15 to 251.12 g.cm (Figure 2). The breaking force decreased in decreased NaCl content as a function of duration of ultrasound exposure, while it was enhanced as

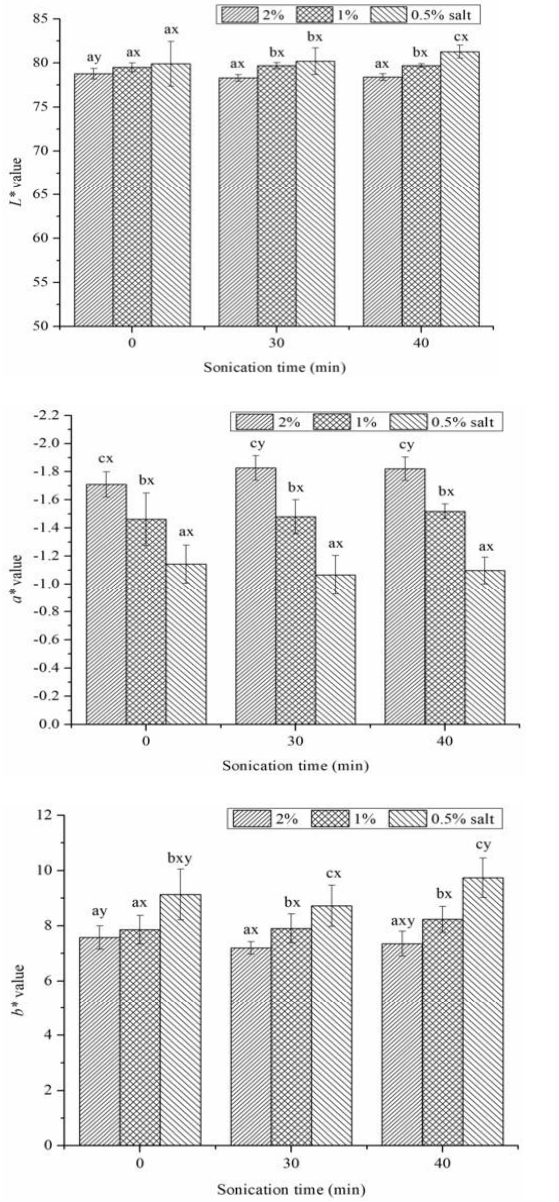

Figure 1: Color of fish meat gels. Different letters (a, b and c) indicate significant differences ($p < 0.05$) among different salt levels at the same sonication time. The letters x and y indicate significant differences ($p < 0.05$) between the sonication times at the same salt level

a function of ultrasound exposure duration in samples with normal NaCl content.

The distance of rupture of samples decreased with reducing salt levels at the two ultrasound treatments. Moreover, the distance of rupture increased significantly with ultrasound treatments in samples with normal salt levels, such that a sample with 40 min ultrasound had higher distance of rupture than the control.

The gel strength value of samples decreased in sample with decreased NaCl content and with increase in ultrasound exposure time, but increased with ultrasound exposure period at normal NaCl content.

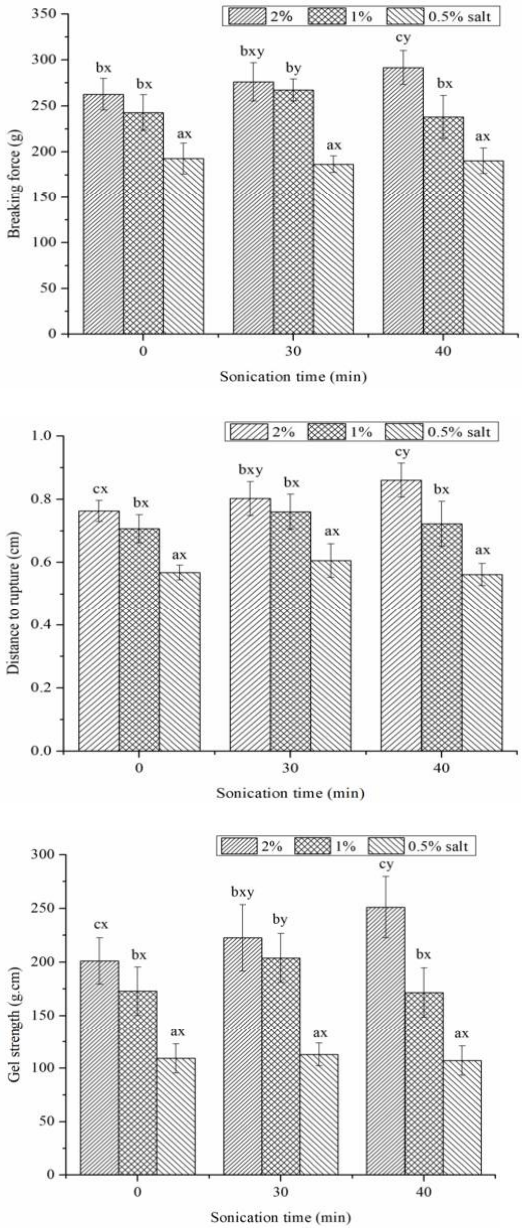

Figure 2: Distance to rupture, breaking force, and gel strength of the fish meat gels. Different letters (a, b and c) reflect significant differences ($p < 0.05$) among samples with different salt contents at the same sonication time. The letters x and y indicate significant differences ($p < 0.05$) between the two sonication periods at the same salt level

Cooking loss

The results of cooking losses in fish meat gels are presented in Figure 3. The values ranged from 10.76 to 16.16 %. Cooking loss was enhanced with reduction in NaCl content of fish meat gels as a function of duration of ultrasound exposure. In samples with normal NaCl contents,

cooking loss was markedly enhanced by ultrasound exposure.

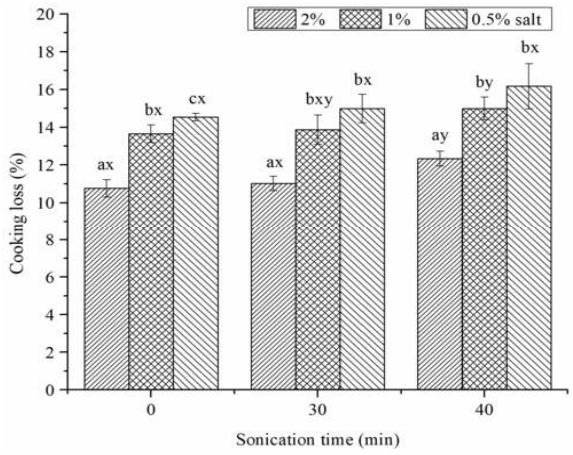

Figure 3: Cooking loss of fish meat gels. The letters a, b and c reflect significant differences ($p < 0.05$) among samples with different salt levels at the same sonication time. The letters x and y indicate significant differences ($p < 0.05$) between the two sonication times at the same salt level

Structural features

The microstructures of the four kinds of fish meat gels are presented in Figure 4. These are: sample containing 1 % salt exposed to 30 min ultrasound, and sample containing 2 % salt exposed to 0, 30 and 40 min of ultrasound.

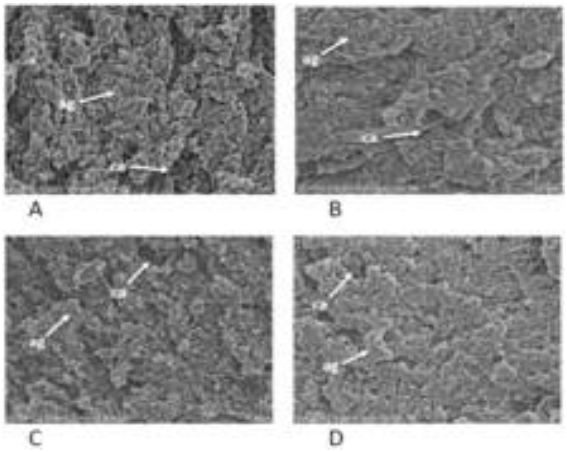

Figure 4: SEM images of ultrasound-treated fish meat gel with different salt level: (A) 1 % salt exposed to 30-min ultrasound; (B) 2 % salt unexposed to ultrasound; (C) 2 % salt exposed to 30-min ultrasound; (D) 2 % salt exposed to 40-min ultrasound (x 3000; bar = 10.0 µm) (ag = aggregate; ca = cavity)

DISCUSSION

In this study, low frequency ultrasound was used to develop reduced-salt gel of silver carp meat batter. The results showed that ultrasound treatment at decreasing salt levels increased the lightness (*L**) value of fish meat gels. However, ultrasound treatment of samples at regular salt level produced the opposite effect. These results are supported by the findings of Hu *et al* [16], who reported that the whiteness values of squid muscle decreased on exposure to ultrasound treatment due to ultrasound-induced enhancement of browning reaction. On the other hand, reduction of salt level increased the *L** value of samples, which is consistent with other studies [15,17,18]. This was attributed to the differences in levels of protein abstracted during chopping, and differences in salt content. It has been shown that increases in protein content decreased the *L** value of beef meat batters [19]. This means that reduction of salt level might increase the *L** value of samples. In the present study, cooking loss increased with reduction in salt content. Studies have shown that *L** value is positively correlated with cooking loss [15]. This phenomenon might be due to heat-induced loss of water from the samples, resulting in accentuated light-scattering capacity of the meat batters.

The results also showed that with reduced salt levels, ultrasound treatment decreased the greenness (*-a**) of fish meat gels, but increased the greenness in samples with regular salt levels. In addition, ultrasound treatment of samples with 1 % salt content had no significant effect on yellowness (*b**) of fish meat gels. Color is an important factor which determines the quality and acceptability of fish processing products such as surimi gels. Surimi gels with high lightness and low yellowness are generally in high demand [20].

In texture observations, the results showed that breaking force, distance to rupture and gel strength values of samples were affected by salt content and ultrasound treatments.

Ultrasound treatment in reduced salt samples decreased the breaking force and distance of rupture values of the fish meat gels, whereas, the ultrasound treatment gave the opposite effect on the breaking force values at regular-salt level and 1% salt of samples, and on the distance of rupture values of fish meat gels with normal salt content.

Some studies have also showed that salt had a positive effect on the mechanical properties of fish gel [4,21-24]. These studies showed that the breaking force, distance to rupture and gel strength of fish gel decreased at decreased salt concentration. Salt significantly influences the extraction and mechanical attributes of proteins

[24]. Furthermore, other studies have revealed that the gel strength of chicken meat batters [25] and beef myofibrillar proteins [8] are positively influenced by ultrasound exposure. These effects are due to the fact that ultrasound exposure improves gel strength due to its effect on the degree of acidity of proteins, which in turn decreases sample particle size thereby resulting in increased solubility. However, prolonged ultrasound treatment causes denaturation of proteins and adversely influences the texture of products [12]. It has been reported that the gel strength of chicken breast meat batter with salt contents of 1, 1.5 and 2 % tended to increase with 10 and 20 min ultrasound treatment, but decreased after 30 and 40 min sonication (40 kHz, 300 W) [12].

In this study, reduction in salt levels increased the cooking loss value of fish meat gels. This finding is consistent with previous reports [12,15,26]. When salt level is increased, the hydration and water-binding characteristics of processed meats are increased [26]. Thus, decreases in salt level increase the cooking loss of samples. Studies have shown that salt contents of 1.5 - 2 % result in solubilization of active myofibrillar proteins, thereby enhancing hydration and the binding capacity of the protein, resulting in better texture and less water loss during cooking [27]. As shown in the results of the present study, cooking losses of fish meat gels with 1 and 0.5% salt content were significantly higher than those of samples with regular salt level samples (2 %) at the same ultrasound treatment time. Ultrasound treatment significantly increased the cooking loss of fish meat gel samples with regular salt content, and samples with 1% salt content on exposure to 40 min of ultrasound. It has been reported that exposure of chicken meat gels to 20-min ultrasound reduced their water mobility and decreased their cooking loss at NaCl levels of 1 – 2 %, relative to cooking losses seen after their exposure to ultrasound for shorter or longer periods [12].

Results of SEM showed three dimensional networks in the sample of meat gels. The gel of regular-salt samples without sonication had visibly dense aggregates and low population of large cavities unevenly distributed. This sample presented a gel mesh of disorganized and dense aggregates, most likely as a result of disordered agglomeration prior to unfolding. When treated with ultrasound for 30 min and 40 min, there was a reduction in the population of dense aggregates and an elevation in the population of uniformly distributed small cavities. Similar results were also reported by other researchers [9,25]. Furthermore, SEM of reduced-salt sample (1 % salt content, 30 min ultrasound) showed a structure similar to that of non-sonicated, regular salt-containing sample.

CONCLUSION

The results obtained in this study demonstrate that it is feasible to obtain reduced-salt silver carp meat gels using low frequency ultrasound (20 KHz, 500 W). The findings show that reduced-salt fish meat gels (1% salt) subjected to 30 min of sonication have better color (lighter) than control (0 min sonication), better texture (higher gel strength), and minimal changes in cooking loss, relative to samples containing regular salt level (2% salt). These products also have microstructures similar to regular-salt content sample without sonication. Thus, the findings suggest that low frequency ultrasound may be a useful technique for preparing reduced-salt fish meat gels using suitable ultrasonic conditions.

DECLARATIONS

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Conflict of interest

This investigation is not linked to any conflict of interest.

Contribution of authors

The authors declare that this work was done by those mentioned in this manuscript. All liabilities in respect of claims associated with the contents of this manuscript will be shouldered by the authors.

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