Water holding capacity and microstructure of sturgeon (*Acipenser gueldenstaedti*) fillets as affected by low temperature vacuum heating

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**ABSTRACT**

This study was aimed to investigate the effect of low-temperature vacuum heating (LTVH; versus the traditional thermal method– TC - 100°C boiling water) on water content/distributions, microstructure, and tenderness of sturgeon fillets. Although all fillets were well done, those fillets heated at lower temperature/time showed higher moisture content (\(P<.05\)). Furthermore, microstructure by paraffin section and shear force results also indicated LTVH70-30 (LTVH method; 70°C, 30 min) shown the most similar to TC fillets, the less firm and less juicy. Oppositely, LTVH60-15/30 (LTVH method, 60°C, 15/30 min) showed the best tenderness/juiciness among all fillets. Therefore, the LTVH method is a good alternative to the TC for fish processing in the catering industry, with tenderness/juiciness gains.

**INTRODUCTION**

Fish are the main superb source of animal protein, with a high nutritive value owing to their favorable essential amino acid composition which is necessary in diet.\textsuperscript{[1,2]} Although fish are sometimes eaten in raw (e.g., sashimi), fish are usually consumed after cooking in boiling water, steaming, frying, or subjected to other heating treatments. The main advantages of heating treatment are sterilization, which ensures safe consumption, increased digestibility,\textsuperscript{[3]} enhanced flavor and taste, and the increase shelf life.\textsuperscript{[4]} As an auxiliary tradition, boiling is popular in China, the USA, Japan, and other countries and has accepted the most attention in the context of home cooking.\textsuperscript{[5]}

In addition to their valuable caviar, sturgeons are also popular for their boneless flesh with excellent taste; in fact, the sturgeon flesh accounts for approximately 300 g/kg skinless fillets.\textsuperscript{[6]} However, in contrast to marine fish and animal meat, sturgeon, as freshwater fish, has a smaller

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muscle fiber diameter, less connective tissue, and atender taste.[7] This means that the sturgeon fillets have poor thermal tolerance and their fiber muscles are much more easily broken during thermal processing. The natural features of the protein molecules are denatured during thermal treatment, and the reactive groups of proteins are exposed, resulting in loss of water, soluble vitamins, and proteins, and ultimately leading to changes in the surface and taste of food.[3] A number of studies exploring the effects of thermal treatments on the eating quality of cooked fish occurring by protein denaturation have been reported.8, 9, 10 Of note, the changes during processing depended on the processing method used and the heating time and temperature. For instance,11 reported that the heat treatment at high temperatures causes protein oxidation overhand, which consequently leads to changes in the physicochemical and organoleptic characteristics of food, including texture deterioration induced by the loss of water-holding capacity, and alteration of flavor. However, thermal treatment of food at lower heating temperatures usually requires a longer heating time, which is generally not appropriate for industrial applications because of the lower productivity of this cooking method.[12]

Low-temperature vacuum heating (LTVH) is similar to the traditional domestic boiling method. Samples are heated in boiling water, but at mild temperatures (under 100°C) via the use of special self-made equipment that allows controllable vacuum conditions. Our previous study showed that the LTVH method can reduce the oxidation of protein and lipid in fillets, compared to those observed in the context of traditional domestic cooking (100°C boiling water).[13] Additionally, the LTVH fillets had a great shelf life after 7 days storage, performing a better storage quality than the control groups thermal treatment at 100°C boiling water.[14] However, there is still limited data available on the water-holding capacity and tenderness of sturgeon fillets after processing via different heating time/temperature combinations.

Hence, the aim of this study was to evaluate the effect of heating time/temperature combinations by LTVH method (using once again the traditional heating method as the control condition), on water-holding capacity (WHC), water distributions, and the microstructure of sturgeon fillets. A better understanding of the water mobility and fiber structure changes induced in fillets during heat-mediated processing is essential to elucidate their role in the protein matrix structure and for the development of new healthy aquatic products.

Materials and methods

Sample preparation

Frozen Russian sturgeons, male, 3-year old and 3 ± 1 kg weight, provided by Quzhou Sturgeon Aquatic Food Technology Development Co. Ltd, Zhejiang, China, were transported to the laboratory within 2 h. The middle part of the sturgeon was cut into fillets (approximately 1.0 cm thickness and 65 to 75 g weight) after thawing with running water for half an hour. The fillets were bathed without any package in the boiling water using the self-made equipment under controllable vacuum conditions and randomly divided into 5 groups: traditional thermal method (TC, fillets were treated at 100°C—boiling water— for 15 min under normal pressure), LTVH60 (including LTVH60-15 and LTVH60-30, fillets were treated at 60°C for 15 or 30 min, respectively, under a = 812 mbar pressure), and LTVH70 (including LTVH70-15 and LTVH70-30, fillets were treated at 70°C for 15 or 30 min, respectively, at a = 700 mbar pressure). After thermal treatment, the fillets were collected, placed into with plastic bags, and cooled to room temperature in an ice bath for subsequent quantitative and qualitative analyses.

Cooking loss rate

The cooking loss rate was calculated based on the weight loss (before versus) after heating treatment. Determinations were carried out in triplicate. The performed equation is shown to be the following equation:
Cooking loss rate (%) = \[\frac{(W_1 - W_2)}{W_1} \times 100\]  
\text{Eq. 1}

where \(W_1\) is the mass (g) of the sturgeon fillet before heating and \(W_2\) is the mass (g) of the sturgeon fillet after heating.

**Moisture content**

The moisture content was determined based on the weight loss after drying 5 g of heated fillet in an assisted air circulation oven (GZX-9140MBE, Boxun Industrial Co., Ltd., Shanghai, China) at 105°C to a constant weight (differences of weight limited to 2 mg). Determinations were duplicated in triplicate. The data were calculated with the following equation:

\[
\text{Moisture content} (\%) = \left[\frac{(m_1 - m_2)}{m_1}\right] \times 100
\]

\text{Eq. 2}

Where \(m_1\) is the mass (g) of the sturgeon fillet before drying and \(m_2\) is the mass (g) of the sturgeon fillet after drying to a constant weight.

**Water-holding capacity (WHC)**

The WHC of fillets was determined according to the method described by,15 using an high-speed refrigerated centrifuge (H2050R, Xiangyi Laboratory Instrument Development Co., Ltd, Hunan, China). All determinations were carried out three times, independently. The following equation was used for the determination of the WHC:

\[
\text{WHC} (\%) = \frac{C_2}{C_1} \times 100
\]

\text{Eq. 3}

Where \(C_1\) is the mass (g) of the sturgeon fillet before centrifugation (1000 \(\times\) g for 10 min at 4°C) and \(C_2\) is the mass (g) of the sturgeon fillet after centrifugation (1000 \(\times\) g for 10 min at 4°C), g.

**Low-field nuclear magnetic resonance (LF-NMR)**

The water distribution in sturgeon fillet subjected to thermal treatment was determined via LF-NMR relaxation experiments according to the method of proposed by,16 Briefly, transverse relaxation time (\(T_2\)) measurements were performed using aMesor MR23-060 V–I nuclear magnetic resonance analysis system (Niumag Electric Corporation, Shanghai, China). A standard copper sulfate solution was placed in the RF coil (25 mm diameter) center of the magnet case, under the following conditions: magnet temperature of 32°C, the magnet strength of 0.5 T. Using the FID sequence, the center frequency SF01 was 23.20 MHz, automatically find the 90° and 180° pulse widths, determined the 90° pulse time (P1) was 30.00 \(\mu\)s, and the 180° pulse time (P2) was 56.00 \(\mu\)s. \(T_2\) values were measured by collecting CPMG (Carr-Purcell-Meiboom-Gill) decay signals with the sampling parameters are set as follows: repeat sampling waiting time (Tw) of 2000 ms, analog gain (RG1) of 20, digital gain (DRG) of 2, pre-amplification gain (PRG) of 1, accumulation number (NS) of 8, echo number (NECH) of 1000, and delay (DL1) of 0.1 ms. The Multi-Exp Inv Analysis software (Niumag Electric Corporation, Shanghai, China) was used for the distributed multi-exponential fitting of the CPMG decay curves and visualization of the lateral relaxation spectrum of the fillets. Three independent scan repetitions were conducted for each group, and the average value was recorded.

**Proton density imaging**

The proton density images of fillets subjected to thermal treatment were acquired by using aMINI MR-60 device [Niumag Electric Corporation, Shanghai, China] according to the method of,17 and with minor modifications. The device was operated at a frequency of 23 MHz and 32°C. The main parameters were set as follows: repetition time (TR) of 500 ms, viewing field of 50 mm \(\times\) 50 mm, and echo time (TE) of 19.0 ms. The final images were obtained by using the respective image processing software [Shanghai Niumag Corporation, Shanghai, China].
**Extraction of myofibrillar proteins**

The extraction and calculation of myofibrillar proteins was performed as described by.\(^\text{13}\) The determinations were repeated three times. The extraction rate of myofibrillar proteins was calculated as follows (16):

\[
\text{Extraction rate of myofibrillar protein} = \frac{E_1}{E_2} \times 100 \quad \text{Eq. 4}
\]

where \(E_1\) is the quantity (mg) of myofibrillar proteins extracted from heated sturgeon fillet and \(E_2\) is the quantity [mg] of myofibrillar protein extracted from raw sturgeon fillet.

**Light microscopy microstructural analysis**

The microstructures of the heated fillet were investigated following the method of reported by.\(^\text{18}\) Briefly, the fillet samples were fixed in fixative (including 4% paraformaldehyde), embedded in paraffin, and then cut (5–8 μm thick sections) using amicrotome (RM2016, Leica Instruments Co., Ltd., Shanghai, China). After de-paraffinization with xylene, the samples were stained with Ehrlich’s hematoxylin for 5 min and dehydrated in absolute ethanol for 5 min twice. The microstructures of the samples were observed and photographed using alight microscope (Nikon Eclipse E100, Nikon Co., Tokyo, Japan) coupled to an imaging system [Nikon DS-U3, Nikon Co., Tokyo, Japan).

**Shear force analysis**

According to the method reported by,\(^\text{19}\) but with minor modifications, the shear force analysis in the context of sturgeon fillets was performed using atexture analyzer (TA.XT Express, Stable Micro Systems, Surrey, UK) combined with a Warner Bratzler shearing device and aspeed of 5cm/min and adistance of 25cm. Six replicates were used per fillet.

**Statistical analysis**

All results are expressed as mean values ± standard deviation. Duncan’s multiple comparison test was used to determine statistical significance, set at \(P < .05\). Pearson’s correlation analysis was performed to understand the relationships between the parameters.

**Results and discussion**

**Extraction rate of myofibrillar proteins**

Myofibrillar proteins are the most abundant proteins in the sturgeon (Acipenser gueldenstaedtii) fillets, constituting approximately 50–55% of the total protein content.\(^\text{13}\) Therefore, changes in the myofibrillar proteins during thermal treatment play an important role in the alteration of the characteristics of sturgeon fillets. In daily life, we usually judge the readiness of cooked fillets based on their visual appearance and color. For instance, well-done fillets are pale, grayish-brown, and dry. Of note,\(^\text{16}\) reported that the abalones were effectively cooked when the extraction rate of myofibrillar proteins was limited to less than 10%. As shown in Table 1, the extraction rates of myofibrillar proteins in all heat-treated fillets were below 10%. These results suggest that all fillets (LTVH and TC) were well done. Of note, the extraction rates of LTVH fillets decreased with the increase in the heating temperature \((P < .05)\) and heating time \((P > .05)\) (Table 1). Therefore, altogether, these results suggest that increased heating temperatures have a greater impact on decreased myofibrillar proteins than prolonged heating time.\(^\text{20}\) reported that the myofibrillar proteins were sensitive to heating temperatures and get denatured during thermal treatment, resulting in protein aggregation and decreased extraction rate. Notably, as shown in Table 1, there were no significant differences between the extraction rates of the LTVH70-15, LTVH70-30, and TC groups. The denaturation of myofibrillar proteins may have been completed at the respective temperatures.\(^\text{21}\) reported that proteolysis and aggregation occur together during thermal treatment. The insignificant difference in LTVH70 and TC was presumably due to the combination of protein aggregation and degradation at high.
temperatures, and also reported that protein digestibility decreased as the heating temperature increased, which was attributed to protein aggregation. Overall, in our study, LTVH60 fillets exhibited better protein retention than both LTVH70 fillets and TC fillets.

**Cooking loss rate, moisture content and WHC**

The cooking loss rate, playing an important role in food processing, is responsible for the juiciness and yield of final products. Changes in the cooking loss rates and moisture contents of the different groups of fillets in this study are presented in Table 1. The heating time played a critical role in cooking loss rates. When the fillets were heated at 60°C for 15 min (LTVH60-15), the cooking loss rate was 27.56% ± 0.53%; while significantly increased to 31.26%±1.01% at LTVH60-30 (P<.05). However, there is no significant difference between the cooking loss rate of LTVH70-15 and LTVH70-30 (P>.05). Notably, the highest value of cooking loss rate was observed at TC fillets, and significantly higher than those heated by LTVH method (P<.05, Table 1). Some water-soluble proteins were lost with the water during cooking process, which enhanced the cooking loss in the TC fillets. The results illustrate the advantages of the LTVH method compared with TC in serving better juiciness and yield of final products.

As presented in Table 1, the moisture content of the heated samples tended to decline with the heating time (P<.05) and heating temperature (P>.05) in fillets processed via the LTVH method. However, there was no significant difference between the WHC of LTVH and TC fillets (P>.05). There are three main states of water in muscles: bound water, immobilized water, and free water [introduced in moisture distribution]. reported that immobilized water and free water, entrapped in the myofibrillary network or within the space between the thick and thin filaments, are sensitive to thermal treatment. It is, therefore, not surprising that increased the heating temperature or prolonging the heating time leads to water evaporation, and consequently, shrinking of myofibrils proteins (due to the weakened-binding forces between proteins and water). In line with this notion, here, the conducting the boiling process at low temperature or for less time persevered more water in the fillet structures; the LTVH60-15 samples showed the highest moisture content and WHC (68. ± 0.19%, 75. ± 1.66%, respectively; Table 1). We hypothesize that the capillary water loss during thermal treatment was one of the reasons for the reduction of free water in fillets, as reported by others. Overall, our results suggest that LTVH fillets treated at lower temperature had better moisture content than TC fillets, probably due to the preservation of protein solubility.

**Moisture distribution**

Moisture distribution and migration are influenced by the heating temperature and heating time during thermal treatment. The different of T2 relaxation spectra obtained in this study are shown in Figure 1; of note, these data exhibit astrign correlation with water-holding capacity. As shown in Figure 1, three peaks, T2, T2, and T2, are observed in the sturgeon fillets processed using low-temperature vacuum heating, indicating three distinct water states: T2 (ranging from 0 to 10 ms), T2 (ranging from 25 to 35 ms) and T2 (ranging from 140 to 1000 ms) were assigned to bound water,
Figure 1. Changes on NMR $T_2$ relaxation time of sturgeon fillets by low temperature vacuum heating. LTVH60-15/LTVH60-30, fillets were treated at 60°C for 15/30 min under −812 mbar vacuum pressure; LTVH70-15/LTVH70-30, fillets were treated at 70°C for 15/30 min under −700 mbar vacuum pressure; TC, fillets were treated at 100°C boiling water for 15 min with normal pressure. A and C were enlarged plot of $T_{21}$ peak (green dotted rectangle) and $T_{23}$ peak (black dotted rectangle) in B, respectively;
immobile water, and free water, respectively (Figure 1). These results are in line with previous studies reporting that the existence of three water states in heated muscle tissues,\textsuperscript{30,31} with a predominance of immobile water.

As shown in Figure 1A, the relaxation time $T_{21}$ shifted downward with the heating temperature and heating time, and its amplitude of $T_{21}$ declined. However, there was no significant difference in $T_{21}$, except for LTVH60-30 (Table 2, $P > .05$). As mentioned above, the relation time $T_{21}$ represents bound water components, which bind to protein molecules via hydrogen bonds, having the lowest mobility in fillet muscles,\textsuperscript{32} which is responsible for its resistance to thermal treatment.\textsuperscript{126} Interestingly, Pearson's correlation analysis showed that there was a very significant positive correlation ($R = 0.910$, $P < .01$, Table 3) between the relaxation time $T_{21}$ and the extraction rate of myofibrillar proteins in heated fillets. As shown in Table 2, $A_{21}$, corresponding to the peak area of $T_{21}$, increased with the heating temperature and heating time [$P > .05$], suggesting that thermal treatment indeed influences protein–water interactions.\textsuperscript{33} Also reported that the reduction in protein solubility was responsible for the decreased water-holding capacity due to weakening of the protein–water interactions and strengthening of the protein–protein interactions. In line with this nation, $T_{21}$ of the LTVH70 fillets and TC fillets changed very little while that of LTVH60 fillets changed much more as myofibrillar proteins varied.

As shown by the NMR date, immobilized water, accounting for more than 90% of moisture, was the highest in fillets after thermal treatment (Table 2). Of note, the relaxation time $T_{22}$ and $T_{23}$ decreased, as the relaxation components shifted to the left in Figure 1B and 1C, indicating that water mobility weakened as the heating temperature/time increased. Notably, the peak area proportion of immobilized water ($A_{22}$) was inclined, while that of free water ($A_{23}$) increased with both the heating temperature and time.\textsuperscript{34} Reported that changes in water may be correlated with the shrinkage and toughening of the fillet structure and cooking loss due to heating. In our study, the correlation analysis revealed a very significant positive correlation ($R = 0.779$, $P < .01$) between the peak area proportion $A_{23}$ and the cooking loss rate in heated fillets. These results suggest that immobilized water was transformed into free water, and then released into the broil during thermal treatment, causing an increase in the cooking loss rate.

Magnetic resonance imaging (MRI) is an noninvasive technique that has been used to analyze the water distribution in food.\textsuperscript{35} In this study, the distribution and migration patterns of water in fillets are also presented in the form of MRI-derived proton density images (Figure 2; the high proton densities are highlighted in red, while low proton densities are highlighted in blue). From the red zone ratios of these images, it is clear that the thermal treatment had a significant influence on the content and distribution of water in the fillets. Notably, there were some red lines obviously present in fillets (Figure 2), demarking the connective tissue wrapping the myofibrils in fillet structures.\textsuperscript{36} Suggested that the proteins in the connective tissues can restore main water loss during thermal treatment. Our data suggests that the water, existing between muscle filaments, myofibrils, and membranes, did not easily flow because of the strong interactions with proteins. As shown in Figure 2, the moisture content decreased when the heating temperatures increased. However, no significant difference was observed in fillets heated via the LTVH method at the same temperature but during

\begin{table}[h]
\centering
\caption{Changes on NMR parameters obtained from sturgeon fillets by low temperature vacuum heating.}
\begin{tabular}{lcccccccc}
\hline
Groups & $T_{21}$ & $T_{22}$ & $T_{23}$ & $A_{21}$ & $A_{22}$ & $A_{23}$ \\
LTVH60-15 & 0.89 ± 0.05$^{a, b}$ & 30.69 ± 2.56$^{ab}$ & 338.86 ± 26.16$^{ab}$ & 9.90 ± 3.28$^{a}$ & 398.37 ± 17.20$^{a}$ & 10.06 ± 1.73$^{a}$ \\
LTVH60-30 & 0.99 ± 0.12$^{b}$ & 33.73 ± 1.48$^{b}$ & 400.42 ± 33.46$^{b}$ & 8.66 ± 2.06$^{a}$ & 393.42 ± 10.40$^{a}$ & 13.59 ± 2.02$^{ab}$ \\
LTVH70-15 & 0.60 ± 0.13$^{a}$ & 30.60 ± 1.03$^{ab}$ & 329.28 ± 21.75$^{ab}$ & 9.28 ± 3.11$^{a}$ & 393.19 ± 5.18$^{a}$ & 15.98 ± 3.33$^{ab}$ \\
LTVH70-30 & 0.60 ± 0.04$^{a}$ & 29.75 ± 0.84$^{ab}$ & 343.03 ± 18.60$^{ab}$ & 16.81 ± 0.85$^{a}$ & 396.51 ± 7.65$^{a}$ & 14.53 ± 1.98$^{ab}$ \\
TC & 0.57 ± 0.04$^{a}$ & 26.26 ± 0.90$^{a}$ & 290.76 ± 21.35$^{a}$ & 17.34 ± 2.02$^{a}$ & 376.83 ± 12.38$^{a}$ & 19.40 ± 1.38$^{ab}$ \\
\hline
\end{tabular}
\end{table}

LTVH60-15/LTVH60-30, fillets were treated at 60°C for 15/30 min under -812 mbar vacuum pressure; LTVH70-15/LTVH70-30, fillets were treated at 70°C for 15/30 min under -700 mbar vacuum pressure; TC, fillets were treated at 100°C boiling water for 15 min with normal pressure. Different small letters along the same column indicate significant difference at $P < 0.05$, as analyzed with Duncan’s multiple range tests.
Table 3. Pearson’s correlation measurement of indicators from sturgeon fillets by low temperature vacuum heating.

|                          | Myofibrillar proteins | Moisture content | Cooking loss rate | T21 | T22 | T23 | A21 | A22 | A23 | WHC | Shear force |
|--------------------------|-----------------------|------------------|-------------------|-----|-----|-----|-----|-----|-----|-----|-------------|
| **Myofibrillar proteins** | 1                     | 0.637*           | −0.122            | 0.910** | 0.621 | 0.602 | −0.370 | 0.445 | −0.324 | 0.187 | −0.795**    |
| **Moisture content**     |                        |                  |                   |     |     |     |     |     |     |     |             |
| 0.637*                   | 1                     | −0.412           | 0.430             | 0.365 | 0.206 | 0.452 | 0.437 | 0.214 | 0.178 | −0.648*     |
| **Cooking loss rate**    | −0.122                | −0.412           | 1                 | −0.015 | −0.032 | 0.154 | 0.416 | 0.779** | 0.304 | −0.086      |
| **T21**                  | 0.910**               | 0.430            | −0.015            | 1    | 0.779** | 0.814** | −0.316 | 0.500 | −0.282 | 0.150 | −0.780**    |
| **T22**                  | 0.621                 | 0.365            | −0.032            | 0.779** | 1    | 0.945** | −0.311 | 0.769** | −0.195 | 0.134 | −0.647*     |
| **T23**                  | 0.602                 | 0.206            | 0.154             | 0.814** | 0.945** | 1    | −0.141 | 0.727* | −0.069 | 0.240 | −0.708*     |
| **A21**                  | −0.370                | −0.452           | 0.416             | −0.316 | −0.311 | −0.141 | 1    | 0.146 | 0.690* | 0.749* | −0.003      |
| **A22**                  | 0.445                 | 0.437            | −0.168            | 0.500 | 0.769** | 0.727** | 0.146 | 1    | −0.005 | 0.572* | −0.719*     |
| **A23**                  | 0.324                 | −0.214           | 0.779**           | −0.282 | −0.195 | −0.069 | 0.690* | −0.005 | 1    | 0.649* | −0.088      |
| **WHC**                  | 0.187                 | 0.178            | 0.304             | 0.150 | 0.134 | 0.240 | 0.749* | 0.572 | 0.649* | 1    | −0.589      |
| **Shear force**          | −0.795**              | −0.648*          | −0.086            | −0.780** | −0.647* | −0.708* | −0.003 | −0.719* | −0.088 | −0.589 | 1            |

A21, A22, A23 are corresponding peak area of T21, T22, T23 respectively.

*is indicated significant differences of indicators, *: P < 0.05; **: P < 0.01.
different time, which is in line with the results of $T_2$ relaxation. Of note, TC fillets showed fewer red zones than LTVH fillets, which may be due to the changes in the muscle tissues in the context of high-temperature boiling water.

**Microstructure**

The microstructural changes of fillets subjected to different thermal treatments were observed on transverse and longitudinal sections of paraffin-embedded tissues via light microscopy are shown in Figure 3. The tissue structure of the fillets was significantly affected by thermal treatment. Denaturation and shrinking of myofibrillar proteins and connective tissues were occurred during the heating process, causing a change in the tissue microstructure; gaps were clearly observed in fillets, as reported elsewhere. However, fillets showed better tissue integrity after low-temperature heating processing (LTVH method). As shown in Figure 3, the LTVH60-15 fillets showed the most intact tissue architecture; in contrast, wider intermyofibrillar spaces and expansion of muscle fibers, were clearly observed in the LTVH60-30 fillets. The same trend was observed in the LTVH70-15 fillets versus LTVH60-30 fillets. However, when the heating time was increased to 30 min in the context of the 70°C
LTVH processing, some breaks were observed in the muscle fibers [LTVH70-30]. Notably, the myofibril bundles of the TC fillets were severely broken with adissolved myofibrillar package and larger gaps. These results are in line with the results of the study by,\textsuperscript{38}which reported that treatment at high temperatures caused shrinking and dissolution of the myofibrillar fibers, resulting in the re-arrangement of broken myofibril proteins. Of note, large intercellular spaces were observed in LTVH70-30 fillets and TC fillets and to a greater extent in the latter, in where some solutes produced after the dissolution/degradation of cellular networks were fulfilled. Altogether, these results indicate that fillets treated using the LTVH method have better tenderness and WHC (Tables 1 and 4).

Shear force

Tenderness is one of the most critical characteristics of fillets after thermal treatment. As shown in Figure\textsuperscript{4}, thermal treatment, especially the heating temperature had a significant influence on the shear force value of the fillets. With respect to the LTVH60-15 and LTVH60-30 fillets (treated at 60°C with \(-812\) mbar), the low heating temperature led to low shear force values, which consequently leading to a better tenderness of the fillets. Structural alterations of proteins are associated with simultaneous changes in the physicochemical properties of the water within the fillet structure.\textsuperscript{39}\textsuperscript{4} An earlier study had reported that changes in shear force values were mainly related to the changes in the tissue strength and water-holding capacity, which was responsible for the changes in myofibrillar.\textsuperscript{40} As mentioned previously, fillets were treated at low heating temperatures, which led to the better preservation of water and the tissue structures, resulting in the tenderization of LTVH60-15 and LTVH60-30 fillets.\textsuperscript{41}investigated the tenderness of meat after thermal processing (also using the Warner-Bratzler shearing device) and their results suggested that the tenderness of meat heated at 60°C was better than that of meat heated at 50°C. However, fillets treated at 70°C under \(-700\) mbar or at 100°C under normal pressure, showed higher shear force values (non-statistically difference among the LTVH70-15, LTVH70-30, and TC groups). These results are in line with aforementioned results.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{shear_force.png}
\caption{Changes on shear force of sturgeon fillets by low temperature vacuum heating. LTVH60-15/LTVH60-30, fillets were treated at 60°C for 15/30 min under \(-812\) mbar vacuum pressure; LTVH70-15/LTVH70-30, fillets were treated at 70°C for 15/30 min under \(-700\) mbar vacuum pressure; TC, fillets were treated at 100°C boiling water for 15 min with normal pressure. Different small letters indicate significant difference at \(P<.05\).}
\end{figure}
that the muscle tissues were damaged severely occurring to the decline in water-holding capacity during high processing temperature.

**Conclusion**

Although all fillets investigated in this study were well-done fillets (extraction rate of myofibrillar proteins was below 10%), different heating time/temperature combinations, resulted in different characteristics in sturgeon fillets. Due to treated at lower temperature and vacuum condition with boiling water, LTVH fillets showed better results in protecting the thermal loss in myofibrillar proteins and moisture. As the results of integrity of muscle tissue and moisture retention capacity, LTVH60 fillet performed best tenderness values out of all of the fillets via thermal processing. TC fillets, heated at 100°C boiling water under normal pressure, showed apredominant loss of structure at the micro-structural level. This was closely linked to lower firmness and juiciness, which consumers perceived as undesirable. Therefore, the application of the LTVH method is agood alternative to the conventional processing fish (below 100°C boiling water) in the catering industry; notably, the final product is expected to be higher quality, with better tenderness and lower cooking loss. This would benefit both customers and the food processing industry.

**Highlights**

(1) The comparison between LTVH method and domestic heating at 100°C boiling water.
(2) The effects on water-holding capacity and microstructure of fillets investigated.
(3) The water distribution in fillet was observed by LF-NMR relaxation experiment.
(4) All fillets were well done and LTVH60 samples preserved more moisture.
(5) LTVH method can be served to achieve better tenderness and juicy fillets.

**Acknowledgments**

This work was supported by National Key R&D Program of China (Grant No.2018YFD0400600), Department of Education of Zhejiang Province (Grant No. Y201924135), and Zhejiang Provincial Natural Science Foundation (Grant No. LQ21C200004). And thanks for Quzhou Sturgeon Aquatic Food Technology Development Co. Ltd, Zhejiang China supported sturgeons for this study. The authors declare no conflict of interest.

**Funding**

This work was supported by National Key R&D Program of China (Grant No. 2018YFD0400600), Department of Education of Zhejiang Province (Grant No. Y201924135), and Zhejiang Provincial Natural Science Foundation (Grant No. LQ21C200004).

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