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Protein oxidation analysis based on comparative proteomic of Russian sturgeon (*Acipenser gueldenstaedti*) after sous-vide cooking

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ABSTRACT

Russian sturgeon meat is a highly perishable food product due to the microbial spoilage. Sous-vide cooking (SVC) at 40 °C, 50 °C and 60 °C with short-term time was applied to sturgeon fillets for the extension of shelf-life and the effect was investigated during storage at 4 °C. Results showed that SVC at 60 °C could effectively inhibit the growth of *Aeromonas* and *Pseudomonas* in samples. However, lipid oxidation and protein oxidation and degradation were aggravated with the myosin light chain (MLC), myosin heavy chain (MHC) and sulfhydryl content reduced after the treatment. Proteomics indicated that differentially expressed proteins were mainly involved in cellular component (CC) and biological process (BP). In this experiment, SVC were useful to preserve meat products, and the problem of protein oxidation should be further explored.

1. Introduction

Sturgeons are species of biological and economic values which can greatly simplify processing of fish product due to the lack of intramuscular spines (Feng et al., 2020). At present, the sturgeon farming production in China accounts for more than 86% of the world (Wang, Gao et al., 2019). However, the fish products are still dominated by smoked, dried and salted treatment due to the perishable characteristics of inevitable microbial activity and high moisture content (Chen et al., 2020; Hou et al., 2019). These methods used to extend the shelf life of sturgeon meat will cause the loss of nutrients and fresh flavor (Abel, Rotabakk, & Lerfall, 2020; Nithin et al., 2020).

Sous-vide cooking (SVC) processing is a more popular technology similar to pasteurization in which products are cooked slowly under mild heating conditions with vacuum packaging and retain the natural sensory qualities and nutritional value (Bongiorno et al., 2018; Modzelewska-Kapituła, Pietrzak-Fiećko, Tkacz, Draszanowska, & Więk, 2019). Sous-vide cooked products can be robustly and precisely controlled owing to the precise control of cooking time and water bath temperature with no required repackaging. SVC has been used with a wide range of temperature from 40 °C to 80 °C (Dominguez-Hernandeza, Salasevicieneb, & Ertbjerg, 2018; Hwang, Ismail, & Joo, 2020; Zielbauer, Franz, Viezens, & Vilgis, 2016). Microbiological safety of the products treated with SVC is more concerned during storage (Karyotis, Skandamis, & Juneja, 2017; Sebastiá, Soriano, Iranzo, & Rico, 2010). However, the lipid and protein oxidation induced by heating are inevitable (Dong et al., 2020) and the oxidation of Russian sturgeon fillets after treated with SVC is rarely researched.

Proteomics is a relatively new developing field in systemically exploring the complex proteins mixtures (Lu et al., 2018). It could compare the protein function and interactions caused by heat stress (He et al., 2019). Recently, application of proteomics technology has focused on the protein expression levels and metabolic pathways in different areas or different development stages (Baldina et al., 2016; Chen et al., 2016; Ma et al., 2020). The proteins after treating with SVC had been studied little based on proteomic analysis.

In this study, sous-vide cooking was applied to extend the shelf-life of Russian sturgeon fillets, and the protein oxidation was analyzed by proteomics. The results determined the effect of SVC on microbial growth of sturgeon meat, and evaluated the changes in protein

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oxidation. Moreover, this work would provide useful information for applying SVC in the sturgeon industry.

2. Materials and methods

2.1. Sample preparation

Freshly Russian sturgeon (*Acipenser gueldenstaedti*, 45 ± 5 cm and 3 ± 1 kg) was collected from a mussel farm facilities (Quzhou, China), transported to the laboratory on ice within 2 h and used within two days. Before SVC treatment, the sturgeon was cut into 1.5 cm thick steaks (3 cm × 3 cm) and prepared 350 fillets, and vacuum-packed (P290, Shineye Inc., China) individually in polyethylene bags after washing with sterile water. After that, the fillets were divided into four groups with different temperature treatments of sous-vide cooking (SVC): RS group (raw samples as control), TA group (40 °C water bath for 10 min), TB group (50 °C water bath for 10 min), TC group (60 °C water bath for 10 min). The equipment (C-BT27, Julabo Inc., Germany) was used for water bath. All the samples were stored at 4 °C in a refrigerator for 9 days and measured at 3-day intervals with three independent replicates.

2.2. Measurement of microorganisms indicators

2.2.1. Total viable counts (TVC)

TVC was measured according to Ge et al. (2020). Sturgeon fillet was homogenized by a stomacher blender (Clapping Stomacher SH-400A, Shanghai Hegong Scientific Instrument Co., Ltd. China) for 1 min after diluting 1:10 with 0.85% sterile normal saline. The bacterial collected from homogeneous substance was incubated onto plate count agar (PCA) plates at 37 °C for 48 h after serial decimal dilutions.

2.2.2. Characterization of the microbiota

2.2.2.1. DNA isolation and PCR amplification. Briefly, bacteria on sturgeon samples were extracted by centrifugation. DNA was extracted as reported by Wang, Qin et al. (2019) with the MicroElute Genomic DNA Kit (D3096-01, Omega, Inc., USA). The V3–V4 regions of 16S rRNA gene were amplified by PCR using primers 338F (5′-ACTCCTACGGGAG GCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). PCR reactions were done according to Chen et al. (2020).

2.2.2.2. Illumine MiSeq sequencing and data processing. AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) were used to purify the PCR products which were quantified by Qubit (Invitrogen, USA). The sequencing was performed in the amplicon pools and carried out on NovaSeq PE250 platform. The barcode and primer sequence of Paired-end reads were cut off based on their unique barcode. The sequences were clustered into operational taxonomic units (OTUs).

2.3. Sensory analysis

Sensory evaluation was performed according to Borilova et al. (2016) with some modifications. The same eight people from the laboratory were employed to evaluate the sensory of sturgeon fillets on color, odor, viscosity and elasticity during storage. The scores from 1 to 5 points expressed the assessment of very poor, poor, acceptable, satisfactory and excellent respectively.

2.4. Measurement of freshness indicators

2.4.1. Thiobarbituric acid reactive substances (TBARS)

TBARS value was determined as described by Wu, Ghirmai, and Undeland (2020). It was calculated by the malondialdehyde (MDA) content read from the optical density at of 532 nm and 600 nm wavelengths.

2.4.2. Total volatile base nitrogen (TVB-N)

TVB-N value was determined by the semi-micro kjeldahl method described by Qiao, Tang, and Dong (2017). 5 g of sturgeon fillet was homogenized for 1 min with 50 mL distilled water and stirred for 30 min. Then it was filtered with qualitative filter paper. 10 mL filtrate was mixed with 10 mL MgO (10 g/L) and distilled for 5 min with a Kjeldahl Apparatus (Kjeltec 8100, Foss, Sweden). The volatile base components were determined by the indicator (methyl red-methylene blue) and hydrochloric acid solution (0.01 mol/L).

2.5. Measurement of protein structure and oxidation

2.5.1. Protein cross-linking

Protein cross-linking with or without SVC treatment was investigated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as described by Pan et al. (2020).

2.5.2. Myofibrillar protein (MP) content

The MP was extracted according to Wang, Gao et al. (2020). 5 g of the sturgeon fillet was mixed with 50 mL phosphate buffer A (20 mmol/L, include 1 mmol/L EDTA, 100 mmol/L NaCl, pH 7.0) and homogenized at 15,000 rpm for 60 s. The suspension was centrifuged at 6000 rpm for 10 min in a high-speed refrigerated centrifuge (TGL-16M, Cence, Inc., China) to collect the precipitate. The precipitate was dispersed in phosphate buffer B (25 mmol/L, include 0.6 mol/L NaCl, pH 7.0) and dissolved overnight. The suspension was centrifuged at 10,000 rpm for 15 min to collect the Supernatant. The protein concentration was determined by a Bradford assay (Cai et al., 2020).

2.5.3. Free sulfhydryl (-SH) content

The free –SH content of sample was measured using 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB) method according to Yan et al. (2020). 2 mL MP solution (5 mg/mL) was mixed with 18 mL phosphate buffer (include 0.2 mol/L Na_2HPO_4, 0.2 mol/L NaH_2PO_4, 10 mM EDTA and 0.6 mol/L KCl), and then the mixture was incubated at 4 °C for 1 h with 2 mL 10 mM DTNB added. The absorbance was recorded at 412 nm. The calculation of free –SH content was using molar extinction coefficient as described by Pan et al. (2020).

2.5.4. Proteomics analysis methods

2.5.4.1. Homogenate and SDT lysis. The samples were mixed with SDT buffer (4% SDS, 1 mM DTT, 100 mM Tris-HCl, pH 7.6) and homogenized by MP homogenizer. And then it was sonicated and boiled for 15 min. The supernatant was collected from centrifugation for 40 min at 14,000 g and filtered with 0.22 μm filters (Zhu et al., 2014). The filtrate was quantified with the BCA Protein Assay Kit (Bio-Rad, USA) and stored at −80 °C.

2.5.4.2. Filter-aided sample preparation (FASP). The ultrafiltration (Microcon units, 10 kD) was used repeatedly to remove the low-molecular-weight components of proteins with SDT buffer. Then the cysteine residues of samples was block in darkness with the addition of 100 μl iodoacetamide for 30 min. After centrifuged at 14,000 g for 15min, 100 μl UA buffer (8 M urea, 150 mM Tris-HCl, pH 8.0) was used to wash the filter three times. The peptide content was determined at 280 nm by UV light spectral density (Wiśniewski, Zougman, Nagaraj, & Mann, 2009).

2.5.4.3. Liquid chromatography tandem mass spectrometry (LC/MS). A Q Exactive mass spectrometer (Thermo Scientific) was coupled with Easy nLC (Proxeon Biosystems, now Thermo Fisher Scientific) and used to perform LC-MS/MS analysis (Zhang et al., 2018). The positive ion mode of mass spectrometer was operated. The conditions of automatic gain control (AGC) target, maximum inject time and survey scans were setted

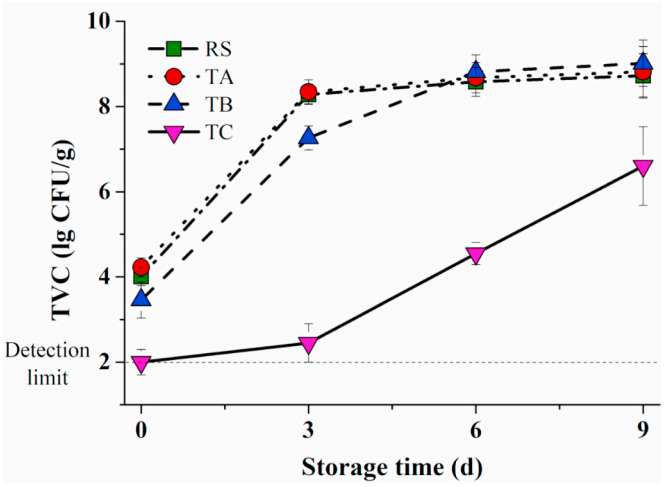


Fig. 1. Numbers (lg CFU/g) of total viable counts (TVC) during storage at 4 °C. RS, raw samples as control; TA, cooked in a 40 °C water bath for 10 min; TB, cooked in a 50 °C water bath for 10 min; TC, cooked in a 60 °C water bath for 10 min.

referred to Ren et al. (2020).

2.6. Data analysis and statistical analysis

Mascot engine (Matrix Science, London, UK; version 2.2) and Proteome Discoverer 1.4 (Chen et al., 2018) were used to perform database identification and quantitative analysis from MS/MS spectra. The homologue protein sequences were retrieved from SwissProt database which could transfer the functional annotation to the studied sequences. Then, the retrieved sequences were loaded into Blast2GO (Version 3.3.5) for GO mapping and annotation (Götz et al., 2008). The target protein sequences were classified to KO (KEGG Orthology) by comparing the KEGG genes database in KAAS (KEGG Automatic Annotation Server) (Moriya, Itoh, Okuda, Yoshizawa, & Kanehisa, 2007). The corresponding KEGG pathways were extracted according to the KOs. Enrichment analysis was performed in GO (Biological process, molecular function, and cellular component) and KEGG pathway based on the Fisher's exact test. The statistical analysis of Russian sturgeon samples in triplicate was performed using SPSS (version 20.0) and reported as the means ± SD.

3. Results and discussion

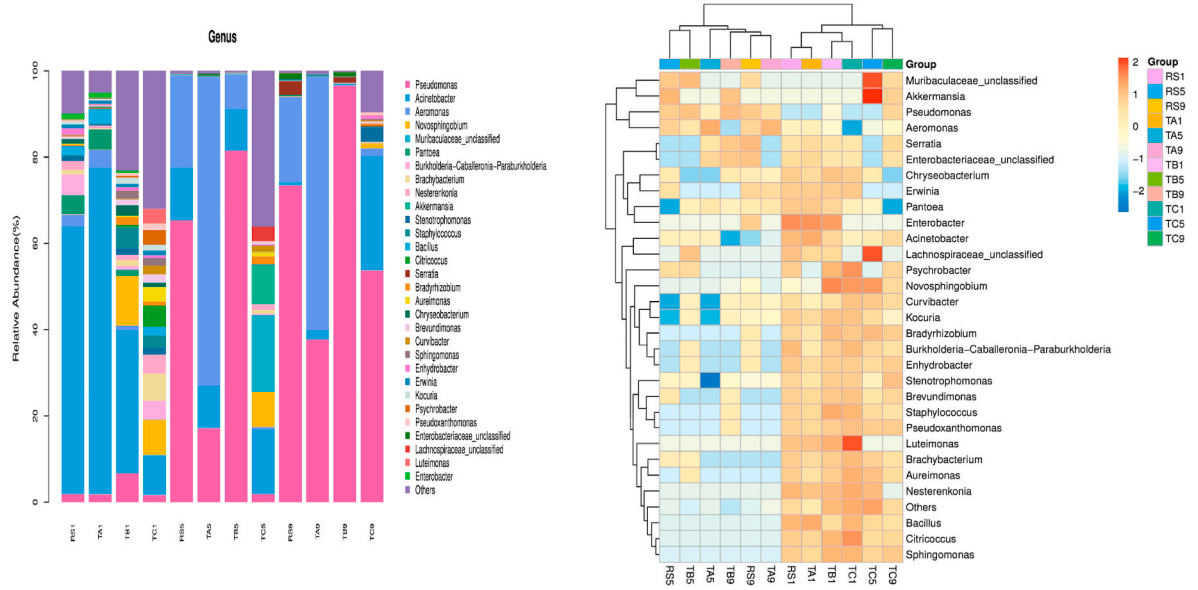
3.1. Microorganisms indicators analysis

3.1.1. Total viable counts (TVC) analysis

It has been known that fish products could be cooked with a wide range temperature and time combinations during sous-vide cooking (Baldwin, 2012). However, the tenderness of meat would be damaged seriously with high temperature and long heating time while reducing consumer desire (Yang et al., 2019). Therefore, looking for a suitable temperature which could effectively extend the shelf life and only have a slight effect on the appearance quality of sturgeon fillets at a short-term heating was necessary. As shown in Fig. 1, the initial TVC decreased with the heating temperature increasing while the temperature was higher than 40 °C. However, TB showed no Significant difference with RS and TA at the storage of 6 d. It may be that the initial TVC decreasing at 50 °C was insufficient, so it could increase rapidly with the favorable conditions creating by the damage of collagenous tissue and texture (Cropotova, Mozuraityte, Standal, & Rustad, 2018). After storing for 9 days, the TVC of RS, TA and TB was higher than 7 lg CFU/g which was as the microbiological limit proposed by ICMSF (1986) while the TC (6.60 lg CFU/g) proved that 60 °C was enough to reduce the number of microorganisms and extend the shelf-life on this side.

3.1.2. Microbiota composition

The shift in microbiota composition of sturgeon fillets at the genus levels including the top 30 genera was analyzed during storage (Fig. 2). According to Fig. 2A, the proportion of *Acinetobacter* was highest at the RS1 and TA1 which could be effectively reduced with heating temperature increase. *Pseudomonas* and *Aeromonas* were still the dominant



A B

Fig. 2. Shifts in composition of microbiota (A) and heat-map on the genus level (B) during storage. RS, raw samples as control; TA, cooked in a 40 °C water bath for 10 min; TB, cooked in a 50 °C water bath for 10 min; TC, cooked in a 60 °C water bath for 10 min. The digital represented days of storage.

Table 1
Changes in sensory scores of Russian sturgeon fillets during storage. RS, raw samples as control; TA, cooked in a 40 °C water bath for 10 min; TB, cooked in a 50 °C water bath for 10 min; TC, cooked in a 60 °C water bath for 10 min.

Storage periods	Groups	Sensory scores			
		Color	Odor	Viscosity	Elasticity
Day 0	RS	4.75 ± 0.15^{aA}	4.60 ± 0.22^{aA}	4.63 ± 0.17^{aA}	4.72 ± 0.11^{aA}
	TA	4.65 ± 0.20^{aA}	4.77 ± 0.13^{aA}	4.71 ± 0.07^{aA}	4.55 ± 0.08^{aA}
	TB	4.3 ± 0.25^{abA}	4.50 ± 0.16^{aA}	4.84 ± 0.09^{aA}	4.63 ± 0.03^{aA}
	TC	4.2 ± 0.18^{bA}	4.45 ± 0.23^{aA}	4.85 ± 0.06^{aA}	4.74 ± 0.17^{aA}
Day 3	RS	4.13 ± 0.04^{aB}	3.91 ± 0.06^{bB}	4.21 ± 0.23^{aA}	4.02 ± 0.05^{aB}
	TA	3.78 ± 0.12^{bB}	4.12 ± 0.10^{aB}	4.17 ± 0.18^{aB}	3.95 ± 0.17^{aB}
	TB	4.01 ± 0.21^{abAB}	4.21 ± 0.13^{aA}	4.34 ± 0.22^{aB}	4.22 ± 0.15^{aB}
	TC	3.98 ± 0.17^{abAB}	4.16 ± 0.05^{aA}	4.40 ± 0.08^{aB}	4.25 ± 0.18^{aB}
Day 6	RS	3.31 ± 0.08^{bC}	2.08 ± 0.23^{cC}	3.17 ± 0.21^{bB}	3.56 ± 0.11^{bC}
	TA	3.55 ± 0.13^{abB}	2.57 ± 0.11^{bC}	3.33 ± 0.05^{bC}	3.61 ± 0.08^{bC}
	TB	3.76 ± 0.17^{aB}	3.56 ± 0.07^{aB}	3.78 ± 0.08^{aC}	4.11 ± 0.04^{aB}
	TC	3.62 ± 0.25^{abB}	3.64 ± 0.14^{aB}	4.00 ± 0.16^{aC}	4.00 ± 0.19^{aBC}
Day 9	RS	2.58 ± 0.15^{bD}	1.15 ± 0.09^{bD}	1.78 ± 0.17^{bC}	2.77 ± 0.21^{bD}
	TA	2.32 ± 0.33^{bC}	1.30 ± 0.16^{bD}	2.15 ± 0.26^{bD}	2.56 ± 0.05^{bD}
	TB	3.41 ± 0.13^{aC}	3.07 ± 0.11^{aC}	3.32 ± 0.12^{aD}	3.52 ± 0.14^{aC}
	TC	3.44 ± 0.06^{aB}	3.22 ± 0.15^{aC}	3.44 ± 0.09^{aD}	3.77 ± 0.13^{aC}

The different superscripts (ãd) denoted significant differences ($p < 0.05$) among different groups in the same storage periods while the capital (Ã D) denoted significant differences ($p < 0.05$) among different storage periods in the same groups.

genus during storage of Russian sturgeon fillets after sous-vide treatment (Chen et al., 2020). However, the different SVC temperature had a great influence on the shift of dominant bacteria during storage. The proportion of *Aeromonas* was higher than *Pseudomonas* at 40 °C which almost disappeared when the temperature exceeded 40 °C. The rapid growth of *Pseudomonas* in the middle and late periods of storage after treatment with SVC at 50 °C was the main factor leading to its TVC growth. The growth of *Pseudomonas and Aeromonas* was obviously inhibited at 60 °C.

The comparison of bacterial composition between different groups was constructed in the heat-map (Fig. 2B). With storage time increasing, the communities of microbiota became less diverse and showed the same trend in the four groups. The color intensity of *Pseudomonas* and *Aeromonas* was increased significantly while most genera such as *Acinetobacter* and *Chryseobacterium* were decreased over time. These data suggested that SVC had a significant effect on the microbiota composition of sturgeon fillets during storage with relatively high temperature at 60 °C and short-trem time of 10 min.

3.2. Changes in the sensory

In addition to the nutritional value and microbial safety, sensory traits were also one of the important points considered by consumers (Cullere et al., 2019). The sensory changes of sturgeon fillets with different temperature SVC treatments were shown in Table 1. At day 0, the sensory of samples almost showed no differences except the scores of color decreased slightly with temperature increasing. It was unavoidable due to the light pinkish coloured fish meat which tends towards white after SVC treatments. However, the sensory decline of samples with 60 °C treatment were much slower that of raw samples especially in odor and viscosity. The main reason was that SVC treatment effectively reduced the initial microbial values of samples when the temperature reached 60 °C and delayed the growth of microorganism during storage. Bongiorno et al. (2018) also found that the odor intensity and meat turgidity decreased significantly as the number of microorganisms increasing during storage. Simultaneously, the elasticity of TB and TC groups decreased slower than RS and TA groups. It was caused by the shrinkage of protein structure in which the muscle fibre diameter

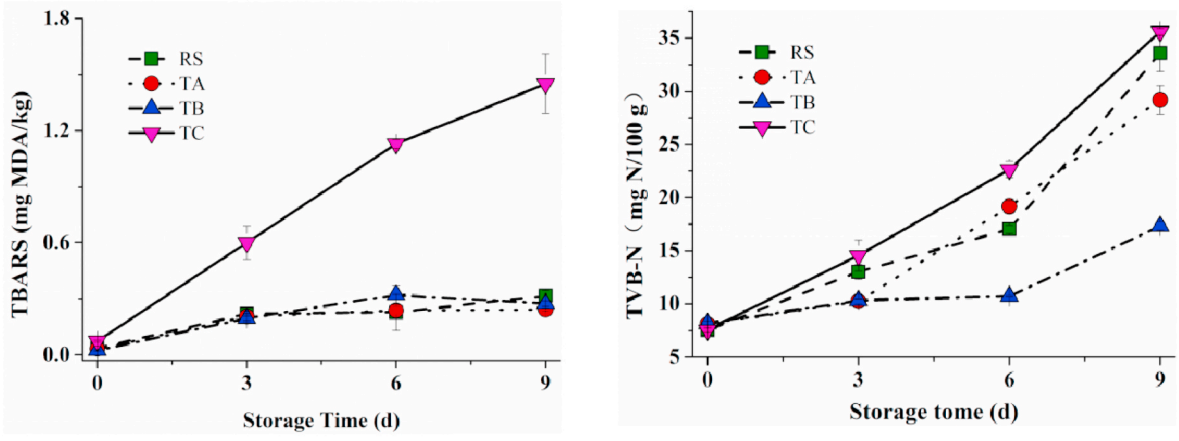


Fig. 3. Changes in TBARS (A) and TVB-N (B) of Russian sturgeon fillets during storage. RS, raw samples as control; TA, cooked in a 40 °C water bath for 10 min; TB, cooked in a 50 °C water bath for 10 min; TC, cooked in a 60 °C water bath for 10 min. MDA, malonaldehyde; N, nitrogen.

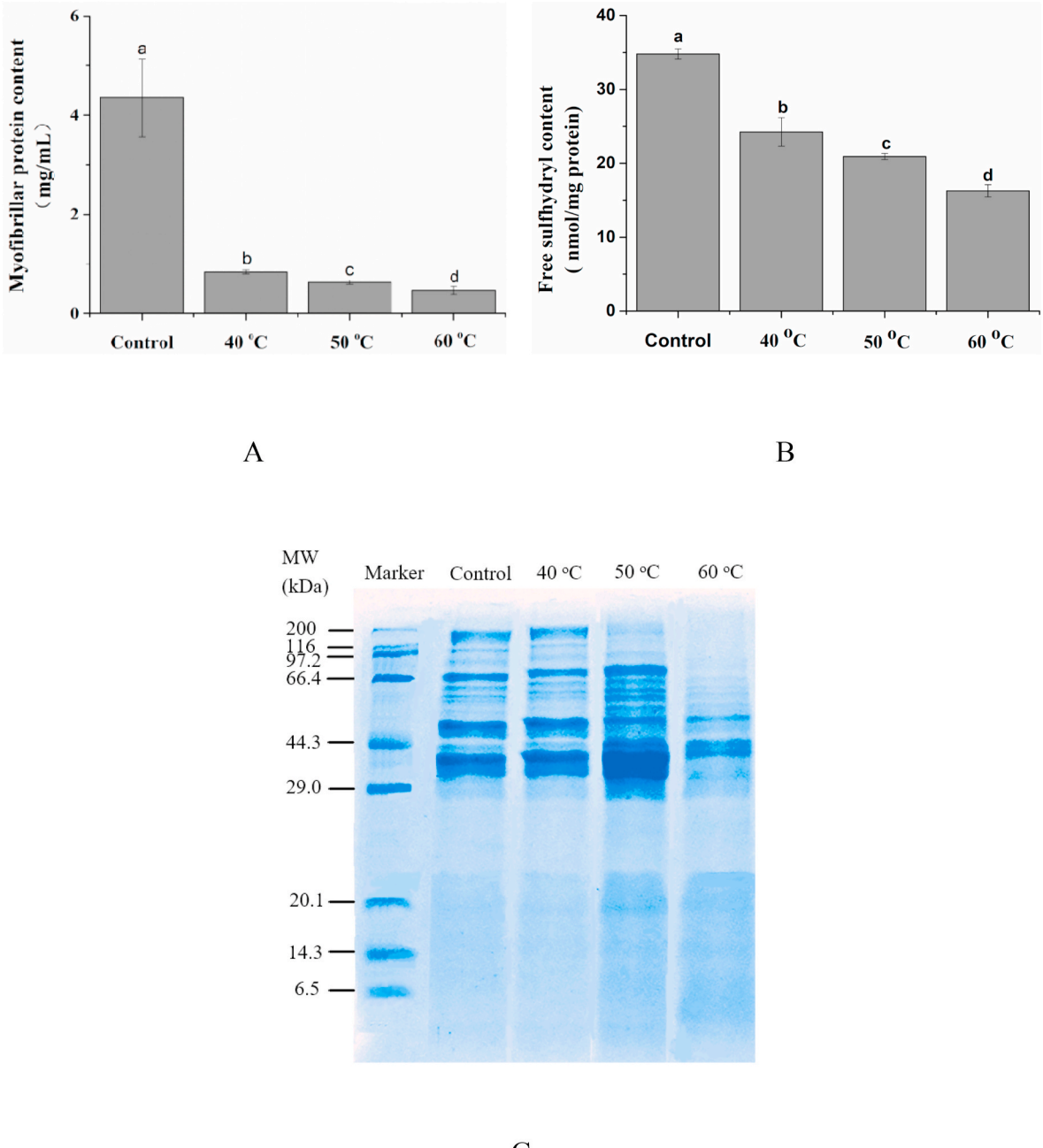


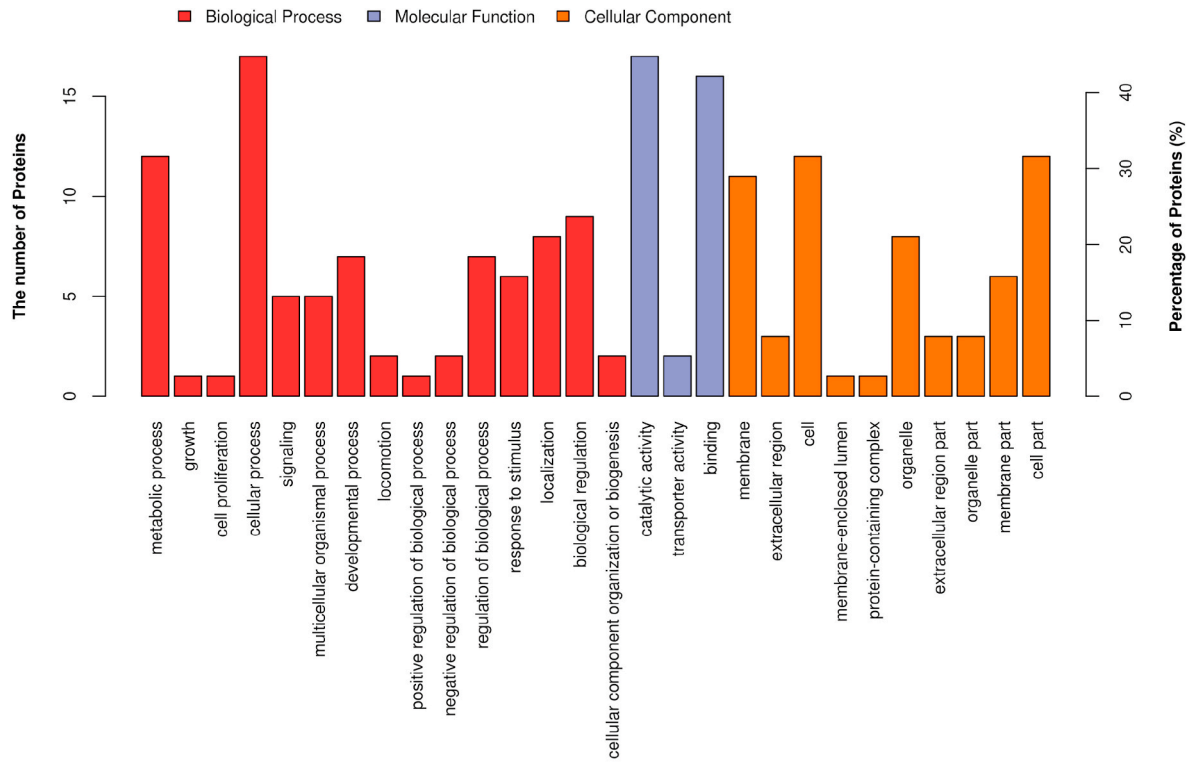
Fig. 4. Myofibrillar protein content (A), Free sulfhydryl content (B) and SDS-PAGE (C) of Russian sturgeon fillets after treating with SVC at 40 °C, 50 °C, 60 °C. The raw sample was as control.

reduced significantly between 50 °C to 60 °C (Dominguez-Hernandeza et al., 2018).

3.3. Freshness indicators analysis

The amount of secondary products produced from lipid oxidation were measured and expressed by TBARS values while 2 mg MDA/kg was regarded as the limit value (Chuesiang, Sanguandeekul, & Siripatrawan, 2020). The initial TBARS values were 0.02–0.04 mg MDA/kg in RS, TA and TB showed no significant difference while it was 0.07 mg MDA/kg in TC (Fig. 3A). The TBARS value of TC group was significantly higher than RS during storage and even close to the threshold value at 9 d while the other three groups only increased to 0.24–0.32 mg MDA/kg. Vacuum packaging of food ingredients in the SVC process was to reduce the contact with oxygen in order to decrease the degree of oxidation (Bhat, Morton, Zhang, Mason, & Bekhit, 2019). However, as a free radical chain reaction, lipid oxidation was easily affected by heating, light, transition metals and other factors (Noon, Mills, & Norton, 2020). Microwave, ozone, ultraviolet and other antibacterial treatments would also increase the degree of lipid oxidation (Feng et al., 2020; Pheomphun, Treesubsuntorn, & Thirayetyan, 2019; Pou et al., 1994).

Protein oxidation was interrelated with lipid oxidation while the oxidation products of one substance would promote the oxidation of another substance (Faustman, Sun, Mancini, & Suman, 2010). TVB-N was produced the degradation of non-protein nitrogenous compounds and proteins from microorganisms and enzymatic activities (Yazgan et al., 2017). As shown in Fig. 3B, the initial TVB-N values of four groups were 7.55–8.16 mg N/100 g which were slightly higher than the results



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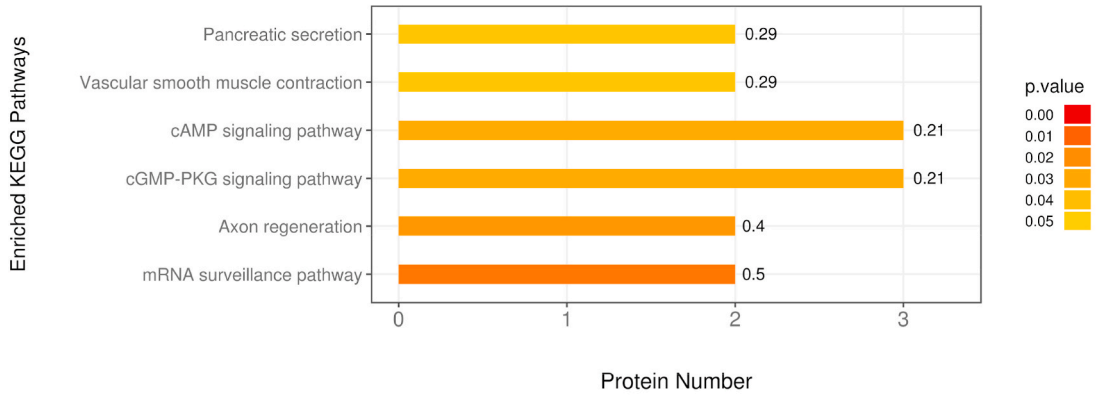

B

Fig. 5. GO enrichment and KEGG pathway analysis of the identified proteins in 50 °C vs 60 °C.

before (Chen et al., 2020). At 6 d, the TVB-N value of TC (22.64 mg N/100 g) already exceeded the upper limit of 20 mg N/100 g (Lu, Liu, Ye, Wei, & Liu, 2009) while the TB showed the lowest content. This might be the protein gel of sturgeon fillets formed at 50 °C to reduce the degree of decomposition while the protein gel was destroyed with temperature increasing to 60 °C (King, Seidel, & Lehrer, 1995; Wang, Xia et al., 2020; Yuan, Liu, Ge, Feng, & Gao, 2017). Although SVC at 60 °C can effectively extend the shelf life of Russian sturgeon fillets at the level of TVC, the shelf life at the level of TVB-N was 6 d. Thus, it was necessary to study the effect of SVC temperature on protein oxidation.

3.4. Analysis of protein structure and oxidation

Myofibrillar proteins (MP) as the main functional components which account for > 65% of total protein in muscle plays a vital role in meat processing (Li, Wang, Zheng, & Guo, 2020; Chen et al., 2017). MP gel formed by heat-induced has a great influence on the oxidation and degradation of protein (Tornberg, 2005). As shown in Fig. 4A, the content of MP was significantly reduced with SVC temperature increase. SVC could promote the crosslinkage of myosin which has a transitional temperature around 47–50 °C in the MP and reduced the solubility of MP (Wang, Zhou et al., 2020; Wang, Lin, Cheng, & Tan, 2020). The changes in free SH contents of samples were reflected in Fig. 4B. Similarly, the content of free sulfhydryl decreased significantly as SVC temperature increased. The sulfhydryl group which was on the surface of the native protein network plays an important role in the formation of gels (Ai et al., 2020; Wang, Kong, et al., 2020). The protein electrophoretic pattern of MP was shown in Fig. 4C. There was little change between the bands of control and 40 °C. The myosin heavy chain (MHC, approximately 200 KDa) was almost lost at 50 °C, while the protein band between actin (approximately 44.3 KDa) and myosin light chain (MLC, approximately 29 KDa) became darker. When the SVC temperature rised to 60 °C, only actin has a slight color on the band. Myosin are sensitive to oxidative attack due to the several cysteine residues (Li & Xiong, 2015). These results indicated that SVC pretreatment would promote the oxidation and degradation of protein which may caused by the potential formation of free radicals (Xiong, Blanchard, Ooizumi, & Ma, 2010).

3.5. GO and KEGG analysis

In order to investigate the reason for the difference in oxidation and degradation of protein during storage after SVC treatment at 50 °C and 60 °C, differential and comparative proteomics methods were used to analyze the complex protein composition from Russian sturgeon meat at a molecular level. GO enrichment on three categories (biological process (BP), cellular component (CC), and molecular function (MF)) was classified in Fig. 5A. The main differences between the protein treated with 50 °C and 60 °C were BPs and CCs, especially the cell proliferation and metabolic process which may be due to the different stress responses of the protein to different temperature (He et al., 2019). The MFs of differentially expressed protein in 50 °C versus 60 °C were highly enriched in binding and catalytic activity. The CCs were mainly involved in cell, cell part and membrane. KEGG pathway enrichment analysis was considered the whole quantified proteins as background dataset which was applied on the basis of Fisher's exact test (Zhang et al., 2018; Ma et al., 2020). The significantly affected metabolism and signal transduction pathways ($P < 0.05$) were focused on pancreatic secretion, cAMP signaling pathway, vascular smooth muscle contraction, cGMP-PKG signaling pathway, axon regeneration and mRNA surveillance pathway (Fig. 5B). The color gradient closer to red corresponded to the higher the significance level of the enrichment of the KEGG channel (Cao, Hou, Hussain, Zhang, & Wang, 2020; Tang, Zhang, Gong, Yan, & Shi, 2019).

4. Conclusions

SVC treatment at 60 °C could effectively extend the shelf-life of Russian sturgeon fillets during storage at 4 °C, especially inhibited the growth of *Pseudomonas* and *Aeromonas*. However, the treatment applied produced some other quality deterioration including lipid oxidation and protein oxidation and degradation. The myofibrillar protein (MP) content was reduced with the degradation of MHC and MLC, and the reduction of the sulfhydryl content meaning that the oxidation increased. Moreover, the cell proliferation and metabolic process of BPs, the binding and catalytic activity of MFs and the cell, cell part and membrane of CCs showed significantly different results between 50 °C and 60 °C of SVC. In conclusion, the high temperature of SVC was efficient to preserve microbial quality during storage. Once the problem of protein oxidation would be solved, SVC could be developed to extend the shelf life of muscle foods.

CRediT authorship contribution statement

Wen-qiang Cai: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Yue-wen Chen:** Methodology, Resources, Supervision, Project administration, Funding acquisition. **Xiu-ping Dong:** Methodology, Writing - review & editing. **Yu-gang Shi:** Methodology, Writing - review & editing. **Jian-ling Wei:** Validation, Investigation. **Fei-jian Liu:** Validation, Investigation.

Conflict of interest

Please check the following as appropriate:

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

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