Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.26423

Analysis of storage quality of crayfish (*Procambarus clarkii*) meat based on sous-vide cooking

Qing ZHAO[1,2], Hailan LI[2], Jun CAI[1], Tao LIAO[2], Liang QIU[2], Xiaoyan ZU[2]*

This study aimed to investigate the effect of different combinations of temperature and time of sous-vide (SV) on the quality of crayfish (*Procambarus clarkii*) meat. The changes in quality indexes and volatile components of crayfish meat after SV treatment were analyzed after refrigeration at 4°C for 1–7 days. The L* values of crayfish meat were higher in the SV group than in the control group ($p<0.05$). Heating at 75° effectively suppressed the decrease in hardness and increase in total volatile basic nitrogen (TVB-N) and total viable count (TVC) of crayfish meat ($p<0.05$). A heating time of 15 min was optimal for most of the indicators. TVB-N, thiobarbituric acid, and TVC were positively correlated with each other and negatively correlated with hardness. Using GC-MS, 59 volatile compounds were identified with a match of >85%. During storage, the highest percentages of volatile alkanes and aromatic hydrocarbons were 44.57 and 14.81%, respectively, with 33 species of aromatic hydrocarbons being identified. In conclusion, SV treatment with a heating temperature of 75° and heating time of 15 min improved the texture and flavor quality of crayfish meat during storage.

Keywords: sous-vide; crayfish; storage; meat qualities; volatiles.

Practical Applications: Sous-vide (SV) technology is effective in reducing the rate of water loss and inhibiting lipid oxidation during cooking. SV treatment at 75° for 15 min can improve the texture and flavor quality of crayfish meat during storage.

1 INTRODUCTION

Sous-vide (SV) involves cooking of vacuum-packed food at low temperatures under temperature- and time-controlled conditions (Thathsarani et al., 2022). SV has been reported to be effective in reducing the rate of loss of water and vitamins during cooking under vacuum bag sealing and prolonged steaming at low temperature (Schellekens, 1996). It has been widely used in restaurants and food industry production processes (Kathuria et al., 2022). Compared to conventional processing, SV processing allows precise temperature control during processing and reduces the loss of nutrients and moisture from the food (Li et al., 2022). At the same time, vacuum packaging isolates oxygen and bacteria from the external environment, which facilitates the standardization of industrial production, ensures food hygiene, and extends the shelf life of food products (Karki et al., 2022).

Kato et al. (2017) showed that SV-treated soy sauce and basil sauce fillets refrigerated at 1±1° resulted in a shelf life of up to 42 and 49 days, respectively, and the flavor was well perceived by tasters. Olatunde and Benjakul (2021) showed that the thiobarbituric acid (TBA) value and peroxide value (PV) of SV-treated crab meat did not change significantly during refrigeration at 4°C, resulting in a shelf life of >60 days. Kurt Kaya et al. (2022) compared the indices of marinated crayfish (*Astacus leptodactylus*) stored at 4±1° for 120 days and reported that the SV group had the lowest TVB-N, TBA, and PV values for crayfish meat (vs. brine, vacuum-packed treatment). Özturan and Ünal Şengör (2022) showed that the SV group had the lowest cooking loss (1.25 g) and the highest yield ratio (97.29%) of crayfish compared to the microwave (MC) and boil-cooked groups. Xiao et al. (2021) investigated the effects of cooking methods such as SV, steaming (ST), and boiling (BO) on squid and showed that the SV group had the lowest water loss. Additionally, the cooking loss was lower in the SV group than in the ST and BO groups by 38.2 and 25.9%, respectively, which was more favorable for squid tenderness, and the quality of the SV-treated squid was the best. In all the above studies, SV treatment of the aquatic products showed superior results in terms of maintaining the flavor, product quality, and prolonging the shelf life of the food compared to the traditional treatment.

Crayfish, *Procambarus clarkii* (Ye et al., 2022), are rich in protein, delicate, and tasty (Abd-Allah & Abdallah, 2006; Cremades, 2003). Currently, only a few studies have reported on the optimal process of SV treatment of crayfish and on the changes in quality and flavor components during storage. This study aimed to investigate the changes in the storage quality of crayfish during 1–7 days of refrigeration at different SV temperatures and times using cooking loss rate (CLR), hardness value, lightness (L*), TBA, total volatile basic nitrogen (TVB-N), and total viable count (TVC) as evaluation indicators. Principal component analysis (PCA) was used to determine the optimal process for SV of crayfish at different temperature-time combinations. And the volatile components of crayfish meat during refrigeration were investigated. The findings of this study could provide data support for the optimization of the crayfish production process and the improvement of quality and flavor during storage.

Received 5 Apr., 2023
Accepted 12 Jun., 2023
[1]*Hubei University of Technology, Ministry of Education, Hubei Provincial Cooperative Innovation Center of Industrial Fermentation, Key Laboratory of Fermentation Engineering, Hubei Key Laboratory of Industrial Microbiology, Wuhan, China.*
[2]*Hubei Academy of Agricultural Sciences, Ministry of Agriculture and Rural Affairs, Institute of Agro-Products Processing and Nuclear Agricultural Technology, Key Laboratory of Cold Chain Logistics Technology for Agro-product, Wuhan, China*
*Corresponding author: zuxiaoyan@hbaas.com

2 MATERIALS AND METHODS

2.1 Materials and reagents

Fresh crayfish (n=150) were provided by Qianjiang Changgui Aquatic Product Co., Ltd. (Hubei Province, China), each weighing 40±5 g and measuring 12.5±0.5 cm in length. Concentrated sulfuric acid, glacial acetic acid, absolute ethanol, methyl red, boric acid, sodium chloride, and magnesium oxide were provided by Sinopharm Chemical Reagent Co., Ltd. Bromide cresol green was supported by Shanghai Yuanye Bio-Technology Co., Ltd. Plate count agar was provided by Qingdao Hi-tech Industrial Park Hope Bio-technology Co., Ltd. Alonondialdehyde (MDA) kit was provided by Nan-Jing JianCheng Bioengineering Institute. Vacuum packaging bags (food-grade PA + PE + CPP, temperature resistance: -20–130°C, size 20×30 cm, monolayer, thickness 0.1 mm) were provided by Wenzhou Hongyi Packaging Co., Ltd. (Zhejiang, China).

2.2 Experimental methods

2.2.1 Single-factor experiments and sample processing

The cooking time was fixed at 10 min, the cooking temperature was set at different temperatures (55, 65, 75, and 85°C), the cooking time was set at different lengths (10, 15, 20, and 25 min), and the quality of crayfish was investigated after SV treatment at 4°C for 1, 3, 5, and 7 days. The CLR and other indicators of the SV group were compared with those of a control group, which included crayfish samples without any heating treatment. Three replicates were used for each group. The fresh crayfish were washed and de-headed, wiped dry, put into vacuum packing bags, and sealed by a vacuum packing machine (Digital Vacuum Packing Machine, Xiamen Jieding Machinery Co., Ltd., Xiamen, Fujian, China). The samples were placed in a thermostatic water bath (Changzhou Zhiborui Instruments Co., Ltd., Changzhou, Jiangsu, China) for SV treatment and then immediately cooled to below 5°C in ice water and stored at 4°C for 1, 3, 5, and 7 days for the determination of relevant indicators.

2.2.2 Determination of CLR

The total mass of the vacuum bag and the sample was recorded as m_1 (g), and then the vacuum bag was cut open, and the juice inside the bag and on the surface of the sample was immediately blotted out with filter paper. The bag and the sample were weighed together as m_2 (g), and the bag alone was weighed as m_3 (g). The process was repeated thrice, and the average value was considered for analysis (Equation 1).

$$\mathrm{CLR}(\%) = \frac{m_1 - m_2}{m_1 - m_3} \times 100\% \quad (1)$$

2.2.3 Determination of hardness

Taking the second to third abdominal segments of crayfish tail meat, samples were placed on a textural loading platform, and the textural properties of the crayfish meat were determined using a P/36R probe (TA-XTplus Texture Analyser, Stable Micro System, UK). The following parameters were used in the texture profile analysis mode: pre-test rate 2 mm/s, test rate 3 mm/s, return rate 3 mm/s, compression distance 5 mm, and trigger force 5 g. All tests were performed in triplicate, and the results were averaged.

*2.2.4 Measurement of L**

A colorimeter (SC-80C, Beijing Kangguang Instruments Co., Ltd., Beijing, China) was used to determine the L* values of crayfish tail meat at the second and third abdominal segments. The L* values of each sample were measured in triplicate, and the measurement was repeated six times.

2.2.5 Determination of TBA

Crayfish meat (5 g) was precisely weighed in a 50-mL centrifuge tube, homogenized with 20 mL of water and 25 mL of 20% TCA for 1 min, allowed to stand for 65 min, and then centrifuged at 4,300×g for 10 min (benchtop high-speed refrigerated centrifuge, Shanghai Anting Scientific Instruments Factory, Shanghai, China). The supernatants were collected and added to 50 mL of distilled water, 5 mL of filtrate was added with 5 mL of a 0.02 mol/L TBA solution in a boiling water bath for 25 min, and then removed and cooled under running water for 5 min. The sample absorbance (A) was measured at a wavelength of 532 nm using a spectrophotometer (UH5300-UV Spectrophotometer, Shanghai Tianmei Scientific Instruments Co., Ltd., Shanghai, China) after cooling (Equation 2).

$$\mathrm{TBA(mg/100g)} = A \times 7.8 \quad (2)$$

2.2.6 Determination of TVB-N

The determination of volatile basic nitrogen in food was conducted according to the automatic Kjeldahl method described in GB 5009.228-2016 "National Food Safety Standard-Determination of volatile basic nitrogen in food". First, the reagent blank was determined using an automatic Kjeldahl analyzer (Shanghai Anting Scientific Instruments Factory, Shanghai, China), and the blank value was measured. After the sample was stirred well, 10 g was taken in a distillation tube, and 75 mL of water was added, shaken well, and allowed to soak for 30 min. Equation 3 was used for the calculation as follows:

$$\mathrm{TVB-N(mg/100g)} = \frac{(V_1 - V_2) \times c \times 14}{m} \times 100 \quad (3)$$

Where:

V_1: the volume (mL) of the hydrochloric acid standard titration solution consumed by the test solution;

V_2: the volume (mL) of the hydrochloric acid standard titration solution consumed by the reagent blank;

c: the concentration (mol/L) of hydrochloric acid standard titration solution;

14: the mass (g/mol) of nitrogen for titrating 1.0 mL of hydrochloric acid [c(HCl) = 1,000 mol/L] standard titration solution;

m: the mass (g) of the sample;

100: the conversion factor.

2.2.7 Determination of TVCs

Colony counts were determined according to the method described in GB 4789.2-2016 "Microbiological examination of food hygiene-Detection of aerobic bacterial count." A sample (25 g) was weighed, and 225 mL of dilution solution was added. The sample plus solution were homogenized and diluted 10 times. Then, 1 mL of diluent sample was added to sterile petri dishes, and 20 mL of plate-count agar medium was added and mixed well. After incubation, the colonies were counted, and the total number of colonies was calculated.

2.2.8 Determination of volatile flavors

To prepare samples, crayfish samples were deveined and mashed, and 2 g of the sample was weighed into a 15-mL extraction vial, which was quickly sealed. The solid-phase microextraction (SPME) extraction fiber head was aged at 250°C in a gas chromatography-mass spectrometry (GC-MS) injection port (Model 7890A-5975C, Agilent Technologies, Santa Clara, California, U.S.) until no spurious peaks were observed. The vial was placed on a SPME device (Supelco, Bellefonte, PA, U.S.) and preheated at 80° for 15 min. The SPME extraction head was inserted into the headspace of the sample through the vial cap, the fiber head was pushed out, and the headspace extraction was performed for 40 min.

Chromatography was performed using an HP-INNOWAX column (60.0 m × 250 μm, 0.25 μm). The temperature of the column was initially maintained at 32° for 3 min, ramped up to 60° at 4°C/min, then ramped up to 100°C at 8°C/min for 10 min, and ramped up to 240°C at 10°C/min for 10 min. The gasification chamber temperature was 250°C, the transmission line temperature was 250°C, the carrier gas was He, the carrier gas flow rate was 1 mL/min, and injection was splitless. Mass spectrometry was performed under the following conditions: the source was an electron impact source; the electron energy was 70 eV; the ion source temperature was 230°C; the quadrupole was 150°C; the scanning mode was "Scan"; and the scanning mass range was 20–500 amu.

The volatile components and relative contents of crayfish samples stored for 1–7 days were analyzed and identified; 180 volatile compounds were detected. Substances with a match of >85% were selected, and their contents were analyzed by row standardization and cluster analysis and then plotted in a heat map.

2.2.9 Statistical analysis

The data were processed and plotted using the Microsoft Excel 2016 software (Microsoft) and Origin 2019 software (OriginLab Corporation). ANOVA was performed using the SPSS 26.0 software (International Business Machines Corporation). Multiple comparisons between groups were performed using the graph-base method. Means within indicators were determined using the least significant difference method with 95% confidence ($p<0.05$). Heat map analysis was performed using the TBtools software (Chen et al., 2020).

3 RESULTS AND DISCUSSION

3.1 CLR of crayfish

Figure 1 shows that the CLRs of most samples of the SV treatment groups were significantly greater than those of the control group ($p<0.05$), and the CLR was proportional to the heating time. The CLR of crayfish meat was not significantly different with increasing heating temperature ($p>0.05$), but the CLR of samples from the 55°C group was higher than the CLR of samples from other SV groups. This may be due to the fact that the gel of the meat products was not fully formed and that the water retention was poor, resulting in a higher rate of juice loss (Liu et al., 2021a).

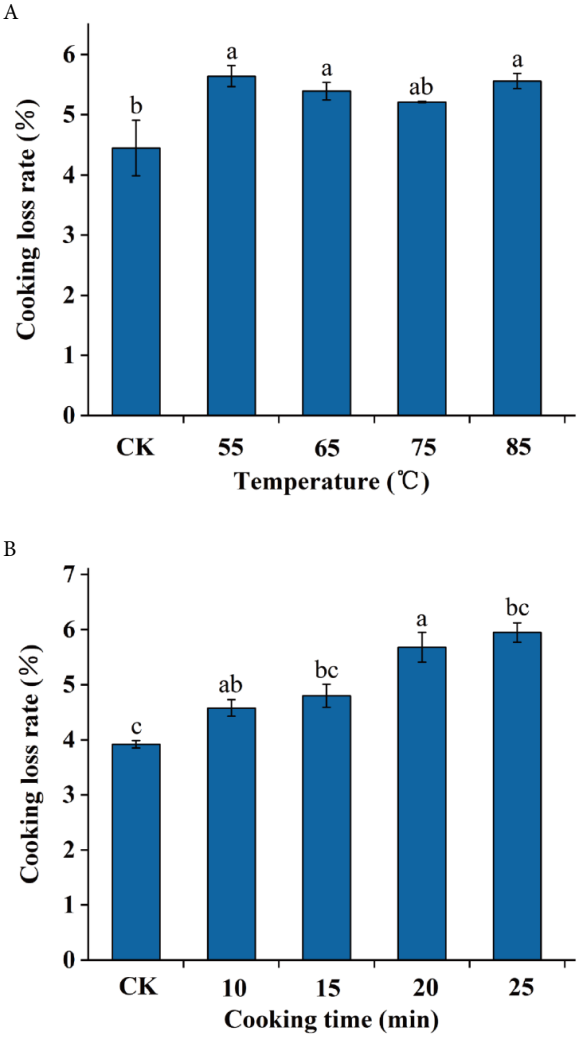

Figure 1. Effects of different SV temperatures (a) and times (b) on the cooking loss rate of crayfish. Different lowercase letters indicate significant differences among groups with different heating temperatures and times ($p<0.05$).

3.2 Hardness and L of crayfish meat*

The hardness of the control samples decreased significantly ($p<0.05$) with increasing storage time, while that of the SV samples first increased and then decreased (Figure 2A). At 1 day of storage, the hardness of most samples of the SV groups was significantly lower than the hardness of samples in the control group ($p<0.05$). However, at 3–7 days of storage, the hardness of the SV groups was significantly higher than that of the control group ($p<0.05$). The hardness of crayfish meat in the SV groups was lower in the early stages of storage due to a large number of products (mainly aldehydes) produced by myofibrillar proteins during cooking, which caused cross-linking of myofibrillar proteins and changes in the protein structure (Karki et al., 2022). At the later stage of storage, crayfish proteins in the control group reacted with enzymes due to the action of microorganisms, resulting in the destruction of the tissue structure of the crayfish meat, which resulted in lower hardness (Wan et al., 2019). At 7 days of storage, the hardness of most SV groups decreased, which may also be associated with the action of microorganisms and enzymes. Overall, treatment at 75°C delayed the decrease in the hardness of crayfish meat.

The hardness of the SV samples was significantly lower than that of the control group samples at 1 day of storage ($p<0.05$), and the hardness of the 20-min group was the highest (Figure 2B). With the increase in heating time, the water content of crayfish meat decreased, the density of myogenic fibers increased, the muscle hardness and cohesiveness increased, and the denatured proteins reaggregated to form a mesh-like structure; thus, the hardness decreased (Gao et al., 2016). At the same time, the hardness of crayfish meat decreases with the extension of storage time under the action of microorganisms and hydrolytic enzymes.

Figures 2C and 2D show that the L* of the samples in the SV group was significantly higher than that in the control group during storage ($p<0.01$), but no significant differences were noted among the SV groups at different temperature times ($p>0.05$). The increase in L* due to heating may be due to light scattering from the denaturation and agglutination of sarcoplasmic and myofibrillar proteins caused by the increase in temperature (Lan et al., 2021). During the storage period, the change in L* values in each SV group was relatively stable, mainly because vacuum sealing increased the proportion of deoxygenated myoglobin, which is thermally more stable than oxidative myoglobin and metmyoglobin (Pulgar et al.,

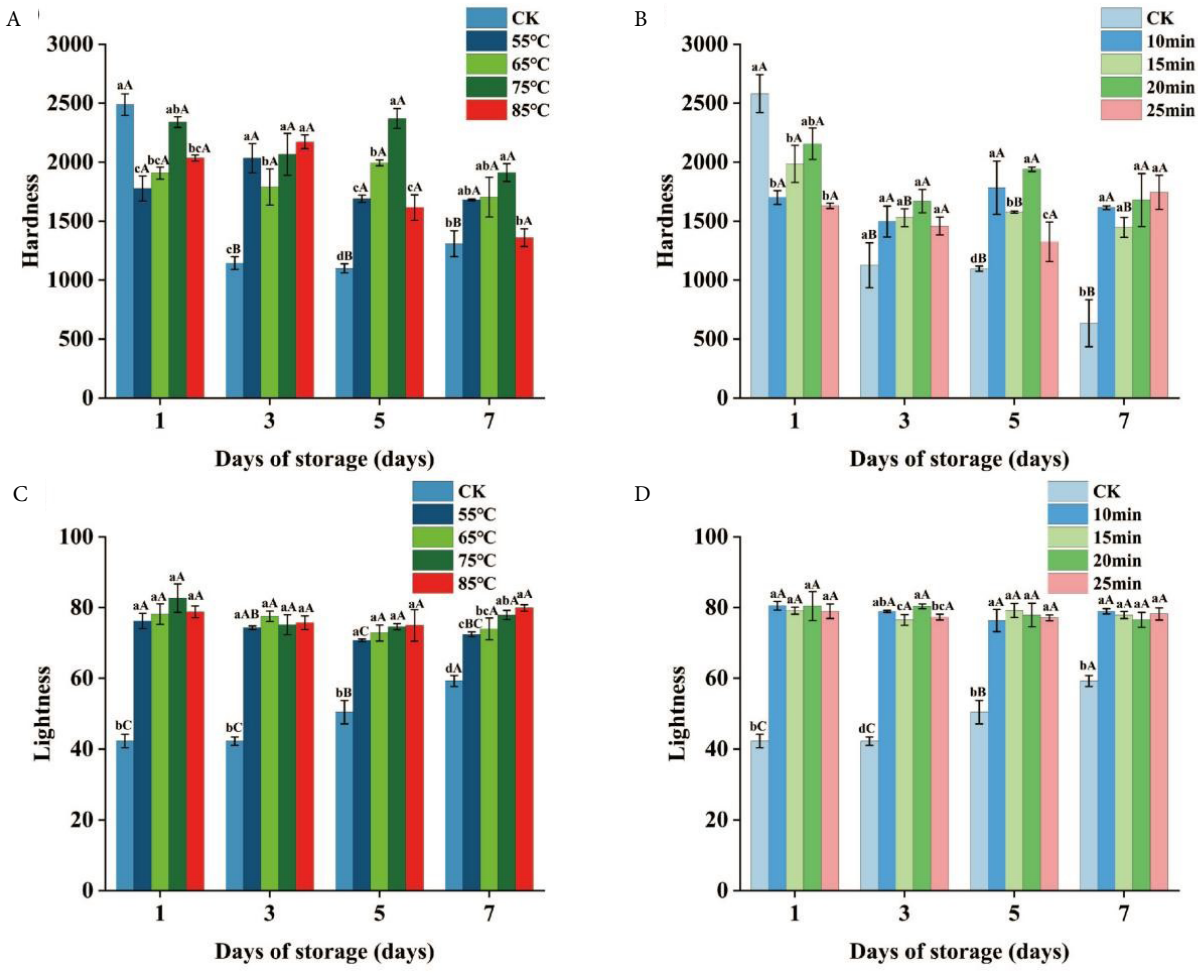

Figure 2. Effect of different SV temperatures and times on the (A) hardness, (B) L* (C), and (D) of crayfish during storage. Different lowercase letters indicate significant differences among groups with different heating temperatures and times and the same storage time ($p<0.05$); different capital letters indicate significant differences among groups with different storage times and the same heating temperature and time ($p<0.05$).

2012). Mohan et al. (2017) compared the effects of ordinary air packaging, vacuum, and SV processing on Indian white shrimp during refrigeration and showed that the color and appearance of the samples during storage were better in the SV group, showing a characteristic bright orange color with a more stable variation over 6 days; this is similar to the results of the present experiment.

3.3 TBA values of crayfish meat

The TBA values of most samples in the SV group were higher than those in the control group, and the TBA values in the 85°C group were lower than those in the other SV groups (Figure 3A). The TBA values of the 10, 15, and 25 min SV groups showed an increasing trend with the increase in the storage time and then showed a decrease by the 7th day (Figure 3B). At the same storage time, the TBA values of crayfish treated with heat for 20 and 25 min were lower on average than those in other SV groups, by 34.93 and 37.31%, respectively. The decrease in the TBA values due to longer heating time may be related to the increase in the central temperature of the samples, close to the heating temperature. Malondialdehyde is a major end-product of lipid oxidation, and TBA is commonly used in experiments to characterize the amount of malondialdehyde in the secondary products of lipid oxidation, thus indicating the degree of lipid oxidation (Weber et al., 2008). Pulgar et al. (2012) showed that pork in the 80°C SV group had lower TBA values than pork in the 60°C SV group. Using systematic simulation, Adams et al. (2008) reported that higher heating temperatures induced a rapid reduction in malondialdehyde content. Roldan et al. (2014) and Ventanas et al. (2007) suggested that this was due to the fact that higher heating temperatures induced a rapid reaction of malondialdehyde with proteins, phospholipids, DNA, or amino acids in the meat, which contain primary amine groups, ultimately lowering the TBA values.

3.4 TVB-N and TVC of crayfish meat

The TVB-N values of the samples were positively correlated with the storage days (Figure 4A). Compared with the control group, the TVB-N values of the SV group samples increased more slowly. At 3–7 days of storage, the TVB-N values of samples from the 75 and 85°C SV groups were significantly lower than those of samples from the 55 and 65°C groups ($p<0.05$). At the same heating temperature, the TVB-N values of the 15, 20, and 25 min groups were significantly higher than those of the 10 min group at 3–5 days of storage ($p<0.05$; Figure 4B). During the storage period, the TVB-N values of the SV group samples did not exceed the limit value of 20 mg/100 g for aquatic products and were at edible levels. TVB-N comprises ammonia and low-level amines produced by the decomposition of proteins in aquatic products by the action of bacteria and enzymes and is usually used as an indicator of freshness in meat products (Li et al., 2019). As the heating temperature increased, the TVB-N values gradually decreased, indicating that the heat treatment at 75 and 85°C effectively inhibited bacterial growth and enzymatic activity, thus delaying the spoilage of meat. However, prolonged heating time resulted in high central temperatures of the samples, destroying some of the proteins in the crayfish meat and leading to higher TVB-N values (Giavasis et al., 2012).

The TVC values of all groups of samples were directly proportional to the storage time (Figures 4C and 4D). During storage, the TVC values of crayfish meat in the control group increased rapidly, while those in the SV group increased slowly and were significantly lower than those in the control group ($p<0.01$). TVC reflects the metabolism of amino acids and proteins, so it is often used to measure the degree of spoilage in meat products. SV treatment was effective in inhibiting the growth of microorganisms, which is consistent with the finding of González-Fandos (2004) that SV treatment of rainbow trout at 90°C for 3.3 min and storage at 2°C resulted in lower colony counts than other treatments. García-Linares et al. (2004) and Paik et al. (2006) reported that the higher the heating temperature of the SV-treated fish, the lower the number of mesophilic and psychrophilic bacteria in the fish. Other researchers have also suggested that heating can significantly reduce the microbial count of the product, but high temperatures can also reduce its

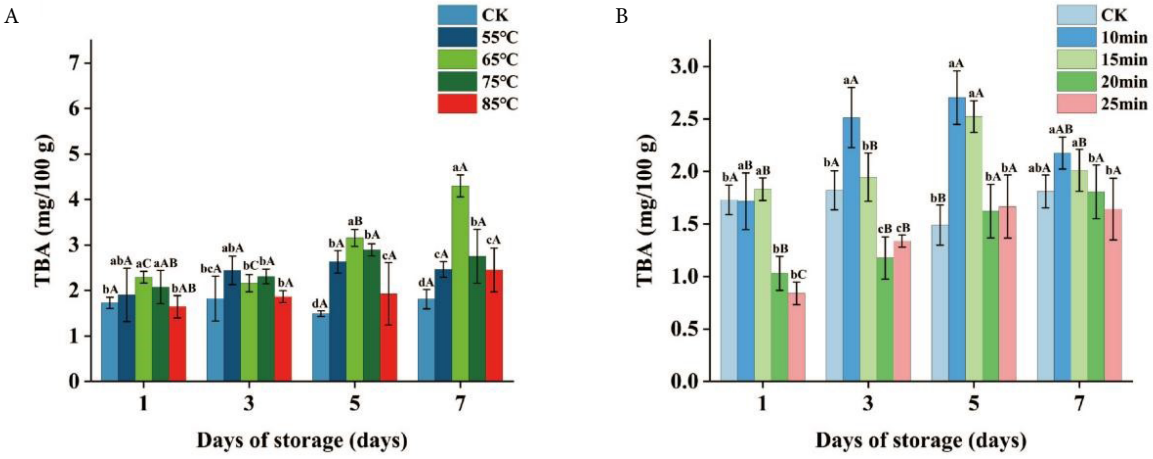

Figure 3. Effect of different SV (A) temperatures and (B) times on TBA values of crayfish. Different lowercase letters indicate significant differences among groups with different heating temperatures and times and the same storage time ($p<0.05$); different capital letters indicate significant differences among groups with different storage times and the same heating temperature and time ($p<0.05$).

quality (Christensen et al., 2011). The optimum temperature should be determined by considering microbial control and product quality comprehensively. Therefore, the optimal process parameters for SV crayfish were further determined by PCA.

3.5 PCA to determine optimal SV temperature and time

Figures 5A and 5B show that the total contribution rates of principal components (PC1, PC2) were 83.3 and 76.8%, respectively. These results indicate that the two components represent

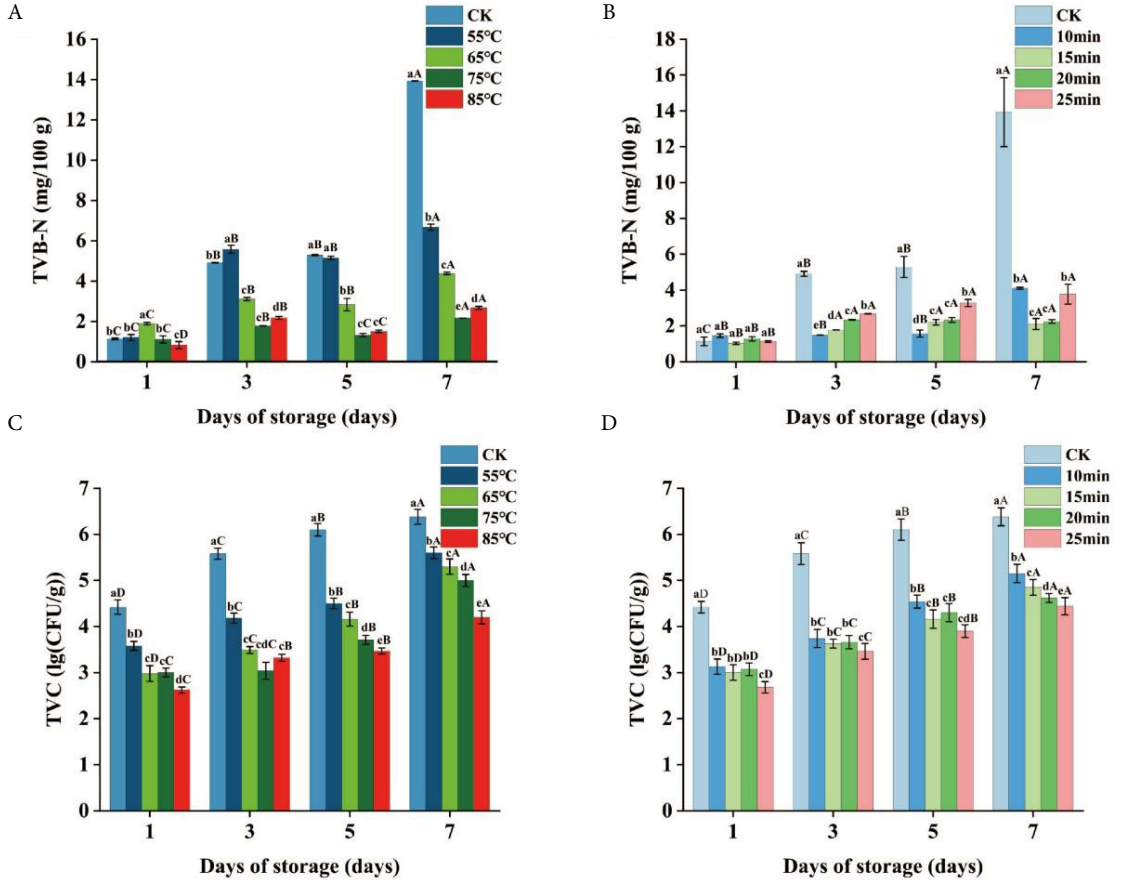

Figure 4. Effect of different SV temperatures and times on (A) total volatile basic (B) nitrogen, and (C) total viable bacterial colony count, (D) of crayfish during storage. Different lowercase letters indicate significant differences among groups with different heating temperatures and times and the same storage time ($p<0.05$); different capital letters indicate significant differences among groups with different storage times and the same heating temperature and time ($p<0.05$).

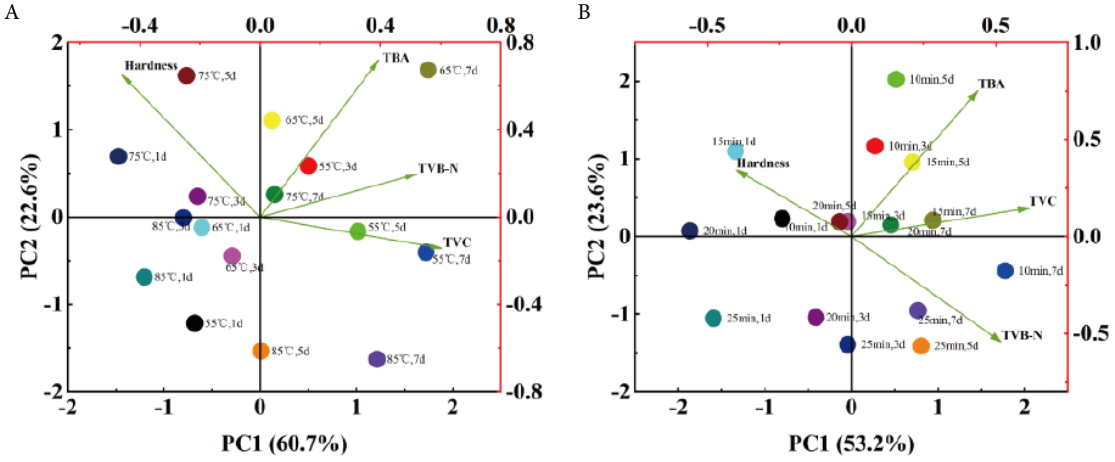

Figure 5. PCA of the effects of SV (A) temperature and (B) time on crayfish meat during storage. (A) PC1 reflects the information on storage time, and PC2 reflects the information on heating temperature. (B) PC1 reflects the information on storage time, and PC2 reflects the information on heating time.

the main characteristic information of the samples. It can be seen from Figure 5A that the TVB-N, TBA content, and TVC were positively correlated with each other and negatively correlated with hardness; the TVC, TVB-N, and TBA content were more influenced by storage time. The closer to the left side of the horizontal axis, the better the quality and freshness of the samples. A comprehensive analysis of the effects of different SV heating temperatures on the quality of crayfish showed that the 75°C treatment group not only slowed the decrease in the hardness of crayfish and reduced the loss of crayfish nutrients but also effectively controlled the proliferation of microorganisms and maintained the freshness of crayfish meat. The 15- and 20-min SV groups showed relatively better results, and the 15-min treatment was effective in controlling the growth of microorganisms.

3.6 Volatile substances in SV crayfish meat during refrigeration

A total of 59 volatile compounds with a matching degree of >85 were detected during sample storage: 17 alkanes, 33 aromatic hydrocarbons, 7 alkenes, 1 ester, and 1 thioether (Figure 6). Acetophenone, ethene, and 1,1-dichloro- were among the compounds with significantly higher contents at day 0 than at other days of storage. At day 1, the compounds with significantly higher contents compared to other days were o-Cymene, benzene, 1-ethyl-3,5-dimethyl-benzofuran, and others. At day 3, the significantly higher contents involved benzene, 2-ethyl-1,4-dimethyl-, and 1-ethyl-4-methylcyclohexane. Moreover, benzene, 1,2,3,4-tetramethyl-, dodecane, and tridecane were significantly higher on day 5, and tyrene, ethylene, 1,2-dichloro-, (E)-, benzene, and 1,3,5-trichloro- were significantly higher on day 7. The variation in the type and content of volatile compounds during storage resulted in changes in crayfish flavor with increasing storage time.

Volatiles are produced during the storage of seafood by the action of endogenous enzymes and microorganisms and have their own unique flavors. As storage time increases, proteins, fats, and carbohydrates are gradually decomposed into small molecules such as ketones and esters (Yuan et al., 2022). Alkanes are generally derived from the degradation of fats and oxidation of amino acids; they have a high odor threshold value and do not have a significant effect on the flavor of crayfish (Geng et al., 2022). Ketones, which have low odor threshold values, impart floral and fruity flavors to foods and are produced by the oxidation of

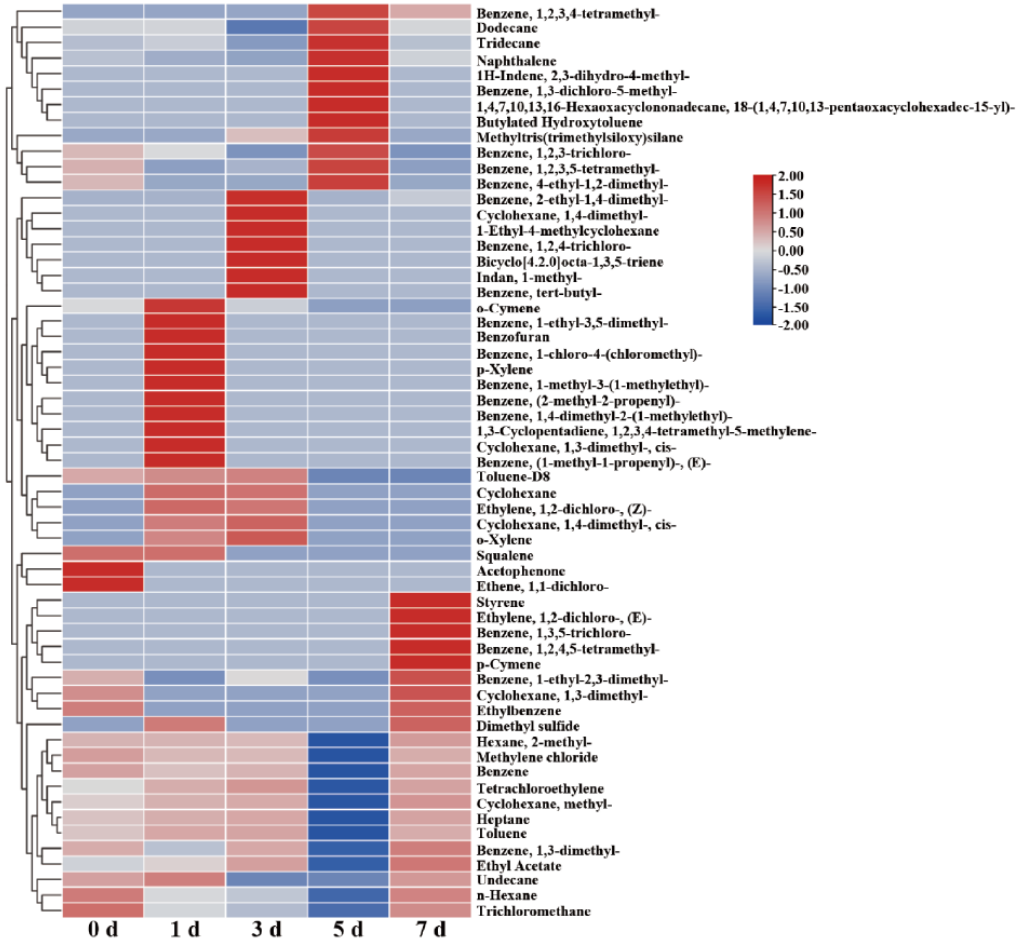

Figure 6. Heat map of volatile compounds of crayfish with a match of >85% at different storage times. The color blocks reflect the content of the compounds at the corresponding storage time, and similar rows are clustered. The red blocks represent high content of the compound at the corresponding storage time, while the blue blocks represent low content of the compound at the corresponding storage time. The row clustering tree is plotted in the figure, as shown on the left side of the heat map.

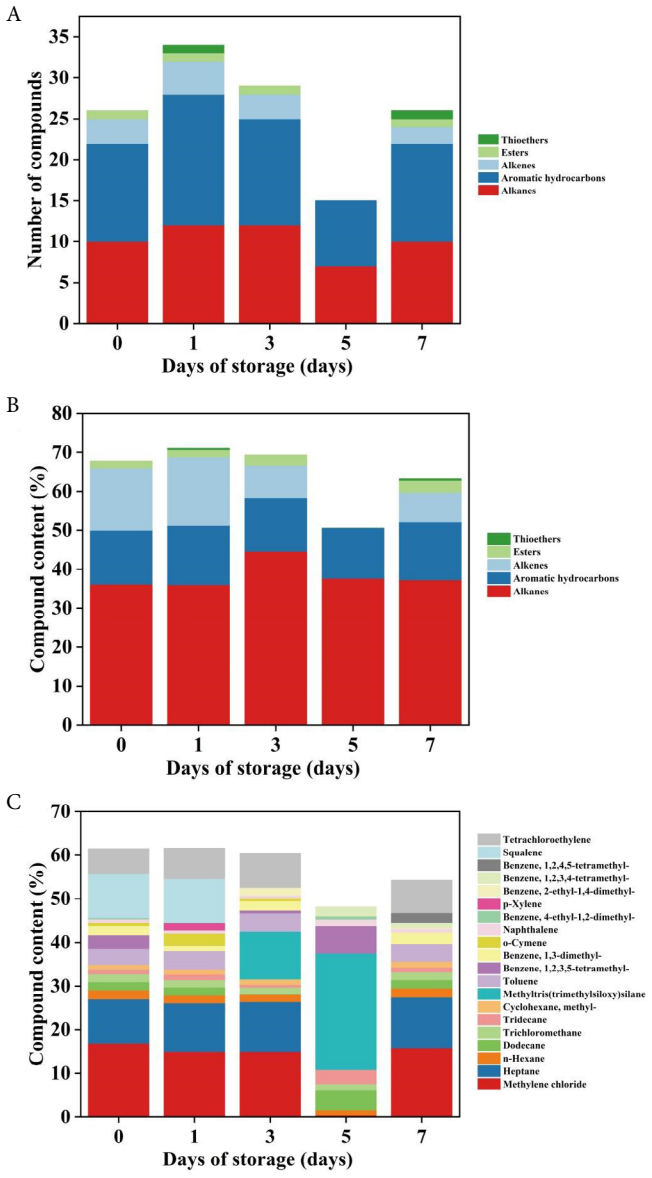

Figure 7. Stacked plots of the (A) number and (B) content of volatile compound species with a different storage time and matching >85% for crayfish, and stacked plots of the contents of top 10 volatile compounds with different storage times and (C) matching >85%.

unsaturated fatty acids, thermal degradation, amino acid degradation, the Maillard reaction, and microbial oxidation (Van Ba et al., 2013). For example, acetophenone has a hawthorn-like odor. Esters are thought to be the products of the lipid metabolism of carboxylic acids and alcohols, and the formation of esters affects odor changes (Yao et al., 2021), with the relative amount of ethyl acetate, which may be a characteristic flavor substance for spoilage of SV crayfish, increasing significantly during storage. The sulfur-containing substances, with sulfur-like aroma, meat flavor, and onion odor, are mainly derived from the Maillard reaction between amino acids and reducing sugars and the degradation of proteins, nucleotides, amino acids, and thiamin in aquatic products (Ruiz et al., 2001). The threshold of sulfur content is low (Liu et al., 2021b), and its content is low in fresh shrimp but increases with storage time, indicating its involvement in the formation of flavor in shrimp meat during the late storage period. Shrimp meat is rich in protein, which is broken down by microorganisms into low-threshold substances such as trimethylamine, ammonia, and methyl sulfide (Lee et al., 2018). Each heterocyclic compound has specific odors. As the number of substituents on the side chain of the benzene ring gradually increases, the odor changes from fruity to fresh and fatty and finally disappears completely. Different functional groups on the benzene ring give different odors, but when there are two or more relatively independent functional groups in the molecule, the odor is not additive. Toluene compounds can cause unpleasant flavors, and several such compounds, such as 1,2,3,5-tetramethylbenzene, m-xylene, and 1,2,3,4-tetramethylbenzene, were detected in this test. Morita et al. (2001) reported that alkyl benzenes may be contaminants present in the environment; therefore, the flavor of SV crayfish may also be affected by environmental contaminants.

The highest number of aromatic hydrocarbons was found at 0, 1, 3, 5, and 7 days of storage, with 12, 16, 13, 8, and 12 species, respectively, and a total of 33 species were detected (Figures 7A and 7B). The highest percentage of alkanes was found during storage, reaching 44.57% on the third day. The percentages of aromatic hydrocarbons were 13.83, 12.84, and 14.81% at 3, 5, and 7 days of storage, respectively, second only to alkanes. Esters were detected at 0, 1, 3, and 7 days of storage, and their contents were proportional to longer storage time, with 3.08% at the 7th day. During storage, oxidation of unsaturated fatty acids and microbial action can lead to changes in the odor of crayfish meat. Hydrocarbons with high threshold values have little effect on food flavor, but some aromatic hydrocarbons have significant effects on the overall flavor of meat (Min et al., 1979; Zhu et al., 2019). The finding about the increase in the ester content with storage time is consistent with the results reported by Yuan et al. (2022).

Figure 7C shows the 20 volatile compounds with a match of >85% and the volatile compounds with the top 10 contents at 0, 1, 3, 5, and 7 days of storage. Methylene chloride had the highest content. Methylene chloride has a sweet taste and chloroform-like odor (Wang et al., 2022), is prevalent in water, and is toxic and a hazardous compound (Chen et al., 2013; Hammoud et al., 2021). Hendra et al. (2021) extracted dichloromethane from dragon fruit peel and showed that dichloromethane has high antiplasmodial activity. Parlapani et al. (2020) detected dichloromethane, which was positively correlated with *Exiguobacterium* and *Lactobacillus* levels, in deep-sea rose shrimp.

4 CONCLUSION

We investigated changes in the physicochemical parameters and volatile substances of crayfish meat treated with SV during storage. The values of hardness, L*, and freshness (TVB-N and TVC) of crayfish meat were higher in the SV group than in the control group. The increase in temperature and time of SV treatment effectively inhibited the lipid oxidation of crayfish meat. Under the refrigeration condition of 4°C, the comprehensive indexes of crayfish meat treated at 75°C for 15 min were optimal. During storage, a high percentage of volatile alkanes were formed, which showed little effect on flavor. Conversely,

the highest number of volatile aromatic hydrocarbons, which showed a significant effect on flavor, was noted. These study results could serve as a reference for developing methods to improve crayfish SV processing and storage quality.

ACKNOWLEDGMENTS

This study was supported by the project of the National Key R&D Program of China (2019YFD0902000).

REFERENCES

Abd-Allah, M. A., & Abdallah, M. A. (2006). Effect of cooking on metal content of freshwater crayfish *Procambarus clarkii*. *Chemistry and Ecology*, *22*(4), 329-334. https://doi.org/10.1080/02757540600812198

Adams, A., De Kimpe, N., & van Boekel, M. A. J. S. (2008). Modification of casein by the lipid oxidation product malondialdehyde. *Journal of Agricultural and Food Chemistry*, *56*(5), 1713-1719. https://doi.org/10.1021/jf072385b

Chen, C., Chen, H., Zhang, Y., Thomas, H. R., Frank, M. H., He, Y., & Xia, R. (2020). TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Molecular Plant*, *13*(8), 1194-1202. https://doi.org/10.1016/j.molp.2020.06.009

Chen, D. Z., Ouyang, D. J., Liu, H. X., Chen, J., Zhuang, Q. F., & Chen, J. M. (2013). Effective utilization of dichloromethane by a newly isolated strain *Methylobacterium rhodesianum* H13. *Environmental science and pollution research international, 21*(2), 1010-1019. https://doi.org/10.1007/s11356-013-1972-7

Christensen, L., Ertbjerg, P., Aaslyng, M. D., & Christensen, M. (2011). Effect of prolonged heat treatment from 48 degrees C to 63 degrees C on toughness, cooking loss and color of pork. *Meat Science*, *88*(2), 280-285. https://doi.org/10.1016/j.meatsci.2010.12.035

Cremades, O. (2003). Isolation and characterization of carotenoproteins from crayfish *(Procambarus clarkii*). *Food Chemistry*, *82*(4), 559-566. https://doi.org/10.1016/S0308-8146(03)00011-6

Gao, R., Feng, X., Li, W., Yuan, L., Ge, J., Lu, D., Chen, B., & Yu, G. (2016). Changes in properties of white shrimp (*Litopenaeus vannamei*) protein during thermal denaturation. *Food Science and Biotechnology*, *25*(1), 21-26. https://doi.org/10.1007/s10068-016-0003-0

García-Linares, M. C., Gonzalez-Fandos, E., García-Fernández, M. C., & García-Arias, M. T. (2004). Microbiological and nutritional quality of sous vide or traditionally processed fish: Influence of fat content. *Journal of Food Quality*, *27*(5), 371-387. https://doi.org/10.1111/j.1745-4557.2004.00676.x

Geng, H., Chen, L., Wang, S., Wu, Y., Gao, P., Jiang, Q., Yang, F., & Xia, W. (2022). Analysis of volatile compounds contributing to distinctive odour of silver carp (*Hypophthalmichthys molitrix*) surimi. *International Journal of Food Science & Technology*, *57*(12), 7774-7786. https://doi.org/10.1111/ijfs.16141

Giavasis, I., Apostolopoulou, A., Deirmentzoglou, A., & Katsanidis, E. (2012). Combined hurdle effects of process parameters on biochemical, microbiological and sensory attributes of trout fillets. *Journal of Food Processing and Preservation*, *38*(1), 466-476. https://doi.org/10.1111/j.1745-4549.2012.00795.x

González-Fandos, E. (2004). Evaluation of the microbiological safety and sensory quality of rainbow trout (*Oncorhynchus mykiss*) processed by the sous vide method. *Food Microbiology*, *21*(2), 193-201. https://doi.org/10.1016/S0740-0020(03)00053-4

Hammoud, N. A., Zervou, S. K., Kaloudis, T., Christophoridis, C., Paraskevopoulou, A., Triantis, T. M., Slim, K., Szpunar, J., Fadel, A., Lobinski, R., & Hiskia, A. (2021). Investigation of the occurrence of cyanotoxins in lake Karaoun (Lebanon) by mass spectrometry, bioassays and molecular methods. *Toxins*, *13*(10), 716. https://doi.org/10.3390/toxins13100716

Hendra, R., Khodijah, R., Putri, R., Amalia, R., Haryani, Y., Teruna, H. Y., & Abdulah, R. (2021). Cytotoxicity and antiplasmodial properties of different *hylocereus polyrhizus* peel extracts. *Medical Science Monitor Basic Research*, *27*, e931118. https://doi.org/10.12659/MSMBR.931118

Karki, R., Bremer, P., Silcock, P., & Oey, I. (2022). Effect of sous vide processing on quality parameters of beef short ribs and optimisation of sous vide time and temperature using third-order multiple regression. *Food and Bioprocess Technology*, *15*(7), 1629-1646. https://doi.org/10.1007/s11947-022-02849-6

Kathuria, D., Dhiman, A. K., & Attri, S. (2022). Sous vide, a culinary technique for improving quality of food products: A review. *Trends in Food Science & Technology*, *119*, 57-68. https://doi.org/10.1016/j.tifs.2021.11.031

Kato, H. C. A., Peixoto Joele, M. R. S., Sousa, C. L., Ribeiro, S. C. A., & Lourenço, L. F. H. (2017). Evaluation of the shelf Life of tambaqui fillet processed by the sous vide method. *Journal of Aquatic Food Product Technology*, *26*(10), 1144-1156. https://doi.org/10.1080/10498850.2014.986593

Kurt Kaya, G. (2022). The effects of different packaging methods and sous vide cooking on chemical, sensory, and microbiological changes of marinated crayfish (*Astacus leptodactylus* Esch., 1823). *Journal of Food Processing and Preservation*, *46*(10), e16919. https://doi.org/10.1111/jfpp.16919

Lan, M., Li, L., Peng, X., Chen, J., Cao, Q., He, N., Cai, J., Li, B., & Zhang, X. (2021). Effects of different lipids on the physicochemical properties and microstructure of pale, soft and exudative (PSE)-like chicken meat gel. *LWT – Food Science and Technology*, *145*, 111284. https://doi.org/10.1016/j.lwt.2021.111284

Lee, K. M., Son, M., Kang, J. H., Kim, D., Hong, S., Park, T. H., Chun, H. S., & Choi, S. S. (2018). A triangle study of human, instrument and bioelectronic nose for non-destructive sensing of seafood freshness. *Scientific Reports*, *8*(1), 547. https://doi.org/10.1038/s41598-017-19033-y

Li, Y., Li, C., Chen, M., Liu, Z., Zeng, M., & Hu, Y. (2022). Sous-vide cooking endows a better microstructure for hairtail (*Trichiurus lepturus*) than traditional cooking: Mechanisms of moisture migration. *Journal of Food Science*, *87*(9), 3953-3964. https://doi.org/10.1111/1750-3841.16260

Li, Y., Tang, X., Shen, Z., & Dong, J. (2019). Prediction of total volatile basic nitrogen (TVB-N) content of chilled beef for freshness evaluation by using viscoelasticity based on airflow and laser technique. *Food Chemistry*, *287*, 126-132. https://doi.org/10.1016/j.foodchem.2019.01.213

Liu, C., Li, W., Lin, B., Yi, S., Ye, B., Mi, H., Li, J., Wang, J., & Li, X. (2021a). Comprehensive analysis of ozone water rinsing on the water-holding capacity of grass carp surimi gel. *LWT – Food Science and Technology*, *150*, 111919. https://doi.org/10.1016/j.lwt.2021.111919

Liu, Z., Liu, Q., Wei, S., Sun, Q., Xia, Q., Zhang, D., Shi, W., Ji, H., & Liu, S. (2021b). Quality and volatile compound analysis of shrimp heads during different temperature storage. *Food Chemistry: X*, *12*, 100156. https://doi.org/10.1016/j.fochx.2021.100156

Min, D. B., Ina, K., Peterson, R. J., & Chang, S. S. (1979). Preliminary identification of volatile flavor compounds in the neutral fraction of roast beef. *Journal of Food Science*, *44*(3), 639-642. https://doi.org/10.1111/j.1365-2621.1979.tb08465.x

Mohan, C. O., Ravishankar, C. N., & Srinivasa Gopal, T. K. (2017). Effect of vacuum packaging and sous vide processing on the quality of Indian white shrimp (*Fenneropenaeus indicus*) during chilled storage. *Journal of Aquatic Food Product Technology*, *26*(10), 1280-1292. https://doi.org/10.1080/10498850.2016.1236869

Morita, K., Kubota, K., & Aishima, T. (2001). Sensory characteristics and volatile components in aromas of boiled prawns prepared according to experimental designs. *Food Research International*, *34*(6), 473-481. https://doi.org/10.1016/s0963-9969(01)00072-2

Olatunde, O. O., & Benjakul, S. (2021). Sous-vide cooking as a systematic approach for quality maintenance and shelf-life extension of crab lump meat. *LWT – Food Science and Technology*, *142*, 111004. https://doi.org/10.1016/j.lwt.2021.111004

Özturan, S., & Ünal Şengör, G. F. (2022). Effects of cooking methods on the quality and safety of crayfish (*Astacus leptodactylus* Eschscholtz, 1823) during chilled storage. *Journal of Food Processing and Preservation*, *46*(10), e16887. https://doi.org/10.1111/jfpp.16887

Paik, H. D., Kim, H. J., Nam, K. J., Kim, C. J., Lee, S. E., & Lee, D. S. (2006). Effect of nisin on the storage of *sous vide* processed Korean seasoned beef. *Food Control*, *17*(12), 994-1000. https://doi.org/10.1016/j.foodcont.2005.07.005

Parlapani, F. F., Ferrocino, I., Michailidou, S., Argiriou, A., Haroutounian, S. A., Kokokiris, L., Rantsiou, K., & Boziaris, I. S. (2020). Microbiota and volatilome profile of fresh and chill-stored deepwater rose shrimp (*Parapenaeus longirostris*). *Food Research International*, *132*, 109057. https://doi.org/10.1016/j.foodres.2020.109057

Pulgar, J. S., Gázquez, A., & Ruiz-Carrascal, J. (2012). Physico-chemical, textural and structural characteristics of sous-vide cooked pork cheeks as affected by vacuum, cooking temperature, and cooking time. *Meat Science*, *90*(3), 828-835. https://doi.org/10.1016/j.meatsci.2011.11.024

Roldan, M., Antequera, T., Armenteros, M., & Ruiz, J. (2014). Effect of different temperature-time combinations on lipid and protein oxidation of sous-vide cooked lamb loins. *Food Chemistry*, *149*, 129-136. https://doi.org/10.1016/j.foodchem.2013.10.079

Ruiz, J., Ventanas, J., & Cava, R. (2001). New device for direct extraction of volatiles in solid samples using SPME. *Journal of Agricultural and Food Chemistry*, *49*(11), 5115-5121. https://doi.org/10.1021/jf0101298

Schellekens, M. (1996). New research issues in sous-vide cooking. *Trends in Food Science & Technology*, *7*(8), 256-262. https://doi.org/10.1016/0924-2244(96)10027-3

Thathsarani, A. P. K., Alahakoon, A. U., & Liyanage, R. (2022). Current status and future trends of sous vide processing in meat industry; A review. *Trends in Food Science & Technology*, *129*, 353-363. https://doi.org/10.1016/j.tifs.2022.10.009

Van Ba, H., Amna, T., & Hwang, I. (2013). Significant influence of particular unsaturated fatty acids and pH on the volatile compounds in meat-like model systems. *Meat Science*, *94*(4), 480-488. https://doi.org/10.1016/j.meatsci.2013.04.029

Ventanas, S., Estévez, M., Delgado, C. L., & Ruiz, J. (2007). Phospholipid oxidation, non-enzymatic browning development and volatile compounds generation in model systems containing liposomes from porcine *Longissimus dorsi* and selected amino acids. *European Food Research and Technology*, *225*(5-6), 665-675. https://doi.org/10.1007/s00217-006-0462-2

Wan, J., Cao, A., & Cai, L. (2019). Effects of vacuum or sous-vide cooking methods on the quality of largemouth bass (*Micropterus salmoides*). *International Journal of Gastronomy and Food Science*, *18*, 100181. https://doi.org/10.1016/j.ijgfs.2019.100181

Wang, W., Shen, X., Zhang, S., Lv, R., Liu, M., Xu, W., Chen, Y., & Wang, H. (2022). Research on Very Volatile Organic Compounds and Odors from Veneered Medium Density Fiberboard Coated with Water-Based Lacquers. *Molecules*, *27*(11), 3626. https://doi.org/10.3390/molecules27113626

Weber, J., Bochi, V. C., Ribeiro, C. P., Victório, A. D. M., & Emanuelli, T. (2008). Effect of different cooking methods on the oxidation, proximate and fatty acid composition of silver catfish (*Rhamdia quelen*) fillets. *Food Chemistry*, *106*(1), 140-146. https://doi.org/10.1016/j.foodchem.2007.05.052

Xiao, H., Li, N., Yan, L., & Xue, Y. (2021). The hydration characteristics, structural properties and volatile profile of squid (*Symplectoteuthis oualaniensis*) mantle muscle: Impacts of steaming, boiling, and sous vide cooking. *Foods*, *10*(7), 1646. https://doi.org/10.3390/foods10071646

Yao, D., Xu, L., Wu, M., Wang, X., Zhu, L., & Wang, C. (2021). Effects of microbial community succession on flavor compounds and physicochemical properties during CS sufu fermentation. *LWT – Food Science and Technology*, *152*, 112313. https://doi.org/10.1016/j.lwt.2021.112313

Ye, T., Zhu, Y., Wang, Y., Liu, R., Lin, L., Zheng, Z., & Lu, J. (2022). Effect of high pressure shucking on the gel properties and in vitro digestibility of myofibrillar proteins from red swamp crayfish (*Procambarus clarkii*). *LWT – Food Science and Technology*, *156*, 113020. https://doi.org/10.1016/j.lwt.2021.113020

Yuan, Y. H., Liu, L. X., Guo, L., Wang, L., Hao, J. W., & Liu, Y. G. (2022). Changes of bacterial communities and volatile compounds developed from the spoilage of white *Hypsizygus marmoreus* under different storage conditions. *LWT – Food Science and Technology*, *168*, 113906. https://doi.org/10.1016/j.lwt.2022.113906

Zhu, W., Luan, H., Bu, Y., Li, X., Li, J., & Ji, G. (2019). Flavor characteristics of shrimp sauces with different fermentation and storage time. *LWT – Food Science and Technology*, *110*, 142-151. https://doi.org/10.1016/j.lwt.2019.04.091