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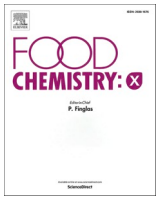

Elucidating the effects of precooked treatments on the quality attributes of red swamp crayfish (*Procambarus clarkia*): Insights from water boiling vs. microwaving

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ABSTRACT

Precooked treatments are essential in food processing, extending beyond mere sterilization to include the enhancement of nutritional value, flavor profile, and digestibility. This research scrutinizes the effects of water boiling and microwaving on red swamp crayfish, two distinct precooked methodologies. A comparative analytical framework has been employed to assess the efficacy of two precooked methods across a spectrum of quality indicators, including aerobic plate counts, texture, nutrient composition, volatile compound characterization, protein oxidation, and digestive properties. The findings revealed that both water boiling and microwaving effectively reduced bacterial counts to a safe level of 500 CFU/g. Microwave precooking facilitated a moderate oxidation of lipids in crayfish, preferentially liberating flavor compounds, thereby enhancing their sensory attributes. The boiling process imparted a pronounced denaturation to proteins, consequently augmenting the hardness of the crayfish. Notably, the enhanced digestibility of boiled crayfish proteins results from the denaturing action of boiling, promoting efficient protein digestion.

Introduction

In the rapidly evolving landscape of modern living, marked by escalating personal standards and an unyielding tempo of professional commitments, a pronounced transformation in consumer dietary inclinations has become evident. This dietary transformation is intricately linked to the burgeoning convenience-centric economy and the pervasive embrace of a lifestyle centered on the home. Notably, there is a discernible inclination among consumers towards a diverse spectrum of convenient, nutritionally enriched prepared meals that prioritize health and efficiency (Yi and Xu, 2023; Xu et al., 2023). The realm of precooked food, which spans both fully processed and semi-finished products, underscores pivotal elements such as nutritional density, flavor profile, quality, convenience, and a diverse culinary experience. Paramount among these is the meticulous maintenance of flavor and nutritional integrity during the reheating process, a factor that substantially influences consumer acceptance. Precooked meals offer a time-saving alternative to traditional fast food and address the issue of culinary skill deficiency among consumers (Khalid et al., 2023; Ying et al., 2024). The transition from fresh ingredients to processed precooked foods involves alterations in sensory attributes, nutrition, and quality. The various heating stages in the processing of fresh materials can lead to meat shrinkage and oxidation, which in turn affect the final product's sensory and nutritional value. A profound comprehension of the mechanisms that govern quality alterations during food processing, in conjunction with precise control measures, is essential for attenuating the degradation of nutritional and flavor characteristics. Such an approach is instrumental in augmenting the intrinsic value of precooked food.

The red swamp crayfish (*Procambarus clarkia*), celebrated for its culinary allure and nutritional bounty, exemplifies a pivotal shift in gastronomic inclinations. This freshwater *gem*, with a commendable protein content of approximately 20 % and a rich profile of eight essential amino acids, has garnered extensive acclaim (Bai et al., 2022).

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Its remarkable fatty acid profile, which includes nearly 50 % unsaturated fatty acids, features notable compounds such as eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA), establishing it as a nutritional powerhouse. Additionally, the crayfish provides a spectrum of essential minerals and vitamins crucial for human health, including selenium and retinol (Peng et al., 2021). The crayfish industry in China has experienced exponential growth since 2016, driven by a burgeoning market for crayfish consumption (Jiang et al., 2023). Currently, the crayfish processing sector is undergoing a renaissance, spurred by the rising demand for prepared dishes. However, a significant portion of the annual crayfish harvest remains underutilized due to regional or seasonal constraints, leading to considerable waste and economic loss (Bai et al., 2022). The strategic deployment of prepared dishes presents a viable solution to the ongoing challenge of year-round availability and holds the potential to enhance accessibility in remote regions (Li et al., 2023a). Interestingly, the scientific examination of the subtleties that govern the quality changes in precooked crayfish products during processing remains scarce. This underscores the pressing need for dedicated research to unlock the full potential of the crayfish industry, while minimizing waste, in accordance with evolving culinary preferences and sustainable food practices.

The meticulous processing of crayfish is a multifaceted endeavor, with precooking being a pivotal step that is orchestrated to enhance visual appeal, neutralize autolysins, and effectively eradicate microorganisms both externally and internally (Khalid et al., 2023). This strategic process serves a dual function: enhancing the permeability of crayfish tissue cells and priming the crustacean for subsequent flavor absorption during curing processes. Water boiling, executed at a precisely controlled temperature of 99 ± 1 °C, is a conventional method that stands as a cornerstone for pathogen inactivation in industrial crayfish production. This method is favored for its convenience, cost-effectiveness, scalability, and ability to preserve natural flavors (Li et al., 2023a). However, it is important to note that thermal processing induces alterations in protein conformation and physicochemical properties, which can impact the appearance, flavor, texture, and chemical composition of the crayfish (Khalid et al., 2023). Despite its efficacy, water boiling raises ecological concerns due to significant water consumption and the generation of wastewater, presenting challenges for sustainability and nutrient retention (Fan et al., 2021). In contrast, microwave technology, which spans the electromagnetic spectrum from 300 MHz to 300 GHz, offers versatility and potential ecological benefits compared to water boiling. However, research on its impact on crayfish quality remains limited (Wang et al., 2023a). Notably, microwave-cooked samples exhibit reduced muscle damage due to shorter cooking times, suggesting the potential for preserving texture and nutrients (Jiang et al., 2023). The narrative of crayfish processing unfolds against the backdrop of contrasting methodologies: the conventional reliability of water boiling juxtaposed with the emerging realm of microwave technology (Wang et al., 2023a). Striking a balance between efficacy, sustainability, and culinary excellence is paramount. Delving into the alterations in crayfish quality induced by microwave technology holds promise for both scientific enlightenment and practical insights, potentially reshaping crayfish processing practices in the culinary domain.

This research embarks on a meticulous scientific odyssey, delving into the complexities of the red swamp crayfish (*Procambarus clarkia*). Through a comprehensive and multifaceted investigation, it scrutinizes the texture, nutrient composition, taste, volatile flavors, and digestion characteristics of this species. The study's primary objective is to elucidate the nuanced interplay between two predominant precooked modalities: water boiling and microwaving. At the crux of this inquiry lies the comparative analysis of the bactericidal efficacies of water boiling and microwaving, as well as the exploration of the intricate mechanistic underpinnings that drive the observed variations in quality. The meticulous examination of these attributes forms the bedrock of this analytical endeavor, which seeks to understand the multifaceted impacts of these precooked methods on the sensory and nutritional dimensions of the red swamp crayfish. By carefully dissecting these attributes, the research aims to reveal the subtle alterations induced by each precooked method. The study aspires to offer invaluable insights that extend beyond the confines of laboratory, poised to guide industrial crayfish processing practices. Strengthening the scientific foundation underpinning crayfish processing, the study envisions enhancing the quality of the final product and optimizing the practical efficacy of industrial operations in this domain. The findings are expected to contribute to the body of knowledge surrounding the processing of aquatic resources, providing a robust framework for future research and innovation in the culinary and food processing industries.

Materials and methods

Materials, reagents and chemicals

Fresh red swamp crayfish, sourced from a local market, exhibited an average weight of 20.70 ± 3.50 g. Essential chemicals, including hydrochloric acid, sodium hydroxide, sulfuric acid, methanol, potassium bromide, petroleum ether, and phenol were procured from Hunan Huihong Reagent Co., Ltd. (Hunan, China). Chromatography-grade *n*-hexane and acetonitrile were obtained from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China).

Sample preparations

The crayfish underwent thorough cleaning, including scrubbing and rinsing with running water, before being weighed. The specimens were divided into two precooked groups: one group was subjected to boiling in water at 100 °C for 5 min (referred to as water boiling), while the other underwent microwave treatment at 210 W (Microwave heating 2450 MHz) for 5 min (referred to as microwaves).

Enumeration of aerobic plate count

The aerobic plate count of caryfish was enumerated as per the stringent protocols outlined in the GB 4789.2–2016 standard. The methodology commenced with the homogenization of the samples, ensuring a uniform distribution of microbial content. This was followed by a systematic serial dilution process, executed in a 0.85 % NaCl solution, which served as a diluent conducive to microbial stability. The diluted samples were then applied onto AOAC 3M Petrifilm™ Aerobic Count Plates, sourced from Kesebai Medical Technology Co., LTD, Shanghai, China. These plates are specifically designed for the isolation and enumeration of aerobic bacteria, providing an optimal environment for their growth. The inoculated plates were incubated for a period of 72 h at a constant temperature of 37 °C, a condition that promotes the proliferation of mesophilic bacteria. Upon completion of the incubation, the bacterial colonies were counted, yielding the aerobic plate count, which serves as a quantitative measure of the microbial load in the carp samples.

Analysis of texture profile

To assess the texture parameters (hardness, elasticity, and chewiness) of crayfish tail meat, a TA. XT plus texture analyzer (Stable Micro Systems LTD, Godalming, UK) was utilized at room temperature (25 °C). The testing parameters included a trigger force of 5 g, pretest speed of 1.0 mm/s, test speed of 3 mm/s, post-test speed of 5 mm/s, compressed depth of 25 %, time interval of 5 s, and a compression ratio set at 50 %. Each treatment was replicated twelve times to ensure robust data.

Analysis of nutrition and flavor

Basic nutrition analysis

The determination of moisture, ash, crude fat, crude protein, and glycogen followed the protocols outlined in GB 5009.3–2016, GB 5009.4–2016, GB 5009.6–2016, GB 5009.5–2016, GB/T 9695.31–2008, respectively.

Fatty acids composition analysis

Crayfish tail meat, after freeze-drying, underwent fatty acid methyl esterification according to the method by Ma et al., (2020). Gas chromatography-mass spectrometer (GC–MS, 7820A GC-5977E MSD, Agilent LTD, Palo Alto, America) utilizing an HP-5-MS capillary column (30 m × 0.25 mm × 0.25 μm) facilitated the analysis. The identification of fatty acid methyl ester relied on MS data obtained from the National Institute of Standards and Technology (N1ST14.L) library database.

Amino acids and free amino acids composition analysis

For the analysis of amino acid and free amino acid composition, a 2 mg sample of freeze-dried crayfish tail meat was placed in a sealed 5 mL vial. Subsequently, 3 mL of either 6 mol/L hydrochloric acid or a 40 % sodium hydroxide solution was added, and the vial was securely sealed. The sample underwent hydrolysis at 110 °C for 24 h. Following the thorough hydrolysis of proteins into amino acid residues, 3 mL of the supernatant, obtained post-membrane filtration, was utilized for analysis employing the Elite-AAK amino acid analysis system (Dalian Elite LTD, Liaoning, China). Amino acid content determination was conducted using high-performance liquid chromatography (HPLC) coupled with the Elite-AAK amino acid analysis column (250 mm × 4.6 mm, 5 μm). The column temperature was maintained at 27 °C, with an injection volume of 10 μL. The mobile phase flow rate was set at 1.2 mL/min, and detection occurred at a wavelength of 360 nm.

For the analysis of free amino acids, the sample solution underwent precipitation with five times the volume of acetone, followed by centrifugation at 10,000 r/min for 15 min. Thereafter, 3 mL of the supernatant, post-membrane removal, was employed for derivatization and measurement using the Elite-AAK amino acid analysis system. The analytical procedure remained consistent with that utilized for amino acid composition.

Flavor nucleotides analysis

The quantification of key flavor nucleotides—inosine monophosphate (IMP), guanosine monophosphate (GMP), adenosine monophosphate (AMP), and cytidine monophosphate (CMP)—in crayfish meat was conducted in strict accordance with the national standard GB 5413.40–2016. The procedure involved a precise extraction method where 5 g of crayfish meat was homogenized with10 mL of deionized water. This mixture was then subjected to centrifugation at 5000 rpm for 15 min to facilitate the separation of the supernatant. A 2 mL aliquot of this supernatant was further diluted to a final volume of 10 mL to ensure optimal conditions for chromatographic analysis. The resulting solution was analyzed using Ultra Performance liquid chromatography (ACQUITY UPLC, Waters, USA).

Volatile compounds analysis

The analysis of volatile compounds in crayfish tail meat followed the methodology outlined by Ma et al. (2020) utilizing GC–MS coupled with an automated solid-phase microextraction (SPME) system. Commercial SPME fibers (50/30 μm DVB-CAR-PDMS, Shanghai Anpel LTD, China) were employed for the extraction of volatiles from crayfish tail muscle. The identification of volatile compounds relied on MS data obtained from the National Institute of Standards and Technology (N1ST14.L) library database.

Calculation of equivalent umami concentration (EUC) and taste activity value (TAV)

Equivalent Umami Concentration (EUC) is defined as a synergistic combination of AMP, GMP, IMP, Glu, and Asp that elicits an umami intensity equivalent to the amount found in monosodium glutamate (MSG). The calculation formula is expressed as follows:

$$\mathrm{EUC(g\ MSG/100\ g)} = \sum a_i b_i + 1218\left(\sum a_i b_i\right)\left(\sum a_j b_j\right) \quad (1)$$

EUC is represented in grams of MSG per 100 g of dried meat. The synergy constant, 1218, plays a crucial role in this calculation. The variables include a_i, the content of umami amino acids (g/100 g dry meat), with b_i representing their relative umami strength compared to MSG (Asp, 0.077; Glu, 1). Additionally, a_j denotes the content of flavor nucleotides (g/100 g dry meat), and b_j signifies their relative strength compared to IMP (IMP,1; GMP,2.3; AMP, 0.18).

Taste activity value (TAV) for each free amino acid and nucleotide is determined by calculating the ratio of their amount to the corresponding taste threshold. A TAV value greater than one indicates a significant impact on the sample's taste (Zhu et al., 2023).

Analysis of microstructure

Microstructure analysis of red swamp crayfish meat adhered to the methodology delineated by Li et al. (2022a). After fixation in a 10 % formaldehyde solution for 24 h, samples underwent paraffin sectioning and were subjected to hematoxylin and eosin staining. Subsequently, imaging slides were prepared for each sample, and their microstructure was observed in a bright field under an inverted microscope (ECLIPSE Ti-S, Nikon, Tokyo, Japan).

Analysis of oxidative status assessment in crayfish meat

Determination of free radical content

The endogenous free radical content within red swamp crayfish (*Procambarus clarkia*) meat was quantified employing an A200 electron spin resonance (ESR) spectrometer, a state-of-the-art device from Bruker Corporation, Karisruhe, Germany. This methodology aligns with the established protocol detailed by Li et al. (2020), ensuring a precise and accurate measurement of free radicals, which are pivotal reactive species in the oxidative processes.

Assessment of thiobarbituric acid-reactive substances (TBARS)

The TBARS assay, a standard procedure for monitoring lipid peroxidation, was conducted on the crayfish meat samples. The TBARS values, indicative of oxidative degradation, were measured following the technique reported by Li et al. (2020) and are expressed in terms of malondialdehyde (MDA) equivalents per kilogram of sample.

Quantification of carbonyl content

The carbonyl content, a biochemical marker for protein oxidation, was determined using a protein carbonyl assay kit procured from Jian cheng Technology Co., Nanjing, China. The assay was performed in triplicate, strictly adhering to the guidelines provided by the manufacturer. This rigorous experimental design ensures the reproducibility and validity of the carbonyl content measurements, offering insights into the extent of protein oxidation in the crayfish samples.

Measurement of sulfhydryl group (SH) contents

The quantification of sulfhydryl groups adhered to the methodology outlined by Li et al. (2022a). In brief, 1.5 mL of crayfish protein solution (5 mg/mL) was initially blended with 10 mL of Tris-glycine buffer (composed of 4 mM EDTA, 8 mM urea, 90 mM glycine, 86 mM Tris, and 0.5 mL of DTNB, with a pH of 8.0) or Tris-glycine buffer without urea (utilized for measuring free SH). Subsequent to the mixing process, the resultant solution was allowed to stand at 25 °C for 1 h and then

centrifuged at 4 °C centrifuged for 10 min. The absorbance of the supernatant was recorded at 412 nm (A_1), with the buffer serving as a blank (A_2). The subsequent mathematical expressions were employed for the computation of total sulfhydryl groups:

$$\text{Total/Free SH}\,(\mu\text{mol/g prot}) = (A_1 - A_2) \times 14.706 \quad (2)$$

Assessment of surface hydrophobicity

Surface hydrophobicity analysis of red swamp crayfish protein was conducted following the methodology outlined by Wang et al. (2019). The determination involved the examination of surface hydrophobicity in an protein solution (2 mg/mL) using a 1 mg/mL bromophenol blue (BPB). As a control, a phosphate buffer without protein was employed. Following agitation at room temperature for a specified duration, all samples and the control were centrifuged at 2000 g for 15 min at 4 °C. Subsequently, the supernatant was separated and diluted 10 times with PBS, and the absorbance at 595 nm was measured. The hydrophobicity index, representing the bound BPB, was calculated using the formula:

$$\text{Bound BPB}\,(\mu g) = 200\ \mu g \times \frac{Ac - As}{Ac} \quad (3)$$

where Ac and As denote the absorbance of the control and samples, respectively.

Fourier transform infrared (FTIR) spectroscopy analysis

Crayfish protein samples were blended with dried KBr powder and compressed into thin slices. Spectra were acquired using an FTIR spectrometer (Perkin Elmer, Salem, MA) within a scanning range of 400–4000 cm^{-1}. The region spanning 1600–1700 cm^{-1} was specifically selected to analyze the secondary structures of proteins, employing PeakFit v4.12 (SeaSolve Software Inc., USA).

Intrinsic fluorescence emission analysis

The intrinsic fluorescence emission spectroscopy of the crayfish protein solution (1 mg/mL) was analyzed using a fluorescence spectrophotometer (Hitachi Corp., Tokyo, Japan). The excitation and emission wavelength were set at 280 nm and 290–450 nm, respectively.

In vitro gastrointestinal digestion analysis

Simulated gastrointestinal digestion procedure

Red swamp crayfish (*Procambarus clarkia*) samples underwent an in vitro gastrointestinal digestion process, following the protocol established by Wang et al. (2023b). The method involved homogenizing 100 mg of sample with 10 mL of simulated salivary fluid (CZ0281, LEAGENE, China) and incubating at 37 °C for 10 min. Subsequently, 10 mL of simulated gastric fluid (CZ0211, LEAGENE, China) was introduced, and the sample was further incubated at 37 °C for 2 h. Post-gastric simulation, the pH of the mixture was adjusted to neutral (pH 7.0), and simulated intestinal fluid (CZ0201, LEAGENE, China) was incorporated, with a final 2-h incubation at 37 °C. The digestion process concluded with the inhibition of enzymatic activity through boiling for 5 min, followed by centrifugation at 10,000 g for 10 min to collect the supernatant for subsequent analysis.

Confocal laser scanning microscopy (CLSM)

The microstructural changes post-gastrointestinal digestion was examined using confocal laser scanning microscopy (CLSM, SP8, LEICA, Germany). A staining procedure with rhodamine B (0.1 % v/w in ethanol) was applied to enhance visualization of the digested sample.

Assessment of hydrolysis degree and soluble amino groups content

The degree of hydrolysis (DH) of the supernatant from the gastrointestinal digestion system was determined through the reaction of o-phthalaldehyde (OPA) with amino groups, utilizing a standard curve generated with L-serine. Additionally, the content of soluble amino groups was quantified using the same OPA-based assay, following the method described by Duque-Estrada et al. (2019).

Particle size distribution analysis

The particle size distribution of the supernatant from the gastrointestinal digestion system was measured using a Zetasizer (3000HSA, Malvern, UK), providing insights into the changes in particle dimensions post-digestion.

Statistical analysis

The statistical evaluation of our data adhered to rigorous standards. Results from three independent and repeated parallel experiments are reported as mean values ± standard deviation unless explicitly stated otherwise. Significance analysis, set at a 5 % level ($p < 0.05$), was conducted using ANOVA along with the student *t*-test, employing SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA). The analysis was further complemented by utilizing OriginPro (version 2023, OriginLab Corporation, Northampton, MA, USA) for a comprehensive examination of the statistical outcomes.

Results and discussion

Proximate analysis of fundamental quality attributes in red swamp crayfish meat

A comprehensive evaluation of red swamp crayfish (*Procambarus clarkia*) meat quality is presented, focusing on aerobic plate count values, texture profile analysis, and basic nutrient composition under two distinct precooking methods: water boiling and microwave treatment. Fig. 1(A) and (B) illustrate the aerobic plate count results for crayfish precooked by water boiling and microwaving, respectively. A clear impact of water boiling for 5 min on microbial reduction is evident. The aerobic plate count after 5 min of microwave treatment was a mere 80 CFU/g, well below the 10^5 CFU/g limit set by the GB 10136–2015 National Standard for Food Safety in Animal Aquatic Products. Both water boiling and microwave treatments maintained aerobic plate counts within acceptable limits, showcasing their efficacy in reducing bacterial presence in crayfish. The mechanism of heat sterilization involves subjecting polymer materials to high temperatures, inducing denaturation and achieving effective sterilization (Barnett et al., 2020). Cooking crayfish at 70 °C successfully eliminated a significant portion of pathogenic bacteria (Li et al., 2023a). The total aerobic plate count after 5 min of boiling or microwaving was significantly below the 500 CFU/g threshold defined by national standards, confirming the robust bacterial reduction achieved by both sterilization methods.

In the analysis of textural properties, as detailed in Fig. 1(C), crayfish precooked by water boiling exhibited significantly higher hardness and chewiness compared to those precooked by microwaving. This can be attributed to the heat-induced denaturation and aggregation of myofibrillar proteins, which enhances muscle fiber density and contributes to a firmer texture (Yu et al., 2021a; Li et al., 2023b; Zhang et al., 2023b). Despite variations in hardness and chewiness, springiness remained statistically invariant between the two precooked methods.

Upon scrutinizing the basic nutrient composition in Fig. 1(D), distinct variations between crayfish precooked by water boiling and microwaving are observed. The water boiling method resulted in a higher moisture content, which is significant ($p < 0.05$), highlighting the necessity for accurate microwave heating to conserve the moisture crucial for the crayfish's edibility. Interestingly, the slight moisture loss during microwaving is associated with improved palatability (Li et al., 2023a). Moreover, the water boiling group showed a significant increase in crude fat and glycogen levels compared to the microwaving group ($p < 0.05$), likely due to the concentration effect caused by moisture depletion during heating (Jiang et al., 2023).

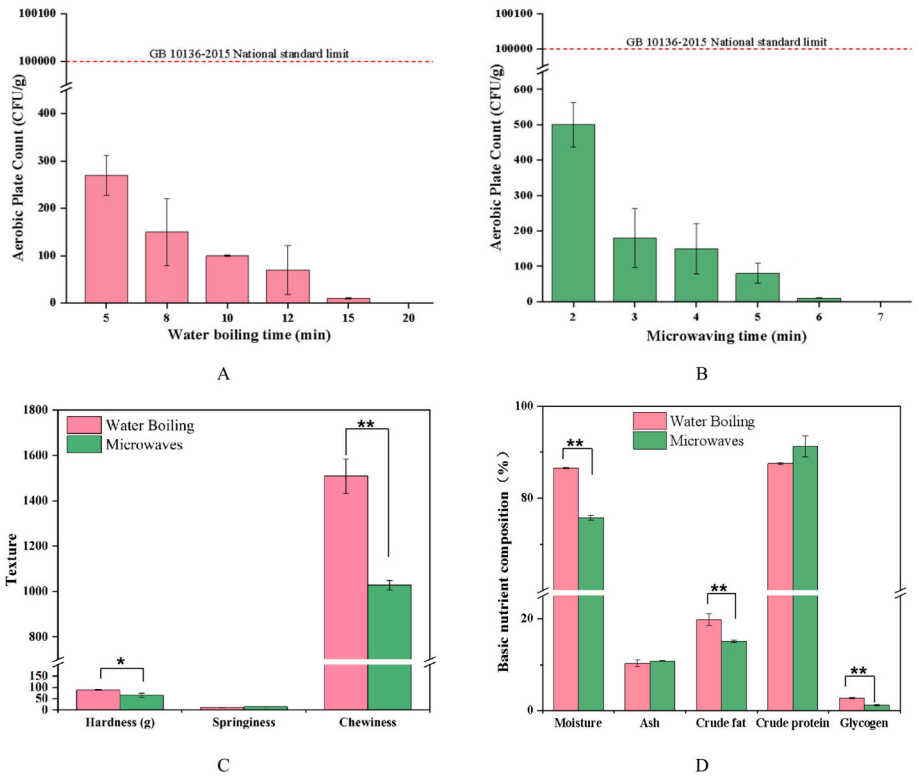

Fig. 1. Comparative analysis of aerobic plate count values for red swamp crayfish (*Procambarus clarkia*) subjected to water boiling (A) and microwave precooking (B) methods. Evaluation of texture (C) and fundamental nutrient composition (D) under the two culinary approaches. Statistical significance is denoted by asterisks, with * indicating $p < 0.05$ for a 95 % confidence interval and ** indicating $p < 0.01$ for a 99 % confidence interval. These markers identify significant mean differences ascertained via one-way ANOVA and *t*-test.

Evaluation of nutrition and flavor compounds

Changes in fatty acid and amino acid profiles

Fig. 2(A) and (B) showcase the comparative results of fatty acid compositions, identifying 21 fatty acids, categorized into 7 saturated fatty acids (SFAs), 7 monounsaturated fatty acids (MUFAs), and 7 polyunsaturated fatty acids (PUFAs). Fig. S1 complements the study by providing chromatograms that illustrate the detailed composition of

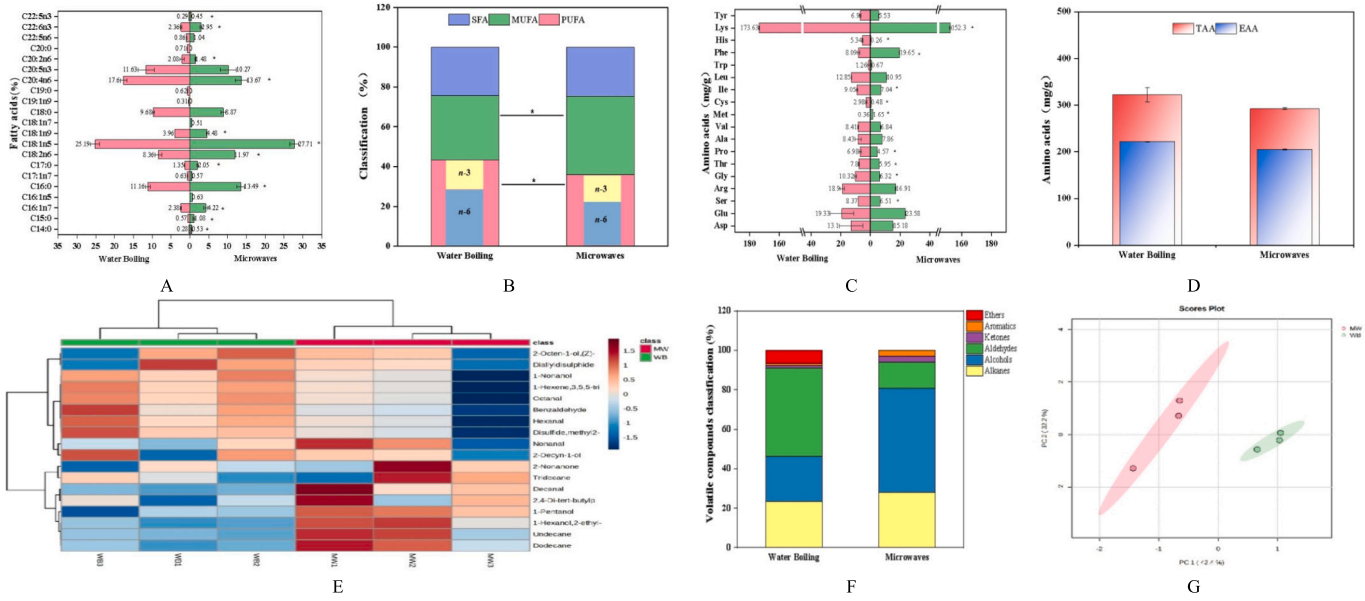

Fig. 2. Comparative analysis of fatty acid composition (A), classification (B), amino acid profile (C), essential amino acid content (D), volatile compounds depicted through heatmap (E), classification (F), and principal component analysis (G) in red swamp crayfish (*Procambarus clarkia*) under water boiling and microwave precooking conditions. Significance levels are indicated by asterisks, signifying mean differences at $p < 0.05$ as determined by one-way ANOVA and *t*-test. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; *n*-3, Omega-3 PUFA; *n*-6, Omega-6 PUFA; TAA, total amino acid; EAA, essential amino acid. WB, water boiling; MW, microwaves.

fatty acids. The MUFAs content in the water boiling group was significantly lower than that in the microwaving group ($p < 0.05$), while the water boiling group exhibited heightened PUFAs content. This decline in PUFA content is attributed to the prevalence of conjugated double bonds and high unsaturation within PUFAs (Li et al., 2023a). As PUFAs undergo oxidation and degradation under elevated temperatures and aqueous conditions, they give rise to small flavor-enhancing molecules such as alcohols, aldehydes, and ketones (Zhu et al., 2023). This dual effect results in a reduction in the nutritional value of crayfish meat but a simultaneous enhancement of its flavor. Notably, essential fatty acids, particularly the n-6 series of linolenic acid (LA) and arachidonic acid (ARA), as well as the n-3 series of eicosapentaenoic acid (EPA), are abundantly present. These essential fatty acids, crucial for preventing coronary heart disease and reducing blood lipids (Mukhametov et al., 2022), exhibit no significant difference in EPA content between crayfish treated by microwaving and water boiling (Fig. 2A). In summary, no substantial variance was observed in n-3 PUFAs and n-6 PUFAs between water boiling and microwaving (Fig. 2B).

Turning to amino acid analysis, Fig. 2(C) and (D) illustrate the comparable compositions in crayfish meat under water boiling and microwave precooking. Fig. S2 further supports this analysis by presenting the chromatograms of the amino acid compositions, offering a detailed visual representation of the data. In both precooked samples, a total of 18 amino acids were identified, encompassing a comprehensive range of nutritionally significant compounds. The Essential Amino Acids (EAA) and Total Amino Acids (TAA) content showed negligible differences between the water boiling and microwave precooking methods, with statistical analysis indicating no significant variation ($p > 0.05$). Amino acids play pivotal roles in maintaining normal human metabolism, improving blood circulation, enhancing oxygen supply, regulating brain nerve cells, promoting brain health, inducing calmness, improving sleep, and regulating blood pressure (Li et al., 2022b). Lysine, the amino acid with the highest content, contributes to immune system strengthening (Huang et al., 2022). According to the ideal model standard proposed by WHO/FAO, the EAA/TAA ratio for both water boiling and microwaving exceeded 40 %, indicating the high nutritional value of processed crayfish protein. This underscores its role as a valuable source of essential amino acids, improving protein utilization. Regardless of the precooking method, crayfish meat demonstrates a diverse array of amino acids, preserving rich nutritional content.

Alterations in free amino acid and nucleotide content

Exploring the intricacy of free amino acid and nucleotide content in crayfish meat, this section sheds light on the influence of water boiling and microwaving methods. The summarized findings are deed in Table 1.

Free amino acids, pivotal precursors to flavor, play a critical role in defining the distinctive flavor profile of crayfish (Zhu et al., 2019). The results underscore the detection of 18 types of free amino acids in both the water boiling and microwaving groups. The total content of free amino acids amounted to 18.300 ± 1.455 g/100 g for the water boiling group and 26.949 ± 0.007 g/100 g for the microwaving group. Substantial differences ($p < 0.05$) were evident in total free amino acids, umami free amino acids (Asp and Glu), and sweet free amino acids (Thr, Ser, Gly, Ala, Arg, and Pro) between the water boiling and microwave methods. The dynamic variations in free amino acid content, influenced by heating time and potential participation in the Maillard reaction, led to a decline in TFAA content during the later stages of cooking (Li et al., 2023a). Notably, the levels of Arg and Lys increased in both groups, with Arg contributing a bitter but pleasantly sweet taste, and Lys offering a sweet but unpleasantly bitter taste. Additionally, Ser, Thr, Lys, Pro, Ala, and Gly were identified as the predominant sweet amino acids, with Asp and Glu serving as primary umami amino acids (Li et al., 2023a). These dynamic shifts in the content of flavor free amino acid contents

Table 1
Comparative assessment of free amino acid and nucleotide content, taste threshold, and total amino acid value (TAV) in red swamp crayfish (*Procambarus clarkia*) subjected to water boiling and microwave precooking methods.

Name	Content (g/100 g)		Taste	Taste threshold (g/100 g)	TAV	
	Water Boiling	Microwaves			Water Boiling	Microwaves
Asp	0.078 ± 0.014^{a}	0.077 ± 0.000^{a}	Umami (+)	1	0.078	0.077
Glu	0.150 ± 0.029^{a}	0.436 ± 0.002^{b}	Umami (±)	0.3	0.499	1.453
Ser	2.002 ± 0.200^{a}	2.883 ± 0.023^{b}	Sweet (±)	1.5	1.335	1.922
Arg	1.445 ± 0.178^{a}	7.016 ± 0.022^{b}	Bitter/sweet (±)	0.5	2.891	14.033
Gly	2.452 ± 0.241^{b}	0.242 ± 0.001^{a}	Sweet (±)	1.3	1.886	0.186
Thr	0.249 ± 0.014^{a}	0.195 ± 0.001^{a}	Sweet (±)	2.6	0.096	0.075
Pro	1.823 ± 0.126^{b}	0.948 ± 0.001^{a}	Sweet/bitter (±)	3	0.608	0.316
Ala	3.028 ± 0.233^{b}	2.168 ± 0.003^{a}	Sweet (±)	0.6	5.046	3.614
Val	0.486 ± 0.026^{b}	0.111 ± 0.003^{a}	Sweet/bitter (−)	0.4	1.216	0.278
Met	0.501 ± 0.030^{a}	0.960 ± 0.003^{b}	Bitter/sweet/sulfurous (−)	0.3	1.669	3.201
Cys	0.129 ± 0.004^{a}	0.188 ± 0.000^{b}	Bitter/sweet/sulfurous (−)	/	/	/
Ile	0.317 ± 0.013^{a}	0.318 ± 0.001^{a}	Bitter (−)	0.9	0.353	0.354
Leu	0.444 ± 0.020^{a}	0.441 ± 0.001^{a}	Bitter (−)	1.9	0.233	0.232
Trp	0.411 ± 0.021^{b}	0.376 ± 0.001^{a}	Bitter (−)	/	/	/
Phe	0.664 ± 0.054^{ab}	0.763 ± 0.002^{b}	Bitter (−)	0.9	0.738	0.847
His	1.743 ± 0.088^{b}	1.058 ± 0.004^{a}	Bitter (−)	0.2	8.716	5.291
Lys	1.664 ± 0.123^{a}	8.130 ± 0.123^{b}	Sweet/bitter (−)	0.5	3.329	16.260
Tyr	0.713 ± 0.043^{b}	0.638 ± 0.131^{ab}	Bitter (−)	/	/	/
AMP	80.704 ± 8.548^{a}	115.056 ± 4.172^{b}	Umami (+)	50	1.614	2.301
CMP	6.396 ± 3.010^{a}	2.818 ± 0.482^{a}	Umami (+)	1293	0.005	0.002
GMP	0.587 ± 0.143^{a}	8.689 ± 0.968^{b}	Umami (+)	12.5	0.047	0.695
IMP	3.480 ± 1.000^{b}	1.671 ± 0.829^{a}	Umami (±)	25	0.139	0.067
TFAAs	18.300 ± 1.455^{a}	26.949 ± 0.007^{b}	/	/	/	/
UFAAs	0.227 ± 0.043^{a}	0.513 ± 0.002^{b}	/	/	/	/
SFAAs	11.000 ± 0.992^{a}	13.452 ± 0.003^{b}	/	/	/	/
EUC	0.038 ± 0.008^{a}	0.232 ± 0.010^{b}	/	/	/	/

The acronyms TFAAs, UFAAs, and SFAAs denote total free amino acids, umami free amino acids (aspartic acid and glutamic acid), and sweet free amino acids (threonine, serine, glycine, alanine, arginine, and proline), respectively. Data not available for certain entries are indicated by '/ '. The symbols '(+)' and '(−)' correspond to the subjective assessment of taste quality, where '(+)' signifies a pleasant taste and '(−)' denotes an unpleasant taste. The term EUC, or equivalent umami concentration, represents a calculated value reflecting the quantitative analysis of free amino acids and nucleotides that contribute to the umami taste. Statistical significance among mean values is denoted by letters in the legend, ascertained through one-way ANOVA and *t*-test with a significance level of $p < 0.05$.

collectively contribute to the overall enhancement of crayfish meat flavor.

ATP-related compounds undergo a transformation into flavor nucleotides, such as IMP and AMP, through thermal treatment. These nucleotides play a significant role in imparting specific taste attributes, with IMP and AMP being closely associated with sweetness and umami taste in aquatic products (Zhu, et al., 2023). The lower AMP content in the water boiling group compared to the microwaving group suggests high solubility of AMP in hot water, leading to increased loss (Liu et al., 2021). Conversely, the higher IMP content in the water boiling group may be attributed to heat-induced decomposition by electromagnetic waves. The microwaving group exhibited higher GMP levels than the water boiling group, consistent with previous reports on flavor nucleotide changes in mussels upon shucking (Liu et al., 2021). Notably, AMP stood out as the predominant taste-active nucleotide in all groups, characterized by TAV values exceeding 1.

Calculating the Equivalent Umami Concentration (EUC) values for crayfish under water boiling and microwaving using the monosodium glutamate equivalent formula (Table 1) revealed a superior taste quality imparted by microwaving to crayfish meat. The EUC for crayfish meat samples in the water boiling and microwaving groups were 0.038 g MSG/100 g and 0.232 g MSG/100 g, respectively. These findings align with the conclusions of Zhu, et al (2023), who reported that the microwave method enhances the taste quality of *Mytilus coruscus* meat compared to water boiling.

Shifts in volatile compounds

The savory essence of cooked crayfish meat arises from intricate reactions involving a myriad of flavor precursors, intermediates, and their resultant interaction products (Sohail et al., 2022). During the precooking process of crayfish, the interplay between lipid oxidation degradation and the Maillard reaction gives rise to a spectrum of volatile flavor compounds, including aldehydes, alcohols, ketones, and others. These compounds collectively contribute to the creation of a distinctive and nuanced flavor experience for consumers (Sohail et al., 2022). Flavor characteristics serve as pivotal indicators for assessing crayfish meat quality and consumer acceptability (Li et al., 2023a). A thorough evaluation of the volatile compounds was undertaken, employing heatmap analysis, classification, and principal component analysis (PCA). This comprehensive study was conducted on crayfish subjected to water boiling and microwaving methods, as depicted in Fig. 2(E–G). Fig. S3 complements the primary analysis by displaying the chromatograms of the volatile compounds. These chromatograms offer a detailed view of the individual peaks, corresponding to the various volatile compounds detected.

The findings uncover notable distinctions in the compositions and proportions of volatile flavor compounds between the microwaving and water boiling groups. Specifically, the water boiling group exhibited the detection of 17 volatile flavor compounds, including 4 alkanes, 5 alcohols, 4 aldehydes, 1 ketone, 2 ethers, and 1 aromatic. In contrast, the microwaving group revealed 9 volatile flavor compounds in crayfish meat, consisting of 3 alkanes, 2 alcohols, 2 aldehydes, 1 ketone, and 1 aromatic. Alcohols, aldehydes, and ketone constituted a significantly higher proportion (69 %) than other detected volatile flavor compounds in both water boiling and microwaving groups. This observation suggests the oxidation and degradation of polyunsaturated fatty acids (PUFAs), enhancing flavor but diminishing nutritional value. Consumer sensory thresholds toward alkane compounds are typically high, suggesting minimal impact on crayfish meat flavor. Unsaturated alcohols, including 2-Octen-1-ol, 2-nonene-1-ol, and 2-Decyn-1-ol, detected in the water boiling group, contribute significantly to crayfish meat flavor. Furthermore, both water boiling (6.67 %) and microwaving (17.66 %) groups exhibited elevated concentrations of 1-Pentanol, a derivative of the oxidation of linoleic acid. Aldehydes, primarily stemming from the oxidative degradation of PUFAs and the Strecker reaction involving amino acids, play a pivotal role in elevating the flavor profile of crayfish meat, owing to their low threshold values (Liang et al., 2022). In the water boiling group, hexanal, nonanal, octanal, and benzaldehyde exhibited high relative contents. The nonanal and decanal were detected as aldehydes in the microwaving group. Both decanal and nonanal contribute orange and fresh scents, respectively, mainly derived from the oxidation of oleic acid and linoleic acid (Li et al., 2023a). Ketone compounds, with relatively high threshold values, exert minimal influence on crayfish meat flavor. Additionally, an aromatic compound was detected in both water boiling and microwaving groups. For a deeper analysis, principal component analysis (PCA) diagrams were generated, displaying two-dimensional scatter plots (Fig. 2G). PCA explained 74.7 % of the total variance, with PC1 contributing 42.4 % and PC2 contributing 32.3 %. This comprehensive coverage of variable information indicates clear distinctions in the results. Zhang et al. (2023a) found that employing a cooking method with reduced water content and prolonged heating duration, such as microwaving, can effectively facilitate the production of desirable tilapia aroma.

While the nutritional differences between microwaving and water boiling crayfish were minimal, the microwaving method displayed enhanced taste and volatile flavor absorption compared to water boiling. The impact of processing conditions on protein structure is central to their flavor adsorption capability, as highlighted by Jiang et al. (2022). The volatilization of flavor compounds from the food matrix is governed by the nature of the compounds and their mass transfer resistance, a point emphasized by Mao et al. (2015). The proteins' structural conformation and surface hydrophobicity, which are critical for flavor adsorption, undergo significant alterations due to the specific processing conditions, as observed by Lv et al. (2017).

Changes in crayfish meat with protein and lipid oxidation

Morphological changes in crayfish meat microstructure

The microstructural analysis of crayfish meat post-treatment is presented in Fig. 3(A-D), revealing the impact of cooking methods on muscle fiber arrangement. In the context of aquatic muscle-based foods, such microstructural modifications are reflective of underlying protein structural changes (Li et al., 2020). The water boiling method resulted in a compact muscle structure with uniform fiber bundle spacing, while microwaving induced the spacing between fibers.

The distinct microstructures are directly associated with the meat's textural properties, as evidenced by the lower hardness and chewiness in the microwaving group compared to the water boiling group, as shown in Fig. 1(C). The temperature response within the microwave field is complex, influenced by the crayfish's composition and shape, which leads to heterogeneous temperature profiles (Fan, et al., 2021). The rapid increase in hotspot temperatures during microwaving plateaus as water evaporates, followed by stabilization through heat conduction (Fan, et al., 2020).

Oxidative degradation of proteins and lipids in crayfish meat

Electron spin resonance (ESR) spectroscopy, a pivotal analytical technique for detecting free radicals, provides a profound insight into the incipient stages of oxidative reactions. Free radicals, as intermediaries in a plethora of chemical processes, are instrumental in instigating protein and lipid oxidation. This study measured the total free radical content, which can assail the α-carbon hydrogens of the protein backbone and side chains, thereby precipitating protein oxidation (Wang et al., 2023b). As depicted in Fig. 3(E), the microwave precooking of crayfish meat induced a slight elevation in radical signal intensities when compared to the water-boiled samples. This observation underscores the subtle yet significant impact of different precooked methods on the generation of reactive species in the meat. The variation in free radical content and ESR spectral characteristics is intricately linked to the progression and accumulation of oxidative reactions, notably lipid oxidation and protein oxidation. Jiang et al. (2023) reported a marginal disparity in free radical content during thermal

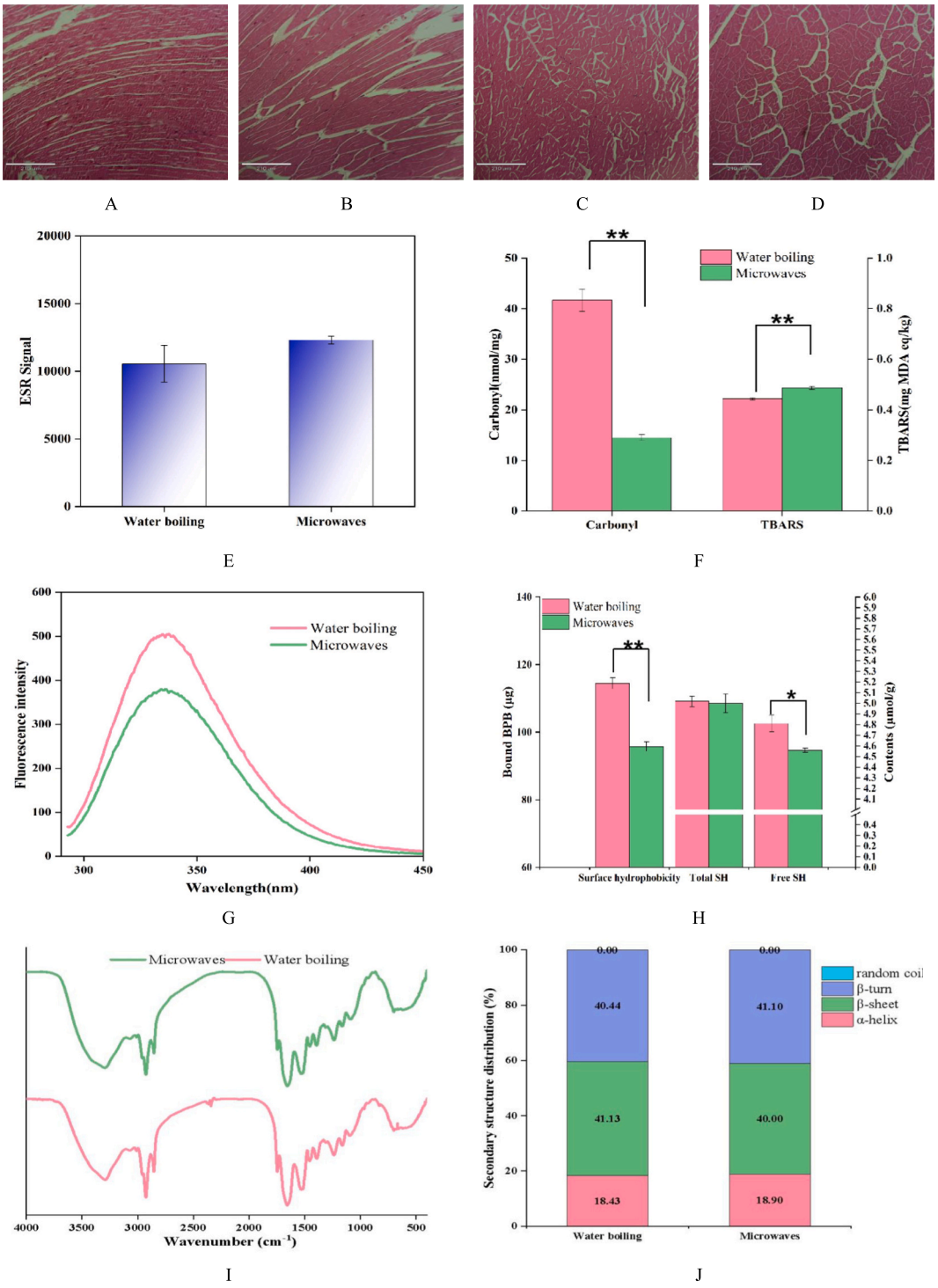

Fig. 3. Comparative analysis of protein and lipid oxidation in red swamp crayfish (*Procambarus clarkia*) prepared by different precooking methods: microstructure post water boiling (A back transverse, C abdomen transverse); microstructure post microwaving (B back transverse, D abdomen transverse); electron spin resonance (ESR) signal (E); oxidation status of protein and lipids (F); fluorescence spectra (G); surface hydrophobicity and molecular force analysis (H); FTIR spectra (I); secondary structure distribution (J). Asterisks in the legend indicate statistically significant mean differences at $*p < 0.05$ and $**p < 0.01$, as determined by one-way ANOVA and *t*-test.

processing, highlighting the nuanced differences in oxidative dynamics.

The TBARS assay is a cardinal indicator of lipid oxidation, primarily quantifying aldehydes generated during the secondary oxidation of polyunsaturated fatty acids (PUFAs) (Jiang et al., 2023). Furthermore, it is instrumental in the formation of flavor precursors. The TBARS values for microwaved crayfish were significantly elevated in comparison to those boiled in water (Fig. 3F). This increase in TBARS values was found to be highly correlated with the crude fat content (Fig. 1D) and alterations in PUFA profiles (Fig. 2B) of red swamp crayfish that underwent different precooked treatments. The higher crude fat content in microwaved crayfish meat, resulting from moisture loss, along with the thermally induced lipid oxidation, contributed to a decrease in PUFA levels. Additionally, the microwaving technique was more effective in enhancing the flavor and volatile profile of crayfish compared to water

boiling methods, offering a superior gustatory experience.

Carbonyl content, a well-established indicator of protein oxidation within meat system (Shi et al., 2020), revealed a significant discrepancy between microwaved and water-boiled crayfish proteins. The microwaved crayfish proteins had a considerably lower carbonyl content compared to those boiled in water, which is in direct contrast to the TBARS values presented in Fig. 3(F). This suggests that water-boiled crayfish are more susceptible to protein oxidation. In contrast, microwaved proteins experience a moderate level of oxidation that results in the cross-linking and compaction of the tissue structure, contributing to the enhanced hardness and chewiness as depicted in Fig. 1(C).

Comparative evaluation of tertiary and secondary protein structures

The intrinsic fluorescence spectrum serves as a valuable indicator of changes in tryptophan residues within the tertiary conformation of crayfish protein. As depicted in Fig. 3(G), the maximum fluorescence intensity (FI) for crayfish protein under both water boiling and microwaving treatments occurred around 337 nm with an excitation wavelength of 290 nm (tryptophan fluorescence). This spectral shift suggests an increased polarity of the environment surrounding tryptophan residues due to protein unfolding. Notably, the maximum fluorescence intensity of crayfish protein under microwaving showed a significant reduction compared to water boiling, implying crayfish protein unfolding and exposure of fluorophores to a more polar environment, leading to fluorescence quenching (Huang et al., 2022).

An effective method for estimating protein denaturation involves assessing surface hydrophobicity through the binding of bromophenol blue (BPB) molecules. The exposure of hydrophobic amino acid residues is a consequence of changes in the chemical and physical states of proteins induced by heat treatment, as elucidated by Yu et al. (2021b). The bound BPB values, as illustrated in Fig. 3(H), were significantly higher in water-boiled crayfish (114.50 μg) than in those subjected to microwaving (95.76 μg). This indicates a stronger surface hydrophobicity in the proteins of water-boiled crayfish, which exposes a larger number of hydrophobic amino acids capable of binding to BPB, compared to their microwaved counterparts (Wang et al., 2019). The reduced hydrophobic interactions resulting from microwaving may be instrumental in the increased liberation of flavor compounds, as evidenced in Fig. 2 and Table 1.

Sulfhydryl groups (−SH), primarily located in the head of myosin, exhibit sensitivity to reactive hydroxyl groups (•OH) and can undergo oxidation, leading to the formation of intramolecular and intermolecular disulfide bonds (Zhu et al., 2022; Huang et al., 2022). As illustrated in Fig. 3(H), the water-boiled crayfish tail meat demonstrated a higher content of reactive sulfhydryl groups at 4.81 μmol/g pro compared to the microwaved crayfish (4.56 μmol/g pro), suggesting that water boiling was more effective at exposing buried sulfhydryl groups within amino acid chains.

Employing FTIR, the study investigated alterations in the secondary structure of crayfish protein under both water boiling and microwaving conditions, as depicted in Fig. 3(I) and (J). The frequencies within the amide I band components (1700–1600 cm^{-1}), extracted from FTIR spectroscopy, were employed to calculate the secondary structural elements of crayfish proteins. Notably, no significant differences were discerned between the water boiling and microwaving in terms of α-helix (1650–1660 cm^{-1}), β-sheet (1600–1640 cm^{-1}), and β-turn (1660–1690 cm^{-1}). Similarly, there were no noticeable changes in random coils (1640–1650 cm^{-1}) (Li et al., 2023b). The substantial modifications in surface hydrophobicity and disulfide bonds (tertiary structure), coupled with minimal changes in the secondary structure of crayfish meat under microwaving and water boiling, suggest that microwaving preserves the spatial structure of proteins with less unfolding (Fan et al., 2021). This preservation could be attributed to the faster heating rate of microwaving, providing ample time for protein denaturation (Wang, et al., 2019, Dong, et al., 2021).

In vitro digestive properties of crayfish protein

Precooking is defined by the attainment of an internal sample temperature of 80°C, ensuring that the product is ready for immediate consumption or subsequent reheating. This process is pivotal in preparing food items for optimal taste and texture. The use of confocal laser scanning microscopy (CLSM) provides a unique lens through which to examine the morphological changes in crayfish protein during the digestive process following water boiling and microwaving. The CLSM images depicted in Fig. 4(A) and (B) reveal a notable reduction in the particle size of water-boiled crayfish protein, accompanied by a more uniform distribution post-gastrointestinal digestion. Upon hydrolysis by pepsin and trypsin, crayfish protein experiences degradation, translating to the transformation of high molecular weight proteins into smaller peptides or amino acids. This biochemical cascade culminates in a diminution of the particle size of the resultant digesta.

Proteins, as macromolecular nutrients, are broken down through enzymatic hydrolysis and digestion in the body, resulting in the release of amino acids. The degree of hydrolysis (DH%) is a critical parameter that quantifies the level of proteolysis, reflecting the efficiency with which proteins are converted into protein hydrolysates (Zhang et al., 2023b). As illustrated in Fig. 4(C), the DH% values for the gastrointestinal digestive solutions of microwaved and water-boiled crayfish were recorded at 46.12 % and 48.68 % respectively. Additionally, the pattern of soluble amino group alteration mirrored this trend, with water-boiled crayfish exhibiting a higher proportion post-digestion compared to microwaved counterparts. These outcomes are closely correlated with the observations in Fig. 3, suggesting that variations in precooked treatment methods could be instrumental in this disparity, as they induce structural modifications in proteins, thereby unmasking additional cleavage sites. The augmentation of surface hydrophobicity, consequent to these structural changes, facilitates enhanced protein unfolding, culminating in improved digestibility (Wijethunga et al., 2024). Fig. 4(D) corroborates these findings, demonstrating that the particle size of water-boiled crayfish protein post-gastrointestinal digestion was significantly smaller than that of microwaved crayfish, a finding consistent with the CLSM observations. The study conducted by Lu et al. (2023) revealed the process of microwaving had little effect on the digestion stability of Chinese mitten crab tropomyosin, comparing with thatultrasound and high temperature–pressure treatments.

Correlative analysis of protein digestibility and physicochemical properties

The susceptibility of protein digestibility to the influences of oxidative and structural modifications is well-established (Dong, et al., 2021, Li, et al.,2021, Wang et al. 2023b). In this study, Pearson correlation coefficients were employed to dissect the interplay between the digestive attributes of proteins and a spectrum of physicochemical indicators, including texture, nutritional content, volatile compounds, and oxidative markers. Fig. 5 utilizes a color-coded system to denote positive (red) and negative (blue) correlations, offering a visual synopsis of the relationships observed. The findings elucidate a significant negative linear correlation between the digestive properties and certain textural attributes, specifically elasticity, as well as with indicators of lipid oxidation and taste-related substances such as free amino acids (FAAs) and nucleotides. Conversely, a positive linear correlation was observed between the digestive properties and parameters associated with protein oxidation, including carbonyl content, reactive hydroxyl groups, and surface hydrophobicity. Additionally, a positive correlation was noted with textural attributes like hardness and chewiness, as well as nutritional components such as fatty acids and amino acids. These correlations underscore the pivotal role of protein structural alterations induced by oxidation in conjunction with nutritional factors in augmenting the digestive properties of crayfish meat. In contrast, lipid oxidation and taste substances appear to exert a detrimental effect on digestibility, potentially hindering the digestive process in red swamp

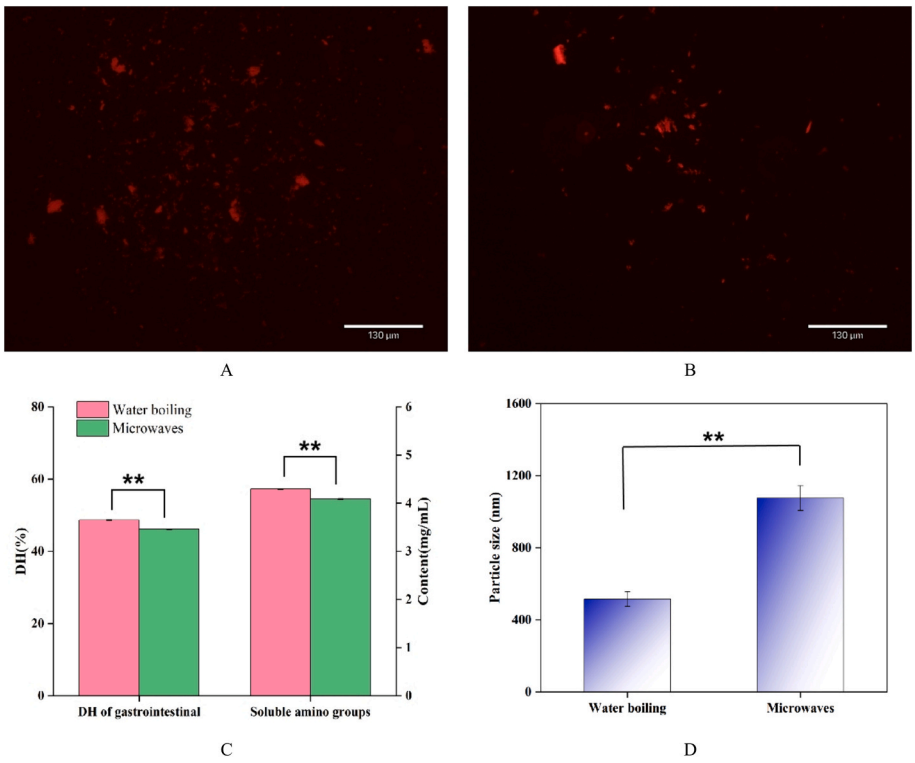

Fig. 4. *In vitro* examination of protein digestive properties in red swamp crayfish (*Procambarus clarkia*) following various precooking methods: Confocal laser scanning microscopy (CLSM) images post gastrointestinal digestion of water-boiled crayfish (A); CLSM images post gastrointestinal digestion of microwaved crayfish (B); degree of hydrolysis (DH) and soluble amino groups from gastrointestinal digestion (C); particle size distribution (D). Statistical significance among means is indicated by asterisks at $*p < 0.05$ and $**p < 0.01$, as determined by one-way ANOVA and *t*-test.

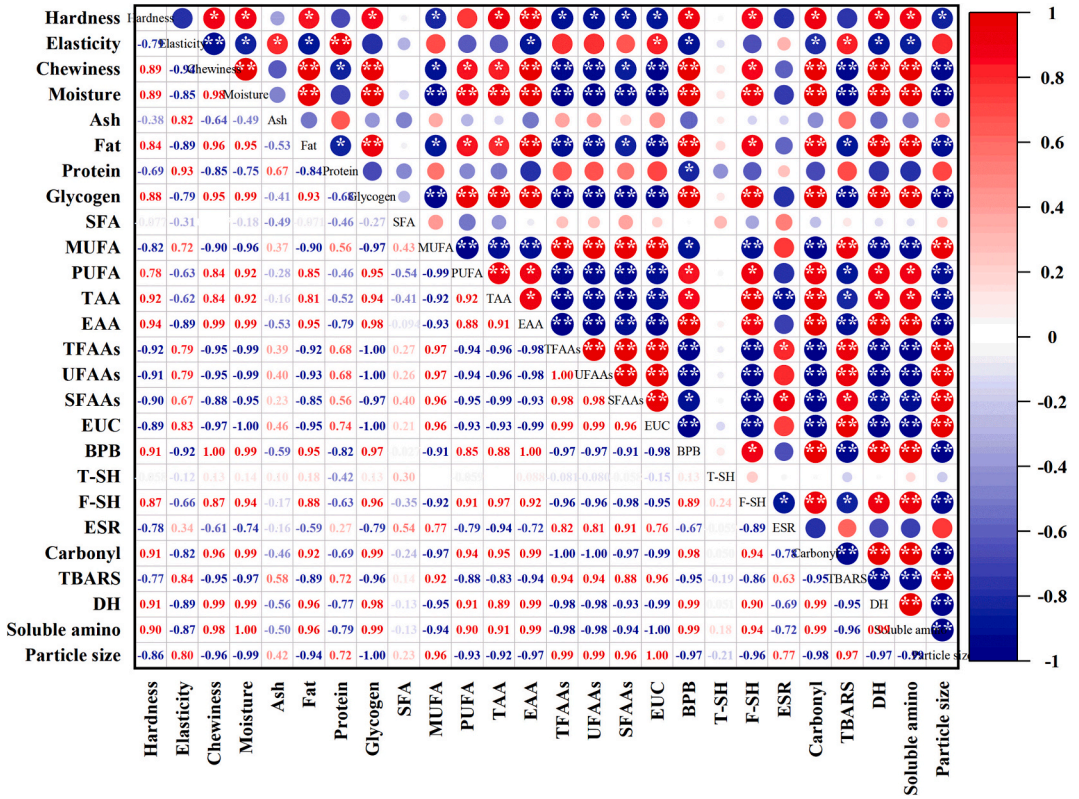

Fig. 5. Correlation analysis between protein digestive properties and physicochemical indicators, encompassing texture, nutrition, volatile compounds, and oxidation. Pearson's correlation coefficients were calculated, with darker shades indicating stronger correlations. Positive correlations are illustrated in red and negative in blue, with significance denoted by $*p < 0.05$ and $**p < 0.01$.

crayfish meat subjected to diverse treatment methods.

Conclusions

The oxidative analysis of precooked red swamp crayfish (*Procambarus clarkii*) meat reveals a nuanced relationship between cooking methods and meat quality. Microwave precooking, while beneficial for flavor retention, leads to moderate lipid oxidation and minimal protein oxidation, subtly altering the meat's lipid profile and potentially its flavor and aroma. In contrast, water boiling is more effective in preserving the meat's digestive properties and protein integrity, crucial for nutritional value and digestibility. The study highlights that precooked treatments significantly influence the nutritional and sensory profiles of crayfish meat, offering valuable insights for food scientists and industry professionals in optimizing cooking techniques. Future research should delve into the protein structural dynamics post-secondary cooking to better understand how these changes impact the meat's texture, flavor release, and consumer appeal. This focused approach will aid in developing food processing strategies that enhance both the nutritional content and sensory experience of crayfish meat, driving innovation and consumer satisfaction in the food industry.

CRediT authorship contribution statement

Wensi Xu: Writing – review & editing, Writing – original draft, Software, Formal analysis, Data curation, Conceptualization. **Qifu Yang:** Writing – review & editing, Visualization, Resources. **Deyang Li:** Visualization, Methodology, Investigation. **Xiaoyang Liu:** Methodology, Investigation, Funding acquisition. **Pinhong Yang:** Supervision, Resources, Funding acquisition. **Liang Song:** Supervision, Software, Methodology. **Dayong Zhou:** Supervision, Resources, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101692.

References

Bai, S., Qin, D., Chen, Z., Wu, S., Tang, S., Gao, L., & Wang, P. (2022). Geographic origin discrimination of red swamp crayfish *Procambarus clarkii* from different Chinese regions using mineral element analysis assisted by machine learning techniques. *Food Control, 138*, Article 109047. https://doi.org/10.1016/j.foodcont.2022.109047

Dong, X., Wang, J., & Raghavan, V. (2021). Impact of microwave processing on the secondary structure, in-vitro protein digestibility and allergenicity of shrimp (*Litopenaeus vannamei*) proteins. *Food Chemistry, 337*, Article 127811. https://doi.org/10.1016/j.foodchem.2020.127811

Duque-Estrada, P., Berton-Carabin, C. C., Nieuwkoop, M., Dekkers, B. L., Janssen, A. E. M., & van der Goot, A. J. (2019). Protein oxidation and in vitro gastric digestion of processed soy-based matrices. *Journal of Agricultural and Food Chemistry, 67*(34), 12521–12526. https://doi.org/10.1021/acs.jafc.9b02423

Fan, H., Fan, D., Huang, J., Zhao, J., Yan, B., Ma, S., & Zhang, H. (2020). Cooking evaluation of crayfish (*Procambarus clarkia*) subjected to microwave and conduction heating: A visualized strategy to understand the heat-induced quality changes of food. *Innovative Food Science and Emerging Technologies, 62*, Article 102368. https://doi.org/10.1016/j.ifset.2020.102368

Fan, H., Huang, J., Zhao, J., Yan, B., Ma, S., Zhou, W., & Fan, D. (2021). Electromagnetic properties of crayfish and its responses of temperature and moisture under microwave field. *Journal of Food Science, 86*(4), 1306–1321. https://doi.org/10.1111/1750-3841.15667

Huang, J., Hu, Z., Hu, L., Li, G., Yuan, C., Chen, J., & Hu, Y. (2022). The modification effects of roselle anthocyanin film on shrimp texture via water distribution controlling and protein conformation maintenance. *Food Bioscience, 49*, Article 101976. https://doi.org/10.1016/j.fbio.2022.101976

Jiang, Q., Shen, H., Gao, P., Yu, P., Yang, F., Xu, Y., Yu, D., Xia, W., & Wang, L. (2023). Non-enzymatic browning path analysis of ready-to-eat crayfish (*Promcambarus clarkii*) tails during thermal treatment and storage. *Food Bioscience, 51*, Article 102334. https://doi.org/10.1016/j.fbio.2022.102334

Jiang, Y., Wang, Z., He, Z., Zeng, M., Qin, F., & Chen, J. (2022). Effect of heat-induced aggregation of soy protein isolate on protein-glutaminase deamidation and the emulsifying properties of deamidated products. *LWT - Food Science and Technology, 154*, Article 112328. https://doi.org/10.1016/j.lwt.2021.112328

Khalid, W., Maggiolino, A., Kour, J., Arshad, M. S., Aslam, N., Afzal, M. F., Meghwar, P., Zafar, K. U., De Palo, P., & Korma, S. A. (2023). Dynamic alterations in protein, sensory, chemical, and oxidative properties occurring in meat during thermal and non-thermal processing techniques: A comprehensive review. Article 1057457 *Frontiers in Nutrition, 9*. https://doi.org/10.3389/fnut.2022.1057457.

Li, D., Li, N., Dong, X., Tan, Z., Na, X., Liu, X., & Zhou, D. (2022a). Effect of phytic acid combined with lactic acid on color and texture deterioration of ready-to-eat shrimps during storage. *Food Chemistry, 396*, Article 133702. https://doi.org/10.1016/j.foodchem.2022.133702

Li, D., Liu, Z., Liu, B., Qi, Y., Liu, Y., Liu, X., Qin, L., Zhou, D., & Shahidi, F. (2020). Effect of protein oxidation and degradation on texture deterioration of ready-to-eat shrimps during storage. *Journal of Food Science, 85*(9), 2673-2680. https://doi:10.1111/1750-3841.15370.

Li, D., Tan, Z., Liu, Z., Wu, C., Liu, H., Guo, C., & Zhou, D. (2021). Effect of hydroxyl radical induced oxidation on the physicochemical and gelling properties of shrimp myofibrillar protein and its mechanism. *Food Chemistry, 351*. https://doi.org/10.1016/j.foodchem.2021.129344. Article 129344.

Li, J., Zhang, Q., Peng, B., Hu, M., Zhong, B., Yu, C., & Tu, Z. (2023a). Exploration on the quality changes and flavour characteristics of freshwater crayfish (*Procambarus clarkia*) during steaming and boiling. *LWT - Food Science and Technology, 190*, Article 115582. https://doi.org/10.1016/j.lwt.2023.115582

Li, M., Wu, Y. Q., & Ye, L. (2022b). The Role of Amino Acids in Endothelial Biology and Function. *CELLS, 11*(8). https://doi.org/10.3390/cells11081372

Li, N., Zhang, K., Dong, X., Xu, Y., Tan, Z., Cao, G., Liu, X., Zhou, D., & Li, D. (2023b). Modification of the structure and function of myofibrillar protein by structurally relevant natural phenolic compounds. *Food Bioscience, 53*, Article 102676. https://doi.org/10.1016/j.fbio.2023.102676

Liang, R., Lin, S., Chen, D., & Sun, N. (2022). Differentiation of *Penaeus vannamei* from different thermal processing methods in physicochemical, flavor and sensory characteristics. *Food Chemistry, 378*, Article 132092. https://doi.org/10.1016/j.foodchem.2022.132092

Liu, C., Ji, W., Jiang, H., Shi, Y., He, L., Gu, Z., & Zhu, S. (2021). Comparison of biochemical composition and non-volatile taste active compounds in raw, high hydrostatic pressure-treated and steamed oysters *Crassostrea hongkongensis*. *Food Chemistry, 344*, Article 128632. https://doi.org/10.1016/j.foodchem.2020.128632

Lu, Y., Cheng, H., Jiang, S., Lin, L., & Lu, J. (2023). Impact of three different processing methods on the digestibility and allergenicity of Chinese mitten crab (*Eriocheir sinensis*) tropomyosin. *Food Science and Human Wellness, 12*, 903–911. https://doi.org/10.1016/j.fshw.2022.09.024

Lv, T., Wang, Y., Pan, D., Cao, J., Zhang, X., Sun, Y., & Liu, Y. (2017). Effect of trypsin treatments on the structure and binding capacity of volatile compounds of myosin. *Food Chemistry, 214*, 710–716. https://doi.org/10.1016/j.foodchem.2016.07.115

Ma, R., Liu, X. H., Tian, H. N., Han, B. Y., Li, Y. P., Tang, C. Z., & Meng, Y. Q. (2020). Odor -active volatile compounds profile of triploid rainbow trout with different marketable sizes. *Aquaculture Reports, 17*, Article 100312. https://doi.org/10.1016/j.aqrep.2020.100312

Mao, L., Roos, Y. H., & Miao, S. (2015). Effect of maltodextrins on the stability and release of volatile compounds of oil-in-water emulsions subjected to freeze–thaw treatment. *Food Hydrocolloids, 50*, 219–227. https://doi.org/10.1016/j.foodhyd.2015.04.014

Mukhametov, A., Yerbulekova, M., Aitkhozhayeva, G., Tuyakova, G., & Dautkanova, D. (2022). Effects of co-3 fatty acids and ratio of co-3/co-6 for health promotion and disease prevention. *Food Science and Technology, 42*. https://doi.org/10.1590/fst.58321

Peng, G., Sun, J., Peng, B., Tan, Y., Wu, Y., & Bai, X. (2021). Assessment of essential element accumulation in red swamp crayfish (*Procambarus clarkii*) and the highly efficient selenium enrichment in freshwater animals. *Journal of Food Composition and Analysis, 101*, Article 103953. https://doi.org/10.1016/j.jfca.2021.103953

Barnett, S. M., Sablani, S. S., Tang, J., & Ross, C. F. (2020). The potential for microwave technology and the ideal profile method to aid in salt reduction. *Journal of Food Science, 85*(3), 600–610. https://doi.org/10.1111/1750-3841.15034

Shi, L., Xiong, G., Yin, T., Ding, A., Li, X., Wu, W., Qiao, Y., Liao, L., Jiao, C., & Wang, L. (2020). Effects of ultra-high pressure treatment on the protein denaturation and water properties of red swamp crayfish (*Procambarus clarkia*). *LWT - Food Science and Technology, 133*, Article 110124. https://doi.org/10.1016/j.lwt.2020.110124

Sohail, A., Al-Dalali, S., Wang, J., Xie, J., Shakoor, A., Asimi, S., Shah, H., & Patil, P. (2022). Aroma compounds identified in cooked meat: A review. *Food Research International (Ottawa Ont.), 157*, Article 111385. https://doi.org/10.1016/j.foodres.2022.111385

Wang, X., Fan, C., Wang, X., Feng, T., Yu, J., Zhang, X., Yu, J., Cui, H., & Xia, S. (2023a). Microwave heating and conduction heating pork belly: Influence of heat transfer modes on volatile compounds and aroma attributes. *Food Bioscience, 52*, Article 102438. https://doi.org/10.1016/j.fbio.2023.102438

Wang, X., Muhoza, B., Wang, X., Feng, T., Xia, S., & Zhang, X. (2019). Comparison between microwave and traditional water bath cooking on saltiness perception, water distribution and microstructure of grass crap meat. *Food Research International, 125*, Article 108521. https://doi.org/10.1016/j.foodres.2019.108521

Wang, Z., Wu, Z., Zhao, G., Li, D., Liu, Y., Qin, L., Jiang, P., & Zhou, D. (2023b). Effect of air frying and baking on physicochemical properties and digestive properties of scallop (*Patinopecten yessoensis*) adductor muscle. *Food Bioscience, 52*, Article 102460. https://doi.org/10.1016/j.fbio.2023.102460

Wijethunga, A. M., Wang, H., & Sun, X. (2024). Starch removal treatment, not the specific thermal processing techniques, improved protein digestibility of corn (*Zea mays*) gluten meal. *Food Hydrocolloids, 149*, Article 109619. https://doi.org/10.1016/j.foodhyd.2023.109619

Xu, S., Zhou, L., & Zou, P. (2023). What influences stakeholders' decision in adopting blockchain-based quality tracking systems in prefabricated construction. *Engineering Construction and Architectural Management*. https://doi.org/10.1108/ecam-06-2022-0501

Yi, B., & Xu, H. Y. (2023). Research and Development Status of Prepared Foods in China: A Review. *Applied Sciences-Basel, 13*(14). https://doi.org/10.3390/app13147998

Ying, X. G., Li, T. Y., Deng, S. G., Brennan, C., Benjakul, S., Liu, H. F., & Ma, L. K. (2024). Advancements in nonthermal physical field technologies for prefabricated aquatic food: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety, 23*(1), 1–34. https://doi.org/10.1111/1541-4337.13290

Yu, M., Li, D., Liu, Z., Liu, Y., Zhou, J., Zhang, M., Zhou, D., & Zhu, B. (2021a). Effects of heat treatments on texture of abalone muscles and its mechanism. *Food Bioscience, 44*, Article 101402. https://doi.org/10.1016/j.fbio.2021.101402

Yu, X., Zou, Y., Zheng, Q., Lu, F., Li, D., & Guo, L. (2021b). Physicochemical, functional and structural properties of the major protein fractions extracted from *Cordyceps militaris* fruit body. *Food Research International, 142*, Article 110211. https://doi.org/10.1016/j.foodres.2021.110211

Zhang, D., Ayed, C., Fisk, I. D., & Liu, Y. (2023a). Effect of cooking processes on tilapia aroma and potential umami perception. *Food Science and Human Wellness, 12*, 35–44. https://doi.org/10.1016/j.fshw.2022.07.016

Zhang, K., Li, N., Li, J., Wang, Y., Liu, C., Liu, Y., Liu, X., Zhou, D., & Li, D. (2023b). Improving the gelation and digestive properties of myofibrillar protein in *Litopenaeus vannamei* by ultra-high pressure. *Food Bioscience, 56*, Article 103402. https://doi.org/10.1016/j.fbio.2023.103402

Zhu, S., Zhu, L., Ke, Z., Chen, H., Zheng, Y., Yang, P., Xiang, X., Zhou, X., Jin, Y., Deng, S., Zhou, X., Ding, Y., & Liu, S. (2023). A comparative study on the taste quality of *Mytilus coruscus* under different shucking treatments. *Food Chemistry, 412*, Article 135480. https://doi.org/10.1016/j.foodchem.2023.135480

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*(2), 142–151. https://doi.org/10.1016/j.lwt.2019.04.091

Zhu, Z., Pius Bassey, A., Cao, Y., Ma, Y., Huang, M., & Yang, H. (2022). Food protein aggregation and its application. *Food Research International, 160*, Article 111725. https://doi.org/10.1016/j.foodres.2022.111725