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Properties of boiled or steamed *Procambarus clarkii girard* and *Procambarus zonangulus* crawfish tail meat during refrigerated and frozen storage

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Abstract

This research was conducted to address the different cooking methods of crawfish processors and determine the refrigerated or frozen shelf-life of cooked product. Live crawfish, (*Procambarus clarkii girard and Procambarus zonangulus*), were either boiled or steamed before storage of 11 days in refrigerated (3°C) conditions or six months in frozen storage (-18°C). There were minimal moisture, ash, protein, and fat differences with cooking type or storage type. There were no *E.coli*/coliforms in samples and aerobic plate counts were less than 3 $\log_{10}$ colony forming untis (CFU)/g after 6 mo frozen storage and higher than 3 log10 CFU/g after 3 days of refrigerated storage. Lipid oxidation by TBARS increased, but was less than 0.53 mg MDA/kg during storage. Peak force, total shear work, pH, L*, a*, b* color values were variable during storage, but not different between cooking treatments at each storage period. Mineral and fatty acid analyses were similarly variable. There were no differences between boiling and cooking crawfish for most variables and natural variation among the samples might explain variability in refrigerated and frozen storage. Processors can use either boiling or steaming to cook crawfish and store cooked crawfish for 3 days in refrigerated storage and for 6 weeks in frozen storage with minimal changes in properties.

Keywords: Crawfish, Boiling, Steaming, Nutrient composition, Shelf-life, Yield.

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Contents

Contribution of this paper to the literature
This research compares two different methods of heating two species of crawfish for consumption and the subsequent properties of the crawfish during refrigerated and frozen storage. It provides data on the composition, texture, color, and microorganism growth during 11 days of refrigerated and 6 months of frozen storage of boiled and steamed crawfish tail meat.

1. Introduction

Crawfish/crayfish have emerged as a highly desirable and valued culinary delicacy because of their distinctive sensory, textural and flavor characteristics [1, 2]. Use of the term "crawfish" in 1817 predates the term "crayfish" used in the Huxley 1880 textbook [3] and so "crawfish" will refer to the crustacean in this manuscript. Of the many species of crawfish around the world, only two species, *Procambarus clarkii*, the red swamp crawfish, and *Procambarus zonangulus*, the white river crawfish, are commercially important for harvest in Louisiana, USA [4-6]. The white river crawfish is less tolerant of poor water conditions and warmer temperatures than the red swamp crawfish [5]. Consumers and processors prefer the red swamp crawfish to the white river crawfish for appearance and flavor [4, 7].

Crawfish may be purchased live or boiled, if for immediate consumption, depending upon the preference of consumers and restauranteurs [8] and more recently as peeled tail meat, fresh or frozen, of cooked crawfish. Crawfish tail meat possess a high protein content [approximately 20%] and eight essential amino acids for human consumption [9]. Crawfish, like other seafood, are a perishable product with postmortem changes occurring rapidly that cause off-odors and flavors even before spoilage Miget [10] and Zeng, et al. [11]. Davis [5] suggested that crawfish tail meat with adhering hepatopancreas can be frozen, but typically becomes rancid within 30 days of storage. A mushy texture may occur after relatively short periods of iced storage, but deterioration of texture has also been observed in crawfish through extended frozen storage [12]. Freezing rate, frozen storage conditions and thawing rate influence frozen food properties [13, 14]. Frozen seafood can experience color changes [15] protein denaturation [16] lipid oxidation [17] and ice sublimation and recrystallization [1, 18] which may result in drip loss, dehydration, toughening, off-flavor, and lipid oxidation [18, 19]. Drum/belt mechanically separated mince of boiled crawfish had 22% less lipid oxidation with cryogenic liquid nitrogen freezing than with air blast freezing after 180 d of frozen storage [20].

The two most prevalent commercial thermal treatments for cooking of crawfish are steaming and boiling because of their low cost and large scale efficiency [2]. Raw crawfish meat may have only faint fishy odors while the desirable flavor of cooked crayfish meat is mainly from complex reactions of flavor precursors, intermediates and their interaction products [21]. Crawfish cooking can produce lipid oxidation degradation, the Maillard reaction and the interaction between the two reactions, resulting in volatile flavor compounds, such as aldehydes, alcohols, ketones and other compounds, that provide a unique flavor [21].

Microorganisms are also a major concern in the spoilage and safety of crawfish [11, 22] as farmed or wild caught crawfish are apt to come in contact with harmful organisms in their habitats [10]. An added microbiological concern with crawfish tail meat is microbial contamination by hand-peeling from the shells if proper sanitation practices are not practiced [23]. An increase in APC to levels exceeding 10^6 colony forming units(CFU)/g is generally a sign of a long period of time at refrigerated temperatures or temperature abuse before freezing [24]. Aerobic plate count (APC) and *E. coli*/coliform limits have been suggested by the International Commission on Microbiological Specifications for Foods [24]. The limits are appropriate for both refrigerated and frozen cooked crawfish. Any sample having an APC count exceeding log 7 CFU/g should result in the product being discarded. For *E. coli*/colifoms, any sample exceeding 500 CFU/g should result in product being discarded.

Procambarus clarkii crawfish harvested from rice fields had higher aroma and texture acceptability scores from a consumer panel than those from moist-soil wetlands, but there were no differences in flavor, overall acceptability, composition, or fatty acid contents [25]. The total amounts of protein, fat, and ash increased in tail meat after both microwave and frying cooking while water content decreased, especially in the fried samples [26].

Steaming and boiling significantly influenced the fatty acids and free amino acids and were highly correlated with flavor and key flavor compounds in pulverized freshwater crawfish (*Procambarus clarkia*) tail meat [2]. Color, texture, flavor components, and microorganism growth were measured through 11 days of refrigerated and 6 months of frozen storage of boiled and steamed crawfish tail meat from *Procambarus clarkia* and *Procambarus zonangulus* species.

2. Materials and Methods

2.1. Procurement and Initial Preparation

Live freshwater crawfish consisting of both red swamp crawfish (*Procambarus clarkia*), and white river crawfish (*Procambarus zonangulus)* were obtained from the Louisiana State University AgCenter Aquaculture Research Station in Baton Rouge, Louisiana, USA and from Tony's Seafood Market and Deli of Baton Rouge, Louisiana, USA. The live crawfish were transported to the Food Science building in Baton Rouge, Louisiana in mesh sacks varying in weight from 12 to 18 kg and stored two days at 4°C until processed.

Prior to cooking the crawfish were removed from the mesh sacks and placed in 1.5m x 0.6m x 0.6m metal containers. The crawfish from the different locations were mixed and washed with municipal tap water to remove any mud and debris. Dead crawfish were separated from the live crawfish and discarded. The crawfish were then kept moist by regularly spraying with municipal tap water until they were processed.

Two lots of 28.8 kg of crawfish destined for boiling were removed from the metal lugs and placed in a stainless steel basket. Temperatures were obtained with an T-type thermocouple and data logger (Omega OM-DAQPRO-5300, Omega Engineering Inc., Stamford, CT). The crawfish were placed in approximately 75.7 L of unseasoned municipal tap water that had been brought to a boil at atmospheric pressure in a 150 L jacketed steam kettle (B.H. Hubbert & Son, Inc., Baltimore, MD) with university-generated 206.85 cm Hg food-grade steam as the steam source. The temperature of the water in the kettle was measured at periodic (~30 second) intervals during the cook process (Comark C28 K-type thermocouple, Comark Instruments, Norfolk, England). Crawfish were lowered into the steam

kettle after the water had been brought to a boil, completely submerged in the water, and continuously stirred with a plastic paddle to ensure that the crawfish were evenly heated. It took seven minutes for the water to return to a boil. The crawfish were then boiled for exactly three minutes. The cook time of ten minutes (as used by the D&T Crawfish Company in Abbeville, Louisiana) satisfied the adequate cook time of seven minutes or more [4] that would ensure the deactivation of proteolytic enzymes. At the end of the three-minute boil, steam was immediately turned off and several crawfish were retrieved to measure internal temperature using the Comark C28 K-type thermocouple inserted into the thickest part of the tail. The internal temperature of the crawfish from the boiling trials ranged from 86.7 to 91.1°C. Once the steam was turned off, crawfish were submerged in a sanitized plastic lug (1.2m x 1.2m x 0.9m) containing ice-water to rapidly cool the crawfish and prevent continued cooking. After five minutes of cooling crawfish temperatures were measured using the Comark C28 K-type thermocouple before pouring into plastic lined, waxed fish boxes (0.6m x 0.3m x 0.3m) for cooling at 5°C for 4 hr prior to being separated into treatments of 3.5°C refrigerated storage and frozen storage at -18°C.

A commercial batch vegetable blancher was used to steam crawfish. Eight trials of approximately 7.3 kg of live crawfish were conducted because of the limited blancher capacity. Live crawfish (~7.3kg) were placed in stainless steel trays and inserted into the chamber of the blancher. Thermocouples (Omega OM-DAQPRO-5300 Type-T) connected to a data logger (Omega Engineering Inc., Stamford, CT) were placed in the tails of two crawfish per trial. Initial temperatures (uncooked) and final temperature of the crawfish during the cook process were recorded. The crawfish were steamed in the blancher using food-grade steam at 206.86 cmHg to the same internal temperature (90 to 92.2°C) reached during the boiling process. The crawfish were immediately removed from their trays and placed in an ice bath similar for five minutes of cooling with periodic stirring. Crawfish were placed in plastic containers [0.6m x 0.3m x 0.3m] and stored in a cooler at 5°C for 4 hr prior to allotment of refrigerated storage at 4°C for 11 d and frozen storage at -18°C for 6 mo. At each month of frozen storage, crawfish were removed from the boxes, placed in shallow trays and thawed at 4°C for analyses.

Deactivation of proteolytic enzymes by the heating treatments was determined by a gelatin test [4]. Following the 4-hour cooling period at 5°C, five g of hepatopancreas from each treatment including raw crawfish hepatopancreas were obtained by peeling the tails of 5-10 crawfish of each treatment. The hepatopancreas was then minced using a metal spatula and set aside at 5°C until used. Then, in triplicate for each treatment, 0.2 g of mince was placed into labeled 22 mm tubes (Pyrex®, Corning Corporation, Tewksbury, MA). Concurrently, blanks in triplicate, which contained no hepatopancreas, were prepared. Five ml of cooled 12% unflavored gelatin (Knox®, Kraft Foods Group, Inc., Northfield, IL) in water were then added to each tube and the contents homogenized using a Vortex-Genie® 2 mixer (Scientific Industries Inc., Bohemia, NY). The samples were then allowed to incubate for 1h at room temperature followed by holding at 3°C for 23 h. After the 23 hour refrigerated storage, the samples were removed and analyzed subjectively to determine the presence of a firm gel. A loose gel or no gel formation at all indicated that proteolytic enzymes present in the hepatopancreas had not been deactivated whereas firm gels revealed that the enzymes had been deactivated by an adequate cooking process.

2.2. Microbiological Analysis

The microbiological analyses conducted on the crawfish included the quantification of the aerobic plate count (APC) and *E.coli*/coliforms of refrigerated samples on days 0, 1, 3, 5, 7, 9, and 11 and on frozen samples at months 0, 1, 2, 3, 4, 5, and 6. Crawfish tail meat with adhering hepatopancreas was removed by hand peeling and placed in 17.78cm x 19.05cm reclosable storage bags (Qwik Seal®, Reynolds®, Lake Forest, Illinois). Twenty-five gram samples [in duplicate for each treatment] were prepared and placed in 1.56kg bags (Whirl-pak®, Nasco, Fort Atkinson, WI) along with 25.0 mL of phosphate buffered saline (PBS), which was composed of 0.24% sodium phosphate monobasic [Sigma-Aldrich Corporation, St. Louis, MO], 0.28% sodium phosphate dibasic (Sigma-Aldrich Corporation, St. Louis, MO), and 0.85% sodium chloride (Amresco LLC, Solon, OH) in distilled water. The 25 g of tail meat and 25 mL of PBS were homogenized for 60 seconds (EasyMix blender, AES Chemumex, Bruz Cedex, France). From each treatment, serial dilutions were prepared and samples plated on both 3M™ E.coli/Coliform and Aerobic Count Petrifilms™ (3M Company, St. Paul, MN), then incubated at 35° ± 1°C. *E.coli* and coliforms were counted and recorded after 24 ± 3 hours as colony forming units and APC was counted and recorded after 48 ± 3 h (Official Methods 991.14 and 998.08 for *E.coli*/coliforms and Official Methods 990.12 for aerobic plate count, [AOAC International, Rockville, MD], 2005).

2.3. Color Analysis

Hunter color scale values (L*, a*, and b*) were measured on ten randomly selected tails with hepatopancreas at the widest point at the back of the peeled tails for both boiled and steamed crawfish [calibrated CM-508d Spectrophotometer, Konica Minolta Sensing Americas Inc., Ramsey, NJ]. Measurements on refrigerated samples were conducted on days 1, 3, 5, 7, 9, and 11 of refrigeration (4°C) and on frozen samples on months 1, 2, 3, 4, 5, and 6 of frozen storage (-18°C).

2.4. Texture Analysis

Texture analysis was conducted using a TA-XT Plus Texture Analyzer (Texture Technologies Corporation, Scarsdale, NY) with a 5-blade Kramer shear attachment and a 30 kg load cell. Texture of peeled tails with adhering hepatopancreas from both boiled and steamed treatments was measured on days 0, 1, 3, 5, 7, 9, and 11 for refrigerated samples and after 0, 1, 2, 3, 4, 5, and 6 months of frozen storage. Peak shear force (N) and work of shearing (N/s) were measured on 100 grams of peeled tail meat from each treatment in triplicate. One hundred grams of sample were placed randomly in the Kramer cell, which filled the cell to roughly 40-50% capacity. The blade was set at 45 mm (~10mm above the sample) and the blade speed was set at 3 mm per second. Following the texture determination, the samples were fully homogenized (Oster® Osterizer 14 speed all metal drive blender, Jarden Consumer Solutions, Providence, RI) and used for subsequent proximate and TBARS analyses.

2.5. Proximate Analysis

Proximate analysis (moisture, ash, protein, and fat) was conducted on refrigerated crawfish tail meat with adhering hepatopancreas on days 0, 1, 3, 5, 7, 9, and 11 and on frozen crawfish tail meat with adhering hepatopancreas at months 0, 1, 2, 3, 4, 5, and 6 for both boiled and steamed samples.

Moisture analysis was conducted in triplicate for each cooking treatment. Three grams of homogenized tail meat were placed in ceramic crucibles, weighed, and then placed in an oven (Model 20 GC Lab Oven, Quincy Lab Inc., Chicago, IL) at 100°C for 24 hours. Samples were removed from the oven and placed in a desiccator to cool before weighing. The percent moisture of tail meat with adhering hepatopancreas was determined by the calculation:

$$\% \text{ Moisture} = ((\text{Wet Weight} - \text{Dry Weight})/\text{Wet Weight}) \times 100.$$

Ash measurements were conducted on the samples after moisture determination. The samples in triplicate were heated to 550°C for 18-24 hours (Type 6000 Furnace, Thermolyne Inc., Dubuque, IA), removed from the furnace and placed in a desiccator to cool before weighing. The percent ash was determined using the calculation:

%Ash = (Weight after ashing – Tare Weight of Crucible)/(Dry Sample Weight after Oven Drying – Tare weight of Crucible) × 100.

Lipid extraction was performed according to a modified method of Bligh and Dyer [27] described by Woyewoda, et al. [28]. Total lipid quantification was performed in duplicate for each treatment. Following lipid quantification on a wet weight basis, fatty acid analysis was conducted on the lipid. Peeled crawfish tail meat with adhering hepatopancreas were homogenized (Oster® Osterizer 14 speed all metal drive blender with a rosin blade, Jarden Consumer Solutions, Providence, RI). A measured amount of homogenized tail meat (approximately 50.0 grams) was added to a commercial blender (Waring® model 51BL31, Waring®, Stanford, CT) along with 100.0 mL of high performance liquid chromatography (HPLC) grade anhydrous methyl alcohol (Avantor™ Performance Materials Inc., Center Valley, PA) and 50.0 mL of HPLC grade Chloroform (Mallinckrodt Baker Inc., Phillipsburg, NJ) and blended for exactly two minutes. An additional 50.0 mL of chloroform was added to the blender and the mixture was blended for an additional 30.0 seconds. The mixture was then filtered through a Buchner funnel containing a #1 filter paper (Whatman,™ GE Healthcare UK Limited, Buckinghamshire, UK). Aspiration was used to expedite the filtering process. The filtered product contained lipid, chloroform, and methanol. Fifty mL of distilled water was added and the mixture was then stirred vigorously. The mixture was transferred to a 250-mL separatory funnel and then allowed to rest at 5°C overnight. The following day, the chloroform-lipid layer was then filtered from the separatory funnel through a glass funnel containing #4 filter paper inside #1 filter paper (Whatman™, GE Healthcare UK Limited, Buckinghamshire, UK) that was filled with American Chemical Society (ACS) grade anhydrous sodium sulfate (ThermoFisher Scientific, Waltham, MA) into a pre-weighed 250 ml round bottom boiling flask (Corning Corporation, Tewksbury, MA). The chloroform was then removed from the round bottom flask using a rotoevaporator (Buchi Rotovapor R114, Buchi Corporation, New Castle, DE). To ensure that all solvent was removed and only crude lipid remained, ultra- high pure (UHP) nitrogen (Air Liquide Corporation, Paris, France) was sprayed into the flask for 10-15 minutes or until no odor or appearance of solvent remained. The crude lipid remaining was weighed and the percent fat was determined by the calculation: % Fat = ((Weight of Flask Containing Lipid – Weight of Empty Flask)/ Weight of Sample Used) × 100.

Protein analysis was conducted on homogenized tail meat with adhering hepatopancreas from each cooking method. The samples were dried in the same manner as samples dried for moisture analysis. The dried samples were then blended to a fine powder using a mortar and pestle and coffee grinder (Custom Grind™, Hamilton Beach Brands Inc., South Pines, NC). Approximately 1.5 grams of finely ground sample were placed in 15 ml clear, screw top vials (Supelco®, Bellefonte, PA) and transported to the Soil Testing & Plant Analysis Laboratory on the LSU campus for combustion and total nitrogen determination. Total nitrogen determination was conducted on 0.25 gram samples in triplicate using a LECO® TruSpec Micro CHNS analyzer (LECO Corporation, St. Joseph, MI). The results were reported as percent total nitrogen in the sample. The total percent protein was determined by multiplying the percent total nitrogen by the appropriate conversion factor of 6.25 and converting from a dry weight basis to a wet weight basis.

2.6. pH Analysis

The pH of crawfish tail meat with hepatopancreas was measured in triplicate for each treatment using a pH meter (SMS115, Milwaukee Instruments Inc., Rocky Mount, NC) that had been calibrated at pH values of 4,7, and 10. Ten grams of homogenized crawfish tail meat were blended with 90 mL of distilled/de-ionized water for one minute using a commercial blender (Waring® model 51BL31, Waring, Stanford, CT). The samples were then transferred to 400 mL beakers (Pyrex, ®Corning Corporation, Tewksbury, MA) and the pH was measured and recorded.

2.7. TBARS Analysis

Thiobarbituric acid reactive substances (TBARS) analyses were conducted on boiled and steamed crawfish tail meat refrigerated for 0, 1, 3, 5, 7, 9 and 11 days and on frozen samples at months 0, 1, 2, 3, 4, 5, and 6 using a modified method of Vyncke [29] by Lemon [30]. Solutions were prepared the day prior to analysis. The extraction solution consisted of 7.5% trichloroacetic acid (TCA) (ThermoFisher Scientific, Waltham, MA), 0.1% propyl gallate (Sigma-Aldrich Corporation, St. Louis, MO), and 0.1% ethylene diaminetetraacetic acid (EDTA) (Sigma-Aldrich Corporation, St. Louis, MO) in 92.3% deionized water. The thiobarbituric acid (TBA) solution consisted of 2.883 g/L (0.02M) of TBA (Sigma-Aldrich Corporation, St. Louis, MO) in deionized water. The standard solution for standard curve determination was prepared by dissolving 0.22 grams of 1,1,3,3-Tetraethoxypropane (TEP) (Sigma-Aldrich Corporation, St. Louis, MO) in one L of water. The working solution to actually obtain the standard curve was formed by diluting the standard solution 100 fold. Fifteen grams of tissue from each treatment [in triplicate] were blended with 30 ml of extraction solution for 30 seconds using a Waring® blender. The samples were then filtered through a Whatman #1 filter paper into a 100 ml Pyrex® beaker. Five mL of the clear filtrate in the beaker was added to five ml of TBA reagent in Pyrex® (120 x 10 mm) test tubes with screw caps. The test tubes were then heated in boiling water in 1000 ml beakers (Pyrex®, Corning Corporation, Tewksbury, MA) on a hot plate (Corning PC-420D, Corning Corporation, Tewksbury, MA). Test tubes containing only five mL of water and five ml of TBA

reagent were added to beakers as blanks. After boiling for exactly 40 minutes, the tubes were removed from the beaker and cooled in running tap water. Using a transfer pipette, each sample was transferred to a cuvette and their optical density was measured at 530nm against the blanks of water and TBA reagent (Thermo Spectronic Genesys™ 2 spectrophotometer, ThermoFisher Scientific, Waltham, MA). The TBARS values were calculated from the standard curve obtained from the TEP working solution and the values were reported in mg malonaldehyde (MDA) equivalent/100 grams of tissue.

2.8. Mineral Analysis

Mineral analyses were conducted on the ashed samples remaining from the proximate analysis of boiled and steamed crawfish tail meat that had been refrigerated for days 0,1,3,5,7,9, and 11 and frozen samples on months 1,2,3,4,5, and 6. Ten mL of 10% nitric acid (Avantor™ Performance Materials Inc., Center Valley, PA) solution in distilled water were added to each crucible for ten minutes to solubilize the ash. The solubilized ash samples were drawn into a sterile 10 mL tip syringe (Luer-Lok™, Becton Dickinson & Company, Franklin Lakes, NJ). A 0.2µm, 25-mm surfactant free cellulose acetate membrane, acrylic housing, syringe filter was placed on the syringe (Nalgene™, ThermoFisher Scientific, Waltham, MA) and the sample was filtered into labeled 15 ml clear, screw top vials (Supelco®, Bellefonte, PA). Two of the three samples were then transported to the LSU AgCenter's W.A. Callegari Environmental Center Central Research Station in Baton Rouge, Louisiana for analysis in duplicate via inductively coupled plasma optical emission spectroscopy (ICP-OES, Varian Vista-MPX CCD Simultaneous ICP-OES unit, Varian Medical Systems Inc., Palo Alto, CA). The results for minerals present in the samples were reported in parts per million [ppm] of crawfish tail meat with adhering hepatopancreas on a wet weight basis.

2.9. Fatty Acid Analysis

Fatty acid profiles were obtained from samples of lipid collected during proximate analysis (2.5). In duplicate, 1.3-2.0 grams of crude lipid was solubilized in exactly ten ml of HPLC grade hexane (Honeywell, Morristown, NJ) and transferred to a 15 mL clear, screw top vial (Supelco®, Bellefonte, PA). Samples were then placed in the freezer (-18°C) until they were transported to the LSU AgCenter's W.A. Callegari Environmental Center Central Research Station in Baton Rouge, Louisiana for analysis. Fatty acid analysis was conducted using a gas chromatograph with an ion trap mass spectrometer (Varian 450 and Varian 240, respectively, Varian Inc., Palo Alto, CA) with a 75 m, 0.18 mm diameter, 0.14 µm film thickness, silica capillary column (SP2560, Supelco®, Bellefonte, PA) with hydrogen as the carrier gas at a flow rate of 40 cm per second.

2.10. Statistical Analysis

Results were expressed as least squares means (LS-Means) ± standard deviation. The experimental design used was a 2x7 factorial design for both refrigerated and frozen studies. Statistical analysis was performed using analysis of variance (ANOVA). Separation of means and difference between control and treatments were determined by the generalized linear model (GLM) procedure with a T comparison for least squares means (SAS Institute, version 9.3). Statistical significance was set at $P < 0.05$.

3. Results and Discussion

3.1. Cooking, Cooling, and Gelatin Test

Crawfish were submerged in an ice bath immediately after cooking to retard the cook process and to cool the crawfish within recommended guidelines [31] which slowed the rate of spoilage reactions that include bacterial and autolytic enzyme activity and reduced the rate at which bacteria multiply [32].

An average internal temperature of 87.6° C was obtained [5 crawfish per each of 2 replications] for the boiled crawfish, which killed pathogen *Listeria monocytogenes* [31]. The average chilled temperature of the crawfish after the ice bath for the two trials was 30.11°C. After four hours of cooling in refrigeration, the temperature of all the crawfish was reduced to an average of 3.5°C.

The average internal temperature of the cooked tails in the eight replications of steaming was 86.9°C. After the ice bath, the average internal temperature of the steamed samples was 29.6°C. The slightly lower internal temperature of the boiled samples might be due to the smaller batch size of ~7.26 kg versus the boiled batch sizes which were ~ 27.22 kg. Once the steamed samples had been boxed and allowed to cool under refrigeration for four hours, the average internal temperature was 5.0°, similar to that of the boiled samples.

Boiled and steamed crawfish samples contained cooked hepatopancreas and formed stable, firm gels after cooking, which indicates that the cook time was sufficient to deactivate the proteolytic enzymes in the hepatopancreas [33].

The heat-labile proteolytic enzymes present in the hepatopancreas of crawfish promote the development of mushiness in the tail meat [34]. It is important to limit texture deterioration before freezing of crawfish tail meat as Godber, et al. [12] found extended frozen storage would cause poor texture.

3.2. Tail Meat Yield

The edible tail meat yields for the boiled and the steamed crawfish through refrigerated and frozen storage are in Table 1. It was expected that the edible yield would decrease during storage, especially frozen storage from freeze-thaw transitions and thawing due to purge. Slow freezing causes large ice crystal formation that damages cell walls and promotes water loss upon thawing [1, 35].

Since crawfish are generally hand peeled, there can be variations in the yield obtained from peeling depending on the technique and experience of the peeler. The average abdominal [tail] meat yield for cooked crawfish is about 15% of the live weight of the crawfish [8]. The yield also depends on the sexual maturity of the crawfish, with immature crawfish having a higher yield because they have smaller claws and thinner shells [8]. This is typical of crawfish early in the season, when yields can be as high as 22-23%. Later in the season, when the crawfish have matured and have larger claws and thicker shells, the yield can be as low as 10 to 11% [8]. The yields obtained in this study averaged around 18%. This was higher than expected because these crawfish were harvested later in the season and their shells were quite thick. A reason why the yields may have been higher than expected is that the

crawfish were very carefully peeled. Also, the hepatopancreas was left attached as well as the vein/intestine that runs along the dorsal side of the abdomen/tail. Typically, this is removed during commercial peeling, but was left on in this research because the goal was to mimic how Louisiana consumers typically ingest boiled crawfish. Cooking losses of 31.17% and 18.33% from fried and microwave cooking, respectively, were reported [28].

Table 1. Percent (%) edible yield of peeled crawfish tail meat with adhering hepatopancreas during refrigerated (3.5°C) storage or after frozen (-18°C) storage and thawing.

Refrigerated	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Avg. % yield
Boiled	19.14	18.41	20.16	18.09	18.02	19.46	17.43	18.67 ± 0.95
Steamed	18.58	18.63	18.60	17.84	17.63	19.19	15.80	18.03 ± 1.11
Frozen	Mo. 0	Mo. 1	Mo. 2	Mo. 3	Mo. 4	Mo. 5	Mo. 6	Avg. % Yield
Boiled	19.14	17.69	17.36	19.16	17.28	18.13	17.52	18.04 ± 0.81
Steamed	18.58	17.08	17.98	18.71	16.84	18.92	19.45	18.22 ± 0.96

3.3. Proximate Analysis Results

The proximate analyses LS-means for the boiled and steamed refrigerated and frozen crawfish tail meat with the attached hepatopancreas are in Table 2. The proximate analyses from the USDA [36] and Sidwell [37] are also shown for comparison. It was not specified whether or not the tail meat samples included hepatopancreas from in the USDA [36] or Sidwell [37] studies.

Table 2. Means for % moisture, ash, protein, and fat of crawfish tail meat during refrigerated (3.5°C) and frozen (-18°C) storage. Proximate values from references 26, 36, and 37 are shown for comparison.

Proximate composition	Boiled	Steamed	USDA [36] **boiled**	Sidwell [37] **boiled**	Sidwell [37] **raw range**	Abou-Taleb, et al. [26] **raw**
% moisture (3.5°C)	79.29	79.40				
% moisture (-18°C)	77.91	77.86	80.8	75.0	(72.1 - 83.4)	78.61
% ash (3.5°C)	1.26	1.31				
% ash (-18°C)	1.22	1.32	1.07	1.5	(0.7 - 3.60	1.33
% protein (3.5°C)	15.89	15.30				
% protein (-18°C)	16.69	16.30	17.5	16.3	(11.9 - 24.10	18.46
% fat (3.5°C)	2.73	2.96				1.58
% fat (-18°C)	3.00	3.22	1.3	0.8	(0.5 - 2.5)	
Total (3.5°C/-18°C)	99.17/98.82	98.97/98.70	100.67	98.9		99.98

Moisture, ash, and protein in crawfish boiled for 10 m were 80%, 1.2%, and 17%, respectively, [25] while the moisture for crawfish boiled for 5 m was 86% [20]. Lower moisture (38.2 and 58.2%) and higher protein (35.8 and 35.2), fat (18.9 and 2.73%), and ash (6.35 and 3.25%) were in cooked crawfish by frying and microwaving [26].

The moisture content of the peeled crawfish tail meat with adhering hepatopancreas through refrigerated and frozen storage from boiled and steamed treatments did not vary greatly. There was little change in the moisture content for the boiled samples through 0 to 11 d refrigerated storage (Table 3). The moisture content for the steamed samples varied a bit more than the boiled samples throughout the 11-day storage. The differences in moisture could be from natural variation, which corresponds to previously reported large range of moistures (72.1 - 83.4%) [37].

Table 3. Percent (%) moisture of peeled crawfish tail meat with adhering hepatopancreas stored during refrigerated (3.5°C) storage.

Treatment	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Boiled	78.23d ± 0.08	78.77cd ± 0.12	78.93cd ± 0.05	80.33ab ± 2.04	80.19ab ± 0.08	78.46c ± 0.10	80.11ab ± 0.14
Steamed	79.00cd ± 0.17	79.30abcd ± 0.02	78.22d ± 0.05	79.84abc ± 0.36	79.77abc ± 0.02	79.27bcd ± 0.15	80.38a ± 0.11

Note: LS-means ± SD of 3 measurements at each time period. LS-mean values with the same letter (abcd) are not different ($P < 0.05$).

A greater difference in moisture content with cooking treatments was observed between frozen samples (which were thawed prior to moisture determination) than for samples in refrigerated storage (Table 4). Ice crystal formation, protein denaturation, and increases in salt concentration during frozen storage can decrease the moisture content of the thawed product [38, 39]. It was thought that ice crystal formation may have been a factor because slow freezing rates cause larger ice crystal formation, which would result in cellular disturbance and rupturing [1, 40] and increased water loss or drip-loss from the crawfish upon thawing. There was a 13 to 20% drip loss during freeze-thaw cycles of crawfish previously boiled for 15 m [41]. There was a significant difference between the boiled and steamed samples at all time intervals except for months four and five.

Table 4. Percent (%) moisture of peeled crawfish tail meat with adhering hepatopancreas after frozen (-18°C) storage and thawing.

Treatment	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	78.23bc ± 0.08	77.05j ± 0.13	78.36bc ± 0.04	78.21cd ± 0.07	77.90efg ± 0.06	77.65h ± 0.17	77.95ef ± 0.10
Steamed	79.00a ± 0.17	77.72gh ± 0.06	77.34i ± 0.14	78.42b ± 0.11	78.02de ± 0.26	77.79fgh ± 0.12	76.71k ± 0.04

Note: LS-means ± SD of 3 measurements at each time period. LS-mean values with the same letter (abcdefghijk) are not different ($P<0.05$).

The ash contents obtained in this study did not exhibit significant differences between the boiled and steamed crawfish samples in refrigerated storage, except on d 9 (Table 5). As Sidwell [37] reported, the range for percent ash in an unspecified species of raw crawfish was 0.7-3.6% which indicates quite a large range of natural variation.

Another study had similar results, an ash content of 1.5% [41]. The values for percent ash content of the refrigerated samples (boiled and steamed) are shown in Table 5.

Table 5. Percent (%) ash of peeled crawfish tail meat with adhering hepatopancreas stored during refrigerated (3.5°C) storage.

Treatment	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Boiled	1.30bcd ± 0.10	1.56a ± 0.16	1.15e ± 0.03	1.25bcde ± 0.08	1.21bcde ± 0.03	1.17de ± 0.06	1.19cde ± 0.08
Steamed	1.25bcde ± 0.04	1.56a ± 0.04	1.23bcde ± 0.12	1.31bc ± 0.02	1.28bcde ± 0.03	1.33b ± 0.01	1.19cde ± 0.08

Note: LS-means ± SD of 3 measurements at each time period. LS-mean values with the same letter (abcde) are not different (P<0.05).

The percent ash contents of the boiled and steamed samples after frozen storage and thawing were slightly higher, on average, than the samples in refrigerated storage (Table 6). This might indicate more leaching of minerals in the boiled samples compared to that of the steamed samples. Blanching, or boiling, of foods has a tendency to cause leaching of vitamins and minerals; steaming has the benefit of less leaching than blanching/boiling [42].

Table 6. Percent (%) ash of peeled crawfish tail meat with adhering hepatopancreas after frozen (-18°C) storage and thawing.

Treatment	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	1.29abc ± 0.10	1.01d ± 0.39	1.31abc ± 0.04	1.15cd ± 0.02	1.21bc ± 0.02	1.22bc ± 0.04	1.33abc ± 0.00
Steamed	1.25abc ± 0.04	1.40ab ± 0.05	1.22bc ± 0.08	1.21bc ± 0.07	1.40ab ± 0.07	1.34abc ± 0.03	1.44a ± 0.02

Note: LS-Means ± SD of 3 measurements at each time period. LS-Mean values with the same letter (abcd) are not different (P<0.05).

The protein contents of boiled samples were significantly higher than for steamed crawfish on day of refrigerated storage, except d 7 (Table 7). Although significant, the actual differences were small. For both the boiled and steamed samples, there was an overall decrease in percent protein from d 0 to 11. This may be due to increased microbial counts and the hydrolysis and consumption of free amino acids and other soluble non-nitrogenous substance in the crawfish that serve as nutrients for microbial growth [11].

Table 7. Percent (%) protein of peeled crawfish tail meat with adhering hepatopancreas stored during refrigerated (3.5°C) storage.

Treatment	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Boiled	16.32c ± 0.04	16.46b ± 0.06	16.93a ± 0.04	15.06g ± 0.00	14.95h ± 0.07	16.23d ± 0.04	15.27f ± 0.05
Steamed	16.15c ± 0.03	15.34f ± 0.06	16.02e ± 0.06	14.56i ± 0.10	15.07g ± 0.04	15.05g ± 0.02	14.91h ± 0.10

Note: LS-means ± SD of 3 measurements at each time period. LS-mean values with the same letter (abcdefgh) are not different (P<0.05).

The protein contents of the boiled samples during frozen storage were higher (P<0.05) than steamed samples at every month except month six. There was a difference between the protein content of the frozen samples and the refrigerated samples. The frozen values were larger by almost one % (Table 8). These values also fall within the range (11.9 - 24.1%) for percent protein in raw samples [37].

Table 8. Percent (%) protein of peeled crawfish tail meat with adhering hepatopancreas after frozen (-18°C) storage and thawing.

Treatment	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	16.32ef ± 0.04	17.31a ± 0.06	16.52d ± 0.03	16.71c ± 0.03	16.21fg ± 0.19	17.26a ± 0.02	16.51d ± 0.04
Steamed	16.15gh ± 0.03	16.02hi ± 0.05	16.32de ± 0.06	16.44de ± 0.16	15.90i ± 0.04	16.37e ± 0.05	16.90b ± 0.01

Note: LS-means ± SD of 3 measurements at each time period. LS-mean values with the same letter (abcdefgh) are not different (P<0.05).

There were some differences in the protein content between boiled and steamed crawfish during refrigerated and frozen storage. The differences could simply arise from natural variation in the samples that were selected. The protein content of frozen samples tended to be higher than that of the refrigerated samples.

The percent fat values obtained in this study (2.12 to 3.76%) were higher than literature values (Table 9). This may be due to the inclusion of the hepatopancreas (fat) with the tail meat in the present study. In Louisiana, crawfish are traditionally consumed with the fat [7] and so the hepatopancreas was kept attached to the tail meat to mimic the way that crawfish are commonly consumed in Louisiana. Most studies delineating the fat content of crawfish tail meat do not include the hepatopancreas. The hepatopancreas is roughly 30% fat by weight [43]. Therefore, the fat content of crawfish with hepatopancreas would be expected to be higher than crawfish tail meat without hepatopancreas.

Table 9. Percent (%) average fat of peeled crawfish tail meat with adhering hepatopancreas during refrigerated (3.5°C) storage.

Treatment	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Boiled	2.99cde ± 0.04	2.91de ± 0.04	2.58f ± 0.08	2.27g ± 0.13	2.24g ± 0.06	3.27bc ± 0.07	2.88de ± 0.11
Steamed	2.76def ± 0.16	2.75ef ± 0.27	3.04cd ± 0.03	2.12g ± 0.11	3.41a ± 0.27	2.90de ± 0.08	3.76a ± 0.06

Note: LS-means ± SD of 3 measurements at each time period. LS-mean values with the same letter (abcdefg) are not different (P<0.05).

The average fat content of the frozen crawfish was higher for both the boiled and steamed treatments than for the same treatments with refrigerated storage (Table 10). This might be explained by the reduced moisture in the frozen samples compared to the refrigerated samples. The variation is much more pronounced for the steamed samples than for the boiled samples.

Table 10. Percent (%) fat of peeled crawfish tail meat with adhering hepatopancreas after frozen (-18°C) storage and thawing.

Treatment	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	2.99def ± 0.04	3.10de ± 0.0	2.72h ± 0.08	3.13cde ± 0.10	3.00def ± 0.08	2.95efg ± 0.01	3.15cde ± 0.04
Steamed	2.76gh ± 0.16	3.07def ± 0.04	3.34bc ± 0.06	3.87a ± 0.10	2.86fgh ± 0.03	3.21bcd ± 0.26	3.42b ± 0.11

Note: LS-means ± SD of 3 measurements at each time period. LS-mean values with the same letter (abcdefgh) are not different (P<0.05).

3.4. Microbiological Results

There were no *E. coli*/coliforms present in any of the water samples or any of the boiled or steamed crawfish at any time. There was no presence of *E. coli*, but there was an average coliform count of 295 colony forming units (CFU)/g in raw crawfish. This suggests that the cook procedure for both boiling and steaming was adequate to kill these organisms. An APC count of log 5.7 CFU/g is suggested by ICMSF [24] as the upper limit of acceptability and, in this study, taken as the upper limit for shelf life determination. As seen in Table 11, the boiled and steamed samples in refrigerated storage did not exceed the limit of log 5.7 CFU/g until after day three. Both the boiled and steamed samples exceeded the chosen limit by day 5, the data point immediately after day 3, so the acceptable shelf life of refrigerated crawfish, either boiled or steamed, was taken as three days. It is not clear why the difference in APC occurred. Further study with analyses conducted on all days may provide better insight. Total bacteria and psychrophilic bacteria counts were 4.6 and 4.7 and 3.3 and 3.5 log CFU/g for fried and microwaved crawfish in refrigerated storage [26].

Table 11. Aerobic plate counts (log_{10} CFU/g) for peeled crawfish tail meat with adhering hepatopancreas during refrigerated (3.5°C) storage.

Treatment	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Boiled	3.03c ± 2.87	3.10c ± 2.87	3.03c ± 2.91	6.10c ± 5.36	6.12c ± 5.05	8.10b ± 7.08	8.01b ± 6.94
Steamed	2.57c ± 2.53	2.78c ± 1.97	5.46c ± 4.89	6.22c ± 5.08	TNC*	8.62a ± 7.53	8.65a ± 8.00

Note: LS-means ± SD of 4 measurements at each time period. LS-mean values with the same letter (abc) are not different (P<0.05). * TNC = Too numerous to count.

The APC counts were constant during frozen storage and below 5.7 log CFU/g at all data points as shown in Table 12 which would be expected during frozen storage at -18°C. This suggests that the shelf life, based upon APC counts, of crawfish in frozen storage is at least six months. This contrasted with results of decreased Ca 2 + -ATPase activity, salt soluble protein content, total sulfhydryl and reactive sulfhydryl content and increased hardness at week 4 to suggest that the recommended shelf-life of frozen crayfish should be 1 month {1].

Table 12. Aerobic plate counts (log_{10} CFU/g) for peeled crawfish tail meat with adhering hepatopancreas after frozen (-18°C) storage and thawing.

Treatment	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	3.02a ± 2.87	2.27d ± 2.18	2.48d ± 1.98	2.90abc ± 2.19	2.69bcd ± 2.00	2.78bcd ± 2.00	2.79bcd ± 2.77
Steamed	2.57cd ± 2.53	2.70bcd ±2.02	2.69bcd ±1.83	2.95ab ± 2.56	2.56cd ± 1.70	2.92ab ± 2.41	3.04a ± 2.41

Note: LS-means ± SD of 4 measurements at each time period. LS-mean values with the same letter (abcd) are not different (P<0.05).

3.5. TBARS Results

TBAR values increased during refrigerated storage for both boiled and steamed samples but the level of TBARS measured at the end of the refrigerated storage was lower than anticipated (Table 13). It was anticipated that the values would be higher than previous studies because the crawfish in the current study had the hepatopancreas and vein, increasing the amount of fat that might be oxidized and result in higher TBARS values. Surprisingly, the TBARS values obtained in this study were lower than literature values. An average TBARS value of 2.24 mg MDA/kg after 7 days [44] and 0.113 after 9 days of refrigerated storage [26] were obtained on crawfish tail meat.

Table 13. TBARS (mg MDA/kg) for peeled crawfish tail meat during refrigerated (3.5°C) storage.

Treatment	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Boiled	0.19fg ± 0.02	0.15h ± 0.01	0.24e ± 0.00	0.33cd ± 0.03	0.34c ± 0.01	0.42b ± 0.04	0.45b ± 0.02
Steamed	0.16gh ± 0.01	0.14h ± 0.02h	0.22ef ± 0.00	0.29d ± 0.03	0.30d ± 0.04	0.32cd ± 0.01	0.53a ± 0.03

Note: LS-means ± SD of 3 measurements at each time period. LS-mean values with the same letter (abcdefgh) are not different (P<0.05).

During refrigerated storage, TBARS values of the boiled and steamed samples gradually increased from d 0 to 11. It was unexpected that TBARS on d 0 were more than on d 1. One possible explanation is that the samples on d 0 remained at room temperature slightly longer before TBARS analyses than on the other days of the study. TBARS values, as expected, increased with refrigerated storage with boiled samples having slightly higher values than the steamed samples on all days except d 11. TBARS of 1.5 mg MDA/kg was the point at which humans can detect any off flavors and 3.0 mg MDA/kg of TBARS was the level at which crawfish are considered rancid and not desirable for consumption [44]. Using these criteria, the fat in the crawfish tail meat and hepatopancreas was not rancid at any time during the course of the study. However, it was noticed that the crawfish in the last 5 to 6 days of refrigeration had an odor that was unpleasant. The TBARS values were similar to those of Abou-Taleb, et al. [26] but Cremades, et al. [45] identified much higher levels of TBARS of crawfish in refrigerated storage.

Table 14. TBARS (mg MDA/kg) for peeled crawfish tail meat with adhering hepatopancreas after frozen (-18°C) storage and thawing.

Treatment	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5
Boiled	0.19f ± 0.02	0.22def ± 0.02	0.16g ± 0.02	0.21def ± 0.01	0.23cd ± 0.01	0.2cde ± 0.01
Steamed	0.16g ± 0.01	0.25bc ± 0.00	0.20ef ± 0.00	0.22cde ± 0.01	0.19f ± 0.01	0.26b ± 0.04

Note: LS-means ± SD of 3 measurements at each time period. LS-mean values with the same letters (abcdef) are not different (P<0.05).

After frozen storage (Table 14), the TBARS values for both boiled and steamed samples, although having statistically significant differences, were essentially stable in contrast to the TBARS values of the refrigerated samples. [46] observed an average TBARS value of 4.00 mg MDA/kg over the course of 10 months of frozen storage, with a maximum value at month ten of 5.60 mg MDA/kg in crawfish tails containing the hepatopancreas. TBARS in crawfish tail meat greatly increased after 30 d of frozen storage [20] and a linear increase in TBARS was found with repeated freeze-thaw cycles [41].

3.6. Texture

Texture analyses were conducted on crawfish tail meat with adhering hepatopancreas over the course of refrigerated and frozen storage. Table 15 shows the peak shear force for boiled and steamed peak shear force values for crawfish during 11 days of refrigerated storage.

Table 15. Peak shear force (kg) for peeled crawfish tail meat with adhering hepatopancreas after refrigerated (3.5°C) storage.

Treatment	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Boiled	231.54abcd ± 8.63	240.66abc ± 20.89	262.43a ± 28.24	218.59bcde ± 11.77	230.95bcd ± 5.28	248.99ab ± 33.73	228.10bcd ± 8.73
Steamed	224.57cd ± 30.30	208.39de ± 14.51	217.41cde ± 19.22	217.81bcde ± 14.71	236.14abcd ± 7.75	220.65bcd ± 15.98	188.19e ± 14.12

Note: LS-means ± SD of 3 measurements at each time period. LS-mean values with the same letter (abcde) are not different (P<0.05).

The peak shear force (kg) for the boiled samples in refrigerated storage gradually increased from day zero to day three even though the values were not different (P<0.05); there was greater variability from day three to eleven. The trend was somewhat similar for steamed samples, but the final value for steamed samples was lower than for boiled crawfish on d 11. The frozen shear force values (Table 16) are statistically similar but the shear force value on day eleven was the lowest which follows previous results [47] that proteolytic enzyme activity and an increased bacterial presence through the refrigerated storage caused the deterioration in the texture of the crawfish. The results indicated that toughening due to moisture loss or mushy or deteriorative texture due to residual activity of native proteinases in the hepatopancreas or tail that had not been deactivated by the thermal treatment (cooking) process did not substantially influence texture. However, d 7 variations in texture may also be due to the inherent nature of crawfish and the Kramer analysis. The uniformity of the sample and direction of the muscle fibers plays a role in the outcome of the analysis [48]. That the crawfish were placed in the Kramer cell at random and did not completely cover the bottom of the cell in the present study may have had an impact. The weight of crawfish added to the cell was kept constant for all samples, however the number of crawfish changed since the weights of individual crawfish tails varied.

Table 16. Peak shear force (kg) for peeled thawed crawfish tail meat with adhering hepatopancreas after frozen (-18°C) storage and thawing.

Treatment	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	231.54f ± 8.63	277.23abc ± 24.12	266.15abcde ± 19.81	266.05abcde ± 16.97	291.06a ± 26.08	272.72abcd ± 3.63	282.23ab ± 18.93
Steamed	224.57f ± 30.30	236.14e ± 18.63	242.42def ± 7.75	247.52cdef ± 9.71	252.62bcdef ± 27.75	265.17abcde ± 16.18	285.86a ± 8.34

Note: LS-means ± SD of 3 measurements at each time period. LS-mean values with the same letter (abcdef) are not different (P<0.05).

The peak shear forces (kg) for the thawed previously frozen crawfish tail meat with attached hepatopancreas were more consistent than for the refrigerated samples, with the boiled samples generally tougher than the steamed samples. However, at month six, the steamed sample had a higher peak shear force than the boiled sample. The hardness, springiness, and chewiness of crawfish varied among freeze-thaw cycles [41].

The work of shearing was chosen as a measure of toughness perceived when biting into a food product by measuring the force needed to shear the sample as a product of time. The results of the work of shearing mimic the values of peak shear force, which might be expected considering the measurements were taken simultaneously. Values for work of shearing of boiled and frozen crawfish tails through refrigerated storage are in Table 17 and through frozen storage are in Table 18. The hardness, resilience, cohesiveness, and chewiness changed with the times of steaming and boiling of crawfish tail meat [2].

Table 17. Total shear work (N/s) for peeled crawfish tail meat with adhering hepatopancreas after refrigerated (3.5°C) storage.

Treatment	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Boiled	1059.31bcde ± 86.00	1081.67abcd ± 152.40	1241.42a ± 52.56	1045.29bcde ± 133.57	1045.49bcde ± 57.07	1092.56abc ± 35.01	1011.07bcdef ± 89.34
Steamed	1105.11ab ± 147.59	907.31ef ± 15.59	994.69bcdef ± 110.62	923.59def ± 118.56	1105.80ab ± 70.51	933.20cdef ± 86.00	849.26f ± 101.99

Note: LS-means ± SD of 3 measurements at each time period. LS-mean values with the same letter (abcdef) are not different (P<0.05).

Table 18. Total shear work (N/s) for peeled thawed crawfish tail meat with adhering hepatopancreas after frozen storage (-18°C) and thawing.

Treatment	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	1059.31cd ± 86.00b	1157.48bc ± 138.57	1130.41bc ± 115.72	1073.44bcd ± 117.29	1347.63a ± 36.38	1226.71ab ± 33.05	1101.48bc ± 76.3
Steamed	1105.11bc ± 147.59	832.19e ± 43.64	1008.71cde ± 33.34	895.94de ± 52.86	1170.52abc ± 181.33	1182.68abc ± 200.45	1138.75bc ± 54.43

Note: LS-means ± SD of 3 measurements at each time period. abcdeLS-mean values with the same letter (abcde) are not different (P<0.05).

3.7. pH Results

There was no general trend for the pH in the boiled crawfish during refrigerated storage, presented in Table 19. For the steamed samples, the initial pH declined during storage to a pH value of 7.77 on day 11. The variable pH for boiled and steamed samples through the refrigerated storage can be attributed to changes in production of basic compounds such as ammonia and other biogenic amines [49, 50]. The pH of fried and microwaved crawfish meat were 5.75 and 5.80, respectively, for refrigerated samples [26]. There were also significant decreases from month zero to month six (which had the lowest pH values) for both boiled and steamed crawfish tail meat in frozen storage (Table 20). Reported pH values were 7.66 to 7.79 during 180 d frozen storage [20] and from 7.35 to 7.85 during repeated freeze-thaw cycles [41].

Table 19. pH of peeled crawfish tail meat with adhering hepatopancreas during refrigerated (3.5°C) storage.

Treatment	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Boiled	8.10ef ± 0.00	8.20cd ± 0.00	8.10ef ± 0.00	8.17de ± 0.06	8.40a ± 0.00	8.27bc ± 0.06	8.03f ± 0.06
Steamed	8.30b ± 0.00	8.20cd ± 0.00	8.10ef ± 0.00	8.13de ± 0.06	8.17de ± 0.06	8.03f ± 0.06	7.77g ± 0.06

Note: LS-means ± SD of 3 measurements at each time period. LS-mean values with the same letter (abcdefg) are not different (P<0.05).

Table 20. pH of peeled thawed crawfish tail meat with adhering hepatopancreas after frozen storage (-18°C) and thawing.

Treatment	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	8.10de ± 0.00	8.20bc ± 0.00	8.27ab ± 0.06	8.23abc ± 0.06	8.20bc ± 0.00	7.87fg ± 0.06	7.87g ± 0.06
Steamed	8.30a ± 0.00	8.03e ± 0.0	8.20bc ± 0.00	8.17cd ± 0.06	8.20bc ± 0.00	7.93f ± 0.06	7.83g ± 0.06

Note: LS-means ± SD of 3 measurements at each time period. LS-mean values with the same letter (abcedfg) are not different (P<0.05).

3.8. Color Results

The results of the color analyses for lightness (L*), green/red (a*), and blue/yellow (b*) showed only slight differences throughout refrigerated and frozen storage.

For boiled samples during refrigerated storage, the L* values were similar except for the difference (P<0.05) in L* between d 7 and 11 (Table 21).

Table 21. L* of peeled crawfish tail meat with adhering hepatopancreas during refrigerated (3.5°C) storage.

Treatment	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Boiled	53.30bc ± 6.26	54.06abc ± 3.45	53.23bc ± 6.39	50.61c ± 6.23	54.73abc ± 4.28	57.84ab ± 6.70
Steamed	53.65bc ± 5.57	52.91bc ± 5.85	51.34c ± 3.85	55.10abc ± 3.91	56.06abc ± 7.20	59.33a ± 10.64

Note: LS-means ± SD of 3 measurements at each time period. LS-mean values with the same letter (abc) are not different (P<0.05).

For the steamed samples, lightness values slowly decreased from day one to day five, then increased to day eleven. There were no differences (P<0.05) between the two treatments at each individual day.

Table 22. L* of peeled thawed crawfish tail meat with adhering hepatopancreas after frozen (-18°C) storage and thawing.

Treatment	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	52.32ab ± 4.28	54.45ab ± 5.76	53.74ab ± 6.66	56.60a ± 7.43	48.63b ± 5.91	48.58b ± 6.92
Steamed	52.12ab ± 9.18	53.11ab ± 4.45	52.46ab ± 6.58	52.84ab ± 7.29	49.86b ± 6.72	54.23ab ± 7.71

Note: LS-Means ± SD of 10 measurements at each time period. LS-Mean values with the same letter (ab) are not different (P<0.05).

The L* values of the boiled samples through frozen storage were all similar except that month 4 was higher (P<0.05) than months 5 and 6 and the boiled and steamed frozen crawfish L* values were not different (P<0.05) from each other at any month, as shown in Table 22.

These L* values through refrigerated storage were slightly higher than the samples in frozen storage. L* values showed a general decrease with increased frozen storage time in the study by Bonilla, et al. [20].

The a* values or green/red scale of color showed little differences (P<0.05) in Table 23 for boiled crawfish samples throughout refrigerated storage with day 3 having the highest (P<0.05) a* value.

Values for the steamed samples fluctuated and on day 11 the steamed samples had much smaller values a* values than the boiled samples.

Table 23. a* of peeled crawfish tail meat with adhering hepatopancreas in refrigerated (3.5°C) storage.

Treatment	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Boiled	32.02bcd ± 6.39	35.52ab ± 5.73	32.58bcd ± 4.63	31.65bcd ± 3.74	29.35cd ± 3.63	31.06bcd ± 5.77
Steamed	34.18abc ± 8.04	38.23a ± 6.94	31.47bcd ± 5.27	27.35de ± 4.03	29.35de ± 3.63	22.91e ± 10.45

Note: *LS-means ± SD of 10 measurements at each time period. LS-mean values with the same letter (abcde) are not different (P<0.05).

The a* values for the boiled and steamed frozen samples were similar at each month except month 6, where the a* values of the boiled samples were larger than the steamed samples (Table 24).

Table 24. a* of peeled thawed crawfish tail meat with adhering hepatopancreas after frozen (-18°C) storage and thawing.

Treatment	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	25.80bc ± 4.08	26.58bc ± 5.04	27.24bc ± 7.27	24.55bc ± 8.27	30.15ab ± 6.23	32.93a ± 5.77
Steamed	23.82c ± 8.18	30.12ab ± 3.48	27.21bc ± 6.21	26.99bc ± 4.83	28.93abc ± 7.80	25.39c ± 7.40

Note: LS-means ± SD of 10 measurements at each time period. LS-mean values with the same letter (abc) are no different (P<0.05).

The b* values [blue/yellow color scale] fluctuated during the eleven days of refrigerated storage (Table 25). The b* value of boiled samples increased (P<0.05) from day one to day three, then decreased from d 3 to 7 when the lowest value of 22.91 occurred.

The b* values then steadily increased to d 11. Steamed crawfish tail meat also had variable b* values in refrigerated storage, with a high value of 40.24 on day three and values that decreased from day five through day 9. It is not understood what caused the fluctuation in the b* values during the refrigerated storage.

Table 25. b* of peeled crawfish tail meat with adhering hepatopancreas in refrigerated (3.5°C) storage.

Treatment	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Boiled	32.48c ± 4.66	37.78ab ± 5.99	30.78cd ± 4.88	22.91f ± 3.40	26.29def ± 4.63	32.35c ± 4.30
Steamed	34.68bc± 6.31	40.24a ± 5.35	30.10cde ± 4.10	26.77def ± 4.98	25.49ef ± 3.30	27.60de ± 8.46

Note: LS-means ± SD of 10 measurements at each time period. LS-mean values with the same letter (abcdef) are not different (P<0.05).

During frozen storage, the b* values were lower than the b* values during refrigerated storage (Table 26) which corresponds to the differences in the L* and a* values with type of storage. The steamed and boiled samples were the same throughout the six months of storage at every month. Cryogenically frozen crawfish showed minimal changes in b* values while blast frozen crawfish had variation in b* values during 180 d frozen storage [20].

Table 26. b* of peeled crawfish tail meat with adhering hepatopancreas after frozen (-18°C) storage and thawing.

Treatment	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Boiled	22.57ab ± 5.37	24.30ab ± 5.04	24.24ab ± 4.73	25.60a ± 3.86	26.12a ± 5.56	25.59a ± 5.13
Steamed	20.94b ± 7.84	25.03ab ± 2.22	22.50ab ± 2.22	25.82a ± 2.13	24.86ab ± 4.47	25.11a ± 3.78

Note: LS-means ± SD of 10 measurements at each time period. LS-mean values with the same letters (ab) are not different (P<0.05).

The L*, a*, and b* values all decreased during frozen storage in comparison to their refrigerated samples. L*, a*, and b* values varied greatly during consecutive freeze-thaw cycles of crawfish during frozen storage [13].

3.9. Mineral Results

Mineral analyses shown in Table 27 were conducted to determine the presence and quantity of 29 different minerals and metals that might have caused differences in crawfish properties throughout the refrigerated and frozen storage.

The amount of arsenic is pertinent as many crawfish are farmed in rice ponds, as in this study, and levels of inorganic and organic arsenic are of concern in rice and rice products [51, 52]. The arsenic levels observed in the crawfish in the present study are higher than that which is approved by the Environmental Protection Agency (EPA) for allowable limits in drinking water. The limit for arsenic in drinking water is 10 parts per billion (ppb) or 0.01 parts per million (ppm).

The mineral values varied greatly from those of frozen crawfish in the Bonilla, et al. [20] possibly due to the inclusion of the hepatopancreas and veins in the samples analyzed in the current study.

Table 27. Selected minerals (ppm) in tail meat with adhering hepatopancreas.

Mineral	Boiled avg. ± SD (ppm)	Boiled range (ppm)	Steamed avg. ± SD (ppm)	Steamed range (ppm)	Other sources (ppm)
Al	9.67 ± 2.98	4.72 – 13.78	8.54 ± 3.68	4.01 – 16.83	_
Mg	86.08 ± 4.86	78.02 - 90.36	81.76 ± 4.73	70.97 – 87.64	330 [35]
Mn	1.04 ± 0.24	0.41 – 1.29	1.06 ± 0.24	0.36 – 1.29	4.2-7.28 [36]
K	576.32 ± 46.15	641.46 – 494.79	647.13 ± 64.06	535.89 – 738.37	5,000 [36]
Na	341.98 ± 21.80	300.21 – 364.26	383.51 ± 39.82	325.93 – 431.24	1,820 [36]
Si	15.77 ± 2.91	11.18 – 21.30	15.71 ± 3.75	10.22 – 21.58	_
B	4.27 ± 0.32	3.73 – 4.77	4.43 ± 0.28	3.84 – 4.85	_

Mineral	Boiled avg. ± SD (ppm)	Boiled range (ppm)	Steamed avg. ± SD (ppm)	Steamed range (ppm)	Other sources (ppm)
Cu	2.60 ± 0.81	1.48 – 4.47	2.40 ± 0.92	0.73 – 3.98	7-11.21 [36]
Fe	14.55 ± 2.68	10.32 – 18.61	12.80 ± 2.57	6.53 – 16.22	9-373 [36]
Ca	416.16 ± 68.71	333.74 – 545.91	371.20 ± 71.10	297.09 – 515.43	650-2,700 [36]
P	435.74 ± 51.60	346.44 – 506.66	426.01 ± 64.05	308.32 – 524.12	1,010-1,920 [36]
Zn	5.92 ± 0.72	4.08 – 6.91	5.62 ± 0.90	3.68 – 6.76	16.38 [36]
As	0.08 ± 0.05	0.01 – 0.15	0.08 ± 0.04	0.01 – 0.13	0.02 [36]

3.10. Fatty Acid Results

Table 28 gives fatty acids and their levels (%) observed in boiled and steamed crawfish samples through 11 days of refrigerated storage. Table 29 represents the fatty acid values (%) of boiled and steamed crawfish through six months of frozen storage. This analysis was conducted to add to the body of knowledge regarding crawfish and the types and levels of fatty acids in crawfish containing hepatopancreas fat. The fatty acid contents of steamed crawfish were determined to be 25.77, 30.86, and 43.36 per cent for SFA, MUFA, and PUFA, respectively [2]. SFA varied from 24.37 to 27.00 while MUFA increased from 29.82 to 33.31 and PUFA decreased from 45.42 to 39.68 with increased steaming time for crawfish, but SFA, MUFA, and PUFA were variable among boiling times with similar ranges in values [2]. Fatty acid composition varied between commercially harvested crawfish and those from moist-soil wetlands [25].

Table 28. Percentages of fatty acid groups in boiled crawfish fat in refrigerated (3.5°C) storage.

Fatty acid group	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
% SFA boiled	38.33	33.51	36.20	72.86	63.00	42.78	26.05
% SFA steamed	41.20	24.93	58.39	56.81	56.94	27.55	16.18
% MUFA boiled	23.34	25.32	24.43	8.47	7.90	18.16	29.67
% MUFA steamed	24.69	26.29	9.60	9.80	13.71	41.23	37.95
% PUFA boiled	38.33	41.47	40.36	18.67	29.20	39.07	44.28
% PUFA steamed	34.10	48.78	32.01	33.39	19.35	31.22	45.86

Note: Saturated fatty acids [SFA], Mono-unsaturated fatty acids [MUFA], And polyunsaturated fatty acids [PUFA].

Table 29. Percentages of fatty acid groups in boiled crawfish fat in frozen (-18°C) storage.

Fatty acid group	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
% SFA boiled	41.04	44.58	36.50	45.83	42.21	36.17
% SFA steamed	32.95	38.24	33.32	37.15	29.67	35.50
% MUFA boiled	16.01	22.73	26.51	27.05	29.00	26.19
% MUFA steamed	27.87	24.98	28.32	29.76	30.39	27.12
% PUFA boiled	42.95	32.65	36.99	27.11	28.78	37.64
% PUFA steamed	39.18	36.79	38.36	33.09	39.94	37.38

Note: Saturated fatty acids [SFA], Mono-unsaturated fatty acids [MUFA], And polyunsaturated fatty acids [PUFA].

5. Conclusions

The quality and shelf life of whole cooked crawfish through refrigerated and frozen storage was similar in steamed and boiled crawfish tails with adhering hepatopancreas. These observations suggest that commercial crawfish processors would not benefit by investing in commercial steamers and steaming their crawfish, but they should continue the traditional boiling processes for crawfish. A further study with analyses conducted on each day of storage would allow for a more precise determination of the exact shelf life of the crawfish regarding aerobic counts.

With either method of cooking, the refrigerated shelf life is very short for whole cooked crawfish in most marketing channels. Further research on sensory properties would be advantageous to determine consumer acceptability on the refrigerated and frozen crawfish to compare with the analytical results observed in this study. Also, sensory analysis may reveal a difference between boiling and steaming from consumer perspectives even though analytical results in this study do not suggest there would be major differences.

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