Quality Changes in Australian Red Claw Crayfish, Cherax quadricarinatus, Stored at 0°C

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ABSTRACT. Shell-on tails of 70 juvenile Australian red claw crayfish, Cherax quadricarinatus, were separately packaged in seven sealed plastic freezer bags (10 in each) and stored on ice (0°C) for 0, 1, 3, 5, 7, 10, and 14 days. At the end of each storage period, raw muscle homogenate from five tails (pooled) were analyzed for lipid peroxidation, proteolysis (electrophoreis), and thermal stability (differential scanning calorimetry). The other five tails were individually cooked (2 minutes boiling) to determine cooking yield and toughness (Warner-Bratzler shear). Lipid oxidation occurred during storage (e.g., TBA values increased from 0.341 mg/kg on day 0 to 1.492 mg/kg on day 14, $P < 0.05$). Myofibrillar and sarcoplasmic proteins were generally resistant to proteolysis, but were destabilized during storage. Transition temperatures for myosin head and actin decreased ($P \leq 0.05$) from 50.2°C and 72.6°C on day 0, to 39.4°C and 60.3°C by day 14, respectively; while enthalpy of denaturation for myosin head reduced from 0.324 J/g to 0.116 J/g during this pe-

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riod. Storage also resulted in a gradual loss in cooking yield, which correlated ($R = -0.82$, $P < 0.05$) with progressive toughening of cooked meat. The results indicate that red claw muscle is susceptible to protein denaturation and lipid oxidation, and these chemical changes may be responsible for decreased cooking yield and reduced tenderness of meat during extended refrigeration storage.

KEYWORDS. Red claw crayfish, Cherax quadricarinatus, storage quality, culture, meat quality

INTRODUCTION

Australian red claw crayfish, Cherax quadricarinatus, also known as freshwater lobster or red claw, is a robust freshwater crustacean species native to the rivers and streams of northern Australia. They are commonly known as red claw because mature males are easily recognized by the red mark shown on the outer margin of the claws. Red claw have been cultured in Australia since 1985, and in the United States research on red claw began at about 1989. Compared to native American crayfish, red claw show some important advantages including larger potential size, higher percentage of dress-out (meat), and better tolerance of crowed culture conditions (Masser and Rouse 1997). Nutrient requirements, diet formulations, and production characteristics of red claw are currently being investigated by several universities around the world; however, there is no information on meat quality, especially during storage.

In most retail seafood markets, crustaceans are usually stored on ice and some are kept on display for days before being sold. Studies on freshwater prawn, Macrobrachium rosenbergii, have shown that storage of intact freshwater prawn on ice resulted in muscle "mushiness" due to diffusion of proteolytic and collagenolytic enzymes from the hepatopancreas (Baranowski et al. 1984; Lindner et al. 1988; Nip and Moy 1988). However, such texture deterioration was completely eliminated when prawn "heads" were removed prior to storage (unpublished results). Currently little information is available about the storage stability of the tail muscle from red claw cultured in the United States.

The objective of our study was to evaluate quality changes in red claw muscle stored at 0°C, simulating retail handling conditions. Chemi-

cal and physical reactions, including lipid oxidation, proteolysis, protein denaturation, and cooking yield were monitored in order to identify possible causes for textural changes in red claw muscle. The goal was to provide information for estimating the shelf-life of red claw under typical refrigerated retail-storage conditions.

MATERIALS AND METHODS

Materials

Juvenile red claw were raised in 0.02-ha ponds at Kentucky State University Aquaculture Research Center, Frankfort, Kentucky, for 8 weeks to a mean live weight of 40 g. A total of 70 red claw were randomly collected from a large sample pool. After stunning by submerging in an ice slurry, red claw were manually processed. The tails (muscle, plus exoskeleton), averaging 10.4±3.0 g in weight, were placed in iced coolers and shipped to the University of Kentucky's Food Protein Research Laboratory within 2-3 hours of collection. On receipt, the red claw were rinsed with tap water and subjected to treatments as described below.

Storage and Sample Preparation

The 70 red claw tails were divided and packaged in seven plastic freezer bags (10/bag) to prevent dehydration during storage. Bags were sealed and subsequently placed on crushed ice in a 5°C cold-room for 0, 1, 3, 5, 7, 10, and 14 days. At the end of each storage period, 10 tails were removed from the cold-room for analyses. Five of them, after removal of the shell, were homogenized in a Mini Chopper (Black and Decker, Inc., Shelton, Connecticut[1]) for 30 seconds. Raw muscle homogenates were analyzed immediately for physicochemical properties, including lipid oxidation, proteolysis, and protein denaturnation. The other five tails, with the shell on, were cooked (100°C) to measure cooking yield and tenderness.

Lipid Oxidation

Lipid oxidation was measured as increases in thiobarbituric acid-reactive substances (TBARS). The TBARS values were determined by

1. Use of trade or manufacturer's name does not imply endorsement.

using the colorimetric method described by Witte et al. (1970). Briefly, a weighed sample of raw muscle homogenate was blended with trichloracetic and phosphoric acids and then filtered. The color development by warming a portion of the filtrate with TBA under standard conditions was measured at 532 nm and then compared with that obtained by reacting TBA with 1,1,3,3-tetraethoxypropane. The TBARS values were expressed as mg of malonaldehyde/kg of sample.

Electrophoresis

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to detect proteolytic changes in red claw raw muscle during refrigerated storage. The SDS-PAGE system consisted of a 10% acrylamide separating gel and a 3% acrylamide stacking gel, and was run with an SE 250 mighty Small II slab gel electrophoresis unit (Hoefer Scientific Instrument, San Francisco, California) following the procedure described by Srinivasan et al. (1997). Samples for electrophoresis were prepared by homogenizing 1 g of minced muscle in 100 mL of prechilled (5°C) distilled, deionized water with a Polytron (Brinkman Instruments, Inc., Weatbury, New York) for 30 seconds. The homogenate was diluted 1:1 with the sample buffer containing 4% SDS, 0.125 M Tris (pH 6.8), 20% glycerol, and 10% β-mercaptoethanol. This preparation yielded a sample protein concentration of approximately 1 mg/mL, assuming a 20% protein content in raw muscle tissue. To each gel lane, a 20 μg sample was loaded. Protein bands were tentatively identified by comparing their electrophoretic mobility and intensity with published results of standard muscle proteins (Porzio and Pearson 1977). Molecular weights (MW) of unknown proteins were estimated from the regression line generated by plotting log[MW] of protein standards versus their migration distance.

Protein Denaturation

Differential scanning calorimetry (DSC) was used to monitor changes in thermal stability of red claw muscle proteins during storage. A model 2920 modulated DSC machine (TA Instruments, New Castle, Delaware) was calibrated for temperature and baseline using indium as standard. Accurately weighed minced muscle samples (14-17 mg) were placed in polymer-coated aluminum pans (TA Instruments) and hermetically sealed. An empty sealed pan was used as reference. Samples

were scanned from 10° to 100°C at a heating rate of 10°C/minutes. The enthalpy changes (ΔH) for the major thermal transitions were determined by measuring the area above the transition curves with a straight baseline constructed from the start to the end of the endotherms. Temperature at the maximum heat flow (Tm; temperature at peak of the endotherm) was also recorded. Both the ΔH and the Tm values were determined using the Universal Analysis Ver 1.2 N software supplied by the DSC company.

Cooking Yield

Raw, shell-on tails were cooked by immersing in boiling water (100°C) for exactly 2 minutes and then placed on crushed ice to quickly chill before further analysis. Cooking yield (%) was calculated and expressed as cooked shell-on weight divided by raw shell-on weight and then multiplying by 100. After the cooking yield measurement, the tail meat was subjected to textural analysis.

Textural Analysis

A Model 4301 Instron Universal Testing Instrument (Instron Corp., Canton, Massachusetts) with a Warner-Bratzler shearing device attached to the load cell (100 kg capacity) was used to measure the shear force required to rupture the first intact major muscle segment from the anterior of the cooked tail meat. The tail was placed in a transverse position to the blade to enable cutting across the muscle fibers. The crosshead speed of the Instron was set at 20 mm/minute, and the first major peak (usually the highest, which represented the maximal shear force required to cut the muscle fibers) was recorded.

Srinivasan et al. (1997) showed that shear force correlated either with the diameter (width) of the muscle portion sheared or with the weight of prawn tail. The positive correlation of prawn size with shear force indicated that the size of muscle or bundles had a major effect on meat tenderness. Thus, it is important to measure the shear force of individual red claw of known weight and normalize values for meaningful comparisons between samples. Accordingly, in the present study, shear force values (N) of individual tails were normalized based on the weights (g) of the tails, and were expressed as force per sample unit weight (N/g) to eliminate size effects.

Statistical Analysis

Data were analyzed using the General Linear Models procedure of the Statistix 3.5 software package (Analytical Software, Inc., St. Paul, Minnesota) for microcomputers. Analysis of variance was performed to determine the significance of the main effect (storage time). Significant ($P < 0.05$) differences between means were identified by Least Significant Difference procedures (Sendecor and Cochran 1989).

RESULTS AND DISCUSSION

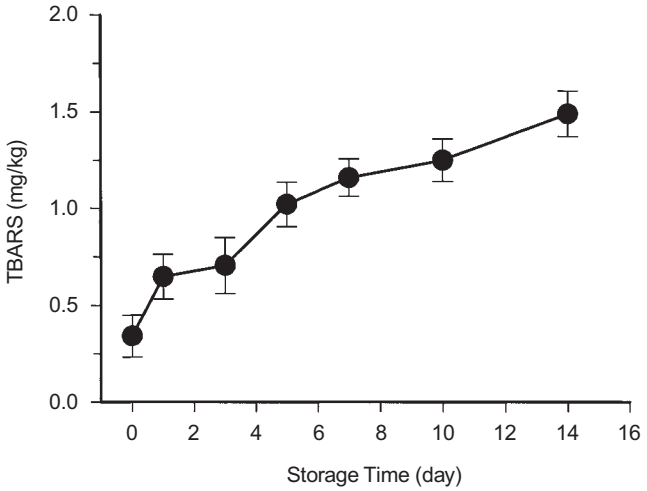
Lipid Oxidation

One of the main quality changes in post-harvest seafood is lipid oxidation that can lead to off-flavor development and may contribute to texture deterioration of the muscle tissue (Shenouda 1980). When stored at refrigerated temperatures, lipids in meat oxidize and unsaturated fatty acids form hydroperoxides that are subsequently cleaved to secondary reaction products, including aldehydes (e.g., malonaldehyde), ketones, alcohols, and acids (Frankel 1996). These secondary products are the primary basis of off-flavors in muscle foods (Kanner 1994). The increase in lipid-derived off-flavor compounds is usually accompanied by a simultaneous loss of desirable flavor compounds, a process described as meat flavor deterioration (MFD) (St. Angelo and Spanier 1993). In our study, TBARS formed during storage, and the values increased steadily ($P < 0.05$) from 0.341 mg/kg on day 0 (fresh) to 1.492 mg/kg on day 14 (Figure 1). The oxidation occurred despite low (< 6%) muscle lipid content. Phospholipids are located mostly in the membrane and are more susceptible to oxidation relative to other forms of lipids found in muscle (Kanner et al. 1986; Frankel 1996). They were probably responsible for much of the TBARS formation in red claw in the present study.

Proteolytic Changes

Electrophoretic analysis showed no major proteolytic changes in red claw muscle over the 14-day storage period (Figure 2). Both the myosin heavy chain and actin seemed to be remarkably resistant to proteolysis. However, a close examination of the electrophoteric patterns revealed a number of minor changes. These included decreased band intensities in

FIGURE 1. Formation of thiobarbituric acid-reactive substances (TBARS) as an indicator of lipid oxidation in Australian red claw crayfish muscle during iced (0°C) storage.

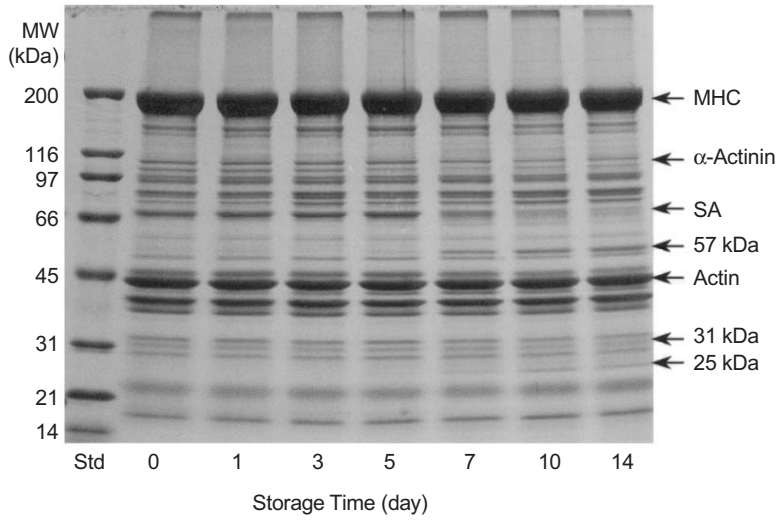


a protein doublet at the-actinin position, a 69 kDa polypeptide presumably serum albumin, and a 31 kDa component. The gradual disappearance of these proteins ostensibly resulted from proteolytic degradation and possibly also the formation of insoluble protein aggregates. Corresponding to the progressive losses of these polypeptides were the appearances of a 57 kDa and a 25 kDa new product (Figure 2). Many endogenous enzymes could cause the proteolytic changes, but calpain and cathepsins, two main protease families present in many crustacean species (Jiang et al. 1992; Jiang and Chen 1999), may have played a major role in producing the observed changes. Further studies are needed to identify the exact proteases involved.

Protein Denaturation

Changes in thermal stability of red claw muscle during storage, as determined by DSC, are presented in Figure 3. Because proteins are the only components which would exhibit major endothermic heat flows for their preponderance in the muscle, all DSC transitions above 40°C were likely due to protein structural changes. Transition peaks I and III are tentatively ascribed to denaturation of myosin head or heavy mero-

FIGURE 2. Electrophoretic analysis of proteolytic changes in Australian red claw crayfish muscle during iced (0°C) storage. Lane "Std": protein molecular weight standards. Myosin heavy chain (MHC), serum albumin (SA) and several other selected muscle proteins are marked.

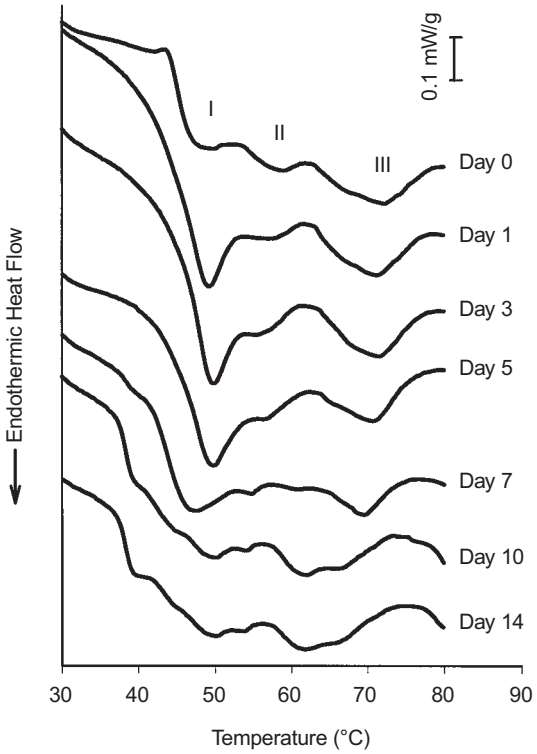


myosin and actin, while peak II to a combination of myosin tail, sarcoplasmic proteins and connective tissue (Stabursvik and Martens 1980).

Compared to muscle samples stored for 1 to 7 days, the height of peak I for fresh (day 0) red claw muscle was quite low. This small peak size for the fresh muscle (< 5 hour post-mortem) could be attributed to an exothermic heat flow (~ 40°C) that partially offsets the endothermic transition in myosin, as has been widely observed in muscle tissue shortly after death. Wright et al. (1977) attributed the exothermic heat flow in pre-rigor muscle to the muscle contraction process, while Park and Lanier (1989) hypothesized the exothermic heat flow as rapid ATP hydrolysis induced with rising temperature. As the storage time was prolonged, the thermal curves started to change, and by day 10, myosin transition (peak I) became less distinctive (Figure 3).

Analysis of the individual transitions and the heat of denaturation indicated major changes in the thermal stability of red claw muscle during storage. The maximum transition temperature (Tm) for myosin head (50.2°C) and actin (72.6°C) both showed a significant decrease ($P <$

FIGURE 3. Differential scanning calorimetry (DSC) of Australian red claw crayfish muscle stored on ice (0°C) for various periods.



0.05) after 7 days (to 46.3°C and 69.7°C); and by day 14, the Tm values dropped to 39.4°C and 60.3°C, respectively (Table 1). The corresponding enthalpy of denaturation also decreased for myosin but remained more or less unchanged for actin. For example, from day 0 to day 14, the ΔH value for myosin head decreased ($P < 0.05$) from 0.324 J/g to 0.116 J/g, and that for actin showed no significant changes.

Overall, red claw muscle proteins, including both myosin and actin, were considerably stable within the initial 5 days when stored at 0°C. After 5 days, they started to denature rapidly. The loss in thermal stability of proteins could result from interactions with oxidized lipids, including both lipid free radicals and secondary products such as reactive aldehydes (Figure 1), and from conformation alterations due to enzyme-induced hydrolysis (Figure 2). Hydrophobic reactions with lipid

TABLE 1. Maximum transition temperature (Tm) and enthalpy of denaturation (ΔH) in myosin (peak I) and actin (peak III) of Australian red claw crayfish muscle stored on ice (0°C) for various lengths of time. Means (n = 4) within the same column with different letters are significantly (P < 0.05) different.

Storage time (day)	Myosin (head)		Actin	
	Tm (°C)	ΔH (J/g)	Tm (°C)	ΔH (J/g)
0	50.2a	0.324a	72.6a	0.260b
1	48.8c	0.296a	71.7ab	0.342a
3	49.5b	0.318a	71.8a	0.338a
5	50.6a	0.308a	71.6ab	0.260b
7	46.3d	0.297a	69.7b	0.254b
10	39.2e	0.135b	62.6c	0.253b
14	39.4e	0.116b	60.3d	0.294ab

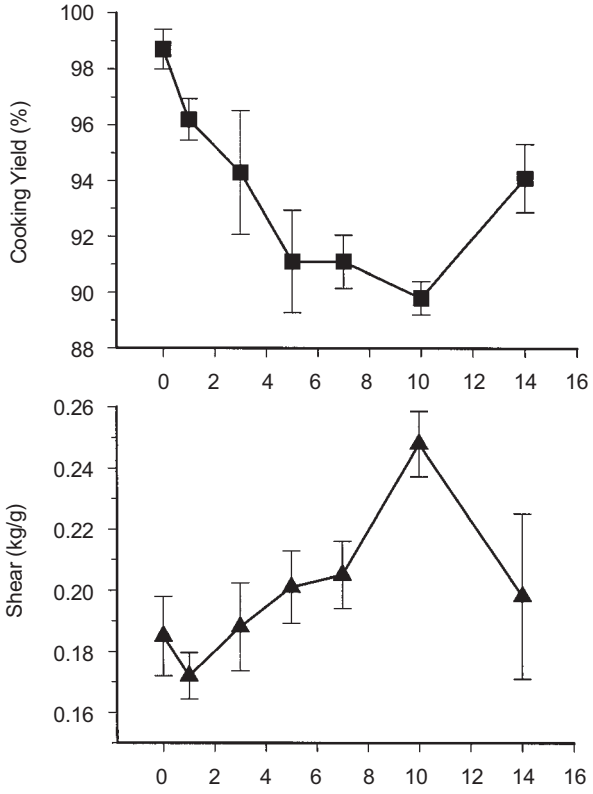
compounds could also lead to a destabilized protein structure in many marine products (Shenouda 1980).

Cooking Yield and Textural Analysis

Cooking yield of red claw muscle gradually decreased up to 10 days and then increased. The values were 98.7% for fresh meat (0 day) and 89.9% after 10 days ($P < 0.05$) (Figure 4). The loss in cooking yield was most likely due to protein denaturation which would result in a reduced water-holding capacity of muscle.

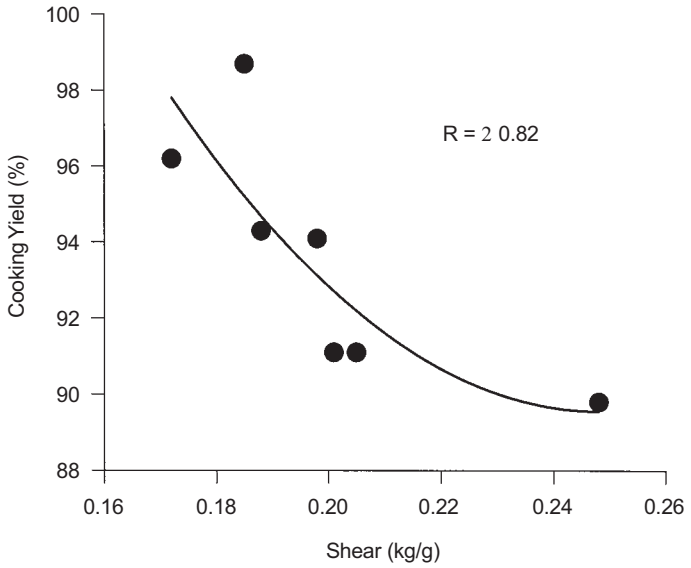
In contrast, shear force of cooked muscle increased during storage, especially between day 7 and day 10. It reached a maximum value at day 10 (0.248 kg/g) (Figure 4). Analysis of variance showed a significant quadratic relationship between cooking yield and shear force ($R = –0.82$, $P < 0.05$) (Figure 5). As muscle fibers and bundles lost their ability to hold water upon extended storage, they would easily form aggregates during cooking, thereby increasing meat toughness.

FIGURE 4. Cooking yield and shear force of cooked Australian red claw crayfish muscle after storage on ice (0°C) for various periods.



Several biochemical processes may be implicated in the textural deterioration and may likely have played an indirect, yet, important role. Both lipid oxidation (Figure 1) and enzymatic hydrolysis (Figure 2) could induce conformation and spatial structure changes in membrane proteins and myofibrils, rendering them less stable and more susceptible to thermal aggregation. This hypothesis may be supported by the observation that these chemical/biochemical changes in the muscle tissue (TBARS, proteolysis, and protein thermal stability) seemed to coincide with one another, e.g., none was particularly remarkable until after 5 or 7 days. Moreover, many of the lipid degradation products, such as malondialdehyde (Buttkus 1970) and 4-hydroxy-2-nonenal (Xiong 2000), could contribute to the production of cross-linked myofibrillar protein

FIGURE 5. A regression plot showing a quadratic relationship between cooking yield and shear force of Australian red claw crayfish muscle.



aggregates that, though not readily detectable by SDS-PAGE, were probably sufficient to increase the shear force value of cooked muscle. Lipid oxidation as an important factor in textural deterioration of muscle in many fish species has been well documented (Shenouda 1980). The lack of an apparent relationship between the physicochemical reactions and shear force of cooked red claw muscle beyond 7 days of storage may be because changes in the textural characteristics were a cumulative effect of physicochemical processes.

The decrease in muscle shear value (i.e., increase in tenderness) after 10 days is unexplained, but may be interpreted by considering two opposing effects. Tissue-toughening (i.e., due to protein denaturation and cross-linking) and tissue-softening (i.e., due to proteolysis) were both likely involved, but at the prolonged storage stage, proteolysis would seem to dominate and off-set the counter-action of the tissue-toughening factors.

Results from the study indicate that Australian red claw crayfish muscle is susceptible to lipid oxidation but resistant to proteolytic degradation and protein denaturation during short storage (< 7 days) at 0°C. However, iced storage did not prevent losses in cooking yield and ten-

derness of the red claw tail muscle. Hence, if red claw tails are to be kept on ice, as in retail situations, the storage time should be limited to less than 7 days.

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