

# Microbiological and Physicochemical Properties of Red Claw Crayfish (*Cherax quadricarinatus*) Stored in Different Package Systems at 2 °C

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**ABSTRACT:** This study compared 3 package systems for their influence on the stability of shell-on red claw crayfish tail meat stored at 2 °C for 14 d. Modified atmosphere packaging (MAP, with 80% CO<sub>2</sub>/10% O<sub>2</sub>/10% N<sub>2</sub>) suppressed ( $P < 0.05$ ) the growth of aerobic bacteria and coliforms when compared with aerobic packaging using polyvinylchloride (PVCP) and vacuum packaging (VP). MAP and VP tended to retard lipid oxidation, inhibited Ca-ATPase activity change, but produced more intense protein denaturation when compared with PVCP. The MAP packaging enhanced proteolysis and resulted in more ( $P < 0.05$ ) cooking losses and a higher ( $P < 0.05$ ) shear force than VP and PVCP for samples stored for 6 and 14 d ( $P < 0.05$ ). Sensory panel results were in general agreement with the physicochemical changes, suggesting that the specific package systems had a significant impact on the quality of refrigerated red claw crayfish raw meat.

**Keywords:** lipid oxidation, modified atmosphere packaging, proteins, red claw crayfish, sensory properties

## Introduction

Red claw crayfish (*Cherax quadricarinatus*), an emerging shellfish species in the global seafood market, has generated considerable interest among aquaculturists in the United States in recent years. Compared with native American crayfish, red claw exhibits some important advantages, including a larger potential size, a higher percentage of dress-out (meat), and better tolerance of crowded culture conditions (Masser and Rouse 1997).

Like any other raw seafood, fresh red claw meat is perishable. Low temperature storage is one of the primary preservation methods to maintain fish freshness, because the rates of microbiological, chemical, and biochemical changes are reduced at decreased temperatures. Several studies have examined meat quality changes during refrigerated and frozen storage of red claw (Tseng and others 2002, 2005) and the gender and spawning effects on meat quality (Kong and others 2006, 2007). These previous investigations showed that raw crayfish tail meat was generally resistant to quality deterioration (oxidation and texture profile). However, it exhibited quality loss when subjected to temperature fluctuations such as repeated freezing-thawing (Tseng and others 2003).

Modified atmosphere packaging (MAP) has been used to extend the shelf life of muscle foods, especially red meats and poultry, due to its microbial inhibition (Phillips 1996). Carbon dioxide in the package atmosphere is extremely effective on inhibiting growth of microorganisms, notably Gram-negative spoilage bacteria, on fresh meats (Dixon and Douglas 1989; Stammen and others 1990; McMillin and others 1999; Dhananjayan and others 2006). The ap-

plication of MAP in the seafood industry has not been adequately explored, and presently, there is no published study on the effect of package systems on the storage stability of red claw crayfish. Research on seafood packaging has mostly focused on microbial inhibition, and relatively few studies have evaluated the physicochemical changes in seafood during MAP storage (Brown and others 1980; Lannelongue and others 1992). However, physicochemical properties such as lipid oxidation, flavor profile, tenderness, and water binding are other important measures of the overall quality of seafood products.

The objective of this study was to investigate 3 different package systems (modified atmosphere packaging or MAP, vacuum packaging or VP, and aerobic polyvinyl chloride packaging or PVCP) for their capability to control microbial growth and their influence on quality preservation of red claw meat during refrigerator storage.

## Materials and Methods

### Sample preparation

Juvenile red claw crayfish were raised in ponds at Kentucky State Univ. Aquaculture Research Center, Frankfort, Ky., U.S.A. for 16 wk to a mean live weight of 45 g. A total of 540 mixed male and female red claws were randomly collected from a large sample pool and spawning females were avoided. After being stunned by submersion in ice slurry for 2 min, red claws were manually beheaded. The tails (muscle, with exoskeleton), averaging 19.5 g in weight, were placed in iced coolers and shipped to the Univ. of Kentucky's Food Protein Laboratory within 3 h of collection. On receipt, the red claw tails were rinsed with tap water and randomly selected for each package system.

### Packaging

A completely randomized design with 2 replications was used in this study. A total of 540 red claw tails were used (270 per replication). These tails were randomized and placed in 36 plastic trays (32 × 26 × 8, length × length × depth, in cm). Each tray contained

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15 tails. In each replication, there were 18 subtreatments arranged in a factorial design with 6 storage times (0, 1, 3, 6, 10, and 14 d postmortem) and 3 types of package systems (MAP, VP and PVCVP).

For PVCVP sample preparation, the trays containing the crayfish tails were double-wrapped with an air-permeable PVC film (15500 to 16275 cm<sup>3</sup>/m<sup>2</sup> per 24 h oxygen transmission rate at 23 °C). For MAP, the trays were filled with a gas mixture of 80% CO<sub>2</sub>, 10% O<sub>2</sub>, and 10% N<sub>2</sub> with a 1.0 bar gas pressure, and packaged with a FoodPack Basic FP372 machine (Ilpra Thermosaldatrici, Vigevano, Italy) using a polypropylene-polyethylene sealing film (1.75 mil, 140 cm<sup>3</sup>/m<sup>2</sup> per 24 h oxygen transmission rate at 23 °C, Amcor Flexibles, Abbotsford, Australia). This particular MAP system was used because it was found to be very effective in extending the shelf life of refrigerated fresh fish fillets (Masniyom and Benjakul 2004). For VP, the trays were placed inside Type B2620 vacuum bags (2.2 mil, 3 to 6 cm<sup>3</sup>/m<sup>2</sup> per 24 h oxygen transmission rate at 23 °C; Cryovac Division, Sealed Air Corp., Duncan, S.C., U.S.A.) and packaged using Model 600A vacuum machine (Sipromac Inc., St-Germain, Quebec, Canada). All packages were placed in a 2 °C walk-in cooler and received approximately 1076 lux of warm white fluorescent light to simulate retail storage conditions, and at the end of 0, 1, 3, 6, 10, and 14 d, 1 pack from each package system was removed for microbial and meat quality analyses.

### Microbiological measurement

For each package system, 5 shell-on red claw tails were randomly selected, each cut into 3 pieces with a sterile scalpel, and then pooled. The mixed sample was analyzed for aerobic plate count (APC), *Escherichia coli*, and *Lactobacillus* spp. following the respective procedures of the FDA (2001). Specifically, chopped samples (25 g) were mixed with 225 mL of 0.1% peptone in a sterile jar and blended 1 min with a Waring blender at the High Speed setting. A serial dilution was made with a standard phosphate dilution buffer (pH 7.20) (BBL, Div. of BioQuest, Cockeysville, Md., U.S.A.). Aliquots of 1 mL of each diluted sample were plated onto proper Petrifilms for coliforms, *E. coli*, and aerobes (3M Microbiology Products, St. Paul, Minn., U.S.A.). Also, 1 mL of each diluted sample was plated onto Rogosa agar for the detection of *Lactobacillus* spp. Coliform and *E. coli* petrifilms were incubated in a 38 °C incubator for 24 h prior to counting, and the APC petrifilm was incubated in a 26 °C incubator for 72 h prior to enumeration. The Rogosa Petri dishes were placed in an anaerobic jar, flushed with N<sub>2</sub>, and sealed before incubation at 38 °C for 72 h. Both non-*E. coli* and *E. coli* colonies (red and blue colonies associated with a gas bubble) growing on the Petrifilm were counted as coliform; only dark blue colonies associated with a gas bubble were counted as *E. coli*.

### Lipid oxidation

The concentration of thiobarbituric acid-reactive substances (TBARS) of muscle samples was determined according to Sinnhuber and Yu (1977). After reaction in the TCA-HCL solution at 100 °C (boiling) to form the pink pigment and cooling to room temperature, 5 mL of the sample supernatant were aspirated into a test tube containing 5 mL of chloroform and vortexed for 1 min. The mixture was centrifuged for 10 min at 1680 g. The absorbance (532 nm) of the supernatant was read, and the TBARS value was calculated from the following equation (Wang and Xiong 2005): TBARS (mg/kg) = ( $A_{520}/W_s$ ) × 9.48, where  $W_s$  is the muscle sample weight and the value 9.48 is a constant derived from the sample dilution and the absorption coefficient (152000/M/cm) of the TBA-malonaldehyde adduct.

### Differential scanning calorimetry (DSC)

At the end of each designated storage day, 3 tails from each package system were randomly selected and deshelled. The tail muscles were pooled and finely chopped by blending for 30 s with a micro Waring blender to produce a homogenous muscle mince for DSC analysis. Protein thermal stability of mince muscle was analyzed in triplicate using a Model 2920 DSC machine (TA Instruments, New Castle, Del., U.S.A.) (Kong and others 2007). The DSC machine was calibrated for temperature and baseline using indium as standard. Samples were thermally scanned from 10 to 100 °C at a heating rate of 10 °C/min. The total enthalpy change ( $\Delta H$ ) associated with protein denaturation was estimated by measuring the area above the DSC transition curve with a straight baseline constructed from the start to the end of the endotherm. Temperature at the maximum heat flow, that is, temperature at the peak of the endotherm ( $T_{max}$ ), was also recorded. Both the  $T_{max}$  and  $\Delta H$  values were determined using the Universal Analysis version 1.2 N software supplied by the DSC Co. (TA Instruments).

### Calcium ATPase activity

Ca-ATPase activity of red claw muscle was assayed based on the method of Wells and others (1979). Briefly, 0.2 mL of diluted muscle homogenate was mixed with 2.0 mL of the reaction solution (7.6 mM ATP, 15 mM CaCl<sub>2</sub>, 150 mM KCl, 180 mM Tris-HCl, pH 7.4). After incubation at 22 °C for 10 min, 1.0 mL of 10% trichloroacetic acid was added to stop the reaction. The liberated inorganic phosphate (Pi) was calorimetrically measured in a 0.75 N sulfuric acid solution containing 0.66% ammonium molybdate, and quantified against a standard curve (0 to 5 μmol/mL phosphate). The Ca-ATPase activity was expressed as μmol Pi/min/mg muscle.

### Gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of muscle from stored red claw tails was performed with a Mini-PROTEAN 3 Cell electrophoresis system (Bio-Rad Laboratories, Hercules, Calif., U.S.A.). A 10% acrylamide resolving gel and a 3% acrylamide stacking gel were used (Laemmli 1970). Homogenized muscle mince was dissolved in an appropriate amount of SDS-PAGE sample buffer (4% SDS, 20% glycerol, 10% β-mercaptoethanol, 0.125 M Tris-HCl, pH 6.8) to obtain a 1 μg/μL protein concentration. Aliquots of 20 μL of the samples were loaded to the sample wells in the stacking gel. A molecular weight (MW) standard, consisting of a cocktail of proteins of known MWs (6.5 to 200 kDa) (Bio-Rad Laboratories) was also run. Protein bands in red claw samples were tentatively identified by comparing their electrophoretic pattern with published results of standard muscle proteins (Porzio and Pearson 1977), and MWs of unknown proteins were estimated from the regression line of protein migration distances compared with logarithm MW of the standard proteins.

### Cooking loss

Weighed shell-on tails were cooked in boiling water (100 °C) for exactly 2 min, chilled to room temperature (21 °C) for 10 min, and weighed again. Cooking loss (percent) was expressed as the weight difference between shell-on raw tails (uncooked) and shell-on cooked tails divided by the weight of the shell-on raw tails. After the cooking loss measurement, the tail meat was subjected to textural analysis.

### Textural analysis

Cooked and deshelled red claw tails were individually weighed before being subjected to textural analysis using a Model 4301

Instron Universal Testing Instrument (Instron Corp., Canton, Mass., U.S.A.) with a Warner-Bratzler shearing device attached to the load cell (1 kN capacity). The tail was placed in a transverse position to the blade so that the blade would cut through the 1st major muscle segment from the anterior of the cooked tail across the muscle fibers (Tseng and others 2005). The crosshead speed of the Instron was 50 mm/min. Shear force (*SF*) values (*N*) were normalized based on the weight (g) of the tails, and were expressed as force per sample unit weight (*N/g*) to eliminate size effects (Srinivasan and others 1997).

### Sensory evaluation

Sensory evaluation of cooked red claw was conducted by an 8-member taste panel that consisted of selected faculty, staff, and graduate students who had prior experience with muscle food sensory tests. The panel was trained to identify the specific sensory traits of red claw using actual cooked red claw meat (Kong and others 2007). A "warm-up" sample at the beginning of every panel session was evaluated as a reminder of the score range for each sensory trait. The following sensory characteristics were evaluated: lobster flavor, off-flavors, rancidity, tenderness, and juiciness. Scores were

assigned using a 7-point scale as follows: for lobster flavor, 1 = mild, 7 = intense; for off-flavors, 1 = nondetectable, 7 = intense; for rancidity, 1 = nondetectable, 7 = intense; for tenderness, 1 = tough, 7 = tender; and for juiciness, 1 = dry, 7 = juicy. The panelists marked on the scale where they felt best described the sensory attributes. As consumers, the panelists were also asked for their opinion on acceptability of the red claw samples evaluated, that is, overall acceptability with 1 = low and 7 = high.

### Statistical analysis

Data were analyzed using the General Linear Models procedure of the Statistix 7.0 software package (Analytical Software, St. Paul, Minn., U.S.A.) for microcomputers. Analysis of variance (ANOVA) was done to determine the significance of the main effects (packaging treatments and storage time). Significant ( $P < 0.05$ ) differences between means were identified using the least significant difference procedures.

## Results and Discussion

### Microbiological analysis

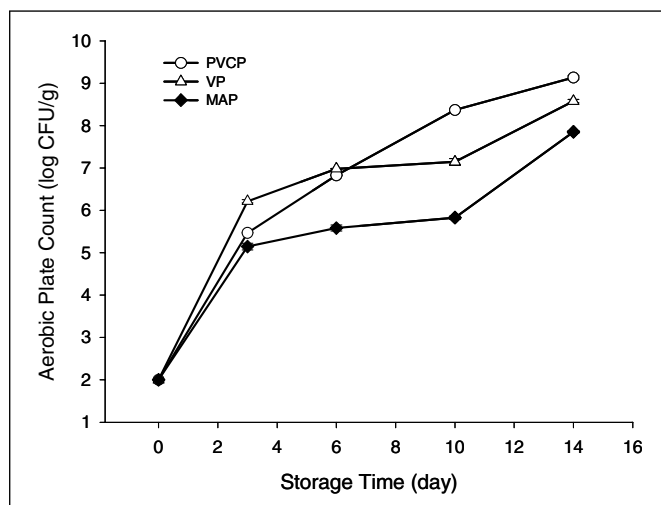
The APC, which could include both aerobic and facultative bacteria, was initially low (2 log CFU/g). However, it increased rapidly within the first 2 d during storage, irrespective of package systems (Figure 1). Microbes that were probably injured from the ice slurry stunning or shocked from changing the ecosystem (live crayfish compared with postmortem samples) rapidly recovered and subsequently multiplied. The neutral pH condition ( $\text{pH } 7.40 \pm 0.16$ ) of the red claw muscle, which was independent of the package systems as observed in our preliminary study, would favor the bacterial growth.

Comparison of the 3 package systems indicated a lower APC (Figure 1) as well as a reduced coliform population (Figure 2) in MAP samples when compared with PV samples after 6 d ( $P < 0.05$ ). The presence of a high level of atmospheric  $\text{CO}_2$  in the MAP system was critical to the inhibition of bacterial growth (Wang and Brown 1983; Phillips 1996), while the VP system would still allow facultative microbes to populate. The mode of  $\text{CO}_2$  action has not been completely elucidated, although it has been postulated that the bicarbonate ion, a dissociation product from  $\text{CO}_2$ , changes cell permeability and affects metabolic processes (Daniels and others 1986). On the other hand, the small amount of  $\text{O}_2$  present in the MAP would inhibit anaerobic microorganisms. It has been recommended that a low level of  $\text{O}_2$  (5% to 10%) be used in any MAP system to inhibit pathogenic anaerobes (Hotchkiss 1988).

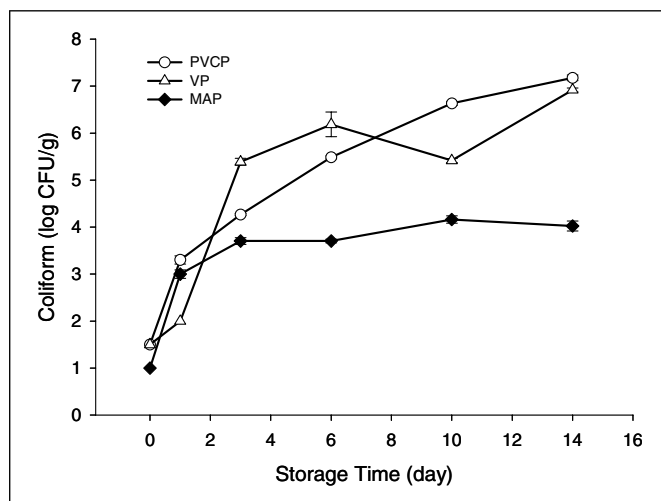
Neither *Lactobacillus* spp. nor *E. coli* was detected in samples stored in any of the 3 package systems over the entire 14 d. Although we cannot conclude that MAP or VP could inhibit *Lactobacillus* spp., the negative result of *Lactobacillus* spp. could provide an extra assurance for the stored red claw to be an unlikely source of histamine. Certain strains of *Lactobacilli* can decarboxylate histidine and produce histamine poisoning during fish storage, and the presence of  $\text{CO}_2$  reduced the incidence (Watts and Brown 1982).

### Lipid oxidation

Red claw tail meat samples stored in the modified atmosphere environment (MAP) or in vacuum (VP) were resistant to oxidation, showing negligible TBARS changes over the storage time tested (10 d) (Figure 3). On the other hand, samples constantly exposed to an aerobic environment (PVCP) had a higher TBARS content on day 1 and day 10 when compared with day 0 ( $P < 0.05$ ). The PVCP sample produced more TBARS than did MAP and PV samples on day 1



**Figure 1—Aerobic plate count (log CFU/g) of red claw crayfish tails stored in 3 different package systems at 2 °C for various times**



**Figure 2—Coliform count (log CFU/g) of red claw crayfish tails stored in 3 different package systems at 2 °C for various times**

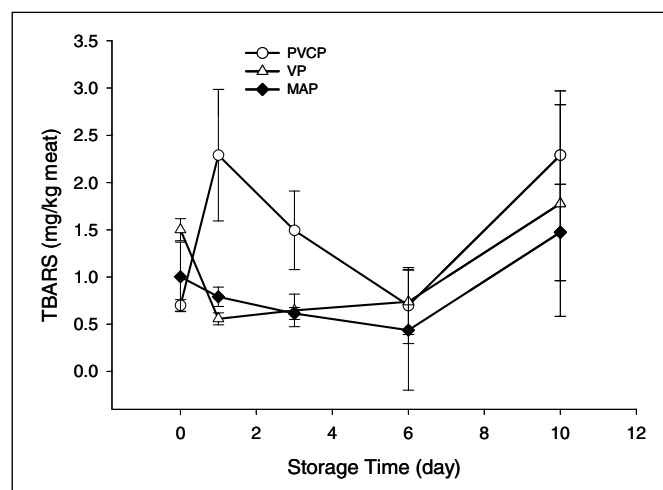
( $P < 0.05$ ), and its TBARS level on day 3 was also slightly higher than that in the other package systems ( $P = 0.1$ ). The overall low levels of lipid oxidation were attributed to the fact that red claw tail muscle was extremely low in lipid content [approximately 0.18% (w/w)] (Kong and others 2006). Notwithstanding, the results demonstrated that PVCP was a more oxidizing system than MAP and VP.

### Thermal stability and myosin ATPase activity

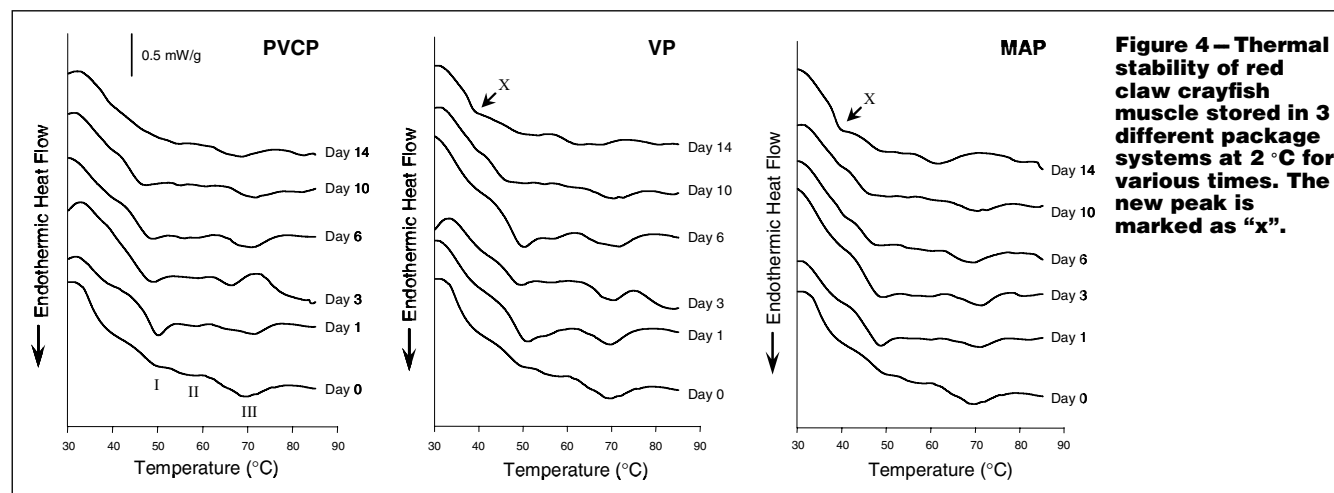
DSC thermal curves of red claw muscle samples are presented in Figure 4. Nonstored muscle samples (day 0) exhibited 3 appreciable thermal transitions (peaks I, II, and III). The overall DSC patterns were essentially identical to those observed in our previous study on red claw muscle (Kong and others 2007), and were similar to those seen in freshwater prawn (Subramanian and others 1997) and mammalian and poultry species (Stabursvik and Martens 1980; Xiong and others 1987). Based on the transitions of the individual, isolated muscle proteins, Wright and others (1977) attributed the 3 transitions to myosin head (peak I), the combination of myosin tail, sarcoplasmic proteins, and connective tissue (peak II), and actin (peak III).

There were several notable changes in the DSC patterns during storage and subtle differences among the 3 package systems. The most appreciable change occurred within the first 24 h when the myosin head transition peak ( $T_{\max-1} = 49.33\text{ }^{\circ}\text{C}$ ) became more prominent after 1 d (Figure 4). Analysis of the specific transition temperatures indicated that  $T_{\max-1}$  in PVCP and MAP samples tended to decrease steadily ( $P < 0.05$ ) during storage up to 10 d but the change in  $T_{\max-1}$  in VP samples was not consistent (Table 1). On the other hand, temperature of actin transition ( $T_{\max-3} = 68.84\text{ }^{\circ}\text{C}$ ) did not change until the last day of storage (day 14) where  $T_{\max-3}$  decreased to 63.24 and 61.29  $^{\circ}\text{C}$  ( $P < 0.05$ ) in VP and MAP samples, respectively, while  $T_{\max-3}$  in PVCP remained unchanged (Table 1). Furthermore, a new peak (marked as "x") with  $T_{\max-x}$  around 39.03 to 39.24  $^{\circ}\text{C}$  emerged in VP and MAP samples after 10 d (Figure 4). It was hypothesized that this particular peak resulted from destabilization or degradation of red claw proteins.

To test the hypothesis, enthalpies ( $\Delta H$ ) associated with transitions I and II were measured. As seen in Table 2, the enthalpy of myosin head transition ( $\Delta H_1 = 0.091\text{ J/g}$ ) increased drastically after the 1st day of sample storage ( $P < 0.05$ ), which was in agreement with a previous finding (Kong and others 2007). The  $\Delta H_1$  value remained high throughout 6 d for MAP samples and 10 d for PVCP



**Figure 3 – The TBARS levels in red claw crayfish muscle stored in 3 different package systems at 2 °C for various times. Each data point represents the mean of 3 measurements.**



**Figure 4 – Thermal stability of red claw crayfish muscle stored in 3 different package systems at 2 °C for various times. The new peak is marked as "x".**

**Table 1 – Transition temperature ( $T_{\max}$ ) for myosin head (peak I), actin (peak III), and the new peak (peak x) of red claw crayfish muscle stored in different package systems at 2 °C for various times**

Storage time (day)	Myosin head ( $T_{\max-1}$ , °C)			Actin ( $T_{\max-3}$ , °C)			Peak x ( $T_{\max-x}$ , °C)		
	PVCP	VP	MAP	PVCP	VP	MAP	PVCP	VP	MAP
0	49.33 <sup>abA</sup>	49.33 <sup>bcA</sup>	49.33 <sup>aA</sup>	68.84 <sup>aA</sup>	68.84 <sup>abA</sup>	68.84 <sup>aA</sup>			
1	49.35 <sup>abB</sup>	50.65 <sup>aA</sup>	48.48 <sup>abB</sup>	71.17 <sup>aAB</sup>	69.71 <sup>abB</sup>	71.40 <sup>aA</sup>			
3	48.82 <sup>aA</sup>	48.62 <sup>ca</sup>	47.98 <sup>abA</sup>	68.57 <sup>aA</sup>	67.14 <sup>ba</sup>	71.11 <sup>aA</sup>			
6	47.70 <sup>abB</sup>	49.58 <sup>ba</sup>	47.12 <sup>bb</sup>	70.31 <sup>ab</sup>	71.47 <sup>aA</sup>	69.46 <sup>aB</sup>			
10	46.63 <sup>bcA</sup>	46.47 <sup>da</sup>	46.68 <sup>ba</sup>	71.30 <sup>aA</sup>	71.30 <sup>aA</sup>	65.81 <sup>abB</sup>			39.16
14	45.69 <sup>cb</sup>	49.16 <sup>aA</sup>	48.11 <sup>abA</sup>	68.86 <sup>aA</sup>	63.24 <sup>cb</sup>	61.29 <sup>bb</sup>		39.03	39.24

<sup>a-c</sup>Means in the same column that do not share a common lowercase letter differ significantly ( $P < 0.05$ ).

<sup>A-C</sup>For the same protein, means in the same row that do not share a common uppercase letter differ significantly ( $P < 0.05$ ).

**Table 2 – Enthalpy of denaturation ( $\Delta H$ ) for myosin head (peak I), actin (peak III), and the new peak (peak x) of red claw crayfish muscle stored in different package systems at 2 °C for various times**

Storage time (day)	Myosin head ( $\Delta H_1$ , J/g)			Actin ( $\Delta H_2$ , J/g)			Peak x ( $\Delta H_x$ , J/g)		
	PVC	VP	MAP	PVC	VP	MAP	PVC	VP	MAP
0	0.091 <sup>bcA</sup>	0.091 <sup>eA</sup>	0.091 <sup>cA</sup>	0.371 <sup>aA</sup>	0.371 <sup>abA</sup>	0.371 <sup>aA</sup>			
1	0.250 <sup>abcB</sup>	0.600 <sup>aA</sup>	0.353 <sup>abB</sup>	0.373 <sup>aA</sup>	0.342 <sup>abA</sup>	0.224 <sup>bcA</sup>			
3	0.318 <sup>aA</sup>	0.235 <sup>cdA</sup>	0.216 <sup>bA</sup>	0.324 <sup>aA</sup>	0.424 <sup>aA</sup>	0.253 <sup>bA</sup>			
6	0.269 <sup>abA</sup>	0.336 <sup>bA</sup>	0.132 <sup>bcB</sup>	0.336 <sup>aA</sup>	0.296 <sup>abA</sup>	0.270 <sup>abA</sup>			
10	0.217 <sup>abcAB</sup>	0.325 <sup>bcA</sup>	0.044 <sup>cB</sup>	0.241 <sup>aA</sup>	0.237 <sup>bA</sup>	0.130 <sup>cB</sup>			0.091
14	0.075 <sup>cA</sup>	0.135 <sup>deA</sup>	0.045 <sup>cA</sup>	0.226 <sup>aA</sup>	0.289 <sup>abA</sup>	0.177 <sup>bcA</sup>		0.077	0.107

<sup>a-d</sup>Means in the same column that do not share a common lowercase letter differ significantly ( $P < 0.05$ ).  
<sup>A-D</sup>For the same protein, means in the same row that do not share a common uppercase letter differ significantly ( $P < 0.05$ ).

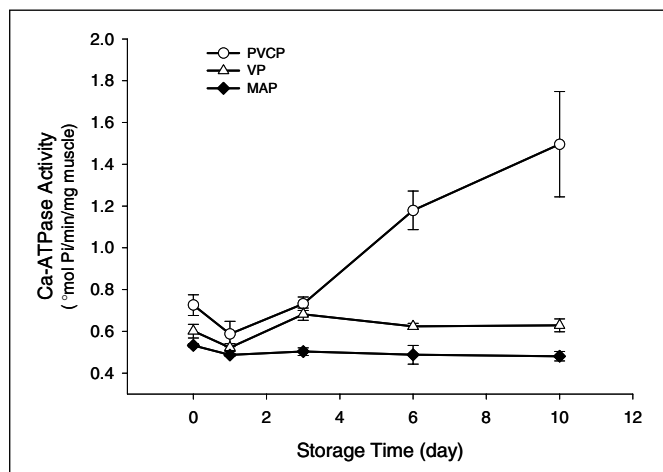
and VP samples. The more rapid and extensive enthalpy reduction for myosin head transition in MAP seemed to coincide with the appearance of the new peak (approximately 39 °C), when compared with PV or PVCP systems. While the enthalpy associated with actin transition ( $\Delta H_2$ ) was more subtle when compared with that of myosin head, the MAP system produced the greatest drop in the  $\Delta H_2$  values, that is, from 0.371 J/g on day 1 to 0.130 J/g on day 10 (Table 2). Both the  $\Delta H$  and the  $T_{max}$  data suggested that proteins in red claw tails in the mixed O<sub>2</sub>/CO<sub>2</sub>/N<sub>2</sub> atmosphere (MAP) were less stable.

The 0-d red claw muscle samples had a Ca-ATPase activity of 0.5 to 0.8  $\mu\text{mol}/\text{Pi}/\text{mg}$  (Figure 5), which can be attributed to the

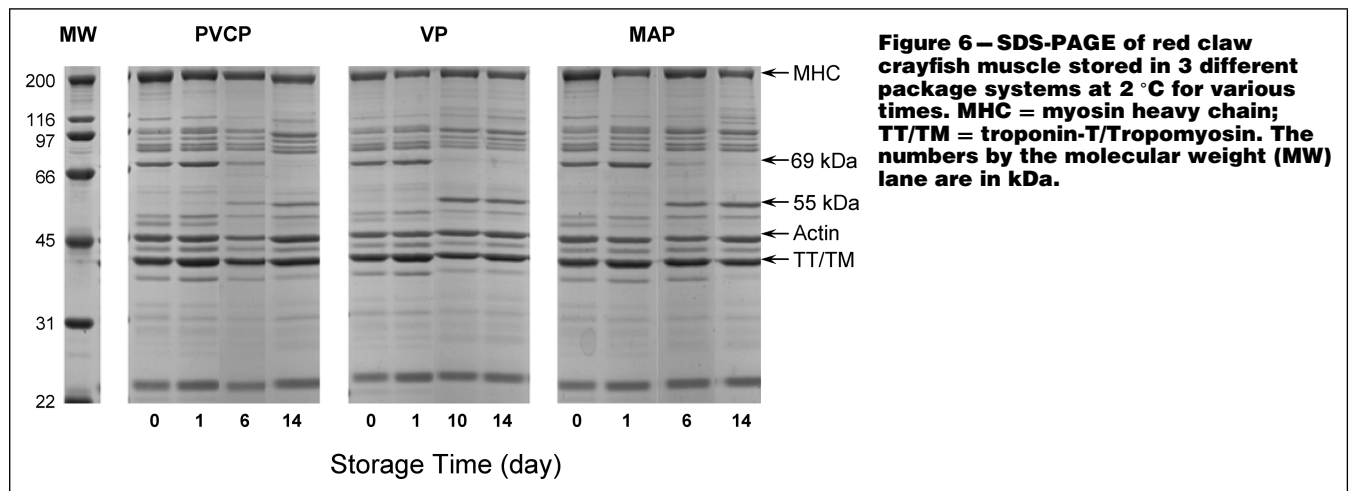
ATPase located at the myosin head (Sekine and Yamaguchi 1963) as well as that at the sarcoplasmic reticulum (Schertzer and others 2005). In contrast with VP and MAP samples that showed negligible change, the Ca-ATPase activity in PVCP samples increased markedly ( $P < 0.05$ ) during storage. Ca-ATPase activity in muscle myofibrils is closely related to the reactivity of sulfhydryl groups in myosin globular head (Sekine and Yamaguchi 1963), and increases under oxidative conditions (Ooizumi and Xiong 2004; Park and others 2006). Thus, the rise in Ca-ATPase activity in the PVCP red claw samples was indicative, at least in part, of oxidative modification of myosin sulfhydryls to form disulfide bonds (Ooizumi and Xiong 2004). The data from the DSC analysis (Table 2) suggested that such modification tended to stabilize myosin, thus explaining the absence of “peak x” in PVCP samples (Figure 4).

**Proteolysis**

Significant proteolytic changes occurred in muscle samples stored in all 3 packaging conditions, with the disappearance of a 69-kDa protein and a concomitant emergence of a 55-kDa polypeptide being the most noticeable events (Figure 6). Because of its close proximity, the 55-kDa polypeptide was presumed to originate from the 69-kDa component. While there was a lack of remarkable differences between the 3 package systems, it was noted that the intensity of the 55-kDa protein band was consistently greater for MAP samples than for PVCP samples stored for 6 d. Preliminary experiments also showed that the 55-kDa polypeptide appeared in 3-d MAP and VP samples but not in 3-d PVCP samples. Calpain may be an endogenous protease producing these proteolytic phenomena and the slight differences associated with the packaging conditions. The relatively high pH of red claw muscle (pH 7.40  $\pm$  0.16) supported the hypothesis because calpain is neutral protease.



**Figure 5 – Ca-ATPase of red claw crayfish muscle stored in 3 different package systems at 2 °C for various times**



Guttman and Johnson (1998) and Rowe and others (2004) demonstrated that oxidative stress could inhibit the calpain activity. Because the PVCPC system was more oxidative than MAP and VP, it can be suggested that the more pronounced proteolysis seen in the MAP or VP resulted from greater calpain activity. However, this hypothesis must be tested in future research.

### Cooking loss

Cooking loss affects meat product weight, appearance, and sensory properties and it is most relevant to water-holding capacity (WHC) (van Laack 1999). The MAP treatment increased ( $P < 0.05$ ) cooking loss of red claw meat when compared with VP and PVCPC throughout storage (Figure 7). Of the 3 packaging conditions, the PVCPC was the best in maintaining a low degree of cooking loss. Although equally capable of retaining moisture, samples stored in the VP system for more than 6 d showed an increasing cooking loss when compared with PVCPC. It was previously shown that elevated concentrations of  $\text{CO}_2$  could induce significant drip loss for packaged fish muscle (Boknas and others 2001). It is reasonable to suggest that the cell wall-rupture effect of  $\text{CO}_2$ , as demonstrated in bacteria (Daniel and others 1986; Park and others 2003), was applicable to red claw muscle tissue; that is, it damaged muscle cell membrane thereby leaching cell fluid from MAP samples. It is well established that meat cooking loss is indicative of reduced ability for muscle proteins or the myofibril matrixes to hold water (Hamm 1986; van Laack 1999). Thus, the higher cooking loss in MAP meat samples may also be construed as a result of increased myosin denaturation as demonstrated in the aforementioned thermal analysis (Figure 4; Table 2), and possibly also weakening of the myofibril lattices due to protein degradation (Figure 6).

### Warner-Bratzler shear force

Significant differences existed among the 3 types of package systems in the shear force values of cooked meat (Figure 8). The MAP samples stored for 6 and 14 d exhibited higher shear forces than VP and PVCPC samples ( $P < 0.05$ ). Compared with respective PVCPC samples, the 10- and 14-d VP samples were also of higher shear values, thus, greater toughness ( $P < 0.05$ ). The higher shear force in MAP probably resulted from a higher degree of protein aggregation upon cooking, which was indicated by the increased cooking loss. The shear force changes could also result from tissue softening

which can be caused by proteolysis. In this case, shear force would decrease. The initial increase followed by the subsequent reduction in the shear force values in VP and PVCPC samples during storage may be explained by these 2 opposing effects. After 10 d of storage, proteolysis would seem to be predominant and offset the counteraction of the tissue-toughening factor, namely, protein aggregation.

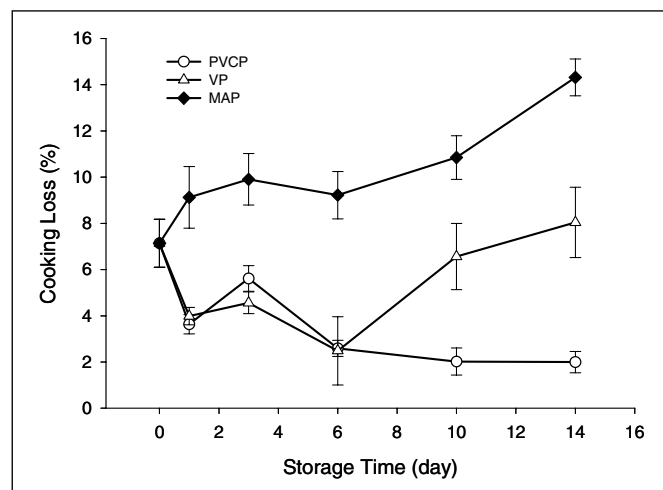
### Sensory properties

Sensory attributes of red claw as related to microbial growth and physicochemical characteristics of meat, which were judged by a trained panel, are summarized in Table 3. Package systems and storage time overall did not affect the lobster flavor, which was perceived to be moderately intense. However, there was a clear tendency ( $P < 0.05$ ) that off-flavors, albeit rather mild overall, became slightly more intense as the storage time increased, and the PVCPC 14-d sample was found to have the greatest off-flavor intensity. Rancidity was largely absent, but of the several slightly detected samples, it was perceived to be the highest also in the PVCPC 14-d sample (Table 3). The extremely low off-flavor and rancidity scores (approximately 1.2 on average on a scale of 1.0 for nondetectable to 7 for intense) agreed with the overall low lipid oxidation, and supported the hypothesis that TBARS can be a reasonable indicator of the degree of off-flavors or rancidity in muscle foods.

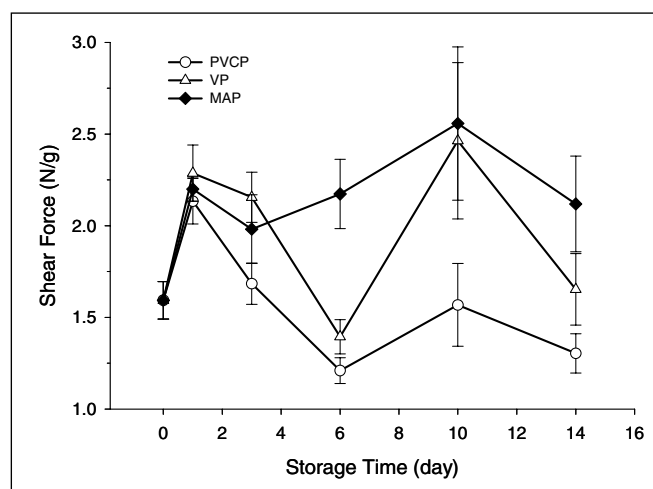
The sensory panel could not detect significant differences in juiciness between red claw samples packaged in different systems during the first 3 d of storage. However, both the MAP and VP 10-d samples and the MAP 14-d sample were found to be less juicy than their respective PVCPC samples ( $P < 0.05$ ). As expected, the result was in accord with the cooking loss analysis that showed a higher cooking yield in PVCPC samples toward the end of storage. Similarly, the panel result of tenderness evaluation supported the Warner-Bratzler shear force measurement; namely, the MAP resulted in less tender meat as the storage time was extended to more than 6 d. Finally, all the red claw samples were considered moderately to highly acceptable. However, slight off-flavors detected on the 14-d PVCPC sample seemed to render it less acceptable when compared with the other 2 packaging conditions, although the difference was non-significant (Table 3).

### Conclusions

Of the 3 package systems compared, the atmosphere that contained a high  $\text{CO}_2$  (80%) and reduced  $\text{O}_2$  (10%) partial



**Figure 7—Cooking loss of red claw crayfish muscle stored in 3 different package systems at 2 °C for various times. Each data point represents the mean of 7 measurements.**



**Figure 8—Shear force of red claw crayfish muscle stored in 3 different package systems at 2 °C for various times. Each data point represents the mean of 7 measurements.**

**Table 3—Sensory evaluation scores of cooked red claw crayfish meat from tails that were stored in 3 different package systems at 2 °C for various times**

Sensory attribute <sup>A</sup>	Package system	Storage time (day)					
		0	1	3	6	10	14
Lobster flavor	PVCP	3.88 <sup>cd</sup>	4.33 <sup>abc</sup>	4.53 <sup>abc</sup>	3.71 <sup>cd</sup>	4.03 <sup>bcd</sup>	4.22 <sup>abc</sup>
	VP	3.88 <sup>cd</sup>	3.85 <sup>cd</sup>	4.55 <sup>abc</sup>	4.34 <sup>abc</sup>	4.14 <sup>bcd</sup>	4.02 <sup>bcd</sup>
	MAP	3.88 <sup>cd</sup>	4.47 <sup>abc</sup>	5.03 <sup>a</sup>	4.81 <sup>ab</sup>	3.93 <sup>cd</sup>	3.28 <sup>d</sup>
Off-flavors	PVCP	1.13 <sup>cd</sup>	1.15 <sup>cd</sup>	1.08 <sup>d</sup>	1.10 <sup>d</sup>	1.14 <sup>cd</sup>	2.31 <sup>a</sup>
	VP	1.13 <sup>cd</sup>	1.15 <sup>cd</sup>	1.25 <sup>cd</sup>	1.35 <sup>bcd</sup>	1.29 <sup>bcd</sup>	1.74 <sup>b</sup>
	MAP	1.13 <sup>cd</sup>	1.27 <sup>bcd</sup>	1.42 <sup>bcd</sup>	1.41 <sup>bcd</sup>	1.60 <sup>bc</sup>	1.74 <sup>b</sup>
Rancidity	PVCP	1.13 <sup>b</sup>	1.05 <sup>b</sup>	1.23 <sup>ab</sup>	1.03 <sup>b</sup>	1.00 <sup>b</sup>	1.48 <sup>a</sup>
	VP	1.13 <sup>b</sup>	1.06 <sup>b</sup>	1.10 <sup>b</sup>	1.25 <sup>ab</sup>	1.14 <sup>b</sup>	1.24 <sup>ab</sup>
	MAP	1.13 <sup>b</sup>	1.05 <sup>b</sup>	1.08 <sup>b</sup>	1.29 <sup>ab</sup>	1.00 <sup>b</sup>	1.02 <sup>b</sup>
Juiciness	PVCP	5.09 <sup>abc</sup>	5.50 <sup>a</sup>	5.42 <sup>ab</sup>	5.60 <sup>a</sup>	5.46 <sup>ab</sup>	4.59 <sup>cd</sup>
	VP	5.09 <sup>abc</sup>	5.60 <sup>a</sup>	5.43 <sup>ab</sup>	4.84 <sup>bcd</sup>	4.46 <sup>cd</sup>	4.23 <sup>de</sup>
	MAP	5.09 <sup>abc</sup>	5.35 <sup>ab</sup>	5.37 <sup>ab</sup>	4.86 <sup>abc</sup>	4.31 <sup>de</sup>	3.79 <sup>e</sup>
Tenderness	PVCP	5.13 <sup>abcd</sup>	5.57 <sup>ab</sup>	5.42 <sup>abc</sup>	5.56 <sup>ab</sup>	5.69 <sup>a</sup>	4.64 <sup>cde</sup>
	VP	5.13 <sup>abcd</sup>	5.53 <sup>ab</sup>	5.58 <sup>abc</sup>	5.09 <sup>abcd</sup>	4.54 <sup>de</sup>	4.34 <sup>de</sup>
	MAP	5.13 <sup>abcd</sup>	5.04 <sup>ab</sup>	5.43 <sup>abc</sup>	4.80 <sup>bcd</sup>	4.11 <sup>e</sup>	3.98 <sup>e</sup>
Overall acceptability	PVCP	5.25 <sup>abcd</sup>	5.82 <sup>ab</sup>	5.87 <sup>a</sup>	5.29 <sup>abcd</sup>	5.29 <sup>abcd</sup>	3.64 <sup>h</sup>
	VP	5.25 <sup>abcd</sup>	5.37 <sup>abc</sup>	5.75 <sup>abc</sup>	4.81 <sup>cdefg</sup>	4.40 <sup>defg</sup>	4.01 <sup>gh</sup>
	MAP	5.25 <sup>abcd</sup>	5.03 <sup>bcd</sup>	5.42 <sup>abc</sup>	4.84 <sup>cdef</sup>	4.40 <sup>efgh</sup>	4.19 <sup>gh</sup>

<sup>A</sup>Lobster flavor = 1 (mild) to 7 (intense); off-flavor = 1 (nondetectable) to 7 (intense); rancidity = 1 (nondetectable) to 7 (intense); juiciness = 1 (dry) to 7 (juicy); tenderness = 1 (tough) to 7 (tender); overall acceptability = 1 (low) to 7 (high).

<sup>a-h</sup>Within the same sensory attribute, means that do not share a common letter differ significantly ( $P < 0.05$ ).

pressure (MAP) gave rise to the best protection of red claw tail meat against microbial growth under a simulated retail display condition. As a result, the MAP treated red claw developed less off-flavors and essentially no rancidity within the storage time tested (14 d). Although vacuum packaging also reduced microbial growth, it was less effective than the MAP system. However, the MAP system tended to promote cooking loss and reduced tenderness of red claw meat. Further research is needed to optimize the atmosphere composition in the MAP system to achieve substantial microbial inhibition and, at the same time, a minimal cooking loss and an overall high product palatability.

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