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Effect of Vacuum Packaging on the Quality of Red Claw Crayfish, *Cherax quadricarinatus*, Tail Muscle during Frozen Storage[1]

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Abstract

This study compared vacuum packaging (VP) and air-permeable polyvinylchloride film wrap packaging (PVCP) for their influence on microbial survival, pH, protein physicochemical properties, cooking loss, and shear force of raw red claw crayfish, *Cherax quadricarinatus*, tail muscle stored at −20 C for up to 12 mo. Overall, frozen storage did not have a detrimental effect on microbial survival; however, aerobic plate counts and total coliforms in 12-mo red claw samples in VP were, respectively, 1.5 and 3.1 log cycles less than those in PVCP ($P < 0.05$). Samples stored in both PV and PVCP exhibited a slight pH decline (0.3–0.5 U, $P < 0.05$) during storage. Similarly, the complex changes in muscle proteins over the course of frozen storage, for example, increases in hydrophobicity in the first 3 mo, reductions of Ca-ATPase activity from 3 to 12 mo, and loss of sulfhydryls from 6 to 12 mo ($P < 0.05$), differed only slightly between VP and PVCP. Muscle samples stored in PVCP experienced a significant cooking loss after 1 mo, and in VP, a major cooking loss occurred after 3 mo. No packaging effect was observed on cooked meat shear force except for samples stored for 12 mo.

Red claw crayfish, *Cherax quadricarinatus*, also known as freshwater lobster, has generated considerable interest among aquaculturists in the USA in recent years (Masser and Rouse 1997); there have been numerous published reports on their culture (Jones 1995; Karplus et al. 1995; Jones and Ruscoe 2000) and nutritional requirements (Thompson et al. 2003a, 2003b, 2006; Metts et al. 2007). Furthermore, studies have shown that red claw meat had good physicochemical stability although mild lipid oxidation and slight muscle tissue toughening were observed in tail meat packaged without vacuum under refrigerated conditions (Tseng et al. 2002, 2003; Kong et al. 2006, 2007). On the other hand, red claw meat was remarkably susceptible to microbial spoilage during refrigerated storage, which was attributed to the high pH of the muscle tissue as well as the abundance of nutrients (amino acids, minerals, etc.) (Chen et al. 2007).

The success in producing sustainable and economically feasible red claw, as well as in its marketing, ultimately depends on the quality of red claw meat that is acceptable by the consumer. Namely, red claw producers and marketers must have a good knowledge on the stability characteristics of red claw meat when stored under specific conditions. Of various preservation technologies available, freezing is by far the best method to curtail microbial growth and retard chemical deterioration of shellfish. However, for most aquatic and mammalian species, frozen muscle tissue remains susceptible to protein denaturation (Jiang et al. 1991; Xiong 1997) and often experiences physical damage because of the ice

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crystal formation as well as to other textural changes from biochemical processes (Haard 1990). Limited research has been conducted to determine the combined effect of freezing and packaging systems on the microbial and physicochemical stability of shellfish.

Currently, shellfish sold in the supermarket are mostly prefrozen and packaged without vacuum; they are either quickly frozen individually or frozen in ice blocks. In a prior study (Tseng et al. 2005), we reported that myosin head was destabilized (reduction in enthalpy of denaturation) and shear values decreased for red claw tail meat after 6 mo of frozen storage under atmospheric or vacuum conditions. However, no specific physicochemical attributes related to muscle protein structures were measured. The objective of the present study was to determine whether vacuum packaging (VP) could complement freezing to preserve both the microbiological and physicochemical qualities of red claw crayfish muscle.

Materials and Methods

Sample Preparation

Juvenile red claw crayfish (mean live weight 6.25 g) were raised in ponds at the Kentucky State University Aquaculture Research Center (Frankfort, KY, USA) for 16 wk to a mean live weight of 45 g. Approximately 250 mixed male and female red claws were randomly collected from a large sample pool excluding spawning females. After being stunned by submersion in an ice slurry for 2 min, red claw were manually beheaded. The tails (muscle, with exoskeleton), averaging 19.5 g in weight, were placed in iced coolers and transported immediately to the University of Kentucky's Food Protein Laboratory. On receipt (within 3 h of collection), red claw tails were rinsed with tap water and then frozen individually for 1 h. Frozen crayfish tails were vacuum packaged twice with double bags (100 tails in each pack) and kept in a −30 C freezer before being used within 3 mo. The proximate analysis, reported elsewhere (Kong et al. 2006), showed an average muscle composition of 81.1% moisture, 17.6% protein, and 0.16% lipid.

Packaging

The experiments were arranged as a completely randomized structure with factorial design. A total of 135 randomly selected red claw tails were used. Frozen crayfish tails in double vacuum package were placed into a 5 C cooler for about 40 h until they were partially thawed. The partially thawed crayfish tails were randomized and placed in nine plastic trays (32 × 26 × 8, length × width × depth, in centimeters) each containing 15 tails (5 for microbial analysis, 3 for physicochemical measurements, and 7 for cooking yield and shear testing). There were nine subtreatments arranged in a factorial design with five storage times (0, 1, 3, 6, and 12 mo) and two types of package systems (VP and polyvinylchloride film wrap packaging [PVCP]). Only one subtreatment (15 tails) was used in Month 0.

For PVCP, the trays containing crayfish tails were double-wrapped with an air-permeable PVC film (15,500–16,275 cm^3/m^2/24 h oxygen transmission rate at 23 C; Bunzl Processor Division, North Kansas City, MO, USA). For VP, the trays were placed inside Type B2620 vacuum bags (2.2-mil, 3–6 cm^3/m^2/24 h oxygen transmission rate at 23 C; Cryovac Division, Sealed Air Corp., Duncan, SC, USA) and sealed using a Model 600A vacuum machine (Sipromac Inc., St-Germain, Quebec, Canada). All packages were stored in a −20 C freezer. At the end of 0, 1, 3, 6, and 12 mo, one pack from each packaging system was removed from the freezer, thawed for 16 h, and then subjected to microbial and meat quality evaluation.

pH

Aliquots of 3 g of minced muscle from three crayfish were dispersed (15 s at setting 6) in 25 mL of deionized distilled water with a low-foam homogenizer (Brinkman Instruments, Inc., Westbury, NY, USA). The pH of the slurry was measured using an AB $15/15^+$ pH meter with a glass pH electrode (Fisher Scientific Co., Fair Lawn, NJ, USA). The measurement was carried out in triplicate.

Microbial Analysis

For each packaging system, five shell-on tails were randomly selected, each cut into three pieces with sterile scalpel, and then pooled. The mixed sample was analyzed for aerobic plate count (APC), total coliforms, *Escherichia coli*, and *Lactobacillus* spp. following the respective procedures of the Food and Drug Administration (2009). Specifically, chopped samples (25 g) were mixed into 225 mL of 0.1% peptone in a sterile jar and blended for 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, Division of BioQuest, Cockeysville, MD, USA). Aliquots of 1 mL of each diluted sample were plated onto appropriate petrifilms for coliforms, *E. coli*, and APC (3M Microbiology Products, St. Paul, MN, USA). 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 before counting, and the APC petrifilm was incubated in a 26 C incubator for 72 h before enumeration. The Rogosa Petri dishes were placed in an anaerobic jar, flushed with N_2, 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*.

Protein Physicochemical Attributes

Surface Hydrophobicity (S_0). The surface hydrophobicity analysis was based on Li-Chan et al. (1984). Carefully weighed tail ($n = 3$) meat (0.25 g) was homogenized in 20 mL of a buffer (0.01 M Na phosphate, 0.6 M NaCl, 1 m M $MgCl_2$, 0.02% Na azide, pH 7.0). After centrifugation at 10,000 *g* for 15 min at 4 C, the supernatant was decanted. The extract was diluted to serial protein concentrations of 0.05, 0.10, 0.15, and 0.20 mg/mL with 0.01 M phosphate buffer (pH 7.0). An aliquot (20 µl) of 8 mM of 8-anilino-1-naphthalenesulfonate (ANS, Sigma Chemical Co., St. Louis, MO, USA) solution was added to each protein sample (4 mL each).

After mixing well by vortexing, the reaction mixtures were sealed from light using aluminum foil and incubated for 15 min at room temperature (22 C). The relative fluorescence intensity (RFI) of each solution was then measured, starting from the lowest to the highest concentration, using a FluoroMax-3® spectrofluorometer (Jobin Yvon Inc., Edison, NJ, USA) with excitation (390 nm) and emission (470 nm) slits set at 5 nm. RFI values of protein dilution blanks (no ANS) and a buffer blank (buffer + ANS) were also measured. The RFI of each protein dilution blank was subtracted from that of the corresponding protein dilution with ANS to provide net RFI. Surface hydrophobicity was expressed as the initial slope of the plot of net RFI values versus percentage protein concentration, computed by least squares linear regression analysis.

Ca-ATPase Activity. Myosin Ca-ATPase activity of red claw muscle from three tails ($n = 3$) was assayed based on the method of Wells et al. (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 $CaCl_2$, 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 (0–5 µmol/mL phosphate). The Ca-ATPase activity was expressed as µmol Pi/min/mg muscle.

Sulfhydryl Content. This was analyzed according to Wang et al. (1997). Tail ($n = 3$) meat samples were homogenized and diluted to 2 mg/mL with 0.1 M phosphate buffer (pH 7.4). The homogenate (0.5 mL) was dissolved in the same buffer containing 8.0 M urea and 3% SDS. The whole solution was reacted with 0.5 mL of 5, 5′-dithiobis 2-nitrobenzoic acid (DTNB, Sigma Chemical Co.) reagent (10 mM DTNB in 0.1 M phosphate buffer, final pH 7.4)

for 15 min at room temperature. Absorbance was read at 412 nm. The concentration of sulfhydryls was calculated using a molar extinction coefficient of 11400/M/cm.

Cooking Loss

Individually weighed shell-on tails ($n = 7$) were cooked in boiling water (100 C) for exactly 2 min, chilled at room temperature (21 C) for 10 min, and weighed again. Cooking loss (%) 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 and then multiplying by 100. After the cooking loss measurement, the tail meat was subjected to textural analysis.

Textural Analysis

Cooked and deshelled red claw tails ($n = 7$) were individually weighed before being subjected to textural analysis using a Model 4301 Instron Universal Testing Instrument (Instron Corp., Canton, MA, USA) 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 first major muscle segment from the anterior of the cooked tail across the muscle fibers (Tseng et al. 2005). The cross-head speed of the Instron was 50 mm/min. Shear force values (in Newtons) were normalized based on the weight (grams) of the tails and were expressed as force per sample unit weight (Newtons per gram) to eliminate size effects (Srinivasan et al. 1997).

Statistical Analysis

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

Results

pH Value

The pH of raw tail samples stored in both packaging systems decreased during the first 6 mo of storage. After Month 6, the pH of PVCP samples continued to decline, whereas the pH of VP samples began to increase, reaching 6.65 and 6.97, respectively, after 12 mo (Fig. 1).

Microbial Survival

The APC, which included both aerobic and facultative bacteria, was 4.04 log CFU/g at the beginning of storage (Table 1). After 1 mo, the microbial population increased to 4.80–4.90 log CFU/g with very little change afterward; the packaging systems did not have a major effect. Overall, the small APC changes (within a log unit) in all red claw samples during storage (except the 12-mo PVCP samples) were practically insignificant, and freezing did not affect the survival of the microbes harboring in red claw tails. The only appreciable change was the APC in PVCP samples that showed a considerable increase from Month 6 (4.64 log CFU/g) to Month 12 (5.98 log CFU/g).

The coliform results were somewhat variable. The counts in PVCP samples increased during the first 3 mo, followed by a drop over the next 3 mo, and a subsequent increase from Month 6 to Month 12 (Table 1). However, for VP samples, the count increase in the first month was followed by a steady decline, reaching the 1.54 log CFU/g minimal level by Month 12 when the frozen storage was completed, indicating susceptibility of coliform bacteria to the frozen temperature in the oxygen-deprived packaging system. Although it was not possible to perform statistical analysis of the data because of the lack of experimental replications (i.e., performed on different days or months), variations within the five repeated measures were small, and the numerical differences in the average values between VP and PVCP were apparent. No *Lactobacillus* spp. or *E. coli*. was detected in samples stored in either packaging system over the entire 12-mo storage period.

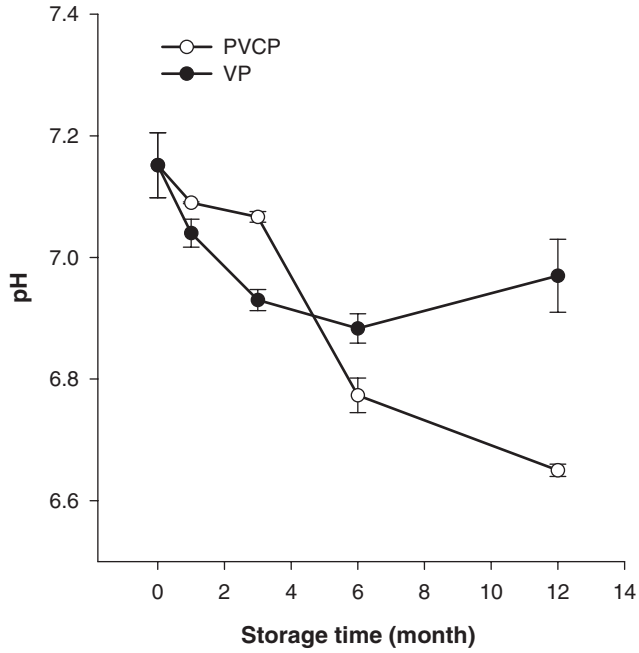

FIGURE 1. *The pH value in frozen red claw crayfish meat stored at −20 C in vacuum (VP) and nonvacuum (PVCP) package systems. Each data point represents the mean (± standard deviation) from three different tail sample measurements. VP = vacuum packaging; PVCP = polyvinylchloride film wrap packaging.*

TABLE 1. *Aerobic plate count and coliform population in red claw crayfish meat packaged in vacuum (VP) and nonvacuum (PVCP) package systems during frozen storage (−20 C).*

Storage time (mo)	Aerobic plate count[a] (log CFU/g)		Coliform[a] (log CFU/g)	
	VP	PVCP	VP	PVCP
0	4.04[a]	4.04	2.00	2.00
1	4.81	4.89	4.16	2.72
3	4.84	4.90	3.32	4.00
6	4.78	4.64	2.95	2.97
12	4.48	5.98	1.54	4.68

VP = vacuum packaging; PVCP = polyvinylchloride film wrap packaging.

[a]Results shown are the average data based on five measurements.

Physicochemical Attributes of Proteins

Lipid oxidation was not assessed in this study because a previous investigation has indicated negligible production of thiobarbituric acid-reactive substances (less than 0.3 mg/kg in 6 mo) in frozen red claw tails under the same aerobic storage conditions as used in the present study (Tseng et al. 2005). The minimal lipid oxidation was apparently because of the extremely low lipid content in red claw muscle tissue (0.16%) (Kong et al. 2007).

All red claw tail samples, irrespective of packaging systems, exhibited the same trend in protein surface hydrophobicity change during frozen storage, that is, increasing during the first 3 mo, decreasing from Month 3 to Month 6, and increasing again subsequently (Fig. 2). At Months 3 and 12, the VP samples exhibited higher surface hydrophobicity than PVCP samples ($P < 0.05$).

Red claw tail samples stored in the two packaging systems also exhibited a similar trend in myosin Ca-ATPase activity change throughout the 12-mo storage period (Fig. 3). A slight decrease in the Ca-ATPase activity was observed in the first month. The enzyme activity increased ($P < 0.05$) for the next 2 mo and then steadily declined for the next 6 mo ($P < 0.05$). There was no significant difference between

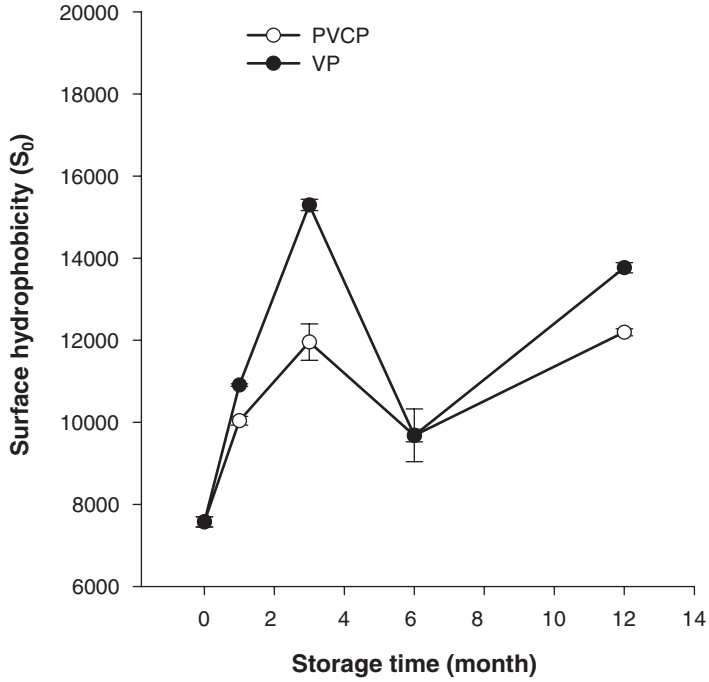

FIGURE 2. *Surface hydrophobicity of red claw crayfish meat stored at −20 C in vacuum (VP) and nonvacuum (PVCP) package systems. Each data point represents the mean (± standard deviation) from three different tail sample measurements. VP = vacuum packaging; PVCP = polyvinylchloride film wrap packaging.*

VP and PVCP except in Month 3 where VP samples had a slightly higher enzyme activity.

Considerably sample-to-sample variations were recorded in quantifying sulfhydryls during the first 3 mo. Only samples stored in VP for 1 mo showed a higher sulfhydryl content than samples in PVCP (Fig. 4). Otherwise, total protein sulfhydryl content remained relatively constant during the first 6 mo for both packaging systems. However, from Month 6 to Month 12, the content decreased sharply for both PV and PVCP. Freeze-induced losses in myosin ATPase activity and sulfhydryl groups have also been observed in grass prawn meat subjected to long-term frozen storage (Jiang et al. 1991).

Cooking Loss

Cooking loss, albeit practically small, was aggravated during the first month for PVCP samples and 3 mo for VP samples, and leveled off thereafter (Fig. 5). There was no significant difference between packaging systems except for Month 1 where PV samples had a lower cooking loss than PVCP samples ($P < 0.05$).

Warner–Bratzler Shear Force

Shear force values of cooked red claw meat samples gradually increased ($P < 0.05$) over the entire 12-mo storage period for VP, and up to 6 mo for PVCP (Fig. 6). From Month 6 to Month 12, samples stored in PVCP exhibited a slight reduction in the shear value.

Discussions

During freezing and subsequent thawing, muscle tissue can undergo substantial structural changes because of the ice crystal formation, accretion, and then dissolution. As a result, cell organelles and proteins will experience physicochemical changes that often lead to deterioration in food quality. Similarly, the bacterial cell wall could be partially damaged during freezing

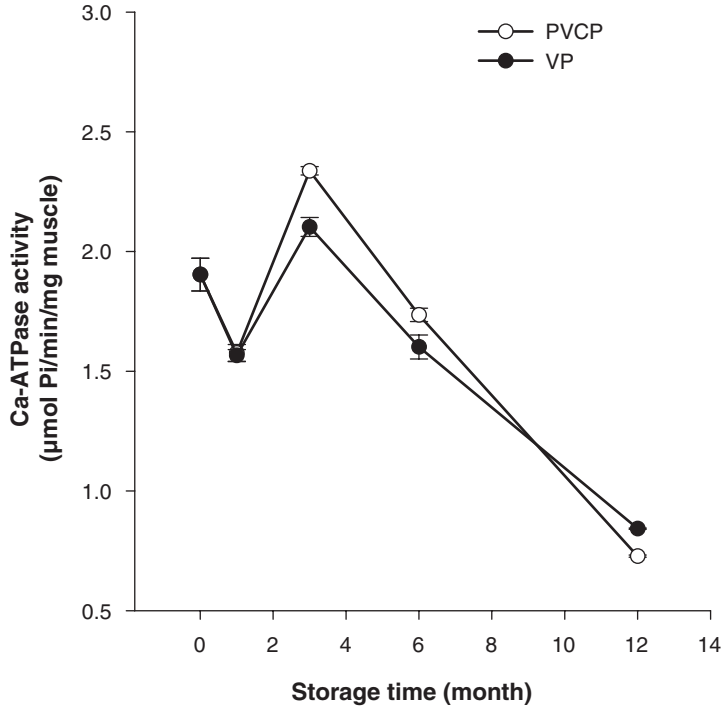

FIGURE 3. *Ca-ATPase activity of red claw crayfish muscle stored at −20 C in vacuum (VP) and nonvacuum (PVCP) package systems. Each data point represents the mean (± standard deviation) from three different tail sample measurements. VP = vacuum packaging; PVCP = polyvinylchloride film wrap packaging.*

and thawing. The results from frozen stored red claw samples reflected such changes. The cause for the slight pH reduction (0.2–0.4 U) in red claw muscle during the first 6 mo of frozen storage was not clear. Presumably, less amine compounds were formed in thawed muscle as they were believed to be the main contributors to increased pH in stored seafood (Emborg et al. 2002; Masniyom et al. 2002). It was also plausible that fewer hydrogen ions were dissociable from proteins because of protein aggregation.

Total APC did not decline as expected during frozen storage, indicating that aerobic bacteria on red claw survived well freezing, storage, and thawing processes. The overall favorable pH (essentially neutral) condition of the muscle tissue, despite some small reductions during storage, probably contributed to the microbial survival. A significant percentage of these microorganisms was comprised of coliform bacteria, which most likely originated from the ponds within which red claw grew. These coliforms survived through the first 6 mo of frozen storage but became susceptible afterward (except in PVCP), presumably because of the ice crystal damage and depletion of nutrients in the muscle exudates. However, as was with the total aerobic bacteria, the population of coliforms increased significantly from Month 6 to Month 12 in the PVCP package.

The result indicated that the presence of atmospheric oxygen in the package aided in the recovery and growth of aerobic bacteria, including coliforms, which probably took place during the thawing process. On the other hand, cell damages that incurred (especially to coliforms) from extended frozen storage under vacuum were not fully recovered even during the culture incubation for microbial evaluation. The results were consistent with our previous finding that both aerobic and coliform counts in red claw tails stored at 2 C for a prolonged period (>10 d) were significantly higher in the PVCP system than in the VP system

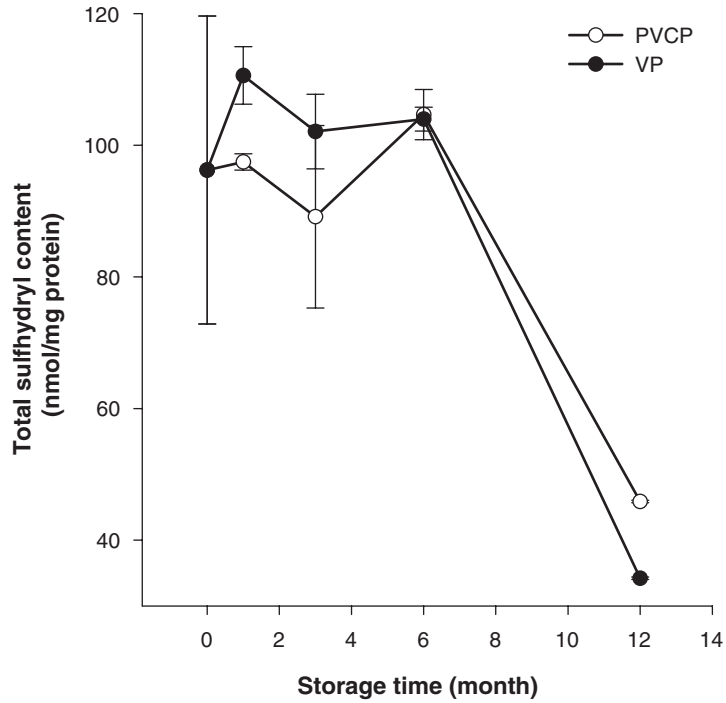

FIGURE 4. *Sulfhydryl content of red claw crayfish muscle stored at −20 C in vacuum (VP) and nonvacuum (PVCP) package systems. Each data point represents the mean (± standard deviation) from three different tail sample measurements. VP = vacuum packaging; PVCP = polyvinylchloride film wrap packaging.*

(Chen et al. 2007). Thus, frozen red claw tails stored in VP would have a longer microbiological shelf-life than the aerobic PVCP system.

The irregular patterns of protein hydrophobicity and Ca-ATPase activity changes, which were manifestations of protein structural alterations, indicated the complex nature of the chemical reactions and possibly also physical interactions that took place during frozen storage of red claw muscle. For protein surface hydrophobicity, the initial increase in its value suggested the exposure of the hydrophobic interior because of protein denaturation (Multilangi et al. 1996), and the subsequent drop probably resulted from hydrophobic aggregation of denatured molecules (Tseng et al. 2005). The slight increase from Month 6 to Month 12 could be the net result for these two opposing processes.

Similarly, denaturing conditions, such as mild oxidation, were found to change the conformation of myosin globular head, rendering it more reactive as a Ca-ATPase (Park et al. 2006). This may explain why red claw muscle stored in the first 3 mo, notably in PVCP, had increased Ca-ATPase activity. However, as myosin becomes more denatured, its Ca-ATPase activity will start to drop (Ooizumi and Xiong, 2004). Because Ca-ATPase activity is closely related to the reactivity of sulfhydryl groups located in the myosin globular head (Sekine and Yamaguchi 1963), the marked loss of sufhydryls, when red claw muscle was stored from Month 6 to Month 12, would explain the corresponding reduction in the Ca-ATPase activity. Hydrophobic aggregation as well as disulfide linkages may be implicated in the Ca-ATPase loss.

Although the trend of the changes in protein surface hydrophobicity, Ca-ATPase activity, and sulfhydryl content during frozen storage showed some agreement within the same type of packaging system, the magnitude of the specific changes was slightly influenced by the packaging atmospheric conditions. For

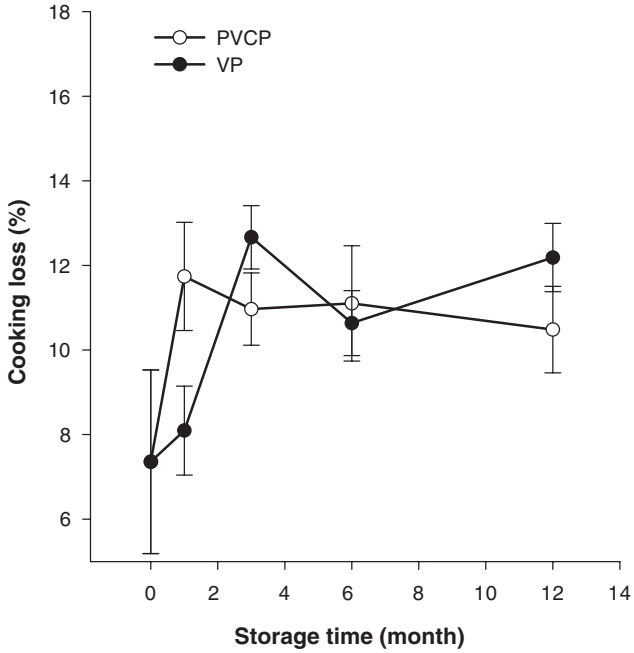

FIGURE 5. *Cooking loss (%) of red claw crayfish muscle stored at −20 C in vacuum (VP) and nonvacuum (PVCP) package systems. Each data point represents the mean (± standard deviation) from seven different tail sample measurements. VP = vacuum packaging; PVCP = polyvinylchloride film wrap packaging.*

example, during the last 6 mo of storage, surface hydrophobicity of VP muscle increased more rapidly, and the loss of sulfhydryls in VP samples was also slightly more extensive when compared with that in PVCP. Leelapongwattana et al. (2005) also observed a pronounced decrease in the sulfhydryl content of frozen whole fish stored under vacuum. It is plausible that the pressure gradient established in the VP package destabilized muscle cells and proteins, rendering proteins more susceptible to unfolding.

Aggregates formed from muscle protein could be a determining factor for water binding in frozen red claw crayfish. The observed physicochemical changes, which were indicative of protein structural unfolding, appeared to predispose myofibrillar proteins to increased aggregation upon heating, thereby driving water out of the myofibril matrix. The possible disruption of muscle cell membrane by frozen storage would also contribute to the increased cooking loss. Both the increase in protein aggregation and the reduction in water-holding ability of muscle were responsible for the increased tendency of red claw muscle to become tougher upon extended storage.

Conclusions

The combination of vacuum and freezing was more effective than nonvacuum freezing for the inhibition of microbial survival in red claw tail stored at −20 C for an extended period. However, vacuum packaging, overall, did not appear to have a remarkable effect on physicochemical attributes of proteins as related to meat texture of frozen red claw crayfish. Thus, it was not surprising that vacuum packaging did not significantly complement freezing for reducing cooking loss and textural changes in red claw meat stored for up to 12 mo. On the other hand, the identification of coliform bacteria on thawed red claw tail muscle reminds the consumers to thoroughly cook the food so as to avoid possible health problems. These findings should be of value to red claw producers and processors

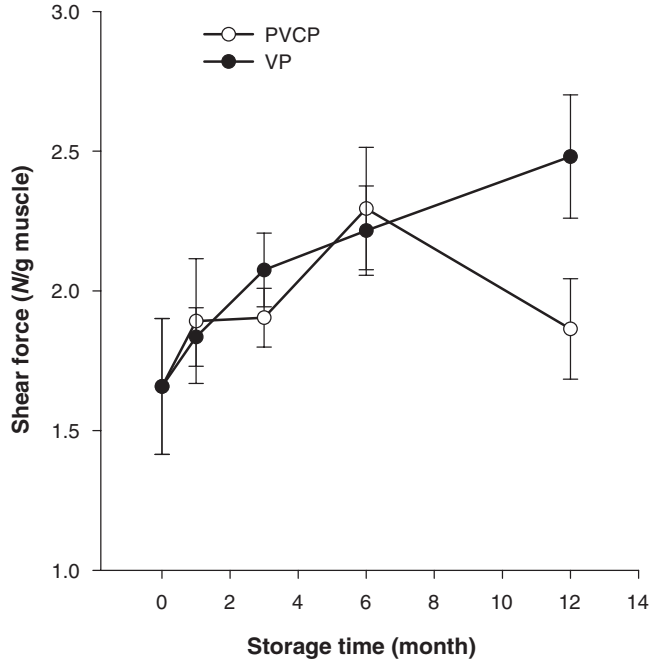

FIGURE 6. *Shear force of red claw crayfish muscle stored at −20 C in vacuum (VP) and nonvacuum (PVCP) package systems. Each date point represents the mean (± standard deviation) from seven different tail sample measurements. VP = vacuum packaging; PVCP = polyvinylchloride film wrap packaging.*

in choosing the proper packaging and freezing methods for a high-quality output of the product. Perhaps, even with the small gain from inhibiting microbes, vacuum packaging can be desirable for frozen red claw meat destined for exporting markets which necessitate extended transportation, distribution, and storage.

Acknowledgments

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