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Original article

The preservation of the quality of the muscle in frozen Australian red claw crayfish (*Cherax quadricarinatus*) by pre-storage anti-oxidant dipping treatments†

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Summary Australian red claw crayfish tails were dipped in either 0.06% (w/w) anti-oxidant solutions (tocopherols, propyl gallate, or rosemary extract) or in water and subsequently stored in a −20 °C freezer. At the end of 0, 1, 3, and 6 months of storage, the raw muscle samples were analysed for lipid oxidation, the thermal stability of proteins, cooking yield and shear force. Red claw treated with anti-oxidants showed a lower ($P < 0.05$) thiobarbituric acid-reactive substances (TBARS) production than the non-anti-oxidant control (dipped in water). Enthalpy of denaturation (ΔH) for the myosin head and shear values for the tails decreased ($P < 0.05$) in all samples after 6 months of storage, while cooking yield was unaffected by storage. Overall, red claw muscle was quite stable and the oxidation, measured as TBARS values, was small (< 0.3 mg kg^{-1}) but could be further stabilized by anti-oxidant dipping treatments. However the anti-oxidant dipping did not prevent texture softening in red claw muscle during frozen storage.

Keywords Lipid oxidation, tenderness.

Introduction

Australian red claw crayfish (*Cherax quadricarinatus*) is a robust freshwater crustacean species native to remote areas of tropical northern Australia. Compared with the native American crayfish, red claw has several important advantages, these include larger potential size, higher percentage of dress-out (meat) and better tolerance of crowded culture condition (Masser & Rouse, 1997). The amount of tail meat in red claw is approximately 22% of the total weight (Jones, 1989). The texture and flavour of red claw meat compares favourably with other common marine crustaceans. They are similar in appearance to lobster, and are, thus, highly regarded crustaceans commanding premium prices.

In our previous studies (Tseng *et al.*, 2002, 2003), the quality changes of red claw tail meat subjected to refrigerated storage or freeze-thaw cycles were investigated. The results indicated that red claw muscle is susceptible to protein denaturation and lipid oxidation at refrigerated temperatures and upon repeated freezing and thawing; these chemical changes seem to be responsible for the decreased cooking yield and reduced tenderness of meat in post-harvest handling/storage conditions. Currently, no information is available about the stability of red claw meat under frozen storage conditions.

Frozen storage essentially stops the growth of micro-organisms in or on muscle foods, thereby

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eliminating microbial spoilage and extending the shelf-life. However, deterioration of quality caused by chemical or physical causes can occur. Studies have shown that lipid oxidation is one of the primary causes of quality losses in frozen stored shrimp and prawns that are packaged under aerobic conditions (Bottino *et al.*, 1979; Reddy *et al.*, 1981; Riaz & Qadri, 1990; Srinivasan *et al.*, 1998). Secondary oxidation of lipid products (e.g. carbonyls) can cause cross-linking and oxidative modification of proteins (Gardner, 1979), thereby adversely affecting the texture of the muscle tissue. In addition, the production of strong rancidity and other off-flavours from lipid oxidation and hydrolysis also greatly reduces consumer acceptance of stored seafood. In crustacean species it is found that most of the lipids are located underneath the shell. These lipids may be readily exposed to light and oxygen, initiating the oxidation of unsaturated fatty acids.

In the meat and poultry industry, dip treatments have been used to retard microbial growth and minimize colour changes or lipid oxidation in the products prior to storage, display, or packaging (To & Robach, 1980; Cunningham, 1981; Mitsumoto *et al.*, 1991; Samelis *et al.*, 2001). It has been reported that dipping with Vitamin E and C solutions improves colour and lipid stability of beef (Harbers *et al.*, 1981; Okayama *et al.*, 1987; Mitsumoto *et al.*, 1991). In the seafood industry, dipping is done primarily for glazing. The presence of a thin layer of ice on the surface of fish helps minimizing dehydration during frozen storage. Studies have also shown that adding anti-oxidants to the glazing water protects the fish against lipid oxidation (Colakoglu & Kundakci, 1983; Stodolnik & Matyjasczcyk, 1990).

This study was done to determine the stability of quality of the red claw tail meat during extended frozen storage and to investigate whether the shelf-life could be prolonged by dipping the tails in anti-oxidant solutions prior to storage. Rosemary extract, tocopherols and propyl gallate were used to evaluate their anti-oxidative effect. Our goal was to provide a simple and feasible processing procedure that could be easily applied to frozen stored red claw so as to retard adverse quality changes during storage. Accordingly, chemical and physical reactions, including lipid oxidation, protein denaturation, cooking yield, and tenderness were monitored in an attempt to identify possible causes for textural changes.

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 200 red claw were randomly collected from a large sample pool. After stunning by submerging in ice slurry, red claw were manually processed. The tails (muscle, with 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 3 h of collection. On receipt, the red claw were rinsed with tap water and treatmented as described later. Rosemary extract (RFI Ingredients, Blauvelt, NY, USA) was a commercial product containing 4.0–4.5% rosmarinic acid. A 30% mixed tocopherols powder (ADM ARKADY, Olathe, KS, USA) was used. Each gram contained 300 mg of total tocopherols, of which a minimum of 240 mg was not α-tocopherols. Propyl gallate was purchased from Sigma Chemical (St Louis, MO, USA).

Treatment, packaging and storage

Red claw were equally divided into five lots (40 in each). All were dipped for 5 min, two being dipped in water and three in one of the following anti-oxidant solutions: 0.06% (w/w) propyl gallate, 0.06% (w/w) tocopherols, or 0.06% (w/w) rosemary extract. After dipping without draining, tails were placed separately on plastic plates, immediately frozen in a −20 °C blast freezer for 1 h, and subsequently packaged in Cryovac® BG bags (Cryovac North America, Sealed Air Corporation, Duncan, SC, USA). The bags were specially designed for packaging bone-in fresh meat. The oxygen transmission rate of the bag is 3–6 cm^3 at 4 °C (m^2, 24 h, 1 atm), and its water vapour transmission rate is 0.4–0.5 g at 3.3 °C, 100% RH (15.5 cm^2, 24 h). One lot of water-treated tails was vacuum-packaged; all other treatments were heat-sealed without vacuum. After 0, 1, 3, and 6 months of storage at −20 °C, tail samples were

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removed from the freezer and thawed for 30 min in a tank filled with running tap water (18–20 °C) before analyses. The tails for month zero were frozen-stored for 2 h before thawing and analyses.

Sample preparation for analysis

At the end of each storage period, 10 randomly selected shell-on tails from each treatment were analysed. Five of them, after removal of the shell, were homogenized in a Mini Chopper (Black and Decker, Inc., Shelton, CT, USA) for 30 s. The pooled raw muscle homogenates were analysed immediately for lipid oxidation and protein thermal stability. The other five tails, with the shell on, were cooked (100 °C) so as to allow measurements of cooking yield and tenderness.

Measurement of lipid oxidation

Lipid oxidation was measured as there were increases in thiobarbituric acid-reactive substances (TBARS) using the colorimetric method described by Sinnhuber & Yu (1977). The TBARS value, expressed as milligram of malonaldehyde per kilogram of muscle sample, was calculated as shown in the following equation:

$$\text{TBARS}(\text{mg kg}^{-1}) = \frac{A_{532}}{W_s} \times 9.48$$

Where A_{532} is the absorbance (532 nm) of the assay solution, W_s the muscle sample weight (g), and '9.48' a constant derived from the dilution factor and the molar extinction coefficient (152 000 M^{-1} cm^{-1}) of the red, TBA reaction product.

Measurement of protein denaturation

Differential scanning calorimetry (DSC) was used to measure the thermal stability of red claw muscle proteins. A model 2920 modulated DSC machine (TA Instruments, New Castle, DE, USA) was calibrated for temperature and base-line by 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 min^{-1}. The enthalpy changes (ΔH) for the major thermal transitions were determined by measuring the area above the transition curves, using a straight baseline constructed from the start to the end of the endotherms. Temperature at the maximum heat flow (T_{max}, i.e. temperature at peak of the endotherm) was also recorded. Both the ΔH and the T_{max} values were determined using the Universal Analysis Ver 1.2 N software supplied by the DSC company. All ΔH and T_{max} measurements were determined at least in triplicate.

Measurement of cooking yield

For each treatment, five randomly selected shell-on tails were cooked by immersing in boiling water (100 °C) for exactly 2 min and then chilled immediately by placing on crushed ice 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.

Textural analysis

After measurement of cooking yield, the shells of all samples were peeled off and the tail meat was used for analysis of texture.

A Model 4301 Instron Universal Testing Instrument (Instron Corp., Canton, MA, USA), with a Warner-Bratzler shearing device attached to the load cell (10 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 (Srinivasan *et al.*, 1997). The tail was placed in a transverse position to the blade to enable cutting across the muscle fibres. The crosshead speed of the Instron was set at 20 mm min^{-1}, and the first major peak (usually the highest overall, which represented the maximal shear force required to cut the muscle fibres) was recorded. In this study, shear force values (kg) of individual tails were normalized based on the weights (g) of the tails and were expressed as force per sample unit weight (kg g^{-1}) to eliminate size effects (Srinivasan *et al.*, 1997).

Statistical analysis

Data were analysed by using the General Linear Models procedure of the Statistix 3.5 software

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package (Analytical Software, Inc., St Paul, MN, USA) for microcomputers. ANOVA was used to determine the significance of the main effects of treatment, storage time and treatment × storage time interaction. Significant ($P < 0.05$) differences between means were identified using the Least Significant Difference procedures (Snedecor & Cochran, 1989).

Results

Lipid oxidation

No difference in TBARS was observed between dipping treatments at time 0 and the values in all samples increased ($P < 0.05$) during subsequent storage (Fig. 1). When comparing the two water-dipping treatments, red claw stored in a vacuum produced less ($P < 0.05$) TBARS than the samples stored aerobically. Moreover, except for a few variations, the TBARS in vacuum-packaged samples were generally lower than those from anti-oxidant-treated counterparts ($P < 0.05$). Figure 1 also shows that red claw tails dipped in anti-oxidant solutions produced less ($P < 0.05$) TBARS than aerobically packaged, water-dipped tails. When comparing the anti-oxidants, propyl gallate showed a better ($P < 0.05$) anti-oxidative effect than rosemary extract but this occurred only in samples stored for 1 month. It was noted that the shells of red claw treated with propyl gallate appeared slightly yellow after 3 months of storage. No significant difference in anti-oxidative effect was otherwise observed between different anti-oxidants during the entire period of frozen storage.

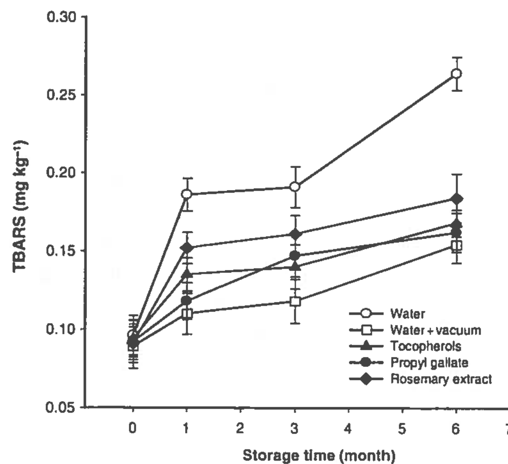

Figure 1 Formation of thiobarbituric acid-reactive substances (TBARS) as an indicator of lipid oxidation in red claw muscle during frozen storage.

Protein denaturation

All muscle samples exhibited two distinct transitions (peaks I and III) and additionally, a less resolved one (II) (Fig. 2). The transition peaks I and III are typical of muscle and have been attributed to the denaturation of the myosin head (or heavy meromyosin) and actin, respectively, while peak II has been attributed to a combination of myosin tail (or light meromyosin), sarcoplasmic proteins and connective tissue (Stabursvik & Martens, 1980; Srinivasan *et al.*, 1998).

Storage of the frozen samples did not alter the overall protein denaturation pattern (Fig. 2). However, an extended storage induced some significant ($P < 0.05$) changes in T_{max} (maximum transition temperature) for both myosin head and actin within each dipping treatment (Table 1). At the end of 6 months of storage, the ΔH (enthalpy of denaturation) for myosin head also decreased ($P < 0.05$) in all samples when compared with the values from 0, 1, and 3 months of storage. Yet, when comparing the different treatments, no predictable pattern indicating specific treatment effects could be established. Statistical analysis also revealed a lack of treatment × storage time interactions for both the denaturation indexes (T_{max} and ΔH).

Cooking yield and textural analysis

The cooking yield was not affected ($P > 0.05$) either by dipping treatments or by storage time (results not shown) and had an overall mean value of 90.3%. Thus, there was no treatment × storage time interaction. However, the length of frozen storage influenced ($P < 0.05$) the muscle shear force. Compared with month 0, samples stored for 6 months had a significant reduction ($P < 0.05$) in the shear value (Fig. 3). For example, the shear force for tocopherol-treated red claw tail meat decreased progressively, from 0.198 kg g^{-1} at month zero to 0.156 kg g^{-1} ($P < 0.05$) after 6 months.

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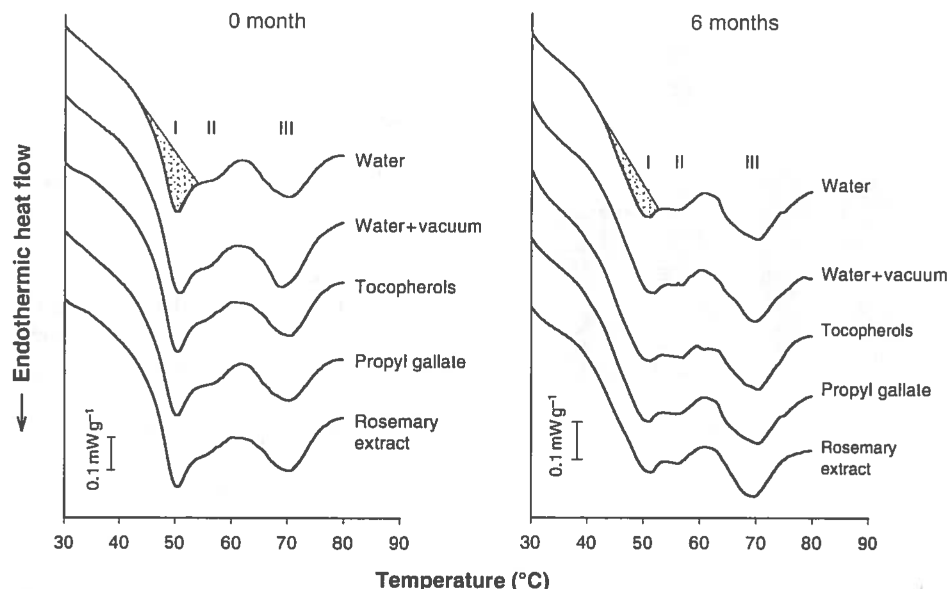

Figure 2 Differential scanning calorimetry (DSC) of red claw muscle subjected to frozen storage. Data for samples after 0 and 6 months of storage are plotted. The shaded area illustrates the region used for calculation of enthalpy of myosin denaturation with the DSC software (see Materials and methods).

Table 1 Differential scanning calorimetric analysis of red claw muscle during frozen storage

		T_{max} (°C)				ΔH (J g^{-1})			
Treatment	**Protein**	**0 month**	**1 month**	**3 months**	**6 months**	**0 month**	**1 month**	**3 months**	**6 months**
Water	Myosin	48.66^{bx}	0.313^{ax}	49.78^{ax}	0.308^{ax}	48.62^{bx}	0.301^{ax}	48.88^{aby}	0.177^{bxy}
	Actin	71.52^{ax}	0.318^{ax}	70.77^{bx}	0.308^{ax}	70.39^{by}	0.313^{ax}	70.34^{by}	0.312^{axy}
Water + vacuum	Myosin	49.47^{ax}	0.311^{ax}	49.58^{ax}	0.307^{ax}	48.98^{ax}	0.303^{ax}	49.43^{ax}	0.189^{bx}
	Actin	71.56^{ax}	0.310^{ax}	70.08^{bx}	0.307^{ax}	70.45^{by}	0.306^{ax}	70.49^{bxy}	0.320^{ax}
Tocopherols	Myosin	48.63^{abx}	0.315^{ax}	48.95^{ay}	0.306^{ax}	48.45^{abx}	0.297^{ax}	48.04^{byz}	0.178^{bxy}
	Actin	71.57^{ax}	0.312^{ax}	70.50^{bx}	0.304^{ax}	71.14^{axy}	0.291^{ax}	70.74^{abxy}	0.299^{ay}
Propyl gallate	Myosin	48.49^{ax}	0.317^{ax}	48.45^{ay}	0.300^{ax}	48.32^{ax}	0.305^{ax}	47.99^{az}	0.175^{bxy}
	Actin	71.14^{ax}	0.309^{ax}	70.92^{ax}	0.298^{ax}	71.35^{ax}	0.298^{ax}	71.10^{ax}	0.296^{ay}
Rosemary extract	Myosin	49.06^{ax}	0.314^{ax}	48.43^{aby}	0.303^{ax}	48.38^{abx}	0.298^{ax}	47.83^{bz}	0.184^{bx}
	Actin	71.24^{ax}	0.310^{ax}	70.32^{bx}	0.306^{ax}	71.44^{ax}	0.296^{ax}	70.90^{abx}	0.308^{axy}

^{ab}Means within the same row for the same parameter (T_{max} or ΔH) without a common superscript differ ($P < 0.05$).
^{xyz}Means within the same column for the same protein (myosin or actin) without a common superscript differ ($P < 0.05$).

Correlation analysis

No correlation was observed, either between TBARS and T_{max} or between TBARS and ΔH for protein denaturation. The plot of enthalpy of myosin denaturation against shear force showed two distinct clusters of data (Fig. 4), reflecting the marked reduction in the ΔH for myosin from month 3 to 6 (Table 1). While lacking a continuous relationship over the entire 6-month storage period, correlations between myosin ΔH and shear force were significant ($P < 0.05$) within the first 3 months ($r = -0.79$) as well as in the last month ($r = -0.94$) (Fig. 5).

Discussion

The overall low lipid oxidation rate (TBARS increased by < 0.2 mg kg^{-1} in 6 months) indicated an excellent oxidative stability of muscle in dipped

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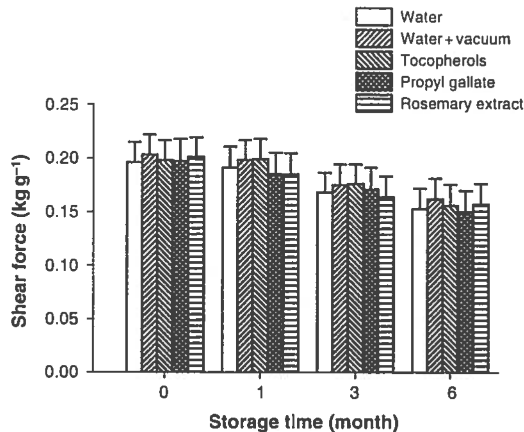

Figure 3 Changes in shear force value of red claw muscle during frozen storage.

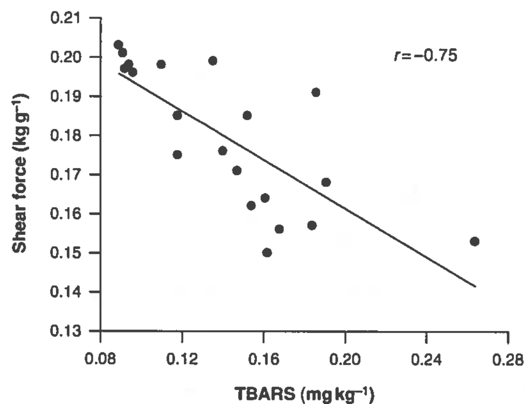

Figure 4 Regression plot of shear force vs. lipid oxidation (TBARS) for frozen stored red claw muscle.

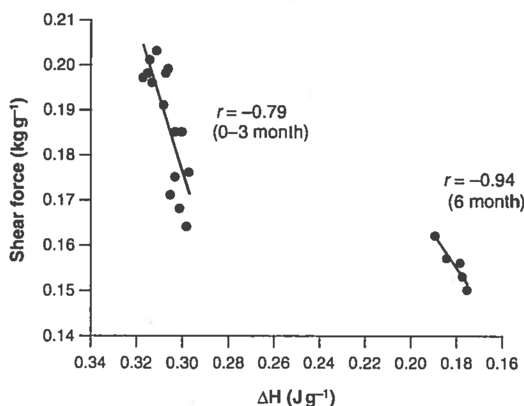

Figure 5 Regression plot of shear force vs. myosin denaturation (ΔH) for frozen stored red claw muscle.

shell-on red claw during frozen storage, irrespective of anti-oxidant or packaging treatments. This stability may be attributable to the presence of the shell that prevented direct contact of the muscle tissue with molecular oxygen (Srinivasan *et al.*, 1997). The formation of a glaze provided an additional barrier to the diffusion of oxygen into the muscle. The inclusion of anti-oxidants in the dipping solution increased the inhibition of lipid oxidation. However, as the measured TBARS values were low (<0.3 mg kg^{-1}) anti-oxidant treatment would not seem to be essential. The light yellow stain appearing on shells of propyl gallate-treated samples may have been caused by the yellow and green coloured compounds produced either by the reaction of copper and iron or their salts with propyl gallate (Xue, 1985), but it should not affect the product palatability as the shell will be removed prior to consumption. The liquid form of the rosemary extract was in black colour and also produced a dark brown colour on red claw treated with this product. Further studies may be required to determine consumer perception of this unpleasant condition if anti-oxidants such as propyl gallate and rosemary extract are to be used.

Protein denaturation is one of the principal factors contributing to quality deterioration in many frozen muscle foods (Shenouda, 1980; Mackie, 1993; Rahman, 1999; Badii & Howell, 2002). The formation and accretion of ice crystals and the increase in salt concentration because of the diminishment of the water phase, can cause damage to the protein structure and convert protein into a metamorphic state (Xiong, 1997). For example, fibre shrinkage, exudation of fluid (drip) and texture toughening are frequently observed in thawed frozen meat products. Fast freezing (e.g. >4 °C min^{-1}) facilitates the formation of small, intracellular ice crystals and, therefore, adverse protein changes can be minimized. The marked reduction in myosin ΔH between month 3 and 6 indicates a decrease in the protein's conformational stability that was independent of dipping or packaging treatments. The lack of correlation between TBARS and T_{max} or ΔH for myosin suggested that lipid oxidation had little, if any, role in destabilization of myosin under the dipping and storage conditions used in the present study.

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In the seafood industry, glazing is used to protect fish from desiccation during frozen storage (Bottino *et al.*, 1979; Nilsson & Ekstrand, 1994). Glazing is usually done by immersing the frozen product in water or by spraying it with water. The final product is coated with an ice layer that provides the protection. The amount of water bound in the ice layer depends on factors such as product size, environmental temperature and water temperature (Santos & Regenstein, 1990). In this study, all the dipped samples, without draining, were immediately subjected to individually quick freezing (IQF). The formation of a thin layer of ice apparently prevented the samples from dehydration during storage, resulting in less moisture loss and essentially unchanged cooking yield.

The progressive decrease in the muscle shear value (i.e. increase in softness or tenderness) during frozen storage may be interpreted as being caused by protein denaturation weakening the myofibril network. The existence of two distinct shear–enthalpy relationships (0–3 months; 6 months) suggests that two different mechanisms are involved in the softening of muscle and the associated changes in texture. In the first 3 months, during which shear force rapidly reduced and this was accompanied by a small decline in myosin ΔH, a subtle change in protein conformation may initiate weakening of the myofibril structure. From 3 to 6 months, when myosin itself underwent a major structural change (both T_{max} and ΔH), major disruptions in the myofibril ultrastructure may have occurred, resulting in texture softening. The low accumulation of TBARS in all samples suggests that oxidation of lipid-protein products, if it occurs at all, would have only a minimal role in textural change within the muscle. Instead physical damage to proteins by ice crystals may have been a major cause of muscle weakening; this has been reported previously (Tseng *et al.*, 2003). Further research is needed to elucidate possible ultrastructural changes in muscle fibres.

Conclusions

Dipping shell-on red claw meat in anti-oxidants retarded lipid oxidation, even though the TBARS values in all samples was low (< 0.30 mg kg^{-1}), but did not influence protein destabilization and muscle tissue softening of red claw crayfish when stored at −20 °C for up to 6 months. Thus anti-oxidant dipping did not seem to be crucial to the preservation of red claw glazed with water ice before long-term frozen storage. The overall small changes in all the muscle quality parameters evaluated, regardless of anti-oxidant treatments or vacuum packaging, indicated that red claw tail meat was very stable during frozen storage under the experimental conditions used in this study. Further work evaluating the sensory properties of the samples and simulating commercial storage conditions is recommended.

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