Original article

Influence of gender and spawning on thermal stability and proteolytic degradation of proteins in Australian red claw crayfish (*Cherax quadricarinatus*) muscle stored at 2 °C

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Summary Thermal stability and proteolytic degradation of male (M), nonspawning female (F) and spawning female (SF) red claw crayfish (*Cherax quadricarinatus*) muscle proteins during refrigerated storage (2 °C) were investigated. The thermal transition temperatures (T_{max}) of myosin and actin remained relatively constant during storage, but their enthalpies of denaturation (ΔH) increased, especially in SF samples. SF muscle proteins were more heat-stable (greater T_{max} and ΔH values, $P < 0.05$) than M and F muscle proteins. Protein degradation occurred in all muscle groups, more rapidly in M and F muscles than in SF muscle. The diminishments of a 69-kDa component and troponin-I and the appearance of a 55-kDa polypeptide represented the most salient proteolytic changes. The results suggested that the spawning status was a more significant factor than gender in affecting the quality of red claw muscle proteins and their changes during refrigerated storage.

Keywords Proteolysis, red claw crayfish, spawning, thermal stability.

Introduction

Australian Red Claw crayfish (*Cherax quadricarinatus*) is a promising aquaculture species that can grow in a diverse environment. It is similar to the native American species, except that it can grow to a much larger size, almost to that of a lobster. Female red claw can also breed year round, and each spawn often produces over 700 eggs. Furthermore, red claw have a non-aggressive nature, which allows a high stock density (Masser & Rouse, 1997; Jones, 1998).

There have been a number of studies on crayfish nutrient requirements (Figueiredo & Anderson, 2003), diet formulation (Muzinic *et al.*, 2004), diseases control (Edgerton *et al.*, 2002), reproduction and hatching (Jerry *et al.*, 2005), and production characteristics (Nguyn & Austin, 2004). A few reports have also been published on meat quality and protein stability of red claw during postharvest storage. Tseng *et al.* (2002, 2003) studied quality changes of red claw tail meat subjected to refrigerated storage or freezing–thawing cycles, showing that red claw muscle was somewhat resistant to protein denaturation and lipid oxidation at refrigerator temperatures, but was susceptible to repeated freezing–thawing. Our more recent study demonstrated that dipping in antioxidant solutions almost completely inhibited lipid oxidation and texture deterioration of red claw muscle stored in a −20 °C freezer for up to 6 months (Tseng *et al.*, 2005).

In a typical aquacultural facility, juvenile red claw are stocked before sex differentiation. Hence, by the time of harvest, the population of mature red claw will consist of a mixture of males, females and spawning females. In our preliminary study, we have found spawning female red claw representing as much as 25% of the total female population in certain ponds. Therefore, understanding the meat quality and protein differences between male and female red claw and between spawning and nonspawning females would be of practical significance.

The objective of this study was to determine muscle protein thermal stability and postharvest protein degradation of male (M), nonspawning female (F) and spawning female (SF) red claw crayfish during storage

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at 2 °C. The influence of gender and spawning on textural properties of red claw meat had been investigated in a preceding study (Kong *et al.*, 2006).

Materials and methods

Materials

Juvenile red claw crayfish (6.3 ± 3.0 g stocking weight) were grown in 0.04-ha ponds at Kentucky State University Aquaculture Research Center, Frankfort, Kentucky for 12 weeks (2 June 2005–22 September 2005) to a mean live weight of 58.9 ± 3.6 g. A total of 180 red claw (sixty M, sixty F and sixty SF) were randomly collected from a large sample pool of over 2000 live red claw. The SF red claw, that comprised 14% (average) of the total female population, were readily identified by the eggs adhered to the underside of the cephalothorax. The F red claw were identified by a pair of genital pores at the base of the third pair (counting from the head) of walking legs, and the M were recognised by a pair of small genital papillae (projections) at the base of the fifth pair of walking legs. After stunning by chilling in an ice slurry for 2 min, the cephalothorax were removed manually, and the tails (muscle, plus exoskeleton) were placed in iced coolers and shipped to the University of Kentucky Food Protein Research Laboratory within 2–3 h of harvest.

Storage and muscle sample preparation

For each crayfish group, the sixty tails were equally divided into six portions and placed in styrofoam trays (ten in each). The trays were double-wrapped with an air-permeable PVC film and stored in a 2 °C walk-in cooler for 0, 1, 3, 5, 7 and 10 days. During storage, fluorescent lights in the cooler were kept on to simulate supermarket retail display. At the end of each storage period, three tails were randomly picked from one tray per crayfish group and deshelled. The tail muscles were pooled and finely chopped by blending for 30 s with a micro Waring blendor to produce a homogenous muscle mince for thermal and electrophoretic analyses as described below.

Differential scanning calorimetry

The minced muscle was analysed immediately for thermal stability using a Model 2920 modulated differential scanning calorimetry (DSC) machine (TA Instruments, New Castle, DE, USA). The DSC machine was calibrated for temperature and baseline using indium as standard. Accurately weighed muscle samples (14–17 mg) were placed in polymer-coated aluminium pans (TA Instruments) and hermetically sealed. An empty, sealed pan was used as reference. A heating rate of 10 °C min^{-1} was used to scan samples thermally from 10 to 100 °C. The total enthalpy change (ΔH) 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, i.e. temperature at the peak of the endotherm (T_{max}), was also recorded. Both the T_{max} and ΔH values were determined using the Universal Analysis Ver 1.2 N software supplied by the DSC Company.

Gel electrophoresis and laser densitometry

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with a Mini-PROTEAN 3 Cell electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA) to detect proteolytic change in red claw muscle during refrigerated storage. A 100 g L^{-1} acrylamide resolving gel and a 30 g L^{-1} acrylamide stacking gel were used. Homogenised muscle mince was dissolved in an appropriate amount of SDS-PAGE sample buffer (40 g L^{-1} SDS, 200 g L^{-1} glycerol, 10 g L^{-1} β-mercaptoethanol, 0.125 M Tris–HCl, pH 6.8) to obtain a 1 mg mL^{-1} protein concentration. Aliquots of 20 μL of the samples were loaded to the sample wells in the stacking gel. A protein standard, consisting of myosin heavy chain (MHC) (200.0 kDa), β-galactosidase (116.3 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa), and aprotinin (6.5 kDa) (Bio-Rad Laboratories), was also run.

Protein bands were tentatively identified by comparing their electrophoretic pattern with published results of standard muscle proteins (Porzio & Pearson, 1977). Molecular weights (MW) of unknown protein bands were estimated from the regression line of protein migration distances vs. logarithm MW of the proteins. The weight percentages of protein components in red claw muscle were estimated by scanning the SDS-PAGE protein bands with an Ultrascan XL laser densitometer (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) that had the capability to integrate the peak areas.

Statistical analysis

Data were analysed 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 done to determine the significance of the main effects (gender, spawning, storage time). Significant ($P < 0.05$) differences between means were identified using least significant difference procedures.

Results and discussion

Thermal stability

Thermal curves of red claw muscles produced by the DSC are presented in Fig. 1. All muscle samples exhibited two major transitions (peaks I and III). For M and F red claw, there was also a minor transition (peak II). As proteins are virtually the only components that would exhibit such distinctive endothermic heat flows, all the DSC transitions presumably resulted from protein structural changes. Peaks I and III are tentatively ascribed to myosin head (or heavy meromyosin) and actin, while peak II may be attributed to a combination of myosin tail (or light meromyosin), sarcoplasmic proteins and connective tissue (Stabursvik & Martens, 1980; Subramanian *et al.*, 1997b).

The T_{max} of an endothermic peak designates the maximum transition temperature during thermal denaturation of a protein. Thus, the smaller the T_{max}, the lower the thermal stability of the protein. Compared with muscle samples of different genders and spawning status (Table 1), the T_{max-1} of SF red claw (peak I, 50.0–52.1 °C) was consistently higher ($P < 0.05$) than that of F (47.5–48.4 °C) and M (47.0–48.7 °C) red claw throughout storage, while no significant gender effect was observed. This suggested that myosin in SF muscle had a greater thermal stability than myosin from the other two groups of red claw. For the transition associated with actin denaturation (peak III), the T_{max-3}

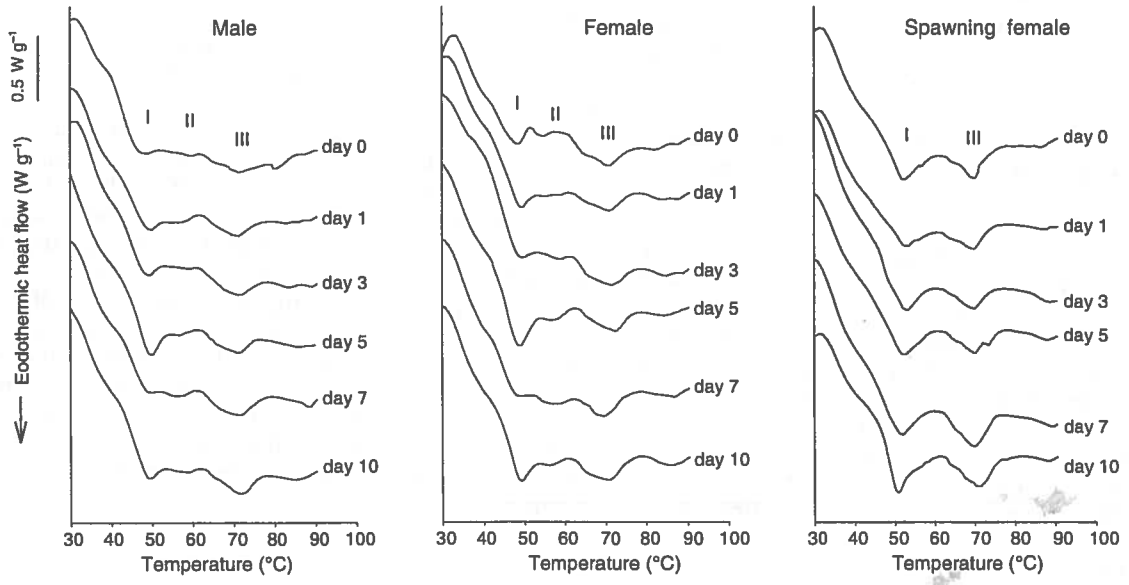

Figure 1 Differential scanning calorimetry (DSC) of red claw crayfish muscles stored at 2 °C for various periods. Each thermal curve is a representative of measurements from at least three replicates.

Table 1 Maximum transition temperature (T_{max}) of myosin (peak I) and actin (peak III) of red claw crayfish muscle stored at 2 °C for various times

	Myosin (T_{max-1}, °C)			Actin (T_{max-3}, °C)			$T_{max-3}-T_{max-1}$ (°C)		
Storage time (day)	Male	Female	Spawning female	Male	Female	Spawning female	Male	Female	Spawning female
0	46.97^{bD}	48.11^{bAB}	52.05^{aAB}	71.29^{aAB}	70.23^{aAB}	70.22^{aAB}	24.32	22.12	18.17
1	47.52^{bCD}	48.39^{bA}	52.45^{aA}	70.69^{aAB}	71.15^{aAB}	70.00^{aAB}	23.17	23.63	17.55
3	47.96^{bBC}	48.41^{bA}	52.49^{aA}	71.02^{aAB}	70.45^{abAB}	69.78^{bAB}	23.06	22.04	17.29
5	49.29^{bA}	48.32^{bA}	52.12^{aA}	70.97^{aAB}	71.12^{aAB}	70.91^{aA}	21.68	22.80	18.79
7	48.13^{bBC}	47.49^{bB}	51.36^{aB}	70.59^{aB}	69.90^{aB}	69.75^{aB}	22.46	22.41	18.39
10	48.68^{bAB}	48.44^{bA}	49.95^{aC}	71.67^{aA}	71.63^{aA}	70.17^{aAB}	22.99	23.19	20.22
Average	48.09^{b}	48.19^{b}	51.74^{a}	71.04^{a}	70.75^{ab}	70.14^{b}	22.95^{a}	22.70^{a}	18.40^{b}

$^{a-b}$For the same protein, means in the same row with different letters differ significantly ($P < 0.05$); $^{A-D}$means in the same column with different letters differ significantly ($P < 0.05$).

was essentially neither influenced by spawning nor by the gender difference. Overall, T_{max} values of red claw transitions were notably lower than those of mammalian muscles (Wright *et al.*, 1977; Wagner & Añón, 1986). A general lower transition temperature for fish muscle proteins, when compared with mammalian species, has also been widely observed (Howell *et al.*, 1991; Paredi *et al.*, 1994).

The cause for the lack of a middle endotherm (peak II) as well as the narrower temperature gap between peak I and peak III transitions for SF muscle samples (18.40 °C), when compared with those for M and F muscles (22.70–22.95 °C), was not clear. Although the pH of the SF red claw muscle (pH ~ 6.51) was slightly lower than that of F and M counterparts (pH ~ 6.77) during the first 3 days (data not shown), it seemed unlikely that such a small discrepancy could explain the significant protein stability difference due to spawning. It is plausible that the physiological condition entailed by the reproductive cycle in live spawning red claw predisposed the muscle to a different denaturing ionic environment, i.e. with different amounts of stabilising and destabilising ions. For example, Ca^{2+} has been shown to destabilise muscle proteins (Stabursvik & Martens, 1980; Xiong & Brekke, 1991). As a note, the mineral content in SF muscle, determined in our preliminary experiment, was 53 mg g^{-1}, which was significantly less than minerals in M and F muscles (62 mg g^{-1}). Paredi *et al.* (2002) studied thermal denaturation of myofibrillar proteins of striated and smooth adductor muscles of scallop and showed that as pH (5.5–8.0) and ionic strength (0.05–0.50) increased, the thermal stability (ΔH) of whole muscles decreased. A similar pH effect on myofibrillar protein thermal stability (T_{max}) of hake muscle was also reported (Beas *et al.*, 1990).

The temperatures associated with the individual transitions within each red claw group showed some small, inconsistent changes during storage (Table 1). In M, the T_{max-1} increased at the first 5 days, reaching a maximum at day 5 ($P < 0.05$); in F, the T_{max-1} was relatively constant throughout storage. In SF, the T_{max-1} showed no change in the first 5 days, but steadily decreased after 7 days ($P < 0.05$). For actin, except for a few variations, the T_{max-3} values of all three red claw groups were overall unaffected by storage.

The ΔH denotes the amount of energy absorbed by a sample during melting or denaturation. Comparison of muscle samples from different genders and from females with or without spawning indicated significantly higher ($P < 0.05$) ΔH_1 (myosin head) in SF (0.300–0.989 J g^{-1}) than in M (0.147–0.391 J g^{-1}) or F (0.174–0.390 J g^{-1}) after 3 days of storage (Table 2). Yet, there was no significant difference between M and F. The result was in accord with that of the T_{max-1} measurement. There was no consistent trend in ΔH_1 change for myosin over storage although the ΔH_1 values of SF in general continued to increase with the storage time. For actin, there was little difference in ΔH_3 between the three groups of red claw.

Paredi *et al.* (1996) studied thermal denaturation of muscle proteins from M and F squid (*Illex argentinus*) at different sex and sexual maturation stages, noting that gender was not a factor affecting T_{max}. Beas *et al.* (1991) compared thermal stability of myofibrillar proteins from pre- and post-spawning hake using DSC. The ΔH values of the protein extracts, notably for that corresponding to myosin transition, were found to be less for pre-spawning hake than for post-spawning hake. Results from these previous investigations, notwithstanding on saltwater species, were consistent with our present findings on a freshwater shellfish. It appears that the physicochemical conditions of aquatic animal muscle proteins were regulated differently before and after spawning, and the physicochemical state of the proteins was probably similar between male and female fish or shellfish.

Proteolytic changes

Postmortem protein degradation occurred in all three red claw groups, with the disappearance of a 69-kDa protein and a concomitant emergence of a 55-kDa polypeptide being among the most noticeable changes

Table 2 Enthalpy of denaturation (ΔH) of myosin (peak I) and actin (peak III) of red claw crayfish muscle stored at 2 °C for various times

Storage time (day)	Myosin (ΔH_1, J g^{-1})			Actin (ΔH_3, J g^{-1})		
	Male	Female	Spawning female	Male	Female	Spawning female
0	0.340^{aA}	0.246^{aBC}	0.300^{aD}	0.097^{bC}	0.072^{bC}	0.212^{aC}
1	0.312^{aA}	0.309^{aAB}	0.350^{aCD}	0.162^{aBC}	0.194^{aBC}	0.150^{aC}
3	0.147^{bB}	0.174^{bC}	0.568^{aB}	0.250^{aAB}	0.217^{aB}	0.248^{aBC}
5	0.357^{bA}	0.237^{bBC}	0.733^{aB}	0.329^{aA}	0.235^{aB}	0.390^{aB}
7	0.209^{bB}	0.220^{bBC}	0.539^{aBC}	0.364^{aA}	0.247^{aB}	0.378^{aB}
10	0.391^{bA}	0.390^{bA}	0.989^{aA}	0.350^{aA}	0.404^{aA}	0.494^{aA}
Average	0.293^{b}	0.263^{b}	0.580^{a}	0.257	0.228	0.312

$^{a-b}$For the same protein, means in the same row with different letters differ significantly ($P < 0.05$); $^{A-D}$means in the same column with different letters differ significantly ($P < 0.05$).

	Storage time (day)	MHC	69 kDa	55 kDa	Actin	TM/TT	LC1	TI	LC2
Male	0	31.7^{a}	5.8^{ab}	1.9^{d}	10.9^{d}	8.5^{c}	2.3^{a}	4.8^{a}	4.3^{a}
	3	31.3^{a}	6.2^{a}	2.3^{cd}	11.4^{c}	10.3^{a}	2.0^{a}	5.0^{a}	3.7^{ab}
	5	27.7^{b}	4.4^{bc}	4.0^{bc}	10.4^{e}	9.8^{b}	1.5^{b}	4.9^{a}	3.6^{b}
	7	32.2^{a}	3.7^{c}	6.2^{a}	15.0^{a}	8.4^{c}	1.7^{b}	2.8^{c}	3.5^{bc}
	10	32.6^{a}	3.6^{c}	5.3^{ab}	14.1^{b}	8.3^{c}	2.0^{a}	3.2^{b}	2.8^{c}
	% change*	+2.8	−59.1	+179	+29.4	−2.4	−13.0	−33.3	−34.9
Female	0	21.5^{b}	6.6^{a}	1.3^{c}	8.3^{c}	13.2^{a}	2.0^{b}	7.4^{a}	3.5^{b}
	3	19.2^{b}	5.5^{a}	2.9^{bc}	9.3^{c}	14.0^{a}	2.2^{ab}	7.6^{a}	3.4^{b}
	5	26.9^{a}	3.6^{b}	4.6^{ab}	11.6^{b}	8.6^{b}	2.2^{ab}	5.1^{b}	4.9^{a}
	7	30.9^{a}	2.9^{b}	5.1^{a}	14.1^{a}	8.1^{b}	2.8^{a}	4.0^{c}	4.2^{ab}
	10	28.7^{a}	2.7^{b}	5.8^{a}	12.1^{b}	8.3^{b}	2.6^{ab}	4.3^{bc}	4.8^{a}
	% change*	+33.5	−59.1	+346.2	+45.8	−37.1	+30	−41.9	+37.1
Spawning female	0	27.4^{a}	4.9^{a}	1.5^{c}	9.0^{b}	9.3^{c}	2.7^{ab}	5.5^{c}	3.1^{c}
	3	23.2^{b}	3.6^{b}	2.3^{b}	9.2^{b}	12.4^{a}	2.3^{b}	6.6^{a}	3.8^{b}
	5	23.8^{b}	3.9^{b}	2.2^{b}	9.2^{b}	11.9^{b}	2.6^{ab}	6.0^{b}	3.6^{b}
	7	28.1^{a}	2.2^{c}	3.3^{a}	10.8^{a}	8.3^{e}	2.8^{ab}	4.2^{e}	4.7^{a}
	10	27.7^{b}	2.2^{c}	3.2^{a}	10.8^{a}	8.7^{d}	2.9^{a}	4.5^{d}	4.7^{a}
	% change*	+1.1	−55.1	+113.3	+20.0	−6.5	+7.4	−18.2	+51.6

Table 3 Relative concentration (%) of selected proteins in red claw crayfish muscle stored at 2 °C for various times as determined by laser densitometric scanning

MHC, myosin heavy chain; TM/TT, tropomyosin/troponin T; LC1, myosin light chain 1; TI, troponin I; LC2, myosin light chain 2. $^{a-e}$Means in the same column with different superscript letters differ significantly ($P < 0.05$).
*Percentage change from day 1 to day 10.

with each successive freeze–thaw cycle (Subramanian *et al.*, 1997a).

Muscle proteins of marine species are generally susceptible to proteolysis, and enzymic degradation of myofibrillar proteins negatively affects fish quality (Kinoshita *et al.*, 1990; Osatomi *et al.*, 1997). The calpain system, which is present in crustacean species, is believed to be the main endogenous proteolytic enzyme complex responsible for postmortem degradation of muscle proteins in headed tails (Jiang *et al.*, 1992; Jiang & Chen, 1999). The calpain system presumably played a significant role in producing the proteolytic changes observed in all three red claw muscle groups in the present study.

Pérez-Borla *et al.* (2002) investigated proteolytic activity of muscle in pre- and post-spawning hake (*Merluccius hubbsi Marini*) after frozen storage and found that the autolytic activity of muscle extracts from pre-spawning fish was significantly higher than that of post-spawning fish. Montecchia *et al.* (1997) claimed, based on the analysis of protein solubility, viscosity and hydrodynamic properties of muscle, that fillets from pre-spawning hake stored at −20 °C deteriorated more rapidly than post-spawning hake. Our results also showed an overall slower proteolytic process in SF muscle than in F muscle during storage, suggesting a slower enzyme activity present in the SF muscle samples. For example, from day 1 to day 10, the concentrations of the 69-kDa protein, tropomyosin/troponin T, and troponin I in SF were lowered by 55.1%, 6.5% and 18.2%, compared with 59.1%, 37.1% and 41.9% in F muscle. Corresponding to the slower protein degradation, the SF muscle exhibited a slower increase in the 55-kDa component (113%) and in myosin light chain 1 (7.4%) when compared with those in F muscle (346.1% and 30% increases for the 55-kDa protein and myosin light chain 1, respectively). If calpain indeed was involved in the proteolysis, then the presumably low level of calcium in the SF muscle (so surmised because of the lower total mineral content in SF than in F muscle) would limit the enzyme activity, thus, explaining the slower protein degradation in SF muscle.

Conclusion

Results from this study indicated that spawning was a significant factor affecting postmortem protein stability and enzyme degradation of Australian red claw crayfish meat. Overall, spawning female red claw muscle proteins were more heat stable and less susceptible to proteolytic breakdown than muscle proteins from nonspawning female red claw as well as from the male counterpart during refrigerated storage. Further research is required to identify the specific biochemical mechanisms (e.g. proteases involved and the intracellular chemical environment) that would more clearly explain the observed protein differences between the different red claw groups.

Acknowledgments

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	Storage time (day)	MHC	69 kDa	55 kDa	Actin	TM/TT	LC1	TI	LC2
Male	0	31.7^{a}	5.8^{ab}	1.9^{d}	10.9^{d}	8.5^{c}	2.3^{a}	4.8^{a}	4.3^{a}
	3	31.3^{a}	6.2^{a}	2.3^{cd}	11.4^{c}	10.3^{a}	2.0^{a}	5.0^{a}	3.7^{ab}
	5	27.7^{b}	4.4^{bc}	4.0^{bc}	10.4^{e}	9.8^{b}	1.5^{b}	4.9^{a}	3.6^{b}
	7	32.2^{a}	3.7^{c}	6.2^{a}	15.0^{a}	8.4^{c}	1.7^{b}	2.8^{c}	3.5^{bc}
	10	32.6^{a}	3.6^{c}	5.3^{ab}	14.1^{b}	8.3^{c}	2.0^{a}	3.2^{b}	2.8^{c}
	% change*	+2.8	−59.1	+179	+29.4	−2.4	−13.0	−33.3	−34.9
Female	0	21.5^{b}	6.6^{a}	1.3^{c}	8.3^{c}	13.2^{a}	2.0^{b}	7.4^{a}	3.5^{b}
	3	19.2^{b}	5.5^{a}	2.9^{bc}	9.3^{c}	14.0^{a}	2.2^{ab}	7.6^{a}	3.4^{b}
	5	26.9^{a}	3.6^{b}	4.6^{ab}	11.6^{b}	8.6^{b}	2.2^{ab}	5.1^{b}	4.9^{a}
	7	30.9^{a}	2.9^{b}	5.1^{a}	14.1^{a}	8.1^{b}	2.8^{a}	4.0^{c}	4.2^{ab}
	10	28.7^{a}	2.7^{b}	5.8^{a}	12.1^{b}	8.3^{b}	2.6^{ab}	4.3^{bc}	4.8^{a}
	% change*	+33.5	−59.1	+346.2	+45.8	−37.1	+30	−41.9	+37.1
Spawning female	0	27.4^{a}	4.9^{a}	1.5^{c}	9.0^{b}	9.3^{c}	2.7^{ab}	5.5^{c}	3.1^{c}
	3	23.2^{b}	3.6^{b}	2.3^{b}	9.2^{b}	12.4^{a}	2.3^{b}	6.6^{a}	3.8^{b}
	5	23.8^{b}	3.9^{b}	2.2^{b}	9.2^{b}	11.9^{b}	2.6^{ab}	6.0^{b}	3.6^{b}
	7	28.1^{a}	2.2^{c}	3.3^{a}	10.8^{a}	8.3^{e}	2.8^{ab}	4.2^{e}	4.7^{a}
	10	27.7^{b}	2.2^{c}	3.2^{a}	10.8^{a}	8.7^{d}	2.9^{a}	4.5^{d}	4.7^{a}
	% change*	+1.1	−55.1	+113.3	+20.0	−6.5	+7.4	−18.2	+51.6

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with each successive freeze–thaw cycle (Subramanian *et al.*, 1997a).

Muscle proteins of marine species are generally susceptible to proteolysis, and enzymic degradation of myofibrillar proteins negatively affects fish quality (Kinoshita *et al.*, 1990; Osatomi *et al.*, 1997). The calpain system, which is present in crustacean species, is believed to be the main endogenous proteolytic enzyme complex responsible for postmortem degradation of muscle proteins in headed tails (Jiang *et al.*, 1992; Jiang & Chen, 1999). The calpain system presumably played a significant role in producing the proteolytic changes observed in all three red claw muscle groups in the present study.

Pérez-Borla *et al.* (2002) investigated proteolytic activity of muscle in pre- and post-spawning hake (*Merluccius hubbsi Marini*) after frozen storage and found that the autolytic activity of muscle extracts from pre-spawning fish was significantly higher than that of post-spawning fish. Montecchia *et al.* (1997) claimed, based on the analysis of protein solubility, viscosity and hydrodynamic properties of muscle, that fillets from pre-spawning hake stored at −20 °C deteriorated more rapidly than post-spawning hake. Our results also showed an overall slower proteolytic process in SF muscle than in F muscle during storage, suggesting a slower enzyme activity present in the SF muscle samples. For example, from day 1 to day 10, the concentrations of the 69-kDa protein, tropomyosin/troponin T, and troponin I in SF were lowered by 55.1%, 6.5% and 18.2%, compared with 59.1%, 37.1% and 41.9% in F muscle. Corresponding to the slower protein degradation, the SF muscle exhibited a slower increase in the 55-kDa component (113%) and in myosin light chain 1 (7.4%) when compared with those in F muscle (346.1% and 30% increases for the 55-kDa protein and myosin light chain 1, respectively). If calpain indeed was involved in the proteolysis, then the presumably low level of calcium in the SF muscle (so surmised because of the lower total mineral content in SF than in F muscle) would limit the enzyme activity, thus, explaining the slower protein degradation in SF muscle.

Conclusion

Results from this study indicated that spawning was a significant factor affecting postmortem protein stability and enzyme degradation of Australian red claw crayfish meat. Overall, spawning female red claw muscle proteins were more heat stable and less susceptible to proteolytic breakdown than muscle proteins from nonspawning female red claw as well as from the male counterpart during refrigerated storage. Further research is required to identify the specific biochemical mechanisms (e.g. proteases involved and the intracellular chemical environment) that would more clearly explain the observed protein differences between the different red claw groups.

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