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COMPARATIVE STUDY OF DSC PATTERN, COLOUR AND TEXTURE OF SHRIMPS DURING HEATING

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Shrimp continues to be the most important commodity traded in value terms, accounting for 16.5% of the total value of internationally traded fishery products in 2004. Despite this importance of shrimp, literature is almost lacking on reports dealing with changes in functional properties and quality caused by heating shrimps while influence of freezing has been investigated more in depth. Therefore, objective of the study was cooking shrimp to different core temperatures in the range 30–80°C and monitoring changes in quality by measuring colour and texture attributes. DSC curves taken on differently heated shrimp differed markedly. With increasing temperature the enthalpy of denaturation decreased significantly.

Keywords: *colour, differential scanning calorimetry, heating, shrimps, texture*

Introduction

Shrimp continues to be the most important commodity traded in value terms, accounting for about 19 and 16.5% of the total value of internationally traded fishery products in 2000 and 2004, respectively [1, 2]. Regarding trends by species groups catches of shrimps increased impressively in the decade to 2004 (by 47.2%) and at the end of the decade they attained the highest ever totals at about 3.6 million tons. Aquaculture production has pushed the demand and consumption for several high-value species such as shrimps. The per capita availability of crustaceans increased more than threefold, from 0.4 to 1.5 kg (mainly as a result of the increased production of shrimps and prawns from aquaculture). The substantial increase in the quantity of shrimp traded coincided with the strong expansion in aquaculture shrimp production, which has grown rapidly since 1997, with an increase of 165% during the period 1997–2004 (annual growth of 15%). In 2004, more than 41% (or 2.5 million tons) of total shrimp production was of farmed origin. During 2005, shrimp imports in several key markets reached new highs. Thanks to aquaculture development, even species which used to be considered 'luxury' species such as shrimps are now more affordable as the surge in volume through improved technology has brought down prices, as reflected in the value data [2, 3].

In the scientific literature papers on shrimps quality and processing include mainly work on quality changes [4–16], melanosis inhibition [17–29] and influence of freeze/thaw cycles on quality and thermal stability [30–35]. However, reports on the influence of thermal treatment of shrimps on quality parameters such as texture, colour and thermal stability of shrimps are scarce. This is particularly surprising because both brown shrimp (*Crangon crangon*) and northern shrimp (*Pandalus borealis*) are mainly cooked directly after catching.

The objective of the present study was therefore, heating of shrimps to defined temperatures and measuring changes in texture, colour and DSC pattern as result of heating steps.

Experimental

Materials

Shrimps

Shrimps used for investigation were deepwater pink shrimp (*Parapenaeus longirostris*), brown shrimp (BS) and northern shrimp (NS). The deepwater pink shrimp (DWPS) were caught in August 2005 in the northern part of the Aegean Sea (Turkey). Prior to peeling shrimps were dipped for 10 min in sodium metabisulphite solution (150 mg L^{-1}) according to the Turkish Food Codex. Peeled shrimps were frozen at –40°C for 14 h and kept frozen at –18°C. They were transported as airfreight in an isolated box to the institute in Hamburg. BS were caught in the North Sea by a commercial shrimp trawler in April 2007 and delivered refrigerated to the institute in Hamburg

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immediately after returning to port. There BS were peeled and frozen at –30°C. NS were processed commercially in December 2006 on board trawler in Greenland for Royal Greenland A/S, Aalborg, Denmark, and sold uncooked frozen as whole shrimp (1 kg portions) under the name 'Ama Ebi' by Kagerer & Co. GmbH, Heimstetten, Germany.

Thermal treatment

The thermal treatment of shrimps was performed as recently described [36]. Shrimps were packed in cooking bags and heated in a water bath (Haake 6P, Karlsruhe, Germany) at temperatures ranging from 30 to 80°C in 10°C intervals until the core temperature was equal to that of the water bath. Temperatures were recorded during heating of the samples using a temperature controller (model 3150, Beckmann+Egle Industrieelektronik, Kernen, Germany) with automatic measured data storage unit 800 equipped with a temperature probe (NTC type, model 841, Beckmann+Egle Industrieelektronik, Germany).

Methods

DSC measurement

For DSC measurements a MicroDSC VII (SETARAM, Caluire, France) was used. Measurements were performed at least in duplicate as recently reported [36, 37]. Results are presented as average curves in the figures. The average curves are used to record the onset and transition temperatures (T_{ons} and T_{max}) and to calculate the transition enthalpy (ΔH) expressed as J g^{-1} of the sample material from the peak area using the SETARAM software SETSOFT 2000 (version 1.6, rev. 4).

Instrumental measurements for colour and texture

Colour measurements were carried out on comminuted shrimp muscle using a spectral colorimeter spectro pen (Dr. Lange, Düsseldorf, Germany) as recently described [36]. In the CIE Lab system, L^* denotes lightness on a 0-to-100 scale from black to white; a^* , (+) red or (–) green; and b^* , (+) yellow or (–) blue. ΔE, the colour difference, denotes the square root of ($\Delta L^2+\Delta a^2+\Delta b^2$).

Texture was characterised as tenderness measured by using a modified Warner-Bratzler shear cell attached to an SMS Texture Analyser TA.XT2 (Stable Micro Systems, Godalming, UK) [38] to cut shrimp tails across to length axis.

SDS-gel-electrophoresis

The protein pattern of the shrimp muscle was verified by sodium dodecylsulphate (SDS)-PAGE [39].

Proximate composition

The proximate composition of the shrimps was determined by using the respective standardized German methods [36]. Measurements were performed in duplicate.

The results were statistically evaluated by using STATISTICA (StatSoft, Inc. 1996) (Tulsa, OK).

Results and discussion

Proximate composition

Table 1 shows the proximate composition of untreated shrimp samples. It becomes obvious that DWPS was much higher in moisture and lower in protein content than the other two samples. NS was highest in protein and lowest in moisture content. For DWPS even lower protein and moisture contents [40], but also higher protein and lower moisture contents [7, 28, 41] were reported, this possibly suggests an influence of fishing area on proximate composition. The NS used for investigating the influence of different cooling conditions had a moisture content of 81.1%, a protein content of 17.4%, a fat content of 0.4% and a NaCl content of 0.7% [14].

DSC measurements

The DSC pattern taken on DWPS heated to different temperatures prior to measurement display three peaks in both the untreated shrimp and in the sample

Table 1 Proximate composition of shrimps investigated (n=2)

	Deep water pink shrimp	Brown shrimp	Northern shrimp
Moisture/%	84.65	78.9	77.2
Protein/%	13.83	16.4	20.8
Fat/%	1.13	1.1	1.0
Ash/%	0.2	–	1.9
NaCl/%	–	1.05	0.95
pH	–	7.77	7.26

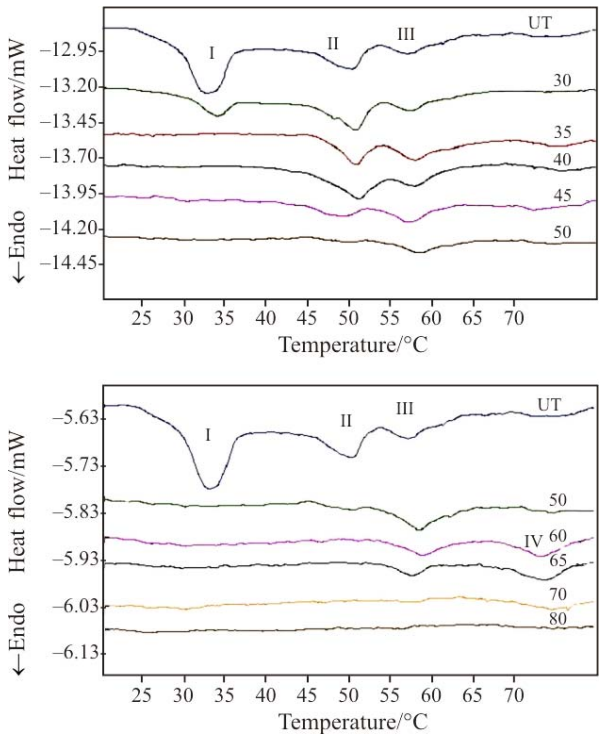

Fig. 1 DSC curves taken on deep-water pink shrimp muscle as affected by elevated temperature (UT – untreated, 30–80°C to which samples were heated prior to DSC measurement)

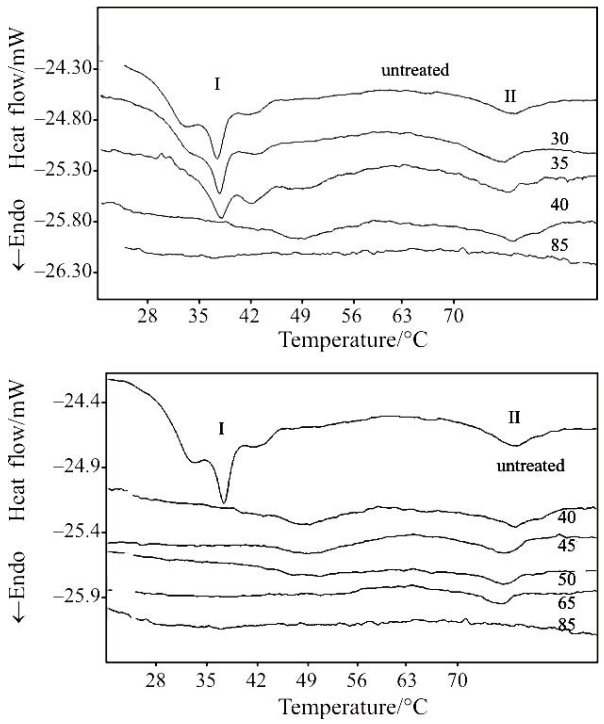

Fig. 2 DSC curves taken on brown shrimp muscle as affected by elevated temperature (30–85°C to which samples were heated prior to DSC measurement)

heated to 30°C (Fig. 1). It could be assumed that these peaks represent myosin or myosin heavy chain (I), sarcoplasmic proteins and connective tissue proteins (II) and actin (III). In the samples heated to 35°C peak I has disappeared already and only peaks II and III are visible. In samples heated to 50°C an additional peak IV appeared at higher temperature, while peak II became almost invisible. In samples heated to 60 and 65°C, only peak III and IV were to be seen, whereas in the 70°C sample all protein fractions were thermally denatured as displayed by a straight line. An increase in temperature was connected with reduction in ΔH in most cases (Table 2).

In Fig. 2 the DSC pattern taken on BS were displayed. In contrast to DWPS only two peaks characterised the protein fraction of these shrimp muscle. The major first peak was characterised by an initial and a finalising shoulder, which broadens the first (myosin) peak (I) remarkable. A second much smaller peak (II) appeared at about 76–78°C. This peak did not change very much with elevating temperature except a slight reduction in enthalpy. Heating up to 35°C did not change substantially the

Table 2 Transition temperatures (°C) and enthalpies (J g^{-1}) calculated from DSC curves taken on heat-treated deep-water pink shrimp muscle dependent on the heating temperatures used

Heated to °C	Peak I			Peak II			Peak III			Peak IV		
	T_{ons}	T_{max}	ΔH	T_{ons}	T_{max}	ΔH	T_{ons}	T_{max}	ΔH	T_{ons}	T_{max}	ΔH
Untreated	28.9	33.4	1.140	47.3	50.4	0.460	55.0	57.9	0.212	–	–	–
30	31.1	34.0	0.066	47.4	50.7	0.134	54.5	57.2	0.040	–	–	–
35	–	–	–	48.7	50.7	0.090	55.3	57.9	0.050	–	–	–
40	–	–	–	47.8	51.0	0.118	55.6	57.9	0.050	–	–	–
45	–	–	–	45.1	49.0	0.070	54.3	57.2	0.105	–	–	–
50	–	–	–	46.1	49.3	0.020	54.9	58.5	0.156	70.0	72.3	0.050
60	–	–	–	–	–	–	55.3	59.0	0.100	70.0	73.4	0.115
65	–	–	–	–	–	–	54.8	57.4	0.049	69.5	74.0	0.104
70	–	–	–	–	–	–	–	–	–	–	–	–

DSC pattern except the diminishing enthalpy of both peaks. After heating shrimps to temperatures in the range of 40–50°C the main peak with T_{max} of about 37.5°C disappeared and a small peak at about 49°C became visible. However, after heating to 65°C only the last peak at about 76°C was to be seen. Heating BS to 75°C caused an almost complete denaturation of muscle proteins. The changes in T_{max} and ΔH dependent on the heating temperatures are displayed in Table 3. It becomes obvious that elevation in heating temperature was connected with a reduction in ΔH, and T_{max} of all peaks detected were higher than the T_{max} found for DWPS.

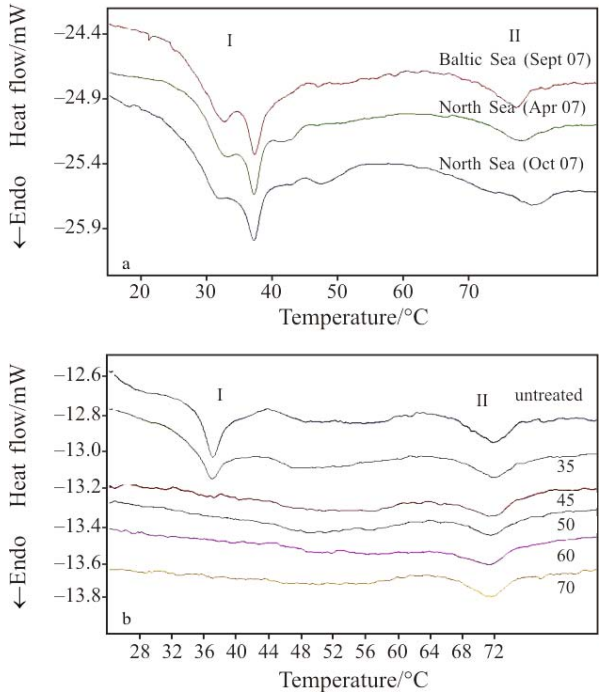

Fig. 3 a – Comparison of DSC pattern of brown shrimp caught at different fishing areas, b – DSC curves taken on northern shrimp muscle as affected by elevated temperature (30–70°C to which samples were heated prior to DSC measurement

Almost no differences in thermal stability of untreated shrimps were found between BS collected in the Baltic Sea and those from the North Sea (Fig. 3). This could be assumed since BS is distributed from the Black Sea over the Mediterranean to the Baltic Sea [42].

The number of peaks characterising the DSC pattern of NS (Fig. 3) was comparable to BS. Furthermore, the first peak (I) had almost the same T_{max} while the one of the second peak (II) was slightly lower. First peak with T_{max} of 37°C disappeared already after heating the shrimps to 45°C while the second one was stable up to 70°C and did not change very much in ΔH as result of heating to respective temperature (Table 3).

When comparing the DSC curves of the different shrimps it becomes obvious that the DSC curve of untreated DWPS is different from those of BS and NS. This difference is manifested by one more peak shown in the curve. Furthermore the denaturation temperatures (T_{max}) of protein fractions detected in DWPS were lower than in BS and NS. Generally, the first peak of the DSC curve (myosin) proved to be susceptible against heating and disappeared in all shrimps at least after heating to 40°C. As already stated above DSC pattern of BS and NS were almost comparable.

Electrophoresis pattern of both BS and NS (Fig. 4) indicate possibly on enzymatic activity particularly in the high molecular mass region that appears to be enhanced by increasing temperature in the range from 35–65°C in the case of BS. Same behaviour became not obvious in NS. Sodium dodecyl sulfate polyacrylamide (SDS-PAGE) is useful for molecular mass analysis of proteins. SDS is a detergent that dissociates and unfolds oligomeric

Table 3 Transition temperatures (°C) and enthalpies (J g^{-1}) calculated from DSC curves taken on both heat-treated brown shrimp and northern shrimp muscle dependent on the heating temperatures used

Heated to °C	Brown shrimp						Northern shrimp					
	Peak I			Peak II			Peak I			Peak II		
	T_{ons}	T_{max}	ΔH	T_{ons}	T_{max}	ΔH	T_{ons}	T_{max}	ΔH	T_{ons}	T_{max}	ΔH
Untreated	34.6	37.3	3.344	73.6	77.8	0.653	35.1	37.1	0.592	68.0	71.8	0.398
30	34.7	37.6	2.084	70.2	76.1	0.602	–	–	–	–	–	–
35	34.1	37.8	1.349	72.9	77.1	0.262	34.6	36.9	0.508	68.3	71.7	0.408
40	38.7	49.3	0.510	75.7	78.3	0.210	–	–	–	–	–	–
45	45.7	49.1	0.224	73.6	76.7	0.346	–	–	–	67.3	72.0	0.367
50	44.8	47.0	0.094	73.7	76.4	0.134	–	–	–	68.2	71.8	0.450
60	–	–	–	–	–	–	–	–	–	65.9	71.4	0.416
65	–	–	–	72.9	76.2	0.141	–	–	–	–	–	–
70	–	–	–	–	–	–	–	–	–	67.2	71.6	0.328

Table 4 Changes in CIE Lab values taken on comminuted shrimp muscle as affected by elevated temperature

Shrimp	Color value	Untreated	Thermal treatment/°C											
			30	35	40	45	50	55	60	65	70	75	80	85
DWPS	L^*	51.78^{a}	50.87^{ab}	49.46^{ac}	50.5^{ad}	↔	56.87^{e}	58.98^{f}	59.02^{f}	↔	60.94^{h}	↔	↔	↔
	a^*	1.84^{a}	1.89^{a}	2.12^{a}	1.43^{b}	↔	1.55^{b}	2.09^{a}	3.04^{c}	↔	3.36^{d}	↔	↔	↔
	b^*	2.44^{a}	2.27^{a}	2.18^{a}	1.49^{b}	↔	2.75^{ab}	3.24^{c}	3.98^{d}	↔	56.2^{e}	↔	↔	↔
BS	L^*	33.94^{a}	↔	36.44^{b}	↔	40.96^{c}	42.93^{d}	↔	↔	50.27^{e}	↔	54.71^{f}	↔	54.27^{f}
	a^*	0.71^{a}	↔	1.09^{b}	↔	2.61^{c}	3.1^{d}	↔	↔	4.99^{e}	↔	6.14^{f}	↔	7.62^{g}
	b^*	0.18^{a}	↔	1.54^{b}	↔	1.11^{b}	0.67^{a}	↔	↔	5.94^{c}	↔	9.78^{d}	↔	12.18^{e}
NS	L^*	49.44^{a}	↔	48.27^{b}	↔	51.6^{c}	53.73^{d}	↔	58.83^{e}	↔	60.26^{f}	↔	61.78^{g}	↔
	a^*	2.66^{a}	↔	2.52^{a}	↔	2.93^{b}	2.02^{c}	↔	1.92^{c}	↔	1.91^{c}	↔	3.43^{d}	↔
	b^*	0.15^{a}	↔	0.27^{a}	↔	0.14^{a}	–0.13^{b}	↔	0.85^{c}	↔	1.94^{d}	↔	3.24^{e}	↔

↔ no treatment; $^{a–g}$ and $^{a–c}$ indicate significant differences ($p<0.05$) ($n=15$)

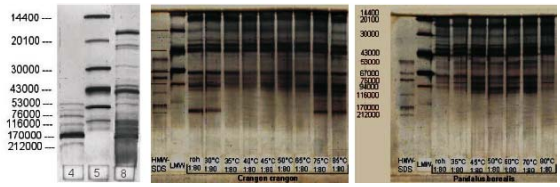

Fig. 4 SDS-gel-electrophoresis pattern of muscle of deepwater pink shrimp (lane 4-HMW standard, lane 5-LMW standard, lane 8-DWPS, untreated), brown shrimp and northern shrimp

proteins into its subunits. The SDS binds to the to polypeptides to form complexes with fairly constant charge to mass ratios. The electrophoretic migration rate through a gel is therefore determined only by the size of the complexes. Molecular masses are determined by simultaneously running marker proteins of known molecular mass.

Thermal stability of protein of freshwater prawns (*Macrobrachium rosenbergii*) decreased after the muscle tissue was frozen and subsequently thawed. While the extent of protein destabilisation was independent of the freezing method and the presence or absence of prawn shells, it varied with the thawing rate [30]. T_{ons} and T_{max} decreased with increasing freeze-thaw cycles as did so ΔH of whole muscle which decreased from 16.6 J g^{-1} for fresh muscle to 11.4 J g^{-1} after five freeze-thaw cycles [31]. When feeds containing 25, 35 and 40% protein were fed to blue shrimp (*Litopenaeus stylirostris*) the DSC curve taken on whole muscle from shrimp fed 40% protein showed lowest ΔH in the myosin peak. The DSC curve exhibited three peaks [43]. When Australian red claw crayfish (*Cherax quadricarinatus*) was stored in ice, the T_{max} for myosin and actin decreased from 50.2 and 72.6°C on day 0 to 39.4 and 60.3°C by day 14, respectively; while ΔH for myosin reduced from 0.324 to 0.116 J g^{-1} during this period [44]. Repeated freeze-thaw cycles caused some minor changes in the thermal stability of red claw muscle. Results were seen as an indication that both myosin and actin were resistant to denaturation, but were destabilised upon extended freeze-thaw abuse [33]. Dipping shell-on red claw meat in antioxidants retarded lipid oxidation, but did not influence protein destabilisation of red claw crayfish when stored at –20°C up to 6 months [45]. A comparison of package systems for their influence on the stability of shell-on red claw crayfish tail meat stored at 2°C for 14 d included DSC measurements. Both the ΔH and the T_{max} data suggested that proteins in red claw tails in the mixed $O_2/CO_2/N_2$ atmosphere (MAP) were less stable [46]. Thermal stability of male (M), nonspawning female (F) and spawning female (SF) red claw crayfish muscle proteins during refrigerated storage (2°C) was investigated. SF muscle proteins were more heat-stable (greater T_{max} and ΔH values) than M and F muscle proteins [47]. DSC curves of black tiger shrimp (*Penaeus monodon*) and white shrimp (*P. vannamei*) showed two major peaks, corresponding to myosin and actin peaks. The two shrimps had similar T_{max} and ΔH values of the first peak (myosin). T_{max} of the second peak, representing actin of black tiger shrimp was lower than that of white shrimp [48]. T_{max} and ΔH of both myosin and actin peaks shifted to lower values when Pacific white shrimp (*Litopenaeus vannamei*) were treated with mixed phosphates. Those changes were generally more pronounced in ice-stored shrimp [49]. Most of the published works on post mortem changes in shrimps and prawns refer to warm water species [50]. Spoilage is due to the action of the shrimp endogenous enzymes on the shrimp tissues and structures and seems to be somewhat different in the Arctic than in the tropical species. The most evident effect in iced stored NS short after death was the decrease in the relative amount of myosin heavy chain (confirmed by SDS-PAGE). It was found that the majority of the proteolytic enzymes in BS may consist of cathepsin-like proteinases rather than trypsin-like enzymes [42]. In Pacific white shrimp the autolytic activity was highest at 35°C and decreased sharply above 40°C, whereas no differences in autolytic activity were observed in the temperature range of 45 to 70°C [51].

Colour and texture

The effect of heating on colour changes measured on comminuted shrimps muscle is displayed in Table 4. In general, lightness (L^*) increased with elevated temperature. This increase appeared to be linearly and was characterised by the following coefficients of determination (R^2): 0.7814, 0.9618 and 0.9367 for DWPS, BS and NS, respectively. Redness (a^*) appeared to be increasing as well however, the linearity of increase was less pronounced as shown by the following coefficients of determination (R^2): 0.4891, 0.00008 and 0.9783 for DWPS, NS and BS, respectively. Yellowness (b^*) increased as well and this increase was almost linearly as shown by the following coefficients of determination (R^2): 0.6294, 0.8231 and 0.6912 for DWPS, BS and NS, respectively. Regarding untreated shrimps it became further obvious that DWPS were lightest followed by NS and BS ($p<0.05$), whereas NS were reddest followed by DWPS and BS ($p<0.05$). In yellowness the following order was found DWPS>BS>NS ($p<0.05$). Table 5 express colour changes caused by heating as colour difference ΔE^* between the different heating steps. A very strong increase in colour difference was characteristic for all of the shrimps

Table 5 Colour differences ΔE^* calculated from measures taken on comminuted shrimp muscle heated to different temperatures

Shrimp	Thermal treatment/°C											
	30	35	40	45	50	55	60	65	70	75	80	85
DWPS	0.90	2.35	1.65	↔	5.11	7.25	7.50	↔	9.81	↔	↔	↔
BS	↔	2.86	↔	7.33	9.32	↔	↔	17.85	↔	23.51	↔	24.59
NS	↔	1.18	↔	2.18	4.35	↔	9.44	↔	10.99	↔	12.74	↔

↔ no treatment

Table 6 Tenderness (N) and normalised tenderness (N g^{-1}) of shrimp tail muscle as affected by elevated temperature

Thermal treatment/°C	Tenderness			Normalised tenderness			
	DWPS	BS	NS	BS	±SD	NS	±SD
Untreated	5.25^{a}	1.88^{a}	5.12^{a}	0.785	0.17	3.831	0.39
30	4.65^{a}	↔	↔	↔	↔	↔	↔
35	3.93^{ab}	2.01^{ab}	5.45^{a}	0.807	0.17	4.094	0.48
40	5.12^{a}	↔	↔	↔	↔	↔	↔
45	↔	2.36^{ab}	4.83^{a}	0.837	0.17	3.996	0.37
50	5.14^{a}	3.16^{b}	4.6^{ac}	0.914	0.19	3.626	0.54
55	2.51^{c}	↔	↔	↔	↔	↔	↔
60	1.37^{d}	↔	1.94^{b}	↔	↔	2.602	0.56
65	↔	1.37^{c}	↔	0.475	0.1	↔	↔
70	2.27^{c}	↔	1.73^{b}	↔	↔	2.003	0.52
75	↔	1.19^{c}	↔	0.444	0.07	↔	↔
80	↔	↔	3.97^{ac}	↔	↔	2.48	0.63
85	↔	2.67^{b}	↔	0.581	0.12	↔	↔

↔ no treatment, $^{a–g}$ and $^{a–c}$ indicate significant differences ($p<0.05$) ($n=16$)

investigated however, it was particularly pronounced in BS.

Changes in texture did not show clear tendencies e.g. texture did not become harder or softer with increasing temperature. Table 6 shows the force necessary to cut through the tail of shrimp across to length axis and commonly called tenderness. Therefore, the lower the force the tender the shrimp was. Untreated DWPS and NS were comparable in tenderness, whereas BS was significantly softer. Heating DWPS tails up to 50°C caused no significant changes ($p>0.05$), while further heating up to 70°C resulted in significant softening. Changes in tenderness of BS were characterised by a decrease up to 50°C, followed by an increase with increasing temperature except at final temperature (85°C). Tenderness of NS did not change significantly when heated up to 50°C. When heated further up to 70°C, tenderness increased significantly. However, final heating temperature of 80°C caused a significant loss of tenderness (Table 6). To see whether tenderness of BS and NS was influenced by shrimp size, the force was related to the mass of specimen. For both BS and NS almost same results regarding the influence of heating temperature were obvious. Species-specific differences in tenderness were underpinned (Table 6).

Shear force to break prawn tails was dependent on prawn size, more on mass than on diameter [31]. They found no change in shear force of raw prawns after 5 freeze-thaw cycles (F/T), whereas it decreased in cooked samples after 3 freeze-thaw cycles. In contrast, it was found that shear force of cooked red claw muscle increased with freeze-thaw cycles reaching a maximum value by cycle 6 [33]. The shear force value of white shrimp was lower, than that of black tiger shrimp, regardless of F/T [52]. When previously frozen shrimp were cooked in water of temperatures ranging from 55 to 95°C temperature significantly affected all texture properties. Small and large shrimp gave the lowest hardness around 65°C. When measuring shear force in large tiger shrimp, only the changes at 55°C were significantly different than the rest [54]. Pre-storage antioxidant dipping treatments did not affect the cooking yield of frozen Australian red claw crayfish during storage. However, the length of frozen storage influenced the muscle shear force. Compared with month 0, samples stored for 6 months had a significant reduction in the shear force value [45]. When stored in ice shear force

of cooked red claw muscle increased during storage especially between day 7 and day 10 [44]. Significant differences existed between males (M), females (F), and spawning females (SF) of Australian red claw crayfish in their shear force values during post-mortem storage. SF muscle had a higher shear force than the M and F muscle during storage at 2°C [55]. After 10-day ice storage, farmed white shrimp fed on commercial feed showed a greater decrease in shear force than those shrimp fed with sardine- and squid-based diet [53]. Regarding the influence of packaging it was found that MAP samples stored for 6 and 14 days exhibited higher shear force values than the vacuum packed samples and those packed in poly-vinylchloride [46]. Kuruma prawn (*Penaeus japop-nicus*) was heat processed at different temperature in the range from 20 to 90°C for 30 min and sub-sequently air-cooled for 10 min. Major macroscopic changes were deformation and discoloration especially for meats heated at higher temperatures (70 to 90°C). The prawn meat became firmer as pro-cessed at higher temperatures, and the penetration resistance of meat heated at 70°C was about double that of the meat heated at 30°C. Deformation value also tended to increase during heat processing, showing high values at 70 and 90°C, in contrast to that of the raw meat. It was assumed that changes were mainly due to heat denaturation of intracellular proteins, which developed gradually from 30 to 60°C. At temperatures >70°C, this denaturation occurred so intensely that the heated meat might show a heavy shrinkage [56].

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