Evaluation of tenderness in prawns (*Machrobrachium rosenbergii*) marinated in various salt and acid solutions†

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Summary Freshly harvested prawns (*Machrobrachium rosenbergii*), with shell removed, were marinated in salt (NaCl, $CaCl_2$) and acid (lemon juice, citric acid, tripolyphosphate) solutions to determine the effects of marination on properties related to the texture of raw and cooked muscle. Sodium chloride and citrate solutions (pH 7.0) slightly increased ($P < 0.05$) the tenderness of cooked prawn, when compared with water-marination control. Tripolyphosphate (pH 7.0) showed no effect, while $CaCl_2$ (pH 7.0), citric acid (pH 3.0), and lemon juice (pH 3.0) increased ($P < 0.05$) the muscle toughness. The decrease of meat tenderness by some of the marinade treatments was attributed to decreases in protein solubility during marination and to cooking losses.

Keywords Marination, muscle, prawn, proteolysis, tenderness.

Introduction

Machrobrachium rosenbergii, a freshwater prawn, has been cultured in water farms to meet increasing consumer demands for shrimp and to provide variety in seafood products. In southeast regions of the continental US including Kentucky, where the climate is relatively mild, freshwater prawns have been successfully grown in farm ponds, and the potential for producing prawns on a larger, commercial scale seems to be high.

Textural properties of the meat are of primary importance for the commercialization of freshwater prawns. Previous studies indicated that freshwater prawns after ice-chilled storage had a maximum shelf-life of about 1 week or less (Angel *et al.*, 1985; Lindner *et al.*, 1988). The development of mushiness in ice-chilled prawns has been described as the gradual, sequential degradation of prawn tissue, including the perimysium and endomysium connective tissue, as well as the proteins that comprise the Z-line and the H-zones, caused by the action of hepatopancreatic enzymes (Baranowski *et al.*, 1984; Lindner *et al.*, 1988; Nip & Moy, 1988; Papadopoulos *et al.*, 1989). On the other hand, freshly harvested prawns, if not subjected to storage, tend to have tough, less palatable meat, compared with those 'aged' for several days. In home and at food services, prawns may be marinated in different salt or weak acid solutions before cooking to enhance flavour characteristics and possibly to improve tenderness. However, the impact of marination on muscle tissue characteristics of prawns has not been thoroughly investigated. The present study was designed to test the hypothesis that salt and acid marination would provide improved textural properties of raw and cooked prawn muscle.

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Materials and methods

Prawns

Juvenile prawns (0.5 g mean live weight) were grown in 0.02-ha ponds for 117 days to a mean live weight of 31.5 g at Kentucky State University Aquaculture Research Center (Frankfort, KY, USA). Harvested prawns were held live in 3000 L flow-through tanks until processed. Prawns (490, randomly picked from a pool of approximately 2000) were killed by beheading, and were immediately cooled on ice before use.

Marination

At 3 h postmortem, prawn muscle was removed from the shell, and the whole shell-less tails were marinated in a 4 °C cooler for 0, 1, 6, 12 and 24 h in the following seven solutions: water, 1% NaCl, 0.5% $CaCl_2$, 0.5% sodium tripolyphosphate, or 0.5% sodium citrate (pH adjusted to 7.0 for all); and 0.5% citric acid or 25% lemon juice (pH adjusted to 3.0 for both). The pH adjustment was performed using 0.1 N HCl or 0.1 N NaOH. For each marination solution, 70 randomly selected prawns were used (14 for each of the five marination time periods). At the end of a marination time for each treatment, seven prawns were randomly withdrawn for proteolytic and tenderness evaluations; and the other seven prawns were cooked and analysed for cooking yield and tenderness. Thus, a total of 490 prawns were used (7 treatments × 5 time periods × 14 animals).

Cooking

Thirty-five (50%) prawns were cooked in boiling water (100 °C) for 2 min and chilled immediately on ice before analysis (the other half were analysed raw). Cooking yield (%) was expressed as the cooked weight divided by the raw weight and then multiplying by 100.

Textural analysis

Shear forces required to rupture the first intact segment from anterior of the tail were measured using a Model 4301 Instron Universal Testing Instrument (Instron Corp., Canton, MA, USA) equipped with a Warner–Bratzler shear device as described by Srinivasan *et al.* (1997). The tail was placed in a transverse position to the blade so that the blade would cut the tail across the muscle fibres. The crosshead speed of the Instron was 20 mm min^{-1}. Shear force values (peak force) for individual prawns were normalized based on the weight of the prawns (force per muscle weight, kg g^{-1}) to eliminate prawn size effect (Srinivasan *et al.*, 1997).

Electrophoresis

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was used to determine proteolytic changes in prawn muscle during marination (Laemmli, 1970). This was done with an SE 250 Mighty Small II slab gel electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA, USA) using a 3% acrylamide stacking gel and a 10% acrylamide resolving gel (Srinivasan *et al.*, 1997). Samples for electrophoresis were prepared by homogenizing 1 g minced raw muscle in 100 mL cold (~5 °C) distilled deionized water with a Polytron (Brinkman Instruments, Inc., Westbury, NY, USA) for 30 s. The homogenate was diluted 1 : 1 in the sample buffer containing 4% SDS, 0.125 M Tris (pH 6.8), 20% glycerol and 10% β-mercaptoethanol, yielding a sample protein concentration of approximately 1 mg mL^{-1}, assuming a 20% protein content in raw muscle tissue. A 20 μg sample was loaded in each gel lane.

Statistical analysis

Data were analysed using the General Linear Models procedure of the STATISTIX 3.5 software package (Analytical Software, Inc., St Paul, MN, USA) for microcomputers. Analysis of variance (ANOVA) was computed to determine the significance of main effects of marinade type and marination time. Significant ($P \leq 0.05$) differences between means were identified using Least Significant Difference procedures (Snedecor & Cochran, 1989).

Results

Raw (uncooked) prawns showed increased ($P < 0.05$) toughness after marination in $CaCl_2$

Table 1 Shear forces (kg g^{-1}) of raw freshwater prawns marinated with different solutions

	Time of marination (h)				
Marinade	**0**	**1**	**6**	**12**	**24**
Water, pH 7.0	0.165^{aA}	0.190^{aBC}	0.188^{aCD}	0.184^{aCD}	0.164^{aD}
NaCl (1%), pH 7.0	0.165^{aA}	0.175^{aC}	0.160^{aD}	0.170^{aCD}	0.156^{aD}
$CaCl_2$ (0.5%), pH 7.0	0.165^{bA}	0.170^{bC}	0.216^{aBC}	0.214^{aC}	0.221^{aC}
TPP (0.5%), pH 7.0	0.165^{aA}	0.163^{aC}	0.178^{aCD}	0.174^{aCD}	0.151^{aD}
Citric acid (0.5%), pH 7.0	0.165^{bA}	0.196^{aBC}	0.166^{abD}	0.156^{bD}	0.158^{bD}
Citric acid (0.5%), pH 3.0	0.165^{dA}	0.220^{bcAB}	0.244^{bcB}	0.280^{abB}	0.321^{aB}
Lemon juice (25%), pH 3.0	0.165^{bA}	0.241^{bA}	0.384^{aA}	0.409^{aA}	0.445^{aA}

^{abc}Means within the same row without a common lowercase letter differ significantly ($P < 0.05$).
^{ABCD}Means within the same column without a common uppercase letter differ significantly ($P < 0.05$).

Table 2 Shear forces (kg g^{-1}) of cooked freshwater prawns marinated with different solutions

	Time of marination (h)				
Marinade	**0**	**1**	**6**	**12**	**24**
Water, pH 7.0	0.328^{bA}	0.335^{bC}	0.415^{abC}	0.355^{bB}	0.450^{aB}
NaCl (1%), pH 7.0	0.328^{abA}	0.359^{aBC}	0.239^{cD}	0.264^{cB}	0.293^{bcC}
$CaCl_2$ (0.5%), pH 7.0	0.328^{dA}	0.443^{cdAB}	0.545^{bcB}	0.675^{abA}	0.706^{aA}
TPP (0.5%), pH 7.0	0.328^{aA}	0.330^{aC}	0.314^{aD}	0.305^{aB}	0.309^{aBC}
Citric acid (0.5%), pH 7.0	0.328^{abA}	0.345^{aC}	0.268^{bD}	0.268^{bB}	0.281^{bC}
Citric acid (0.5%), pH 3.0	0.328^{dA}	0.496^{cA}	0.591^{bcAB}	0.666^{abA}	0.769^{aA}
Lemon juice (25%), pH 3.0	0.328^{cA}	0.513^{bA}	0.649^{aA}	0.705^{aA}	0.699^{aA}

^{abcd}Means within the same row without a common lowercase letter differ significantly ($P < 0.05$).
^{ABCD}Means within the same column without a common uppercase letter differ significantly ($P < 0.05$).

(pH 7.0) and in acidic solutions (citric acid, lemon juice, pH 3.0); marination in other solutions at neutral pH did not affect shear force (Table 1). However, the effect of some of the marinades was manifested by cooking. Cooked prawns that had been marinated in pH 7.0 NaCl and citric solutions became more tender, showing a decrease in shear force from 0.33 to 0.24 kg g^{-1} ($P < 0.05$) by 6 h (Table 2). Tripolyphosphate had no effect, while $CaCl_2$ and the low pH citric acid and lemon juice caused an increase ($P < 0.05$) in cooked muscle toughness with marination time. Analysis of variance indicated a quadratic regression curve ($r = 0.89$) between raw and cooked shear forces (Fig. 1).

To elucidate the possible mechanism for the textural change, the whole muscle homogenates of marinated prawns were subjected to SDS–PAGE. Unexpectedly, samples that had an increased toughness (lemon juice, citric acid, pH 3.0) actually also exhibited the greatest protein changes, as evidenced by the gradual disappearance of the myosin and actin bands (Fig. 2). Prawns that were treated with water or $CaCl_2$ also showed some losses in myosin and actin. However, prawns that were marinated with NaCl and neutral pH citric solutions (both of which increased muscle tenderness) had only slight changes in protein bands (result not shown). On the other hand, the marinade-induced muscle tenderness changes seemed to parallel the loss in cooking yield; the correlation coefficient between shear force and cooking yield was –0.87 (Fig. 3). Further analysis with a multiple regression computation that included both raw muscle shear force and the cooking yield produced the following equation for the prediction (estimation) of shear force of cooked prawns:

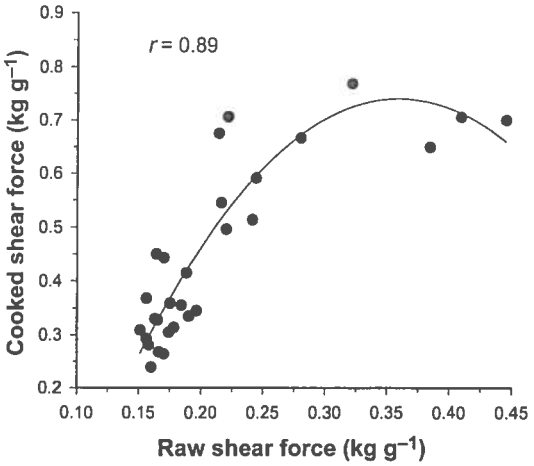

Figure 1 A regression curve showing a quadratic relationship between cooked shear force and raw shear value of marinated prawns.

$$SF_{cooked} = 1.272 + 1.0036\ SF_{raw} - 0.0154\ CY$$

where SF stands for shear force, and CY for cooking yield (Fig. 4). The multiple correlation was 0.96. The equation may be useful for the prediction of tenderness of cooked prawns.

Discussion

Tenderness changes in marinated muscle may be accounted for by proteolysis, altered association of muscle fibres, and/or interactions between proteins and water. The gradual loss of myosin and actin bands without the production of distinctive polypeptides immediately below them seemed to suggest reduced solubility of both proteins (actomyosin) and, less likely, degradation into peptides that were too small to be retained in the gel. For samples that were marinated in the acidic solutions (citric acid, lemon juice), acid-induced protein denaturation could occur, resulting in a decreased water-binding ability of myosin, actin and essentially all other myofibrillar components. Although electrophoretic analysis revealed myosin and actin losses, the result did not correspond to marination-induced textural changes. It is possible that degradation of other proteins or muscle tissue structure may have been involved. Lindner *et al.* (1988, 1989) and Papadopoulos *et al.* (1989) reported that softening of freshwater prawn tissue resulted, in part, from sequential degradation of muscle components, including the

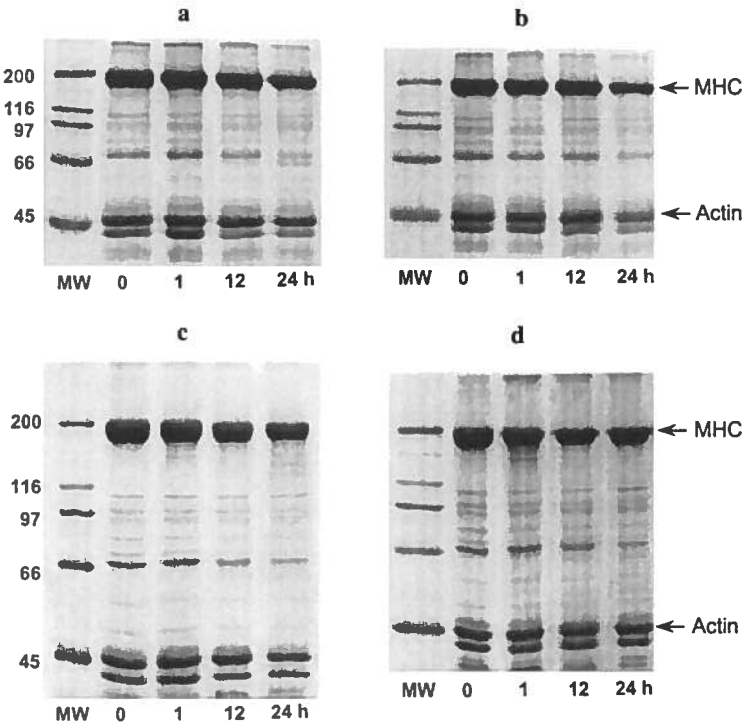

Figure 2 Sodium dodecyl sulphate–polyacrylamide gel electrophoresis of raw muscle homogenates from prawns marinated for various times (0–24 h). Marinade solutions shown in the graph are (a) water, (b) lemon juice, (c) citric acid at pH 3.0 and (d) $CaCl_2$. Numbers shown on the left denote molecular weights (MW, $\times 10^{-3}$) of the protein standard. MHC: myosin heavy chain.

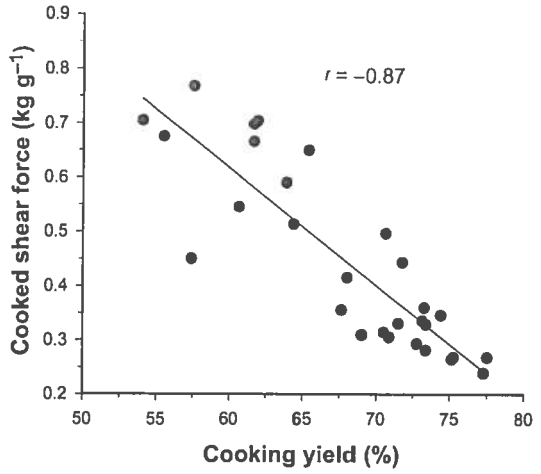

Figure 3 A regression plot showing a linear relationship between cooked shear force and cooking yield of marinated prawns.

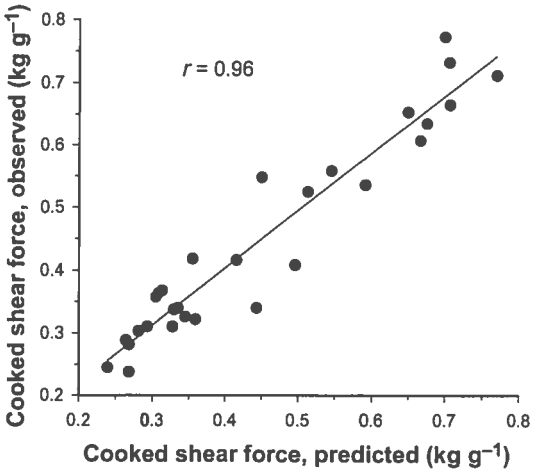

Figure 4 A regression plot showing a linear relationship between observed and predicted cooked shear values of marinated prawns.

perimysium and endomysium connective tissue as well as the proteins comprising the Z-lines and H-zones, caused by the action of hepatopancreatic enzymes such as collagenases. Other proteolytic enzymes that can cause muscle tissue disruption (e.g. in the Z-disc) may include calpain and cathepsins, which are endogenous to muscle and have been demonstrated in black tiger shrimp (*Penaeus monodon*) (Jiang *et al.*, 1992; Wang *et al.*, 1993). Nip & Moy (1988) and Papadopoulos *et al.* (1989) did not observe major structural changes in the Z-lines and H-zones of *M. rosenbergii* until three days postmortem, but in their study, the prawns were stored on ice rather than in marinade solutions. The involvement of calpain, a Ca^{2+}-dependent protease, seemed minimal in the freshwater prawn since the $CaCl_2$-treated samples exhibited less proteolytic changes when compared with other treatments. The inhibition of muscle toughening by NaCl marination was unlikely to be caused by hydration of myofibrils as the level of NaCl used (1%) was below the critical concentration normally required for myofibrillar protein solubilization (~2%).

It is noteworthy that tenderness changes not observed in marinated raw muscle tissue were manifested in cooked prawns, which was indicated by the lack of a linear relationship between the raw and cooked samples (Fig. 1). A similar finding between raw and cooked prawns was also obtained in our previous study (Srinivasan *et al.*, 1997). Alterations in the physical characteristics of raw prawn muscle tissue were not detected by the shear test, and cooking may have brought physical changes into the sensitivity range of the testing device. For example, any factor that promotes protein aggregation (as in the case of $CaCl_2$ treatment) could increase toughness in cooked meat. Conversely, because aggregation of connective tissue proteins (collagen, elastin) occurs during cooking, any proteolytic fragmentation of the connective tissue sheath that may have taken place during marination would become a significant influencial factor on tenderness of cooked prawn. Thus, direct evidence obtained from this study seemed to point to the textural changes in marinated prawns being the result of factors that affected protein–protein interactions rather than because of proteolytic degradation of myofibrillar proteins *per se*. Furthermore, the increase in shear values for some of the marinade treatments can be attributed to increased cooking losses ($r = -0.87$) (Table 3; Fig. 3) which most likely resulted from reduced interfilamental spaces because of fibre shrinkage/protein aggregation and denaturation. The existence of a strong correlation ($r = 0.96$) of predicted (estimated) shear force for cooked prawns (which was based on both raw muscle shear force and cooking yield) with the actual (observed) shear force of cooked samples indeed suggests that protein changes in

Table 3 Cooking yield (%) of freshwater prawns marinated with different solutions

Marinade	Time of marination (h)				
	0	1	6	12	24
Water, pH 7.0	73.4^{aA}	73.1^{aA}	68.0^{bCD}	67.6^{bC}	57.4^{cCD}
NaCl (1%), pH 7.0	73.4^{abA}	73.3^{abA}	77.3^{aA}	75.1^{abAB}	72.8^{bAB}
$CaCl_2$ (0.5%), pH 7.0	73.4^{aA}	71.8^{aA}	60.6^{bE}	55.5^{bE}	54.0^{bD}
TPP (0.5%), pH 7.0	73.4^{aA}	71.5^{abA}	70.5^{abBC}	70.9^{abBC}	69.0^{bB}
Citric acid (0.5%), pH 7.0	73.4^{aA}	74.4^{aA}	75.3^{aAB}	77.5^{aA}	73.4^{aA}
Citric acid (0.5%), pH 3.0	73.4^{aA}	70.6^{aAB}	63.9^{bDE}	61.6^{bcD}	57.5^{cCD}
Lemon juice (25%), pH 3.0	73.4^{aA}	64.4^{bB}	65.4^{bCDE}	61.9^{bD}	61.6^{bC}

^{abc}Means within same row without a common lowercase letter differ significantly ($P < 0.05$).
^{ABCDE}Means within the same column without a common uppercase letter differ significantly ($P < 0.05$).

the raw muscle tissue during marination and those induced by cooking both contributed to the changes in textural quality of cooked prawn muscle.

Overall, although marination can be used to modify sensory properties of freshwater prawns, it does not necessarily lead to desirable changes in meat texture (tenderness). Hence, a careful selection of marinades is essential for assuring desirable flavour as well as textural attributes of cooked prawns.

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