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Gelation properties of previously cooked minced meat from Jonah crab (*Cancer borealis*) as affected by washing treatment and salt concentration

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Abstract

The influence of washing treatment (dewatered only, one wash, and three washes) and sodium chloride (NaCl) concentration (0%, 2%, and 4%) on the gelation properties of crab mince was investigated. This previously cooked muscle mince is a low-value by-product of the crab processing industry, considered to have little or no functional properties. Crab mince gels were produced and tested for water-holding capacity (WHC), gel strength, colour, and electrophoretic profile. Wash treatment and NaCl concentration significantly affected gelation. Washed samples exhibited significantly higher WHC than dewatered samples. The 4% NaCl treatment decreased WHC compared to lower NaCl levels. Multiple washing steps increased the force to gel deformation. Wash treatment and NaCl concentration also affected the colour of gels. Based on these results, cooked crab meat mince treated with three washes and 0% NaCl resulted in the strongest gels with the best water-holding capacity, which can be used in the development of value-added products.

Keywords: Jonah crab; Surimi; Thermal gelation; Denatured proteins

1. Introduction

Last year, 3036 metric tonnes of Jonah crab (*Cancer borealis*) were harvested in the US (National Marine Fisheries Service, 2006), primarily as a by-catch of the lobster industry in Maine and Massachusetts. There has been an increase in consumer demand for Jonah crab claws due to their mild flavour and relatively low cost. Though the claws have become a popular consumer item, much of the remaining crab meat is wasted after removal of the claws. A mechanical deboner can be used to produce a relatively low-value meat mince from the residual muscle that can be added to a variety of food products for the food service industry. However, newer higher value uses for crab mince are needed to increase profitability and total utilization of marine resources.

Underutilized fish species and by-products of fish processing can be used in the development of new products to gain a greater value from the original resource. Processing these meat by-products into surimi is one way of increasing utilization of the resource. Earlier research showed that previously cooked minced crab meat could be used in the formation of surimi-like gels (Baxter & Skonberg, 2006). In traditional surimi processing, sodium chloride is added to raw muscle mince to aid in gel formation. The sodium chloride initiates the conversion of surimi into a *kamaboko* style gel by forming an actomyosin sol from the actin and myosin present (Hall & Ahmad, 1997). Typically, between 2% and 3% sodium chloride has been added to aid in gel formation. However, Lin, Park, and Morrissey (1995) showed that significant

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amounts of myofibrillar proteins in fish are solubilized in very low (near zero) ionic strength solutions. In raw cod muscle, the solubility of proteins in near zero ionic strength solutions was greater than the solubility at high ionic strengths (Stefansson & Hultin, 1994). Low salt concentrations allow proteins to be surrounded by salt ions of opposite charge which decreases the electrostatic free energy leading to increased activity of the solvent resulting in greater solubility of proteins (Debye & Huckel, 1923). This salting-in phenomenon is attributed to nonspecific electrostatic interactions between charged proteins and the ionic environment which leads to solubilization (von-Hippel & Schleich, 1969). As the salt concentration in a solution is increased, the salt ions compete with the proteins for binding sites on the water molecules, resulting in greater protein–protein interactions and leading to the precipitation of proteins. Salting-out occurs at high salt concentrations and is attributed to the loss of a stable hydrophilic surface, causing exposed hydrophobic areas of the proteins to interact, aggregate, and precipitate. The contradictory research showing that proteins are most soluble at both high and low ionic strengths, suggest that the traditionally used levels of sodium chloride of 2–3% may be higher than what is needed for gelation of muscle proteins.

Processing of raw fish into surimi requires several washing steps. The washing steps not only remove fat, sarcoplasmic proteins, and other undesirable substances such as blood, pigments and odorous compounds, but also concentrate the myofibrillar proteins to enhance gelation (Mendes & Nunes, 1992). The number of washing cycles and ratio of water to mince needed varies with fish species, initial condition of the fish, and the type of operation, either continuous or batch processing (Lee, 1984). In general, a five minute agitation in each of two washing cycles using a 3:1 (v/w; water to mince) ratio has been considered adequate for raw fish surimi production (Lee, 1986). Pacheco-Aguilar, Crawford, and Lampila (1989) investigated the effect of washing in Pacific whiting surimi production and reported that removal of lipids was not efficiently achieved using a single wash with a 3:1 ratio (v/w; water to mince) but resulted in high solids and protein recoveries which are often low in multiple exchange washing procedures. Up to 38% of the solids were lost in a third wash cycle (Lin & Park, 1996). Reducing the number of washing cycles is important in overall recovery of solids and maximization of yield as well as decreasing the wash water volume.

Previous studies by other researchers have focused on gelation of raw fish muscle. The purpose of this study was to investigate the impact of washing and sodium chloride addition on the gelation of previously cooked crab muscle proteins. The specific objectives of this study were to determine the effects of washing treatment (dewatered only, one wash, and three washes) and sodium chloride level (0%, 2%, and 4%) on the gel formation of previously cooked Jonah crab meat mince.

2. Materials and methods

Frozen, commercially available Jonah crab meat mince processed in the following manner was obtained from Portland Shellfish (Portland, Maine, USA): crabs had been eviscerated, boiled for 14 min without the top portion of the carapace, and cooled. Cooked crabs were kept on ice until mince was separated later the same day. Claws were removed and the remaining meat was separated using a mechanical deboning machine. The mince consisted of meat from the body and walking legs. Ten pounds of mince were packaged in polyethylene bags and frozen.

2.1. Mince processing

A 10-pound block of frozen Jonah crab meat mince was thawed at 4 °C for 48 h. The thawed mince was subjected to one of three wash procedures: (1) dewatered only, (2) dewatering followed by one wash, or (3) dewatering followed by three washes. Due to the high water content of the thawed mince it was dewatered via centrifugation at 25,800*g* (15,000 rpm) for 10 min. Dewatered mince was then further washed in 200 g batches in a Waring blender for 1 min using a ratio of 4:1 (v/w; water to mince). A cold (4 °C) 0.3% sodium chloride solution (NaCl) was used for the one wash treatment. For the three wash treatment, two washes of cold deionized water were followed by a third wash using a cold 0.3% NaCl solution. Mince was dewatered via centrifugation at 15,000 rpm for 10 min after each wash. The supernatant from each wash was collected and stored at 4 °C for further analysis. Once washed and dewatered, approximately 500 g of the mince was mixed with 0%, 2%, or 4% sodium chloride in a stand mixer (Kitchenaid, St. Joseph, MI, USA) with paddle attachment for 5 min. The moisture of the mince was adjusted to 80%. Mince was then stuffed into 30 mm cellulose casings (Nojax E-Z Peel; Viskase, Kentland, IN, USA) using a hand operated sausage stuffer (The Sausage Stuffer, Buffalo, NY, USA) to form approximately three sausages per treatment. The mince was cooked at 35 °C for 30 min followed by 90 °C for 30 min and then cooled on ice for 1 h. The cellulose casings were removed, gels were cut into 30-mm tall cylinders, and the cylinders were stored in food-grade plastic bags at 4 °C for no more than 24 h before testing.

2.2. Proximate analysis

Gels from each treatment were pooled and ground to create one homogenous sample per treatment for proximate analysis. Fat, protein, ash, and moisture content were measured in all samples. Nitrogen content was analyzed using the Elementar Rapid N III nitrogen analyzer (Elementar Americas, Inc., Mount Laurel, NJ, USA) to determine total protein content. Total fat, ash, and moisture were analyzed using AOAC (2005) methods, methods # 922.96, 938.08, and 950.46, respectively. Moisture, ash,

and protein analysis were performed in duplicate, and fat analyses were performed in triplicate.

2.3. Water-holding capacity

The water-holding capacity of the crab mince gels was determined using the method described by Roussel and Cheftel (1990) and modified by Alvarez, Couso, Tejada, Solas, and Fernandez (1992). The gels in each treatment were pooled and cut into 3-mm cubes. Approximately 2 g of sample were centrifuged for 15 min at 1000g and the exudate was collected on Whatman No. 1 filter paper. Samples were analyzed in triplicate and water-holding capacity was expressed as percentage of water retained with respect to the water present in the gel prior to centrifugation.

2.4. Gel strength

A penetration test was performed on 30 × 30 mm cylinders of each gel using a cylindrical stainless steel ball probe of 5 mm dia with a speed 60 mm per min into 1 end of the sample. The probe was attached to a 25 N load cell connected to TA-XT2i texture analyzer (Texture Technologies, Scardale, NY, USA). Both the breaking force (g) and deformation (cm) were recorded. Nine measurements were taken per treatment.

2.5. Colour

Ground sample was pooled from each of the three gels per treatment and analyzed using the LabScan XE Hunter Lab colorimeter (Hunter Associates Laboratory, Reston, VA, USA). Three sub-samples from each treatment were analyzed three times, resulting in a total of nine measurements for each treatment. Average L^*, a^*, and b^* values were determined from the nine measurements per treatment.

2.6. Protein molecular weight identification

The molecular weight of proteins in each gel sample and wash water supernatant was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE). Samples were solubilized in a SDS-urea solution containing 2% SDS, 8 M urea, 50 mM Tris-HCl (pH 8.0), and 2% 2-mercaptoethanol heated at 100 °C for 2 min and incubated overnight at 25 °C (Ni, Nozawa, & Seki, 1998; Park et al., 2003). SDS–PAGE was performed by the Laemmli (1970) method using a 10% separating and 4% stacking polyacrylamide gel (Esturk, Park, & Thawornchinsombut, 2004). Proteins were separated using a Bio-Rad Mini-PROTEAN III Cell (Bio-Rad Laboratories, Inc., Hercules, CA). The Precision Plus Protein Dual Colour Standard (Bio-Rad Laboratories, Inc., Hercules, CA) was used for comparison. Separated proteins were stained with 0.125% Coomassie brilliant blue R-250 (Mallinckrodt Baker, Phillipsburg, NJ) and destained in a solution containing 10% methanol and 10% acetic acid. The total protein concentration in the wash water supernatant was determined using the method by Lowry, Rosebrough, Farr, and Randall (1951). All analyses were conducted in duplicate.

2.7. Statistical analysis

A 3 × 3 factorial randomized complete block design was used for statistical analysis. Three wash treatments (dewatered only, dewatered followed by one wash, and dewatered followed by three washes) and three sodium chloride levels (0%, 2%, and 4%) were used resulting in nine total treatments. Multi-way analysis of variance of water-holding capacity, breaking force, deformation, L^*, a^*, and b^* values, and proximate composition was conducted on all nine treatments. Statistical analyses were performed using SAS (9.1) using least square means with Tukey mean separation. Significance was established at $p < 0.05$.

3. Results and discussion

3.1. Proximate analysis

Proximate analysis characterized the different crab mince gels as having less than 1% fat, 6.5 to 6.9% ash, 13.6 to 16.8% protein, and 79.6 to 81.0% moisture (Table 1). Based on multi-way analysis of variance, the crude fat content was significantly greater ($p < 0.05$) in the control (dewatered only) samples with an average of 1.0% fat than the other wash treatments at 0.9% fat for one wash and 0.7% fat for three washes. The addition of salt did not affect the fat content of the samples. The ash content was not significantly ($p > 0.05$) affected by either wash treatment or addition of sodium chloride. The moisture content of the gels was significantly ($p < 0.05$) higher in gels with 4% sodium chloride with 80.7% average moisture. Gels with 2% sodium chloride averaged 80.1% moisture and gels with no sodium chloride averaged 80.0% moisture. The protein content of the gels was not significantly ($p < 0.05$) affected by wash treatment but was affected by addition of sodium chloride. Sample with no sodium chloride had a greater percentage of protein (16.6%) than samples with 2% sodium

Table 1
Proximate analysis of cooked crab mince gels treated with varying wash treatments and sodium chloride levels

Sample	% Crude fat	% Ash	% Protein	% Moisture
Control 0% NaCl	1.1 ± 0.2	6.7 ± 0.2	16.7 ± 0.3	79.6 ± 0.7
Control 2% NaCl	1.0 ± 0.1	6.9 ± 0.1	14.9 ± 0.3	80.6 ± 0.4
Control 4% NaCl	0.9 ± 0.1	6.6 ± 0.2	13.6 ± 0.3	81.0 ± 0.3
One wash 0% NaCl	0.9 ± 0.1	6.5 ± 0.0	16.3 ± 0.9	80.5 ± 0.6
One wash 2% NaCl	0.8 ± 0.1	6.5 ± 0.1	15.6 ± 0.6	79.9 ± 0.7
One wash 3% NaCl	0.8 ± 0.1	6.6 ± 0.2	14.2 ± 0.4	80.6 ± 0.6
Three washes 0% NaCl	0.6 ± 0.0	6.6 ± 0.1	16.8 ± 0.2	79.9 ± 0.4
Three washes 2% NaCl	0.8 ± 0.2	6.5 ± 0.1	15.7 ± 0.7	79.9 ± 0.9
Three washes 4% NaCl	0.7 ± 0.5	6.6 ± 0.1	13.9 ± 1.0	80.6 ± 1.1

Values shown are averages of four measurements ±standard deviations.

chloride (15.4%) or those with 4% sodium chloride (13.9%). This difference was due to the increase in non-protein solids which lowered the overall percentage of protein in the sample.

3.2. Water-holding capacity

The water-holding capacity of the gels was significantly ($p < 0.05$) affected by both the washing treatment and sodium chloride levels (Fig. 1). Multi-way analysis of variance showed that gels from mince that was washed, either once or three times, had significantly ($p < 0.0.5$) higher water-holding capacity than gels from the control (dewatered only). Previous research conducted by Baxter and Skonberg (2006) showed that gels from washed crab mince and 2.5% sodium chloride produced gels with 68.5% water-holding capacity. The washed gels from this study were similar in water-holding capacity (70.9%), whereas gels from control mince (dewatered only) retained on average only 60.3% of the original water. The water-holding capacity for these gels is also comparable to that of surimi from raw Pacific whiting at 60–65% retained water (Lin et al., 1995). The washed crab mince gels did not retain as much water as sardine surimi suwari gels, which retained 75–80% water (Alvarez & Tejada, 1997). Karthikeyan, Dileep, and Shamasundar (2006) found that 10% more water was retained in the gel after three washes than with no washing in surimi made from raw threadfin bream (*Nemipterus japonicus*). Differences in water-holding capacity are dependent on the stability of the protein network. Washing of the crab mince removed components, such as fat, that may interfere with the stability of this protein network, therefore increased washing resulted in gels with a higher water-holding capacity.

Sodium chloride level also affected the water-holding capacity of the crab mince gels. The addition of 4% sodium chloride significantly ($p < 0.05$) decreased the water-holding capacity of gels compared to the two other salt treatments (Fig. 1). Addition of sodium chloride above the isoelectric point of the proteins in a meat system caused swelling of the proteins which led to a greater water-holding capacity (Honikel, 1989). In raw fish surimi, the water-holding capacity of the fish increases with increased sodium chloride concentration. Tellez-Luis, Uresti, Ramirez, and Vazquez (2002) found that the water retained by raw silver carp (*Hypophthalmichthys molitrix*) surimi increased as the sodium chloride concentration increased from 0 to 20 g kg^{-1}. The same held true in restructured hams made from raw silver carp. The water-holding capacity of the hams was greater with 2% sodium chloride than with 0% by almost 3-fold (Ramirez, Uresti, Tellez, & Vazquez, 2002). The trend observed in raw fish is opposite of that seen in the washed, cooked, crab mince gels. Gels with 0% added sodium chloride retained an average of 70.1% of the water within the gel, whereas gels with 4% sodium chloride retained only 61.9% water. In raw fish, the increased water-holding capacity is related to the isoelectric point of the fish proteins. Though not investigated in this study, the isoelectric point of the cooked crab proteins may be different from that of raw fish, resulting in the unexpected relationship between salt concentration and water-holding capacity.

3.3. Breaking force and deformation

Breaking force (g of force) was affected by both the number of washes and amount of added sodium chloride in the gels. The force significantly ($p < 0.05$) increased with

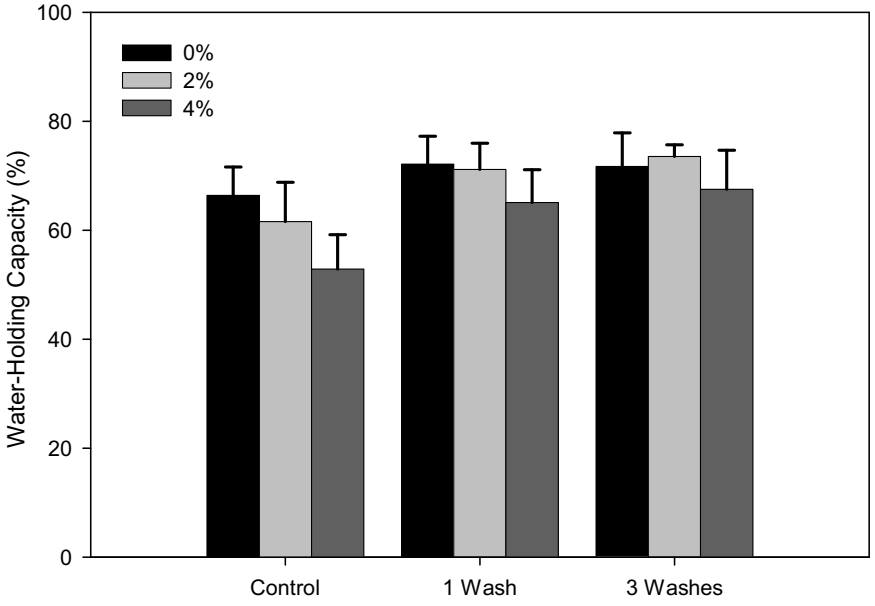

Fig. 1. Water-holding capacity (percent water retained) of crab mince gels as affected by sodium chloride level (0%, 2%, and 4%) and number of washes (0, 1, and 3). Values shown are averages of three treatments with standard deviations.

increasing number of wash treatments. Gels from mince washed three times required on average 175.5 g of force, gels from mince washed once required 117.4 g, and gels from dewatered mince required on average 83.1 g of force (Fig. 2). Previous work showed that gels made from washed crab mince and 2.5% sodium chloride had an average breaking force ranging from 160.3 to 194.6 g (Baxter & Skonberg, 2006). The breaking force of the washed crab mince gels in this study was similar to values of gels from previous work. Washing concentrates the myofibrillar proteins which may result in a stronger breaking force due to a stronger protein network (Chen, Chiu, & Huang, 1997); this correlates well with the results from the washed cooked crab mince gels. Lee (1986) found that the breaking force of surimi made from raw fish increased with up to two washings, at which point the breaking force leveled off.

The breaking force (g of force) significantly ($p < 0.05$) decreased with increasing sodium chloride concentration (Fig. 2). Gels without additional sodium chloride were the firmest (175.9 g) whereas gels with 4% sodium chloride were the softest (72.9 g). This may be due, in part, to the decreased protein concentration resulting from the addition of salt. These results are unique when compared to gels made from raw fish muscle. Kubota, Morioka, Itoh, Tamura, and Matsui (2006) found that walleye pollock (*Theragra chalcogrammma*) surimi breaking force increased with increasing salt concentration, with a maximum breaking force at 3% sodium chloride. The same was found in another study investigating walleye pollock. Akahane and Shimizu (1989) found that the breaking force of walleye pollock surimi increased when up to 3% sodium chloride was added. In the crab mince gels, deformation was affected only by the number of washes and not the sodium chloride content. Gels made from dewatered mince exhibited significantly ($p < 0.05$) greater deformation than either of the washed sample gels (Fig. 2).

3.4. Colour

The L^* values were not significantly affected by the number of washings but increased significantly ($p < 0.05$) with the addition of sodium chloride. The lack of change in L^* values in response to increased washing was surprising, given that washing usually increases L values in raw surimi. Gels with 4% sodium chloride had average L^* values of 62.4 whereas those with no or 2% sodium chloride had average L^* values of 61.2 (Fig. 3).

A^* values (redness) were significantly ($p < 0.05$) affected by both the number of washing cycles and the addition of sodium chloride. Dewatered mince gels were more red (13.9) than those washed once (13.3) and those washed three times (10.8) (Fig. 3). In the previous work done with cooked crab mince, a^* values increased with washing, which is contradictory to the current results. Washing reduced the redness whereas the addition of sodium chloride increased the redness. Gels with 4% sodium chloride were significantly more red (12.9) than those with no sodium chloride (12.4).

Although yellowness (b^* value) was not affected by addition of sodium chloride, it was significantly affected by the

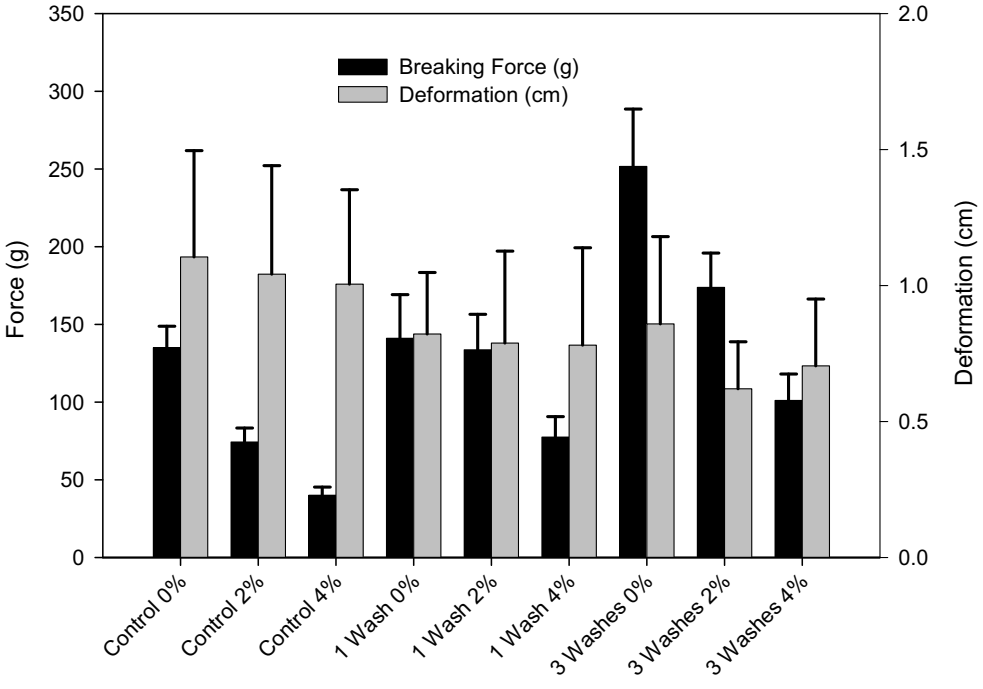

Fig. 2. Breaking force (g) and deformation (cm) of crab mince gels as affected by sodium chloride level (0%, 2%, and 4%) and number of washes (0, 1, and 3). Values shown are averages of 9 measurements with standard deviations.

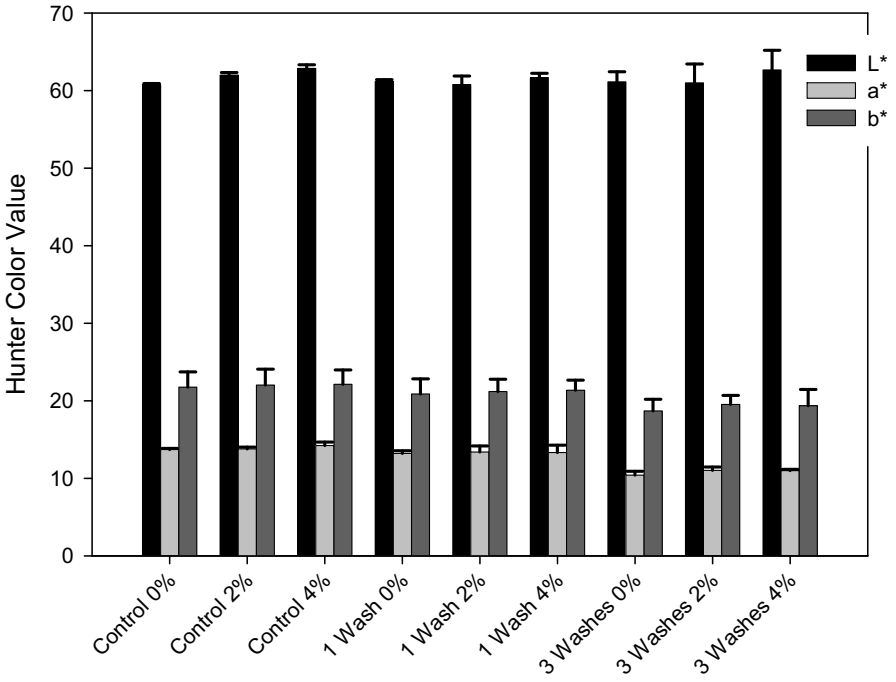

Fig. 3. Hunter colour values (L^*, a^*, b^*) of crab mince gels as affected by sodium chloride level (0%, 2%, and 4%) and number of washes (0, 1, and 3). Values shown are the average of three treatments with standard deviations.

number of washings. Gels made from mince washed three times were significantly ($p < 0.05$) less yellow than those washed once or only dewatered. Average b^* values for gels made from mince washed once or from dewatered mince ranged from 21.1 to 21.9, whereas average b^* values for gels from mince washed three times was 19.1 (Fig. 3).

Muscle tissue colour is dependent on season, age of crabs, and dietary composition. In surimi, colour is an important quality indicator (Lanier, 1992). The lighter the washed muscle, the higher the quality of surimi. In washed crab mince, colour may not be as important. Crabs and other crustaceans contain the blue coloured oxygen-carrying pigment hemocyanin as well as blue and red coloured carotenoproteins, which may affect the colour of the mince (Garcia-Carreno, Gollas-Galvan, del-Toro, & Haard, 1999; Terwilliger, Ryan, & Towle, 2005). Browning may also occur during the cooking process that may influence the colour of the crab meat during processing (Boon, 1975). The colour of the washed crab mince may not be as important of a quality indicator as it is in surimi processing. In fact, for new food product development, a more intense colour of the meat may enhance the product properties.

3.5. Protein molecular weight

Proteins in the crab mince, gels, and wash water analyzed using SDS–PAGE. SDS–PAGE gels (Fig. 4) indicated that the crab mince gels consisted primarily of proteins in the 190 kDa range, 75–100 kDa range, and 36–45 kDa range. The wash water contained a protein

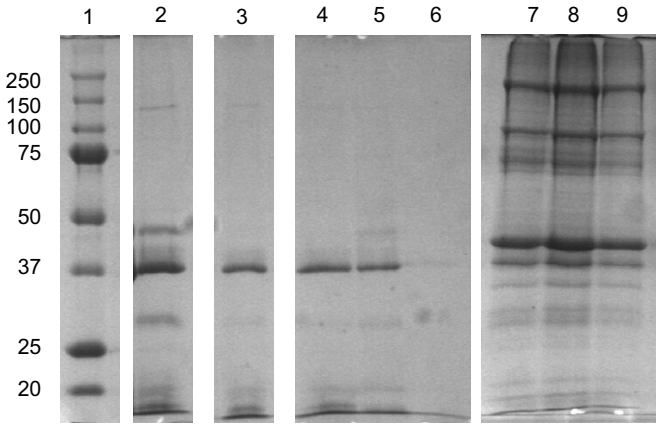

Fig. 4. SDS–PAGE analysis of wash water and gel from previously cooked Jonah crab meat. Lane 1 is the molecular weight standard. Lane 2 is the supernatant from the dewatering step. Lane 3 is the supernatant recovered from one wash. Lanes 4–6 are the supernatants recovered from each of the three washes. Lanes 7–9 are the gels from mince washed three times with 0%, 2%, and 4% sodium chloride added, respectively.

band at 37 kDa, identified as likely one strand of the tropomyosin molecule, with some small proteins in the 18–20 kDa ranges. The band at 190 kDa was probably the myosin heavy chain (Thorarinsdottir, Arason, Geirsdottir, Bogason, & Kristbergsson, 2002). As the major protein involved in meat gelation, the presence of myosin heavy chain in the gels was expected. The banding in the 36–45 kDa range was believed to contain both the actin and tropomyosin. The actin, which is also integral to gelation, was expected in all of the gels. The band identified as likely

tropomyosin, 37 kDa, was found in both the gels and wash supernatant. French (1986) found that the stiffness of the Alaska pollock surimi gels was inversely correlated to tropomyosin content. The relationship between tropomyosin content and breaking force was also seen in the crab mince gels. The breaking force of the gels increased with increasing number of wash cycles, resulting in less tropomyosin within the gel. The protein bands in the 18–20 kDa range in the gels and wash supernatant were correlated with myosin light chain and troponin (Thorarinsdottir et al., 2002).

Interestingly, the major protein component removed in the wash water was tropomyosin, as such, it is suggested that tropomyosin may be the main protein component interfering with gelation of cooked crab proteins. Some smaller muscle components, such as myosin light chain and troponin, were also removed during washing, though at smaller concentrations. The first two washing cycles removed mostly tropomyosin, troponin, and myosin light chain. The myosin was most likely present in the wash water due to the freezing treatment, though further research is necessary to determine the cause. Future research will focus on identifying the specific proteins removed and their function in the crab protein gels.

4. Conclusions

Protein gels were formed using previously cooked proteins from Jonah crab (*C. borealis*) minced meat. A three wash treatment resulted in the gels with the best gel strength and water-holding capacity. Given that little protein was removed in the third washing cycle, it was determined that two wash cycles will be adequate for future gelation studies. The improved characteristics due to washing are likely due to the removal of tropomyosin, troponin, and myosin light chain in the first two washes that may interfere with protein–protein interactions involved in gel formation. The washed gels had similar water-holding capacity and breaking force similar to that of other cooked crab mince gels. Increased sodium chloride concentration decreased the breaking force contrary to gelation results common for raw fish proteins. The addition of sodium chloride was detrimental to all physical gel characteristics and is not recommended for gelation of cooked proteins. With regard to colour, the whiteness values were not affected by washing, whereas the redness and yellowness values decreased. Based on these results, washed crab mince from previously cooked Jonah crab meat can produce gels with water-holding capacity and gel strength similar to those of surimi from some raw fish but without the addition of sodium chloride.

The gelation properties of the previously cooked crab mince suggest that it can be used as a major component in protein gel-based products. The ability of cooked Jonah crab minced meat to form gels upon washing will enable future product development endeavors to develop a value-added product with crab mince as the primary ingredient.

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