Effect of washing treatment and microbial transglutaminase on the gelling properties of blue crab (*Callinectes sapidus*) proteins

Verónica Hernández-Robledo $^a$, Miguel Ángel Martínez Maldonado $^b$, Rocío M. Uresti-Marín $^a$, José A. Ramírez $^{a,a}$ and Gonzalo Velázquez $^{a,b}$

*Dirección General de Innovación Tecnológica, Universidad Autónoma de Tamaulipas. Edificio Centro de Excelencia, Centro Universitario. CP 87040, Ciudad Victoria, Tamaulipas, México; $^a$Instituto Politécnico Nacional. CICATA unidad Querétaro, Cerro Blanco 141. Colinas del Cimatario, CP 76090. Santiago de Querétaro, Querétaro, México

**ABSTRACT**

The effect of washing cycles and microbial transglutaminase on the gelation properties of crab meat was evaluated. The blue crab (*Callinectes sapidus*) was cooked at 120°C for 30 min before obtaining the meat. Cooked meat was homogenized in a cutter with 0% (control) or 0.5% microbial transglutaminase (MTGase), stuffed in steel tubes and set at 40°C for 30 min before cooking at 90°C for 15 min. Control samples were cooked directly at 90°C. Changes in color, mechanical properties (puncture test) and water holding capacity (WHC) were evaluated. Adding MTGase improved the mechanical properties of crabmeat gels after one or three washing cycles in both cooking processes (setting and cooked directly). The results suggest that a single washing process produces crab meat gels with good mechanical properties while maintaining soluble proteins, responsible for the distinctive flavor of crabmeat.

**1. Introduction**

Gelling is one of the most important functional properties of muscular proteins, because of the technological and commercial benefits when developing new products. The main proteins involved in gelling of muscle meals are myosin, actin and actomyosin, which confer the mechanical and textural properties of the gels (Ramírez, Uresti, Velázquez, & Vázquez, 2011). Soluble proteins confer characteristics of flavor, odor and color but also they have been reported for interfering with the gelling mechanism, inducing the formation of weaker gels. Gelling of myofibrillar proteins from most animals involves three consecutive steps: protein solubilization with salt (1 to 2%); denaturation, usually by heating and the ordered aggregation of denatured proteins during the heating process and reinforced during the final cooling (Martínez et al., 2014). There is a generally accepted premise that myofibrillar proteins must be in native state in order to obtain gels with appropriated mechanical properties. Inducing the denaturation/aggregation of muscle proteins before gelling inhibits the formation of the tridimensional structure responsible for the mechanical and functional properties of gels, and in some cases, when aggregation is extensive, the formation of gel is not feasible (Byrem & Strausburg, 2000). Despite this, recently, it has been reported the muscle proteins of several species of crabs can gel after a heating process that is applied to facilitate the removal of the meat from the shell. This behavior is different from other animals commonly used to obtain commercial food gels. In this regard, Baxter (2007) reported that meat extracted from previously cooked Jonah crab (*Cancer borealis*) was able to gel after removing the soluble protein by washing the cooked meat in cold water. The myofibrillar proteins from the Jonah crab, obtained after three washing cycles, did not require solubilization with salt to form a thermally induced gel (Baxter & Skonberg, 2008). Recently (Martínez et al., 2014;
Hernández-Robledo, Uresti, Martínez-Maldonado, and Velázquez, 2013) it has been reported that previously cooked meat from the blue crab (Callinectes sapidus) was able to gel without adding salt, after removing the soluble proteins in three washing cycles. These authors reported that the gels obtained from crabmeat cooked at 120 °C for 30 min showed better mechanical properties than those obtained after cooking the crabmeat at 50–70 °C for 30 min. The improving effect of the mechanical properties of surimi gels has been associated with an increasing effect on the concentration of myofibrillar proteins considered responsible of the gelling phenomenon of muscle proteins. On the other hand soluble proteins do not form gels (Paking and Mataá, 2011).

Mechanical and functional properties of myofibrillar gels can be improved with several additives. The use of microbial transglutaminase (MTGase) has been recognized as an efficient strategy for improving the mechanical properties of protein-based gels (Téllez-Luis, Ramírez, & Vázquez, 2004). MTGase has also improved the mechanical properties of gels from low commercial value fish species such as striped mullet (Mugil cephalus) allowing production of surimi gels with appropriate texture (Ramírez, Rodríguez-Sosa, Morales, & Vázquez, 2000). Also, MTGase has been reported as useful for increasing the mechanical properties of beef gels and restructured products obtained from previously washed and mechanically deboned chicken meat (Castro-Briones, Calderón, Velázquez, Salud-Rubio, Vázquez, & Ramírez, 2009); and it has been used in different species such as squid, rabbit, pork and turkey (Sun & Holley, 2011).

MTGase improved the mechanical properties of gels from blue crab when added at 0.6% (Martínez et al., 2014). The authors reported that an incubation period of 30 min at 40 °C, before cooking at 90 °C for 15 min, improved the efficiency of MTGase to increase the mechanical properties of gel. Also, Galetti (2010) found that MTGase added at 2% was able to improve mechanical properties of patties obtained from raw (non-washed) crabmeat of the European green crab (Carcinus maenas). Blue crab meat has a unique and highly appreciated flavor which should be preserved to obtain restructured products with good commercial acceptance, but three washing cycles remove almost all the soluble proteins and consequently most of the flavor. The objective of this study was to determine the effect of microbial transglutaminase on the gelling properties of previously cooked blue crab meat after one or three washing cycles and setting the treatment before cooking at 90 °C.

2. Material and methods

2.1. Raw material

Meat of blue crab (Callinectes sapidus) was obtained from Integradora Pesquera Comercial Acuícola (IPESCA) located in San Fernando, Tamaulipas, Mexico. Fresh crabs were kept alive and processed in plant within 12 h after being caught. The blue crab was precooked at 120 °C for 30 min in a commercial autoclave and immediately cooled with clean water. Meat was removed manually from the shell, placed in plastic receptacles and transported on ice to the laboratory.

2.2. Washing the crabmeat

Cooked crabmeat was processed within 4–6 h after being received by the laboratory. Crabmeat was washed one or three times with three volumes of ice-cold water (less than 4 °C). Each washing process consisted in stirring the crabmeat gently in water for 7 min. After each washing cycle the water was removed using a cotton cloth and soft manual pressing. Unwashed crabmeat was used as the control.

2.3. Preparation of crabmeat gels

Gels for each treatment were obtained by chopping 0.5 kg of crabmeat in a 5.5 L capacity cutter (Hobart, Model 84145, Troy, OH) for 3 min. MTGase (Active TG-Ti, Ajinomoto USA, Inc., Teaneck, NJ) was added at 0 (control) and 0.5% into the crabmeat paste in a dry form. The temperature of the paste remained below 15°C throughout the chopping operation for all treatments. The homogenized paste was stuffed into stainless steel tubes (1.8 cm inner diameter; 17.7 cm long), which were previously sprayed with commercial vegetable oil to prevent sticking. Tubes were capped before immersion in water at 40 °C for 30 min followed by immersion in water at 90°C for 15 min. Controls heated directly at 90 °C for 15 min were also processed. After cooking, the tubes were placed in a refrigerated water bath, and cooled at 4–5 °C for 30 min. Restructured crabmeat products were removed from the tubes and stored overnight at 4 °C in polyethylene bags before analyzing them.

2.4. Puncture test

The puncture test was performed compressing samples of 30 mm in height and 18 mm in diameter to 75% of the initial height using a spherical probe (P/20) with a 1.2 cm diameter and crosshead speed of 1 mm/s. The breaking force (N), deformation (mm) and work of penetration (N/mm) for each treatment were calculated. Samples were placed on the base of the texturometer (TA-XT2i Stable Micro Systems Texturometer;Vienna Court, UJ), ensuring that the spherical probe reached the sample at the center. Six samples were analyzed for each treatment.

2.5. Expressible water

Expressible water was determined as described by Martinez et al. (2014). The amount of expressible water for each treatment was measured. Cooked gel samples (3 ± 0.1 g) were weighed and put between two layers of filter paper (Whatman No. 1). Samples were placed at the bottom of 50 mL centrifuge tubes and centrifuged at 1 500g for 5 min at 15°C. Immediately after centrifugation, samples were removed and reweighed. The amount of expressible water was calculated as follows:

\[ EW = \frac{(Wi - Wf)}{Wi} \times 100 \]
Where EW is the percentage of expressible water. Wi is the initial weight and Wf is the final weight. Six samples were analyzed for each treatment and averages were reported.

2.6. Color attributes

Spectral reflectance of crabmeat gels was determined using a MiniScan XE Plus spectrocolorimeter (HunterLab, model 45/0-L; Hunter Associates, Reston, VA) calibrated against black and white tiles. Commission Internationale de L’Eclairage (CIE) L*, a* and b* values, chrome (C, sqrt(a^*^2 + b^*^2)) and hue angle (H, arc tan b*/a*) were calculated based on illuminant C and the 2º standard observer.

2.7. Statistical analysis

Data were analyzed using Statgraphics v5 software (Manugistics, Inc., Rockville, MD, USA). A multifactorial analysis of variance was applied. Differences among mean values were established using the least significant difference (LSD) multiple range test and were considered significant when P<0.05.

3. Results and discussions

3.1. Effect of washing cycles on the mechanical properties of gels

The behavior in the puncture test parameters of crabmeat gels obtained by cooking directly at 90 ºC without setting, is shown in Figure 1. Gels obtained from unwashed cooked crabmeat without MTGase showed the lowest values of breaking force (BF) (6.24 N). The washing improved the breaking force of gels, reaching a maximum value of 14.02 N by using a single washing cycle, but there was no difference (P < 0.05) by using one or three washing cycles (Figure 1). Control gels obtained from unwashed crabmeat without MTGase showed a low value of deformation (10 mm) (Figure 1). This mechanical property was improved only by using three washing cycles, reaching a significantly higher value of 15 mm (P < 0.05). The gel strength (GS) was significantly improved (P < 0.05) from 62.87 N/mm in the control unwashed gels reaching a maximum value of 211.1 N/mm by using a three washing cycle treatment.

The mechanical properties of gels obtained from unwashed and washed crabmeat by incubating the samples at 40 ºC for 30 min before cooking at 90 ºC for 30 min are shown in Figure 2. Control gels obtained without MTGase showed low values of BF (6.3 N). This parameter was not significantly improved (P < 0.05) by using one or three washing cycles. Deformation was significantly improved (P < 0.05) from 11 mm in the control gels to 14 mm in the gels obtained by using a three-cycle washing treatment. The GS properties of these gels were not improved significantly (P < 0.05) by the washing treatment before gelling (Figure 2).

Cooked meat from blue crab was able to produce weak gels with no salt added, and the gels could become stronger after a three-cycle cold-water washing process as previously reported by Martínez et al. (2014). Similar results were reported for Jonah crab (Baxter & Skonberg, 2006; Baxter & Skonberg, 2008). Results in this study suggest that it is feasible to obtain gels from cooked crabmeat in a single washing step. However, according to our experience, the mechanical properties of such gels, obtained by cooking directly at 90 ºC or by using a previous setting process at 40 ºC before cooking, are at a lower level than the minimum expected texture for commercial restructured products. In this regard, fish gels obtained using a similar process, with the same geometry and tested under similar conditions, required GS values higher than 294 N/mm to show an acceptable consistency (Téllez-Luis, Uresti, Ramírez, & Vázquez, 2002). The GS value can be improved by using different additives (Uresti, Téllez-Luis, Ramírez, & Vázquez, 2004).

3.2. Effect of washing cycles and MTGase on mechanical properties of gels

Control gels obtained from unwashed crabmeat and without MTGase showed no significant difference in BF, deformation and GS values when compared to gels obtained with a similar treatment but adding 0.5% MTGase (Figure 1). Cooked crabmeat gels showed higher BF, deformation and GS properties (P < 0.05) by combining washing cycles and
Efecto del lavado y la transglutaminasa microbiana en la capacidad de deformación y fuerza de gel de geles de carne de jaiba obtenidos por retención de agua de geles de carne de jaiba obtenidos por cocción.

Para la determinación de deformación y fuerza de gel se realizaron tres ciclos de lavado para cada nivel de MTGase. Las diferencias (P < 0.05) entre tratamientos de lavado se indican con letras distintas. Las barras indican el error estándar. La EW de gels obtenidos por directa cocción a 90 ºC aumentó significativamente (P < 0.05) de 12.8% en los control comparados a 18.6% en gels obtenidos después de tres ciclos de lavado, pero sin MTGase (Figura 3). El EW de gels obtenidos a 90 ºC con 0.5% MTGase no fue significativamente diferente (P < 0.05) que de 9.5 a 12.8% (Figura 3).

3.3. Expressible water

El Water Extracted (EW) es un método indirecto para medir la capacidad de retención de agua (WHC) de gels como el porcentaje mínimo de la expresión de agua correspondiente a la WHC más baja (Ramírez, Rodriguez, Uresti, Velázquez, & Vázquez, 2007). El EW de gels obtenidos por un tiempo de cocción de 90 ºC aumentó significativamente (P < 0.05) un 12.8% en los control comparados a 18.6% en gels obtenidos después de tres ciclos de lavado, pero sin MTGase (Figura 3). El EW de gels obtenidos a 90 ºC con 0.5% MTGase no fue significativamente diferente (P < 0.05) que de 9.5 a 12.8% (Figura 3).

Figure 2. Efecto de la transglutaminasa microbiana y lavado en la fuerza de ruptura, deformación y fuerza de gel de geles de carne de jaiba obtenidas por incubación a 40 ºC por 30 min antes de cocerlas a 90 ºC durante 15 min. Valores promedio de tres repeticiones. Las barras indican el error estándar. Letras distintas indican diferencias significativas (P < 0.05) entre tratamientos de ciclos de lavado para cada nivel de MTGase.

Figure 3. Efecto del lavado y la transglutaminasa microbiana en la capacidad de retención de agua de geles de carne de jaiba obtenidos por cocción directa a 90 ºC por 15 min. Valores promedio de tres repeticiones. Las barras indican el error estándar. Letras distintas indican diferencias significativas (P < 0.05) entre tratamientos de ciclos de lavado para cada nivel de MTGase microbiana.
The EW values of gels obtained after incubation at 40 °C before cooking showed a similar behavior with gels obtained by cooking directly at 90 °C (Figure 4). Gels obtained without MTGase and with no washing treatment showed an EW value of 13.6%. This parameter was improved by using a three-cycle washing reaching an 18.5% value. The EW of gels obtained with 0.5% MTGase ranged from 13.1 to 13.9% without significantly differences (P < 0.05) among treatments.

The values for WHC reported in this study seem to be higher than previously reported. The WHC value for Johan crab gels, obtained after three washings was 68.5% (Baxter & Skonberg, 2006) and 70.9% (Baxter & Skonberg, 2008). Recently, Martínez et al. (2014) reported a 51.2% of expressible water in crabmeat gels from blue crab, using three washings.

### 3.4. Color

The color attributes of crabmeat gels were significantly (P < 0.05) affected by the washing process, but they did not change by adding MTGase or the setting period (Table 1 and Table 2). Using one washing cycle improved the L* attribute and decreased the C* value indicating a less intense color of the gels. The H* attribute of all gels ranged from 74.9 to 76.5, indicating a yellowish color. Recently (Martínez et al., 2014) it has been reported that crabmeat gels from blue crab obtained after a three washing cycle showed values of L*, C* and H* of 66, 9.1 and 93, respectively, indicating that extensive washing removes more pigments and the obtained gels have a paler yellowish color.

### 4. Conclusions

The results obtained demonstrated that it is feasible to obtain gels from cooked crabmeat by using a single cold-water washing step to partially remove the soluble proteins. Although the mechanical properties of gels obtained after a single washing step were lower than the mechanical properties of gels obtained from a three washing cycle, the addition of 0.5% MTGase improved the texture of gels. The effect of adding MTGase was higher when a setting period of 30 min at 40 °C was used before cooking. The reason for the gelling of previously cooked crabmeat remains unclear, as does the inhibiting effect of soluble proteins on the MTGase activity resulting in poorer mechanical properties. Further research work is required to understand both phenomena. This study opens the feasibility of obtaining crabmeat-restructured products, such as nuggets, preserving the unique flavor of this species.
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Disclosure statement

No potential conflict of interest was reported by the authors.

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ORCID

Verónica Hernández-Robledo http://orcid.org/0000-0002-5283-9858
Miguel Ángel Martínez-Maldonado http://orcid.org/0000-0003-1618-4185
Rocio M. Uresti-Marín http://orcid.org/0000-0002-6918-6187
José A. Ramírez http://orcid.org/0000-0001-7971-7044
Gonzalo Velázquez http://orcid.org/0000-0003-1901-9919

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