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Gelling properties of blue crab (*Callinectes sapidus*) meat added with gelatin and microbial transglutaminase

Propiedades de gelificación de carne de jaiba (*Callinectes sapidus*) adicionada con gelatina y transglutaminasa microbiana

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RESUMEN
Las proteínas de la carne de cangrejo generalmente se separan de su caparazón después de la cocción, lo que implica una agregación térmica tipo coágulo, que induce problemas relacionados con su capacidad de retención de agua, lo que limita sus alternativas de procesamiento posteriores. El objetivo de este estudio fue determinar el efecto de la adición de gelatina y transglutaminasa microbiana (MTgasa) sobre las propiedades de geles obtenidos de carne de jaiba azul (*Callinectes sapidus*) previamente cocida. La carne de jaiba que se sometió a 3 ciclos de lavado-prensado con agua fría, utilizando una relación 3:1 agua-carne y se mezcló con 0 a 3 % de gelatina y 0.5 % de MTgasa, se embutió en tubos de acero inoxidable y se cocieron a 90 ºC durante 15 min en agua caliente. Se determinaron las propiedades mecánicas (análisis del perfil de textura, módulo de Young y tensión máxima), color, agua extraíble e imágenes micrográficas (SEM). Los resultados mostraron que la gelatina interaccionó con las proteínas de la carne de jaiba y la MTgasa utilizó ambas proteínas como sustrato para formar una red estructurada. Las propiedades mecánicas del gel dependieron del nivel de gelatina añadido, con mejores resultados a menor cantidad. La cantidad de agua extraible se redujo mediante la adición de 3 % de gelatina.
Palabras clave: carne de jaiba, gelatina, transglutaminasa microbiana proteína muscular, gelificación.

ABSTRACT
Crab meat proteins are usually separated from shells after cooking, which induces a thermal coagulum-type aggregation affecting the water-holding capacity and limiting further processing alternatives. This study aimed to evaluate the effect of adding gelatin and microbial transglutaminase (MTgase) on the properties of gels obtained from cooked blue crab (*Callinectes sapidus*) meat. Crab meat was subjected to 3 washing-pressing cycles with cold water using a 3:1 (water:meat) ratio, mixed with 0 to 3 % gelatin and 0.5 % MTgase, stuffed into stainless steel tubes, and cooked by immersing in hot water at 90 ºC for 15 min. Mechanical properties (texture profile analysis, Young modulus, and maximum stress), color, extractable water, and micrograph images (SEM) were obtained. Results showed that gelatin interacted with crab proteins, and MTgase used both proteins as substrates to form a structured network. The mechanical properties of gels depended on the level of gelatin added, with better results at a lower amount. The amount of extracted water was reduced by adding 3 % of gelatin.
Keywords: crab meat, gelatin, microbial transglutaminase, muscle protein, gelation.

INTRODUCTION
Muscle protein gelling is a widely used technological process for developing different meat, poultry, and fish products. Different conditions used in the process, such as temperature, pH, salt level, or additives, allow modifying the functional properties of the final products to develop or improve quality parameters like texture, color, flavor, or stability (Ramírez *et al*., 2011).

Solubilization with salt and thermal denaturalization (unfolding) of the non-cooked muscle protein are required to form gel products (Uresti *et al*., 2005; Castro-Briones *et al*., 2009). However, meat extracted from crabs cannot follow such steps since these organisms are processed by high thermal treatment, inducing the denaturation and aggregation of proteins before being separated from the shell (Martínez *et al*., 2014). These steps hinder any further processing alternative for this important and abundant natural resource, with particular interest in the blue crab (*Callinectes sapidus*) of the Gulf of Mexico (Rodríguez-Castro *et al*., 2017; Morales-Azpetia *et al*., 2021), different than canning (Bouriga *et al*., 2023) or freezing (Ghribi *et al*., 2023) both with highly appreciated market for consumers. Different processes have been explored to solve this situation.

Baxter and Skonberg (2006) reported that cooked meat from Johan crab (*Cancer borealis*) could form gels using a 2-cycles washing-dewatering process, similar to the widely used technique to obtain surimi products. Similar behavior, but with weak gels, was obtained when cooked crab meat was centrifugated to remove the water-soluble protein fraction (tropomyosin being the most abundant extracted protein) (Baxter and Skonberg, 2008). Other processing techniques have been reported, such as a different cooking

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temperatures of crab meat in the range of 50 to 120 °C, with better mechanical properties in gels obtained at higher precooking temperature (Martínez *et al*., 2014). microbial transglutaminase (MTgase) at 0.5 or 1.0 % has been reported as an alternative to reduce the washing-dewatered pressing cycles to just one, which still affects the characteristic flavor of the crab by removing the water-soluble fraction of meat (Hernández-Robledo *et al*., 2015; 2017). Gels with appropriate texture from cooked crab meat without removing the water-soluble fraction were obtained by mixing crab meat with MTgase at 60 °C (Trejo-Díaz *et al*., 2021). However, all these methods using thermally induced gelling of crab meat result in gels with poor water holding capacity.

The objective of this work was to determine if gelatin combined with MTgase can improve the water holding capacity of cooked crab meat proteins without affecting the mechanical properties of the gels.

MATERIAL AND METHODS

Raw material

The blue crab *(Callinectes sapidus)* was captured in Laguna Madre, Tamaulipas, transported, and processed at the facilities of the company "Integradora Pesquera Acuícola" located in San Fernando, Tamaulipas (México) within 24 h after caught. The crabs were cooked using a commercial autoclave and cooled with clean water. The crab meat was removed from shells, introduced into a plastic recipient, and transported on ice to the laboratory for further analysis.

Additives

Gelatin (GP 28, 290 bloom, Coloidales Duché, S.A DE CV. Ciudad de México, México) was purchased at a local market. The commercial microbial transglutaminase was Active TG-TI (Ajinomoto USA, Inc., Teaneck, NJ).

Meat processing

Cooked and minced crab meat was washed with water at a 3:1 (water: meat) ratio for 10 min at 4 °C to dissolve soluble proteins, drained with a cotton fabric filter to remove the excess water containing the soluble compounds and stored in 250 g portions in polyethylene bags until analyzed.

Preparation of gels

Following the methodology reported by Hernández-Robledo *et al*. (2017), the meat was mixed with gelatin (0, 0.5, 1, or 3 %) and MTgase (at 0 % as control or 0.5 %) using a food processor (Hamilton Beach Model 72860-MX, Glen Allen, VA, USA) for 3 min and stuffed into stainless steel tubes with cap in both extremes, closed, and immersed in hot water at 90 °C for 30 min in a water bath with temperature control (Techne Tempette Junior TE-8J). After cooking, the tubes were immersed in ice water (0 °C) and stored at 4 °C until analyzed (less than 16 h).

Mechanical properties

A texturometer (Model TA Plus, Lloyd Instruments, Ametek, UK) was used to perform the uniaxial compression test. Cylindrical gel samples (25 x 30 mm) were compressed to 75 % at 1 mm/s crosshead speed using a 50 mm aluminum probe (P50). The test was carried out at room temperature. Two compression cycles were used for texture profile analysis (TPA). The hardness, cohesiveness, springiness, and chewiness parameters were calculated from the TPA curves. From the first compression cycle, stress-strain plots were obtained, and the Young modulus and maximum stress were calculated. Six replicates were analyzed for each treatment.

Colorimetry

The L*, a*, b* CIELab color parameters of the samples were determined using a colorimeter (Bluemetric, model BLUE-HP200, SA de CV, Monterrey, Mexico) in four random points on each gel sample. From the measurements, a*, b*, L*, and the color attributes Hue* and Chroma* were calculated.

Expressible water

A 3 g sample from the crab meat gels was wrapped in 5 paper sheets of 6 cm x 6 cm, placed in 50 mL conical centrifuge tubes, and centrifuged at 15,000 rpm for 15 min (Rotofix 32 A. Hettich Zentrifugen, Tuttlingen, Germany) following the methodology reported by Hernández-Robledo *et al*. (2017). Subsequently, the final weight of the sample was registered. Four repetitions per treatment were performed. The EW was calculated using Equation 1.

$$EW = (Wi-Wf\ /Wi) \times 100 \quad \text{(Eq. 1)}$$

Where:
EW = amount of extracted water.
Wi = Initial weight.
Wf = Final weight.

Scanning Electron Microscopy (SEM)

The crab meat gels were freeze-dried and ground. A sample was fixed on an aluminum pin using conductive double-sided tape and then observed in an ambient scanning electron microscope (Phenom Pro., Phenom-World BV, Eindhoven, The Netherlands).

Experimental design and analysis

Data were analyzed using a factorial ANOVA and the post hoc Tukey test was performed to evaluate the significant effects of the treatments. Results are presented as mean ± standard deviation, and differences were considered statistically significant at a $p < 0.05$. Also, multiple regression analysis was performed to model the response of the gel properties as a function of the percentage of gelatin and MTgase using a second-order polynomial equation (Design Expert® v10, Stat-Ease, Inc., Minneapolis, MN, USA). Coefficients of the terms in the polynomial equation were calculated and 3D plots were used to describe the behavior of the analyzed properties.

Table 1. Experimental design.

Factors	Levels
Gelatin concentration	0, 0.5, 1 and 3 %
MTgase concentration	0 % and 0.5 %

RESULTS AND DISCUSSION

The heating process allowed gelling the crab meat despite meat proteins were previously denatured and aggregated due to the processing conditions required to separate of the meat from the shell.

Regression analysis

The correlation values obtained from the multiple regression analysis are shown in Table 2. Hardness, cohesiveness, and chewiness showed R^2 and adjusted R^2 values higher than 0.9, indicating that the model fits adequately to data and that the model could be appropriate for future data.

Young modulus maximum stress, and expressible water fitted appropriately to the model ($R^2 > 0.9$). The differences between R^2 and adjusted R^2 values are lower than 0.2 indicating that the terms included in the polynomial models allow describing the effect of the parameters on these properties.

The model obtained for springiness did not fit well at any of both parameters, indicating that his property was not a good indicator to observe changes in mechanical properties after adding gelatin. Springiness is not the same as elasticity, because this test may involve the fracture of the sample affecting its capacity to return to its original form (Rahman *et al*., 2021). Young modulus, on the other hand, is associated with elasticity because it considers the compression values (stress and strain) involved in the linear region of the test implying only a small deformation (Lam *et al*., 2017) that does not fracture (macro or micro) the sample, thus its deformation theoretically is reversible and the sample should return to its original shape.

The analysis of variance (ANOVA) for the fitting of the gelatin and MTgase effect on mechanical and extractable water is shown in Table 3. Models differ significantly (at $p < 0.05$) for changes induced in hardness, cohesiveness, chewiness, Young modulus, and maximum stress. Gelatin significantly affected these parameter ($p < 0.05$) except for expressible water. However, MTgase did not affect any parameter, and there was no significant effect on the interaction of gelatin and MTgase on any studied property. The quadratic term (A^2) suggests that gelatin had a higher effect on the mechanical properties than in expressible water.

Mechanical properties

Figure 1 shows the changes in the texture profile analysis parameter. The hardness of gels was not affected by the MTgase level (0 to 0.5 %); however, it was negatively affected by the addition of low levels of gelatin (less than 2 % as calculated for the model). Gelatin added at 3 % improved the hardness

Table 2. Coefficients of the regression model for hardness (N), cohesiveness, springiness, chewiness (N), Young modulus (MPa), maximum stress (MPa) and expressible water, of gels from crab meat added with gelatin and MTgase.

Factor	Hardness (N)	Cohesiveness	Springiness	Chewiness (N)	Young modulus (MPa)	Maximum stress (MPa)	EW (%)
				Gelatin			
Intercept	11.77	0.2169	0.7402	0.6142	78.93	17.9	11.72
A-Gelatin	-8.19	-0.0788	-0.0022	-4.14	36.9	9.06	-9.47
B-MTgase	3.39	-0.0059	0.0144	0.7807	7.93	1.2	1.96
AB	-2.56	-0.0163	0.0031	-1.11	4.65	2.55	0.2864
A^2	26.46	0.1092	-0.0122	8.97	4.52	10.49	10.36
R^2	0.9595	0.9658	0.435	0.9595	0.9356	0.9499	0.8933
Adjusted R^2	0.9055	0.9203	-0.3183	0.9055	0.8497	0.8832	0.7511

Table 3. Statistical significance (p-value) for hardness, cohesiveness, springiness, chewiness, Young modulus, maximum stress, and expressible water of gels from crab meat added with gelatin and MTgase obtained from the ANOVA of the regression model.

Factor	Hardness (N)	Cohesiveness	Springiness	Chewiness (N)	Young modulus (MPa)	Maximum stress (MPa)	EW (%)
				Gelatin			
Model	0.0199*	0.0155*	0.7018	0.0199*	0.0393*	0.0272*	0.0815
A-Gelatin	0.0206*	0.0037*	0.881	0.0089*	0.0091*	0.0116*	0.0187*
B-MTgase	0.0946	0.4810	0.2551	0.2333	0.1908	0.4133	0.3009
AB	0.2420	0.1753	0.8265	0.1904	0.4871	0.2045	0.8933
A^2	0.0054*	0.0106*	0.6784	0.0071*	0.7356	0.0491*	0.0843

*Indicates statistical significance.

of gels, but it was not enough to recover the initial parameters of samples obtained without this hydrocolloid (Figure 1A).

Cohesiveness (Figure 1B) and chewiness (Figure 1D) showed a similar behavior to hardness, with an initial decrease as affected by the addition of 2 % gelatin and a slight increase when added at 3 %. As mentioned previously, when modeling was discussed, an interaction between MTgase and gelatin was not observed. Crab gels showed relatively high values of springiness (72 %), and this parameter was not affected by the addition of MTgase, gelatin, or their combination (Figure 1C).

Small changes in mechanical properties of crab meat gels, when 0.6 % MTgase was added and cooked directly at 90 °C (by immersion in hot water), were reported previously. However, better mechanical properties were found when gels from crab meat containing MTgase were incubated at 40 °C for 30 min. This behavior was associated with side-to-side protein aggregations promoted by covalent bondings of adjacent proteins as a result of MTgase activity during incubation at 40 °C (Martínez *et al*., 2014).

The disruptive effect of gelatin on the mechanical properties was also observed in gels obtained from Alaska pollock surimi (Hernández-Briones *et al*., 2009).

Young modulus (Figure 2A) and maximum stress (Figure 2B) showed a different behavior than TPA parameters. These mechanical properties increased when both MTgase and gelatin were added, and showed a combined effect when added at their maximum level (0.5 % and 3 %, respectively). Differences between both mechanical parameters are associated with the basis of each test. TPA simulates the textural perception in the mouth while a meal is being chewed, while Young modulus and maximum stress, as mentioned previously, are fundamental tests (Rahman *et al*., 2021).

Expressible water

The percentage of water extracted was reduced by 20 % by adding up to 1.5 % gelatin (Figure 3). Gelatin is a hydrocolloid that is characterized by the ability to trap water, which can help crab meat gel to improve its water-holding capacity

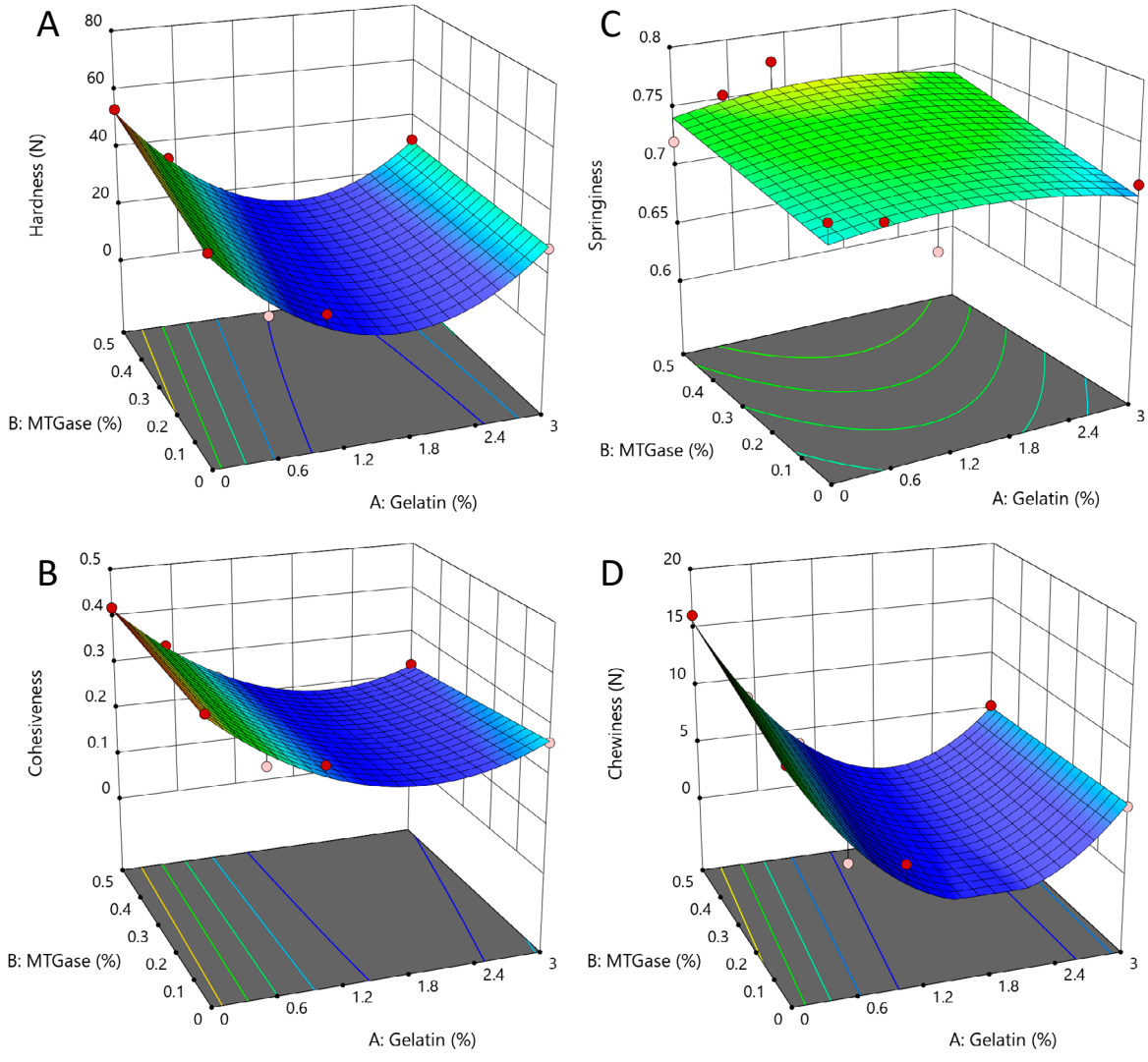

Figure 1. Effect of MTgase and gelatin on the TPA parameter values of crab meat gels.

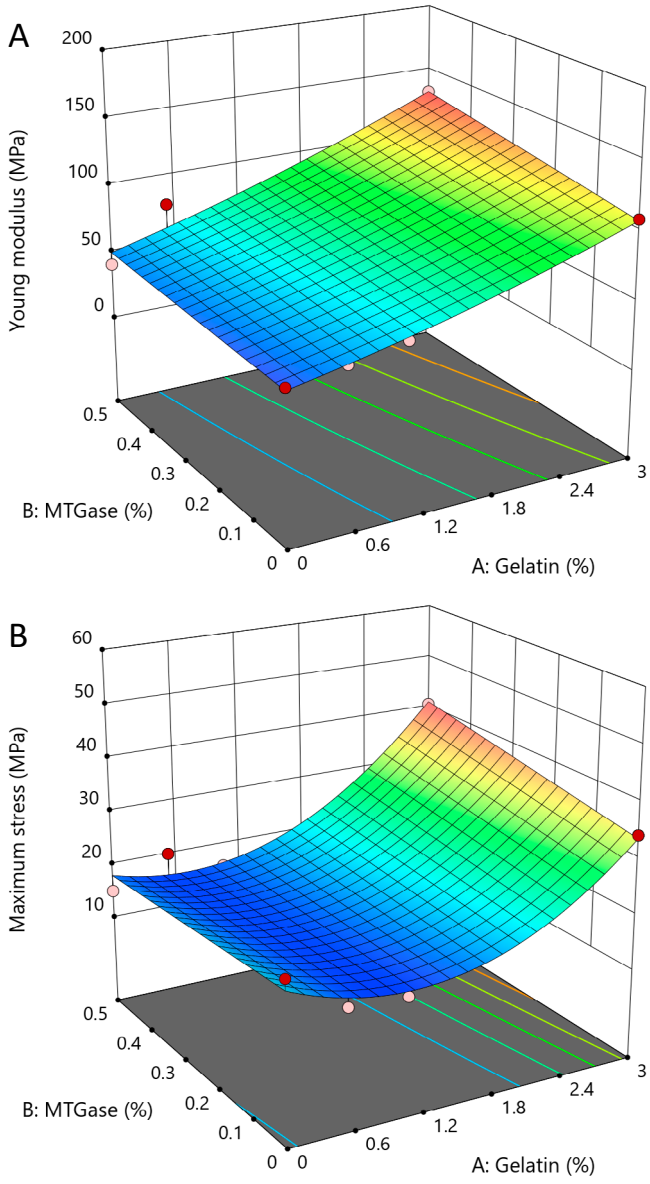

Figure 2. Effect of MTgase and gelatin on Young modulus (A) and maximum stress (B) of crab meat gels.

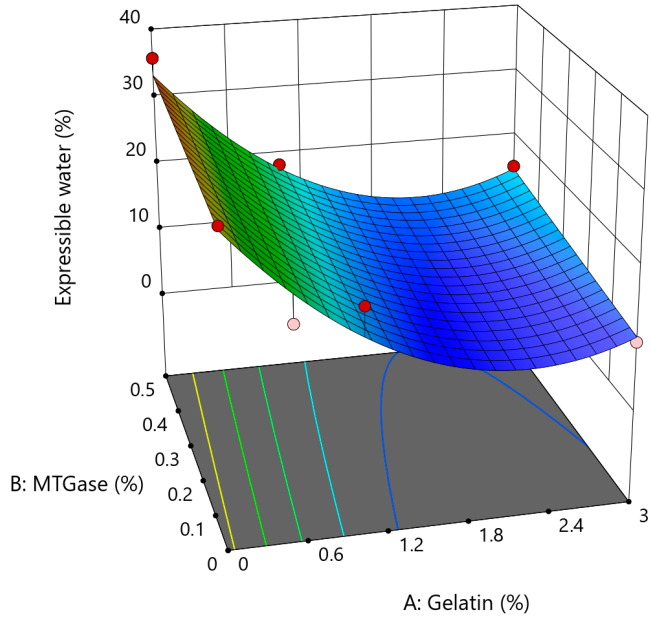

Figure 3. Effect of MTgase and gelatin on the amount of expressible water of crab meat gels.

and thus, decrease the amount of water it can lose. However, previous studies (Martinez *et al*., 2014) with blue crab meat gels, the addition of transglutaminase (6 g/kg), incubated at 40 °C and cooked at 90 °C, showed lower percentages of water extracted than gels without enzyme. In this study, adding MTgase did not affect the amount of extracted water alone or combined with gelatin, even though gelatin also acts as a substrate for the enzyme (Haug *et al*., 2004). Studies on adding MTgase and gelatin to surimi gels reported a decrease in water loss (Hernández-Briones *et al*., 2009; Kaewudom *et al*., 2013).

Color attributes

The effect of gelatin addition on color attributes of crab meat gels is shown in Table 4. No significant differences ($p \leq 0.05$) were observed in these parameters as a function of the level of gelatin or MTgase, indicating that, regardless of the concentration of gelatin or the enzyme, the gels had similar color attributes. Gelatin is a translucent food additive (Siccha and Lock, 1992) and therefore, adding this protein does not affect the color of gels.

Micrographs

Microstructure SEM images of crab meat proteins containing different levels of gelatin or MTgase are shown in Figure 4. Control gels (without MTgase) containing 1 % of gelatin (Figure 1C) were more fibrous, less compact and denser than gels containing 0.5 % or 0.3 % of gelatin (Figure 1A and 1E). These images agree with the results obtained for hardness, cohesiveness, and chewiness, all of which were negatively affected by adding 1 % MTgase but increased slightly by adding 3 % gelatin and had a lower amount of extracted water (higher water holding capacity).

Gels containing MTgase and gelatin seemed denser than control gels, suggesting a better interaction among adjacent proteins by the enzyme effect. Gels containing only 0.5 % gelatin and 0.5 % MTgase seemed more fibrous (Figure 1b) than gels containing 1 % gelatin, which showed lower TPA attributes (except springiness) (Figure 1). Gels containing 3 % gelatin and 0.5 % MTgase were denser and more fibrous than gels with 1 % gelatin, and showed better TPA parameters (Figure 1).

It is important to consider that gelatin is a recognized substrate for MTgase (Haug *et al*., 2004), and it has been reported to negatively affect the mechanical properties of surimi gels (Hernández-Briones *et al*., 2009; Kaewudom *et al*., 2013), especially at levels equal or higher than 1.5 %. However, in this study, adding gelatin improved the texture fundamental properties (Young modulus and maximum stress). Additionally, TPA parameters (except springiness) were improved by adding 3 % of gelatin, even after the negative effect caused by adding 1 % (Figure 1).

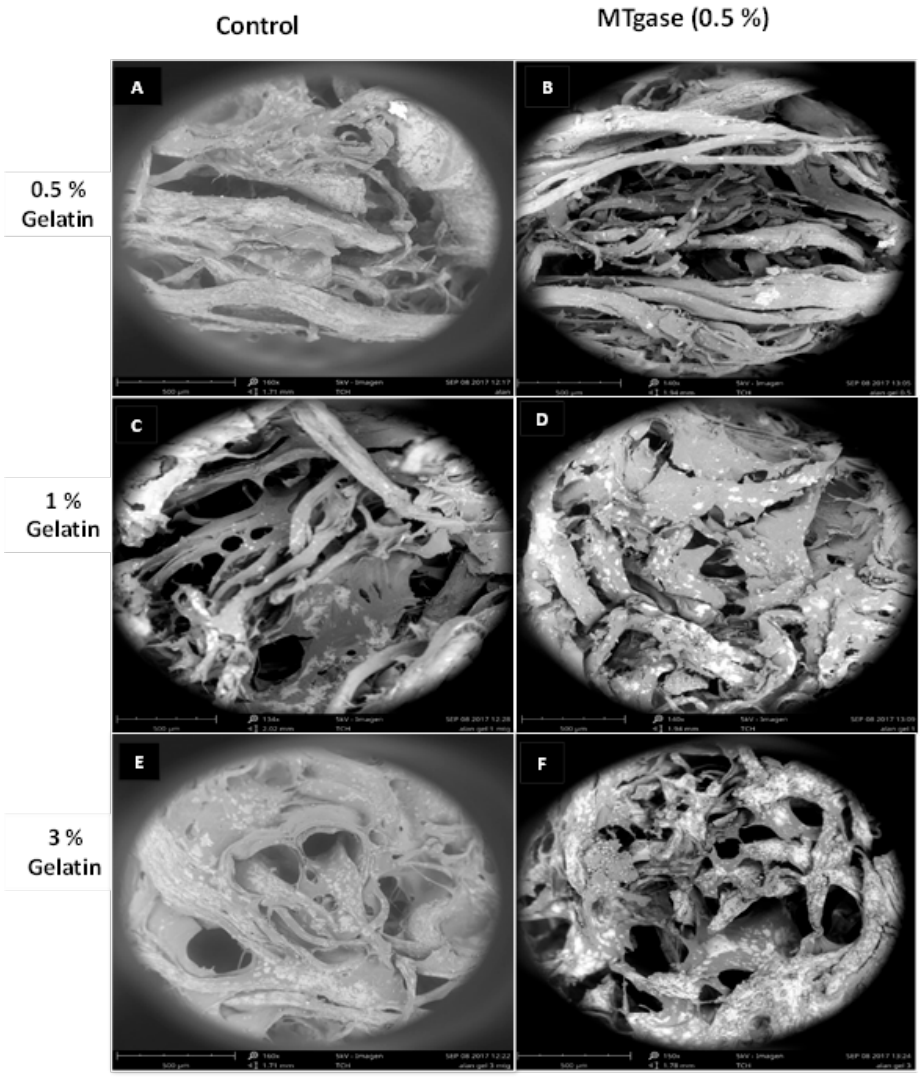

Figure 4. Effect of MTgase and gelatin on SEM micrographs of crabmeat gels.

Table 5. Color parameters of crabmeat gels with MTgase and hydrocolloids.

MTgase (%)	Gelatin (%)	L*	C*	H*
		Control		
0	0	73.73 ± 1.18[a]	8.40 ± 0.88[a]	105.27 ± 1.43[a]
0.5	0	71.16 ± 0.98[a]	8.18 ± 0.62[a]	103.77 ± 2.12[a]
		Gelatin		
0	0.5	73.21 ± 0.66[a]	8.57 ± 0.50[a]	105.01 ± 1.66[a]
	1	72.57 ± 0.78[a]	8.83 ± 0.48[a]	104.47 ± 1.64[a]
	3	74.00 ± 1.81[a]	10.213 ± 1.09[a]	101.39 ± 1.12[a]
0.5	0.5	72.00 ± 0.72[a]	8.76 ± 0.68[a]	104.36 ± 1.95[a]
	1	72.362 ± 1.17[a]	7.51 ± 2.80[a]	102.77 ± 2.32[a]
	3	70.862 ± 2.02[a]	8.28 ± 0.50[a]	102.6 ± 1.17[a]

[a,b] Different letters indicate statistical difference ($P < 0.05$) among treatments (MTgase level for control or % gelatin for each level of MTgase).

Thermally denatured muscle proteins form an opaque and not reversible gel. Gelatin proteins are different as they interact during cooling to form a reversible translucid gel after being thermally denatured. Both of them are recognized as a substrate for MTgase. Meat crab proteins have been reported as a unique and different kind of proteins, which, after being denatured and aggregated by thermal processing, keep the ability of gelling again (Martínez-Robledo *et al*., 2014), suggesting a partially reversible gel. SEM images indicate that gelatin, a protein that forms reversible gels, was able to interact with muscle crab proteins, and both were an appropriate substrate for MTgase, forming a structural network where crab meat proteins-gelatin interactions took place.

CONCLUSIONS

Cooked crab meat proteins formed a tridimensional ordered structure with good mechanical attributes after applying a thermal denaturation-aggregation process. Adding MTgase and gelatin improved the fundamental mechanical properties. However, TPA parameters were negatively affected by the addition of 1 % gelatin, although they were slightly improved by adding 3 % gelatin. Results indicate that both proteins interacted, forming the network that stabilized the gels.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

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