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## Effects of high pressure processing on protein fractions of blue crab (Callinectes sapidus) meat



M.A. Martínez<sup>a</sup>, G. Velazquez<sup>a</sup>, D. Cando<sup>b</sup>, R. Núñez-Flores<sup>b</sup>, A.J. Borderías<sup>b</sup>, H.M. Moreno<sup>b</sup>, and M.A. Martínez<sup>a</sup>, G. Velazquez<sup>a</sup>, D. Cando<sup>b</sup>, R. Núñez-Flores<sup>b</sup>, A.J. Borderías<sup>b</sup>, H.M. Moreno<sup>b</sup>, and M.A. Martínez<sup>a</sup>, G. Velazquez<sup>a</sup>, D. Cando<sup>b</sup>, R. Núñez-Flores<sup>b</sup>, A.J. Borderías<sup>b</sup>, H.M. Moreno<sup>b</sup>, and M.A. Martínez<sup>a</sup>, G. Velazquez<sup>a</sup>, D. Cando<sup>b</sup>, R. Núñez-Flores<sup>b</sup>, A.J. Borderías<sup>b</sup>, H.M. Moreno<sup>b</sup>, and M.A. Martínez<sup>a</sup>, G. Velazquez<sup>a</sup>, D. Cando<sup>b</sup>, R. Núñez-Flores<sup>b</sup>, A.J. Borderías<sup>b</sup>, H.M. Moreno<sup>b</sup>, and M.A. Martínez<sup>a</sup>, G. Velazquez<sup>a</sup>, D. Cando<sup>b</sup>, R. Núñez-Flores<sup>b</sup>, A.J. Borderías<sup>b</sup>, H.M. Moreno<sup>b</sup>, and M.A. Martínez<sup>a</sup>, A.J. Borderías<sup>b</sup>, A.J. Martínez<sup>a</sup>, A.J. Borderías<sup>b</sup>, A.J. Martínez<sup>a</sup>, A.J. Martínez

- a Instituto Politécnico Nacional, CICATA unidad Querétaro, Cerro Blanco 141, Colinas del Cimatario, 76090 Santiago de Querétaro, Mexico
- b Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC), José Antonio Nováis 10, 28040 Madrid, Spain

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#### ABSTRACT

The studies about the effect of high pressure processing (HPP) on the myofibrillar proteins of crab meat are scarce in the literature. The aim of this study is to evaluate the effect of high pressure processing (HPP) at 100, 300 and 600 MPa (10 °C/5 min) on the muscular protein fractions of blue crab meat (*Callinectes sapidus*) and compares the effect of high pressure treatments and the thermal cooking process on the yielding of crab meat. Differential scanning calorimetry analysis of raw crab meat showed two peaks at 48.18 and 76.76 °C corresponding to myosin and actin denaturation. The increasing in the pressure level resulted in a decrease in denaturation enthalpy of both proteins. Data from Fourier transform infrared spectroscopy indicated changes in the secondary protein structures in which a reduction in  $\alpha$ -helix and an increase in  $\beta$ -turn were observed as a result of denaturation induced by HPP. Electrophoresis analysis (SDS-PAGE) showed myofibrillar protein denaturation as the pressure level increased. The HPP at 100 and 300 MPa resulted in a significant increase in the yielding of meat extracted when compared to the thermal treatment (90 °C/20 min). Higher sensory scores were obtained in 300 and 600 MPa suggesting higher acceptance. Results suggest the feasibility of applying HPP as an alternative to the thermal treatment to process crab meat.

Industrial relevance: High pressure processing (HPP) technology has been successfully applied to several seafood products. However, it is important to study the effect of HPP on the food components, mainly proteins in the crab meat to optimize the processing parameters to get high-quality products. In the present study, the benefit of using HPP as an alternative to the commercial thermal processing for extraction of crab meat has been confirmed. Applying 600 MPa (10 °C/5 min) to the whole blue crab resulted in a higher yield of extracted crab meat compared with the other treatments. However, using a range of 100–300 MPa (10 °C/5 min) also increases the yielding of extracted crab meat when compared to the thermal process, and moreover, the extraction procedure is faster. The quality and the functional properties of the crab meat with fresh appearance is preserved after the treatment at 100 MPa. These results could promote subsequent applications of pressurized crab meat in the crab industry, especially with the HPP treatments in a range between 100 and 300 MPa.

#### 1. Introduction

Blue crab (*Callinectes sapidus*) is an important fishery from Canada coast to northern Argentina, and it has also been found in the Mediterranean. In the past decade, the estimated global production of the blue crab was 300,000 ton per year (FAO, 2016). The blue crab meat is a traditional delicacy sold at a high market price reaching an average price of 35.84 US dollars per lb. It can be graded into "jumbo lump", "backfin", "special" or "claw" and that involves the muscle tissue of the crab (Paolisso, 2007). Although the blue crab meat composition can vary depending on the season, age and dietary habits, generally speaking the content of moisture is 76–80%, the protein

content is around 19–14% and lipids are between 0.5 and 0.8%. Also, the crab meat contains a minor amount of carbohydrates, and it is rich in calcium, phosphorous, magnesium, sodium, potassium, manganese, zinc and iron (Gökoolu & Yerlikaya, 2003; Küçükgülmez, Çelik, Yanar, Ersoy, & Çikrikçi, 2006; Lopez, Williams, & Ward, 1981). Industrial processing of blue crab starts with steaming or boiling to facilitate the removal of meat from the shell. The thermal treatment produces the primary flavors compounds of cooked crab meat and destroys pathogenic microorganisms (Ward, Nickelson, Finne, & Hopson, 1983). However, a considerable moisture loss is associated with the cooking and contributes to a significant decreasing in the yield of extracted crab meat. Moreover, Ward et al. (1983) reported that the yield of crab meat

E-mail address: hmoreno@ictan.csic.es (H.M. Moreno).

<sup>\*</sup> Corresponding author.

before cooking is around 10–15%. This is a sensitive problem for crab processors and according to that, new strategies to improve the yield of extracted crab meat are required. Preserving the quality of the extracted crab meat should be the focus of the new processing alternatives (Huang, Lung, Yang, & Wang, 2014).

High pressure processing (HPP) is a non-thermal technology that has been studied in seafood products since several years ago, and the applications of HPP in food industry continue growing. This technology has been used in seafood products for the inactivation of microorganism and enzymes with minimal effects on flavor, color and nutritional quality of foods (Hurtado, Montero, & Borderias, 2000; Hurtado, Montero, & Borderías, 2001: Ramirez-Suarez & Morrisev. Teixeira et al., 2014; Torres & Velazquez, 2005), Moreover, HPP could extend the shelf-life and improve the safety and sensory properties of seafood products as previously reported (Erkan et al., 2011; Alpas & Akhan, 2012; Senturk & Alpas, 2013; Chouhan, Kaur, & Rao, 2015; Reyes, Tabilo-Munizaga, Pérez-Won, Maluenda, & Roco, 2015; Christensen, Hovda, & Rode, 2017). In some cases, as in oysters, HPP can inactivate specific pathogenic microorganisms preventing from some kind of illness in the consumers (Lopez-Caballero, Perez-Mateos, Montero, & Borderias, 2000).

Furthermore, HPP causes conformational changes in proteins, as partial protein unfolding promoting covalent (inter and intramolecular bonds) and non-covalent (hydrophobic, hydrogen and/or ionic bonds) interactions during pressurization and upon release of pressure (Huppertz, Fox, & Kelly, 2004: Perez-Mateos. Lourenco. 1997; Montero, & Borderias, Velázouez. Ramírez. Uresti, Vázquez, & Torres, 2004). This partial denaturation of proteins can modify several functional properties including water holding capacity, gelification, emulsification and foaming (Ayensa, Montero. Borderias, & Hurtado, 2002: Cando. Moreno. Herranz, & Borderias, 2014; Messens, Van Camp, & Huyghebaert, 1997: Perez-Mateos et al., 1997: Uresti et al., 2004). These modifications of the functional properties of proteins could result in important positive changes in the texture of some food products (Ramírez, Uresti, Velazquez, & Vazquez, 2011). However, to the best of our knowledge, no studies based on the effect of HPP on the blue crab meat proteins have been reported. In this connection, an alternative for increasing the yield of the extracted meat in crabs could be HPP that has been used to facilitate shell shucking and meat extraction from several bivalve shellfish including oyster and bay scallop (Murchie et al., 2005; Yi, Xu, Hu, Dong, & Zhang, 2013). This technology has been successfully applied to facilitate the detachment of the adductor muscle in bay scallop with pressures treatments ranging from 200 MPa/3 min to 350 MPa/0 min. In this species, an increase of tissue yields up to 18% compared to manual shucking was observed (Yi et al., 2013). Cruz-Romero, Smiddy, Hill, Kerry, and Kelly (2004) reported that the HPP of 260 MPa/3 min at 20 °C allowed a yielding 15.5% higher than untreated oysters. In all cases, the effect of HPP on macromolecules, like proteins, will depend on the processing conditions i.e. pressure level, temperature and time (Torres & Velazquez, 2005). Besides, the origin of the protein, like beef (Ma, Zhou, Ledward, Yu, & Pan, 2011), rabbit (Cao, Xia, Zhou, & Xu, 2012), pork (Grossi, Olsen, Bolumar, Rinnan, & Øgendal, 2015) or fish (Christensen et al., 2017), will also influence the effects of HPP.

Therefore, the aim of this work was to study the effect of high pressure treatments (100, 300 and 600 MPa at 10  $^{\circ}$ C/5 min) on the myofibrillar proteins of raw crab meat and to compare the yielding of crab meat (*Callinectes sapidus*) extracted after HPP treatment with the typical thermal process (90  $^{\circ}$ C/20 min). In the crab meat, the myofibrillar proteins functional properties were evaluated in order to determine their gelation ability.

**Table 1**Processing conditions for blue crab (*Callinectes sapidus*).

Samples	Treatment
Raw	Ice + water/30 min + 4 °C/1 h
R100	Ice + water/30 min + 100 MPa/10 °C/5 min + 4 °C/1 h
R300	Ice + water/30 min + 300 MPa/10 °C/5 min + 4 °C/1 h
R600	Ice + water/30 min + 600 MPa/10 °C/5 min + 4 °C/1 h
Cooked	90 °C/20 min + 4 °C/1 h

#### 2. Materials and methods

#### 2.1. Raw material and samples preparation

Blue crabs (Callinectes sapidus) were acquired at a local market (Madrid, Spain) and transported in a plastic basket with ice to keep the crabs alive to decrease their metabolism. In the lab, the whole crabs were kept in a mixture of water/ice during 30 min before the application of the different treatments (Table 1). Three specimens for each replicate were used for each treatment. Fresh lot consisted on raw crab meat kept at 4 °C/1 h after the hand-picked meat extraction (hepatopancreas and gonads were removed by washing). The high-pressure processing conditions were selected based on reported parameters for other seafood products (Cruz-Romero et al., 2004; Yi et al., 2013). The raw crabs were processed applying 100, 300 or 600 MPa/10 °C/5 min (Stansted Fluid Power LTD, FPG 7100. Stansted, UK). The treatments were coded as R100, R300 and R600 corresponding to 100, 300 and 600 MPa, respectively. After the HPP treatment, the whole crab was washed and muscle was hand-picked. The crab meat was kept at 4 °C/ 1 h before being analyzed.

To obtain the cooked crab meat, the whole crab was subjected to heating into boiling water (90  $^{\circ}\text{C}$  for 20 min) and then kept at 4  $^{\circ}\text{C}$  for 1 h.

The extraction of crab meat consisted of removing the top carapace and viscera followed by manual picking of the meat attached to the shell. Both raw and cooked meat samples were stored at 4 °C until further use. The crab meat extracted consisted of all the muscle parts including jumbo lump, backfin, special, and claw.

After thermal or HPP treatment, the extracted crab meat was vacuum-wrapped and subjected to a pasteurization treatment (85  $^{\circ}$ C for 3 min) using a water bath to reduce microbiological load after meat extraction by hand. Pasteurized samples were utilized for the sensory analysis and to determine water binding capacity as an indicator of crab meat juiciness.

#### 2.2. Yield determination

Before the applications of the different treatments, the crabs were weighed. After each treatment, the carapace and viscera were removed from the body, and the crab meat was remove manually and weighed; this meat was integrated by the jumbo lump, backfin, special, and claw. The percentage of meat extraction based on live crab weight was calculated using the Eq. (1).

Yield crab meat (%) = 
$$(M1*100)/C1$$
 (1)

where M1 is the weight (kg) of the extracted crab meat and C1 is the weight (kg) of the whole live blue crab.

#### 2.3. Chemical analysis

Crab meat moisture and ash contents were determined following the AOAC (2000) method. The content of crude protein was quantified using a LECO FP-2000 nitrogen determinator (LECO Corporation, St Joseph, MI, USA). Measurements were carried out in triplicate, and all contents were expressed in percentage (%) on a wet basis.

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