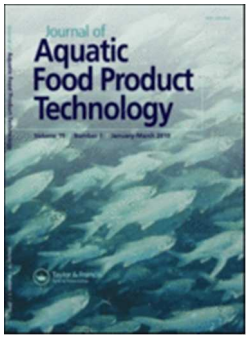

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Pasteurization conditions and evaluation of quality parameters of frozen packaged crab meat

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Abstract

Ovalipes trimaculatus is a crab recognized as a new resource with fishing value to obtain frozen products. Pasteurization conditions of meat crab at temperatures below 85ºC (60, 72 y 82ºC) were established to achieve better quality attributes in the frozen product. The lethality curves of *Staphylococcus aureus* and *Listeria monocytogenes* were measured and the decimal reduction times (D) and Z values were determined. Heat transfer during the pasteurization process of pouches containing crab meat, were simulated using a computational code in finite elements and the mathematical model was experimentally validated. Thermal histories were coupled to the microbial lethality kinetics of the most heat resistant pathogen microorganism in order to establish pasteurization times, necessary for the process system design. The predicted pasteurization conditions were microbiologically validated. The pouches were frozen under industrial conditions and stored at -22ºC during one year. The influence of the type of packaging (vacuum and non-vacuum plastic pouches) on physicochemical and sensory quality parameters of frozen crab meat (color, exudate, lipid oxidation, water holding capacity and overall acceptability) was analyzed observing better performance under vacuum conditions.

Keywords: crab, pasteurization, *Staphylococcus aureus*, vacuum packaging, quality parameters, frozen storage, mathematical modeling.

Running Head: Pasteurization and quality parameters of frozen crab meat

Introduction

The decrease in the traditional fishery resources has led to the search for alternative processes to achieve a better use of the hydrobiological resources and to obtain food products with greater economic interest for the country. In the coastline of Patagonia-Argentina (42°-43°S, 64º-65ºO), there are species of true crabs which are abundant and recognized as resources with fishing value such as the Portunid denominated Southern Swimming crab *Ovalipes trimaculatus* (Fenucci and Boschi, 1975; Wyngaard et al., 2001; Dima et al., 2012). The worldwide crab meat imports have increased steadily over the past 20 years, with over 300,000 tons of products in 2007 (FAO, 2009). However, despite the high acceptability of crab meat, the exploitation of this resource in Argentina is still lagging behind due to lack of scientific and technical information for optimum industrial processing. One of the main ways of commercializing crab meat is as frozen products. The whole process includes: i) primary thermal treatment that permits the detachment of the meat from the exoskeleton; ii) manual extraction of the meat, that is a potential source of contamination; iii) packaging of the crab meat in plastic pouches under vacuum or non-vacuum conditions using films of different gaseous permeabilities; iv) pasteurization process to inactivate pathogenic microorganisms; v) freezing and frozen storage.

The first stage consists in a thermal treatment that allows the detachment of the meat from the exoskeleton and provides the product with the characteristic color and flavor (Tinker and Learson, 1972; Codex Alimentarius, 1983). Previous works have been performed on the numerical simulation of the primary heating process, coupled to the

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denaturation of the myofibrillar proteins to determine adequate conditions for the detachment of the exoskeleton from the meat (Dima et al., 2012).

Crab meat is usually divided into different sections: claws, legs and body (Edwards and Early, 1981; Yomar-Hattori et al., 2006). The meat of the body and claws intended for the manufacture of products based on packaged crab meat is mainly extracted manually. This contact with the human operator allows potential microbial contamination (Senkel et al., 2005). *Staphylococcus aureus* is one of the most common pathogens that can easily contaminate crab meat (Huss, 1997). Crabs can be contaminated by infected handlers. *Staphylococcus aureus* is an organism of easy contagion because it can be found in water, air, and all elements that come into contact with humans. Another pathogen of primary concern is *Listeria monocytogenes* (Gates and Parker, 1992). Since this bacterium is not thermally resistant, it can be eliminated if the products are properly heated. Currently, the Food and Drug Administration of the United States requires the absence of *L. monocytogenes* in ready-to-eat fish products. Pasteurization is a practice currently used for the control of microorganisms in the crab industry since it allows preserving crab meat at low temperatures for several months (Codex Alimentarius, 1983). Selection of the target pathogen is critical to the effectiveness of pasteurization. Acording to FDA (2011), "*C. botulinum* type E and non-proteolytic types B and F are considered as the target pathogens in the pasteurization process if the product is reduced oxygen packaged (e.g., vacuum packaged or modified atmosphere packaged), does not contain a barrier that is sufficient to prevent growth and toxin formation by this pathogen, is not equipped with a time and temperature integrator, and is stored or distributed refrigerated (not frozen)". Therefore in our case, considering that the product is pasteurized, immediately frozen and stored at -22ºC, the inactivation of *C. botulinum* type E and non-proteolytic types B and F would not be necessary during pasteurization.

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To estimate the energy requirements and to properly design the pasteurization process of packed crab meat, it is necessary to predict heating times accurately. The numerical simulation of the heat transfer process in plastic pouches containing crab meat allows calculating the time-temperature distribution by varying different parameters such as the temperature of the heating medium and the heat transfer coefficients. For the pasteurization of packed crab meat, various reports (Codex Alimentarius, 1983; Ward et al., 1983) have recommended heating at 85°C recommend heating at 85°C at least during for 1min in the geometric center of the package. However several works carried out on packed crab meat have reported that the use of pasteurization temperatures above 83°C can cause undesirable changes in color and flavor in the meat during its storage due to the high processing temperatures (Edwards and Early, 1981; Gates and Parker, 1992; Gates et al., 1993). Edwards and Early (1981), Gates and Parker (1992) proposed a pasteurization temperature between 80.6°C and 83°C for packed crab meat in different containers.

Frozen products are one of the main ways in which crabs can be marketed. Portunid crabs (that include *Ovalipes trimaculatus*) are small and not adequate to be stored as whole frozen crabs; crab meat must be removed from the exoskeleton, packed into plastic bags, pasteurized, and then frozen (Gates et al. 1993; Kolbe and Kramer, 2007). In previous works, the freezing process of crabs was studied and mathematically modeled (Dima et al., 2013).The quality problems usually associated with pasteurized - frozen crab meat are lipid oxidation, dehydration, loss of juiciness, loss of color and excessive exudate during thawing (Kolbe and Kramer, 2007; Benjakul and Sutthipan, 2009). To reduce oxidation, the available oxygen should be reduced by packaging under vacuum or inert atmospheres; the product should be protected from light and radiation and very low storage temperatures should be used.

The objectives of this work were: a) to analyze the pasteurization process of packed crab meat (pouches) at different temperatures lower than 85ºC (60, 72, 82ºC) to inactivate representative pathogens such as *Staphylococcus aureus* and *Listeria*

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monocytogenes, b) to quantify the lethality curves of *S. aureus* and *L. monocytogenes* by determining their decimal reduction times (D) and Z values, c) to mathematically simulate the heat transfer during the pasteurization process of pouches containing crab meat, using a computational code in finite elements and to experimentally validate this model by comparing predicted and experimental temperature profiles, d) to couple thermal histories and microbial lethality kinetics of the most heat resistant pathogen microorganism in order to establish pasteurization times, necessary for the process system design, and to validate these results by microbiological analysis, e) to evaluate during frozen storage at -22°C, the effect of packaging type (under vacuum or non vacuum conditions) on physicochemical and sensory quality parameters of pasteurized crab meat frozen under industrial conditions.

Materials and Methods

Sample preparation

Specimens of the Southern Ocean swimming crab *Ovalipes trimaculatus* (Fig. 1a) were collected manually on the sea bottom by SCUBA diving in Golfo Nuevo, Patagonia-Argentina at depths ranging within 0-10m on board of a 4.5-m-long semi-rigid boat. Only male crabs larger than 70mm of carapace width were used in the experiments. The crabs were transported alive to the laboratory within 30 minutes after capture. After that, crabs were sectioned and separated in body and claws and carapace and viscera were removed from the body (Fig. 1a, b). Then both parts were exposed to thermal treatment. Thermal treatment was carried out for the detachment the meat from the crab calcareous layer. Bodies of *O. trimaculatus* (Fig. 1b) were thermally treated by immersion in boiling water at 100ºC, during 10 minutes (Dima et al., 2012). After cooking, crab pieces were submerged in iced water to stop the thermal process; at this stage crab claws were ready to be frozen. In the case of the bodies this procedure facilitates meat detachment from the exoskeleton; meat from the crab bodies was picked manually, using steel devices. The extracted meat was compacted, and portions

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of 250g were immediately packed in pouches made with plastic films with different gaseous permeabilities. In the case of vacuum packed samples, Cryovac (T7335) multi layer film composed by polyethylene and polyamide (PE/PA), 90μm thicknesses with an oxygen permeability of 60cm^3/m^2/day and a water vapor permeability of 9g/m^2/day was used. The vacuum packaging was carried out in a Komet Plusvac 21 Equipment. In the case on non-vacuum packaging, polyethylene film, 90μm thickness was used. In both cases the dimensions of the pouches were 11.5cm width x19.2cm length. The packed samples were then pasteurized at different temperatures (60, 72 and 82ºC) in a thermostatic water bath.

Mathematical modeling of the heat transfer process during pasteurization of pouches

The heat transfer pasteurization process in pouches (Fig. 1c) was mathematically modeled considering the corresponding irregular geometry. The non-stationary partial differential equation representing heat conduction during thermal treatment was solved using the finite element method. The problem was solved considering that the geometry of the pouch containing meat can be represented by an extruded cylinder with an elliptical cross section. The heat conduction equation was as follows:

$$\rho\, Cp \frac{\partial T}{\partial t} = \nabla \cdot (k \nabla T) \qquad (1)$$

where k= thermal conductivity of the crab meat

The initial and boundary convective conditions are as follows:

$$T = T_0 \qquad t = 0 \qquad \text{in } \Omega_c$$

$$-k_c \left(\frac{\partial T}{\partial x} \cdot n_x + \frac{\partial T}{\partial y} \cdot n_y \right) = U(T - T_{ext}) \qquad t \geq 0 \qquad \text{in } \delta\Omega_c \qquad (2)$$

n_x and n_y are the external normal unit vectors at the interphase.

T_{ext} is the external temperature of the heating medium and $\delta\Omega_c$ represents the external surface of the product. U is the global heat transfer coefficient that includes the thermal

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resistances produced by the internal air layer inside the package, the plastic film, and the external heating water.

Comsol Multiphysics Software v 3.5a was used to generate the finite element mesh and to numerically solve the non-stationary heat transfer equation. This is a comercial general-purpose software platform, based on advanced numerical methods (finite element), for modeling and simulating physics-based problems; it provides a full overview of the model and access to all functionality – geometry, mesh, physics settings, boundary conditions, studies, solvers, postprocessing, and visualizations. The geometry was considered as an irregular bidimensional object; the axial heat flow was considered negligible in comparison to the heat transfer through the elliptical cross section.

The convective heat transfer coefficients and the properties of packaging were considered for the simulation. The global heat transfer coefficient (U), was defined as:

$$\frac{1}{U} = \frac{1}{h_{\text{int}}} + \frac{e_p}{k_p} + \frac{1}{h_{ext}} \quad (3)$$

where h_{ext} is the heat transfer coefficient at the interface between the pouch and the water bath; e_p and k_p are the thickness and thermal conductivity of the packaging film respectively and h_{int} is the heat transfer coefficient inside the package that takes into account the internal air layer.

The value of h_{ext} at the interface between the pouch and the water bath was obtained by means of independent experiments using an aluminum rectangular parallelepiped (17cm x 7cm x 5cm) in which a thermocouple type K was inserted at the geometric center. The time-temperature curves at the center of the metallic object, and the external water temperature were recorded by means of thermocouples connected to the acquisition system (Edmund-Buhler, Germany). Different values of h_{ext} were introduced in the numerical model and the simulated thermal histories were compared to the experimental values. The h_{ext} that minimized the residual sum of squares

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(squared differences between the experimental and predicted temperatures) was selected.

Validation of the numerical model: Thermal penetration curves in crab meat pouches

To validate the numerical model, heating experiments were carried out and the experimental thermal histories were compared with the numerical simulations.

Plastic pouches containing 250g of crab meat were thermally treated by immersion in a temperature controlled water bath at 60, 72 and 82ºC. Time-temperature curves were recorded by using a data logger device of reduced size (iButton® Sensors, Maxim products USA). This small sensor was placed at the geometric center of the pouches; the working temperature range of the device is −55ºC to 100°C and the temperature data was recorded every 1min.

The simulation required as inputs the thermophysical properties of the meat, (specific heat, thermal conductivity and density) and the operating parameters including initial temperature and immersion water temperature. The properties of the crab meat were estimated taking into account the proximal composition of crab muscle, and using the equations for k, ρ, Cp proposed by Choi and Okos (1986). Experimental data were compared to the curves predicted by the numerical model.

Determination of the microbial inactivation parameters of Staphylococcus aureus and Listeria monocytogenes.

Staphylococcus aureus and *Listeria monocytogenes*, were selected as the representative pathogens in crab meat pasteurization. Strains of *S. aureus* and *L. monocytogenes* were provided by the Faculty of Veterinary Medicine, National University of La Plata, Argentina. Thermal death curves of these microorganisms previously inoculated in crab meat were carried out at the different assayed pasteurization temperatures (60, 72 and 82ºC). For both pathogenic bacteria the

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decimal reduction time at different temperatures (D value) and the Z value were determined. To obtain these parameters, 50g of packed meat crab was inoculated with 1mL of each pathogenic microorganism ($1x10^7$ UFC/mL).

One gram of the inoculated sample was placed in glass tubes with peptonized solution 0.1%. These tubes were placed in a thermostatic bath at constant temperature; tubes were withdrawn after intervals of 15 to 30 seconds. Viable counts of the surviving bacteria were determined by standard plating technique. Every sample was diluted in peptonated water (dilution 1:10), plated in Petri dishes containing a nutrient medium Baird–Parker Agar, specific for *S. aureus* and UVM (University of Vermont Medium) for *L. monocytogenes*. The plates were incubated at 37ºC for 48 hours before counting.

Colonies were quantified to obtain the inactivation curves for each assayed pasteurization temperature. The results were expressed as $\log(N/N_0)$ versus time, where N_0 is the initial number of cells in the control sample and N is the number of cells in the sample after treatment. The reported results, expressed as the mean values of $\log(N/N_0)$ were obtained by at least three independent experiments. The values of D and Z of *S. aureus* and *L. monocytogenes* were obtained from these thermal inactivation curves. The parameters of the most heat resistant microorganism coupled with the thermal history of the coldest point in the pouches containing crab meat were used to determine the pasteurization times.

Determination of the pasteurization times

Pasteurization times can be obtained by introducing the thermal history of the coldest point in the sample T(t) in the following Equation:

$$\log \frac{N}{N_\theta} = \int_0^t \frac{10^{\frac{T_\theta - T(t)}{Z}}}{D_\theta} dt \qquad (4)$$

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In the present work, pasteurization times based on a 5D reduction time of the pathogenic microorganisms *S. aureus* and *L. monocytogenes* were determined at different thermal bath temperatures (60, 72, 82ºC).

Microbiological validation of the pasteurization process

The microbiological validation of the process time was carried out inoculating the crab meat sample (250g) with 1mL of the most heat resistant microorganism. The sample was mixed in order to allow a good contact between meat and inoculum. The inoculated meat was packed and then submitted to the pasteurization times predicted by Eq. (4) for the different tested water bath temperatures. All the experiments were replicated at least twice. For the determination of bacterial counts, after the pasteurization process, pouches were cooled, and the packages were aseptically open. The meat samples were retrieved for bacterial recovery; 1g of crab meat was aseptically transferred from the package to a glass with peptonized solution 0.1% and the sample was homogenized. After homogenization 1mL of the homogenate was collected, serially diluted, and then surface-plated onto freshly prepared selective agar plates for the target pathogenic microorganism. Each dilution was plated in duplicates and the plated were incubated at 37ºC for 48h.

Freezing and storage of the samples

After the crab meat pouches were pasteurized, they were frozen in an industrial tunnel freezer 6m long with a cross sectional area of 3.6m^2 designed by Refmar S.R.L., in a plant located in Puerto Madryn, Chubut, Argentina. The conveyor belt freezer having six blowers at the upper section operated continuously. The minimum cooling temperature of the tunnel was -40°C and the maximum residence time of the samples was 40min. The air velocity was recorded and the average air velocity in the tunnel

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freezer was 4.16m/s for a frequency air blower of 40Hz (Dima et al., 2013). Freezing times of the pouches were recorded with thermocouples.

The effects of pasteurization, freezing and frozen storage during one year at -22°C on the preservation of the crab meat pouches were studied; changes in physical, chemical, and sensory attributes of the samples were investigated.

Determination of crab meat quality parameters during frozen storage

pH

The crab muscle was homogenized in distilled water in a ratio 1:5 (w/v). The pH of the homogenate was measured using a pH meter (Hanna Instrument) previously calibrated with two buffers.

Water holding capacity

The water-holding capacity was determined as “centrifuge drip” in each crab sample. About 10g of muscle crab was weighed into dry clean centrifuge tubes and centrifuged at 10000 rpm for 15min at −4°C (Sorvall Instruments, Model RC5C). Water-holding capacity was calculated on a wet weight basis as 100× (1−A/*B*), where *A* is the final weight of the sample and B is the initial weight of sample.

Exudate

Exudate was calculated using the following equation:

Dripping% = [(A-B)/A] x100, where A is the weight of the sample after being unfrozen and B is weight of frozen sample.

Lipid oxidation (TBA)

For the measurement of lipid oxidation, the thiobarbituric acid (TBA) test, which determines MDA as an end product of lipid peroxidation, was used. Two grams of muscle crab was homogenized in 16mL of 12% (w/v) TCA solution. The homogenate

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was filtered and 2mL of filtrate was added to 2mL of 0.5% (w/v) TBA. The mixture was incubated in water at 70ºC for 30 min, and the reaction stopped by placing the reaction tubes in an ice bath. After that, the samples was read at 532 nm in a spectrophotometer (HP 8452A). The amount of MDA–TBA complex (red pigment) was calculated from the extinction and expressed as mg malondialdehyde/kg samples.

Color changes during storage

Changes in color (Y, x, y) as a function of storage time were determined by using a Minolta CR14 Instrument (Osaka, Japan) on samples of crab products. Data were transformed into L*, a*, b* (Hunter Lab scale).

$$\Delta E = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2} \quad (5)$$

where: Δa* , Δb* and ΔL* were measured with respect to the initial values (raw muscle).

Sensory analysis

Pasteurization and frozen samples, packaging with and without vacuum, stored at -22ºC were evaluated by 35 panelists (bimonthly) using a 9-point hedonic scale (Sriket et al., 2010): 1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely. Panelists were seafood consumers, with the age of 25-35 years. The panelists were asked to assess samples for: aspect color, odor, flavor and overall acceptability. Panelists were instructed to rinse their mouths with water before starting and between sample evaluations. Evaluations were made individual.

Statistical analysis

For data analysis, standard deviation and ANOVA test were used. Significance of differences was defined at $P< 0.05$. The program Statistica 8.0 (Statsoft Inc.) was used for calculations.

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The Root Mean Square Percentage Error (RMSPE) was used to measure the differences between predicted and experimental temperatures.

$$RMSPE = \sqrt{\frac{\sum (PE)^2}{M}} \quad (6)$$

where PE is the percentage error:

$$PE = \left(\frac{T_{\exp} - T_{predicted}}{T_{\exp}} \right).100$$

Results and Discussion

Mathematical modeling of the heat transfer process during pasteurization of crab meat pouches

For the numerical simulation, the product was represented by an extruded cylinder whose cross section corresponded to an ellipse with a minor axis of 1.7cm and a major axis of 11.5cm.

In order to determine the thermo-physical properties of *O. trimaculatus* crab meat, its composition was analyzed; the obtained results expressed in g/100g (wet basis) were: moisture: 76.01±0.08; proteins: 15.56±2.16; lipids: 1.07±0.12; ash: 1.70±0.11; carbohydrates: 5.66±0.12.

The computer program was fed with the following thermo-physical properties of cooked crab meat: density= 858kg/m^3, specific heat= 3.209kJ/kg, and thermal conductivity= 0.53W/mK, calculated according to the equations proposed by Choi and Okos (1983). The values of the global heat transfer coefficient (U) that best fitted the experimental data were: 40W/m^{2}K for vacuum pouches and 25-27 W/m^{2}K for non- vacuum pouches. Figure 2a shows the elliptical cross section of the pouch, the mesh used for the numerical simulation in finite elements and the temperature distribution in a vacuum pouch exposed to a temperature of 82°C after 1020s. Figs 2b and c show a good agreement between, the proposed model (numerical simulation) and the experimental

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time-temperature data for vacuum and non vacuum crab meat pouches respectively; the RMSPE was lower than 4% for all the tests.

Thermal inactivation parameters of Listeria monocytogenes and Staphylococcus aureus

Figure 3 a,b shows the obtained experimental values of log(N/No) vs. time for *Listeria monocytogenes* and *Staphylococcus aureus* at the different tested pasteurization temperatures. Decimal reduction times (D values) for these microorganisms were obtained from the reciprocal of the inactivation curves slopes (Fig. 3a,b). The obtained D values at 60, 72 y 82°C for S*. aureus* were 3.62, 0.57 and 0.19 min and for *L. monocytogenes* 1.72, 0.52 and 0.14 min, respectively. As can be observed the values of D were higher for *S. aureus* to *L. monocytogenes*. The values of D are within the range published by Huss (1997) for different marine species (D_{60}=1.95-4.48 min for *Listeria monocytogenes* and D_{60}=0.43-7.9 min for *Staphylococcus aureus*).

The experimental Z value (ºC) value for each microorganism was determined from the plot of log D vs. Temperature. The obtained values were: Z=16.6°C for *Staphylococcus aureus* and Z=17.5°C for *Listeria monocytogenes.* Harrison and Huang (1990) and Huss (1997) reported Z values in the range between 5°C and 17°C for different marine species. The most heat resistant pathogen bacteria was *S. aureus,* and therefore it was considered the target microorganism for the calculation of the pasteurization process.

Pasteurization times: coupling thermal histories and inactivation kinetics of the microorganisms

Eq. 4 was discretized and numerically integrated to obtain the heating time required to satisfy the 5D reduction time of the pasteurization process

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$$\sum_{i=1}^{n} 10^{\frac{(T(t) - T_{\theta})}{Z}} \Delta t_i = 5 . D_{\theta} \quad (7)$$

Using Eq. (7) that combines the thermal history T(t) of the coldest point in the pouch (Fig. 2b) with the inactivation parameters of the target microorganism (*S. aureus)*, pasteurization times at different thermal bath temperatures were obtained .

Table 1 shows processing times for vacuum-packed and non-vacuum-packed crab meat pasteurized at 60, 72 and 82ºC. Longer processing times were observed for non-vacuum packed meat due to the presence of an inner air layer that decreases the global heat transfer coefficient. It must be considered that processing times in Table 1 correspond to pasteurization followed by freezing and frozen storage.

The 5D process times considering *S. aureus* as the target microorganism reported in Table 1, are even longer than the 6D *L. monocytogenes* process recommended by FDA (2011). In this case the corresponding values at 82ºC were 15.5 min for vacuum packaging and 20min for non vacuum packaging.

Higher processing times were reported for crab pasteurization followed by refrigeration because more heat resistant microorganisms such as *Clostridium botulinum* were considered as target pathogens. Edwards and Early (1981) proposed the pasteurization of crab meat (*Cancer pagurus*) packed into 0.5 pound can during 30 min at 79.4 °C. Gates and Parker (1992) proposed pasteurization temperatures between 80.6°C and 83°C for packed crab meat in different containers; the recommended pasteurization time- temperatures for meat packed in 2.3 kg, low-density, polyethylene tubes were 3h at 80.6°C and 180 min at 83°C. Gate et al (1993), suggested the use of smaller portion sizes and proposed the following pasteurization conditions at 83ºC: 70 min for barrier and non-barrier plastic pouches(283.5 g), 163 min for steel cans (453.6g); 130min for plastic cans (283.5 g) and 120 min in for aluminum cans.

Microbiological validation of the pasteurization process

In order to validate the predicted pasteurization times, inoculation experiments were

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performed using the most resistant microorganism (*S. aureus*). Pouches with 250g of crab meat were inoculated with 1mL of *S. aureus* ($1x10^7$CFU/mL) and then submitted to the heating times proposed in Table 1 at different temperatures. After heating, the sample was plated on nutritive medium Baird-Parker Agar, specific for *S. aureus*. The pasteurization process designed was adequate because no surviving colonies were found. It is important to highlight that, to ensure the microbiological quality of food, the product must be packed and pasteurized immediately after detaching the meat, always maintaining a temperature lower than 10°C, in order to prevent the growth of microorganisms that could survive the pasteurization process (Mossell and Moreno Garcia, 1982; Codex Alimentarius, 1983).

Effect of the type of packaging on the quality parameters of the pasteurized and frozen crab meat.

Crab meat samples packed in vacuum or non-vacuum pouches were pasteurized at 82ºC and then frozen in an industrial tunnel freezer. The cooling temperature of the tunnel was −40ºC and the residence time of the samples was 40min. The local characteristic freezing times (tc: time necessary to change the temperature in the center of the product from -1.8ºC to -10ºC) were tc= 15. 5min for vacuum pouches and tc= 22min for non-vacuum pouches. For both types of packaging quality parameters were determined during storage time (exudate, water holding capacity, water content, lipid oxidation, pH, surface color, sensory analysis).

Figure 4a shows the monthly values of the loss by exudate for the pouches analyzed. A progressive increase of the exudate can be seen with storage time. After eight months of storage, the non- vacuum packed meat showed exudate values significantly higher than the vacuum-packed meat. In the non-vacuum samples, the weight loss reached values of 14%, similar to those observed by Murakami (1994) for packed crustacean meat. Figure 4b shows the water holding capacity (WHC) of the samples. These values presented a marked decrease with time, (ANOVA, $P<0.05$). In the case of non vacuum

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packed samples, this decrease in WHC was significantly higher than in the vacuum packed samples. The decrease in WHC during freezing and thawing is consistent with the higher exudate values due to the cellular damage and aggregation of proteins which take place during the freezing and thawing of food (Benjakul et al., 2003).

Figure 4c shows the values of percent moisture of the stored frozen crab meat .In general, the moisture content remained constant along the storage time.

Figure 4d shows the values of thiobarbituric acid (TBA), a parameter related to lipid oxidation. The TBA values remained stable during the 12 months of the analysis for both packed products; the samples showed normal values of oxidative rancidity.

In contrast, Benjakul and Sutthipan (2009) reported that lipid oxidation can be induced during the storage of fish meat, since during the freezing of food, the ice crystals formed may alter the cells and cause the release of pro-oxidant compounds such as iron. These authors also reported an increase in TBARs after three months of storage in frozen claws of the crab *Scylla serrata*. Our results are in agreement with those reported by Valls et al. (2004) for vacuum-packed frozen sardine, which showed no variations in TBA values during storage.

Figure 4e shows the pH values for the products studied. The pouches showed a gradual increase in pH along time. No significant differences were found between the non-vacuum and vacuum pouches. The increase in pH can be due to the decomposition of nitrogenous compounds and to the conversion of trimethylamine oxide to dimethylamine, together with the release of inorganic phosphate and ammonia, which give a basic character to the meat (Benjakul and Sutthipan, 2009). A similar behavior has been reported for shrimp (*Penaeus brasiliensis*) by Moura et al., (2003), who reported a maximum pH of 8.1 during storage, and by Martinez-Alvarez et al. (2009) for pink shrimp *(Parapenaeus longirostris)*, who reported pH values of 7.5.

The color is one of the quality parameters affected by storage (Gates et al. 1993) Figure 4f shows the change in color (ΔE) vs. storage time. Factors such as the exposure of the products to light, the storage temperature and the presence of oxygen

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can increase the discoloration on the meat (Boon, 1975). The color varied significantly over time in the products studied. This phenomenon is mainly due to the previous heating suffered by the crab meat before freezing, which results in discoloration during storage (Boon, 1975; Gates et al. 1993). The non-vacuum packed samples presented a greater color change than the vacuum-packed samples. This can be attributed to the presence of oxygen in the non vacuum samples. For both products, the L* parameter decreased more markedly toward the last month of storage, for which it can be inferred that the meat becomes more opaque or brown (Maillard reaction).

Figure 5 shows the results of the sensory evaluation. The average sensory score of the vacuum-packed meat (full line) remained constant throughout the months, whereas the non-vacuum packed meat (dotted line) significantly decreased the acceptability after eight months of storage (ANOVA, $P<0.05$). The vacuum-packed frozen meat had a better acceptance than the controls towards the last months of storage.

These results are reinforced by the physical and chemical parameters such as exudate, water holding capacity and instrumental color; the non vacuum pouches showed unfavorable values with respect to vacuum-packed meat. A similar pattern was reported by Songsaeng et al. (2010) during the sensory analysis of oyster *(Crassostrea belcheri)*, in which all the parameters were disadvantaged after eight months of storage.

Conclusions

In the processing of frozen crab meat one of the stages is the manual separation of the meat from the exoskeleton, that is a potential source of contamination. In the present work the pasteurization process before freezing to inactivate pathogenic microorganisms and to assure microbial safety was evaluated. Crab meat pasteurization was carried out at temperatures lower than 85ºC out using two types of packaging films (plastic pouches with or without application of vacuum) in order to improve the quality of the final frozen product. D and Z parameters of *Staphylococcus*

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aureus and *Listeria monocytogenes* microorganisms were determined.

Heat transfer during pasteurization process of the pouches containing crab meat, was mathematically simulated using a computational code in finite elements. The problem was solved considering that the geometry of the pouch can be represented by an extruded cylinder with an elliptical cross section. Thermo-physical properties of crab meat were evaluated and the heat transfer coefficients were determined from independent experiments. The heat transfer numerical model was experimentally validated by comparing temperature profiles.

By coupling the kinetics of microbial inactivation to the heat penetration curves, pasteurization times in the final packaged product, were determined using the 5D reduction time. The obtained values at 82ºC were Fp= 18min for vacuum packaging pouches and Fp= 22min for non-vacuum pouches. Microbiological validation of the process was performed on pouches inoculated with *S. aureus*; after the proposed heating times there were no surviving colonies of this pathogenic microorganism.

After the analysis of the pasteurization process, the evaluation of the crab meat quality frozen and stored during one year at -22°C was carried out. Physical, chemical and sensory properties were determined during storage. The quality parameters in both types of packaging were stable for 9 months of frozen storage, gradually decreasing from the tenth month. The physical, chemical and sensory attributes showed significant differences between the vacuum packed frozen product and the non-vacuum one at the final storage period. The packaging method produced significant differences in exudate, water holding capacity and instrumental color parameters observing that vacuum packaging improved the quality parameters of the frozen crab meat. Sensory analysis, showed a better score for the frozen vacuum packed meat thus reinforcing the obtained results based on physical and chemical properties.

The study provides new information on quality and safety requirements for processing pasteurized and frozen *Ovalipes trimaculatus* crabs. The authors need to recognize that the processing conditions determined in this study may not be adequate for

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meeting safety requirements of other countries. To ensure regulatory compliance the identified process conditions would need to be independently verified by the crabmeat processors in consultation with their regulatory agency prior to use.

At the same time this study provides information about the quality of the frozen pasteurized product and on the packaging alternatives during frozen storage of a product with high economic potential

Acknowledgments

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FIGURE CAPTIONS

Figure 1. a) *Ovalipes timaculatus* whole crab; b) *Ovalipes timaculatus* body of the crab after removing crab back shell, viscera, claws and legs and c) Photograph of the vacuum pouch of crab meat.

Figure 2. a) Elliptical cross section of the vacuum pouch of crab meat and the corresponding mesh. Model validation: comparison of the experimental data with the predicted thermal penetration curves in the pouch for different heating temperatures (■) 82ºC, (▲) 72ºC and (●) 60ºC: b) vacuum pouches, c) non- vacuum pouches.

Figure 3. Microbial inactivation curves at the different tested pasteurization temperatures for: a) *Listeria monocytogenes* and b) *Staphylococcus aureus.*
Reciprocal of the slopes were used to determine D values.

Figure 4. Changes in quality parameters of frozen crab meat vacuum packed (full line) and non-vacuum packed (dotted line) stored at -22ºC during 12 months. a) Exudate (%); b) Water Holding Capacity (HWC-%); c) Moisture (%); d) Thiobarbituric acid (TBA-mgMDA/Kg sample); e) pH ; and f) surface color (ΔE).

Figure 5. Variation of the average sensory score of all attributes of vacuum packed (full line) and non-vacuum packed (dotted line) crab meat stored at -22ºC during 12 months.

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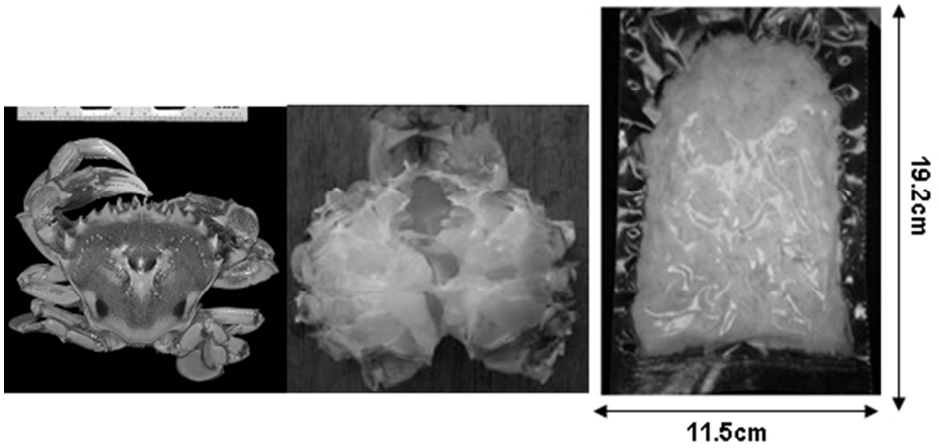

Figure 1. a) Ovalipes timaculatus whole crab; b) Ovalipes timaculatus body of the crab after removing crab back shell, viscera, claws and legs and c) Photograph of the vacuum pouch of crab meat.
156x78mm (96 x 96 DPI)

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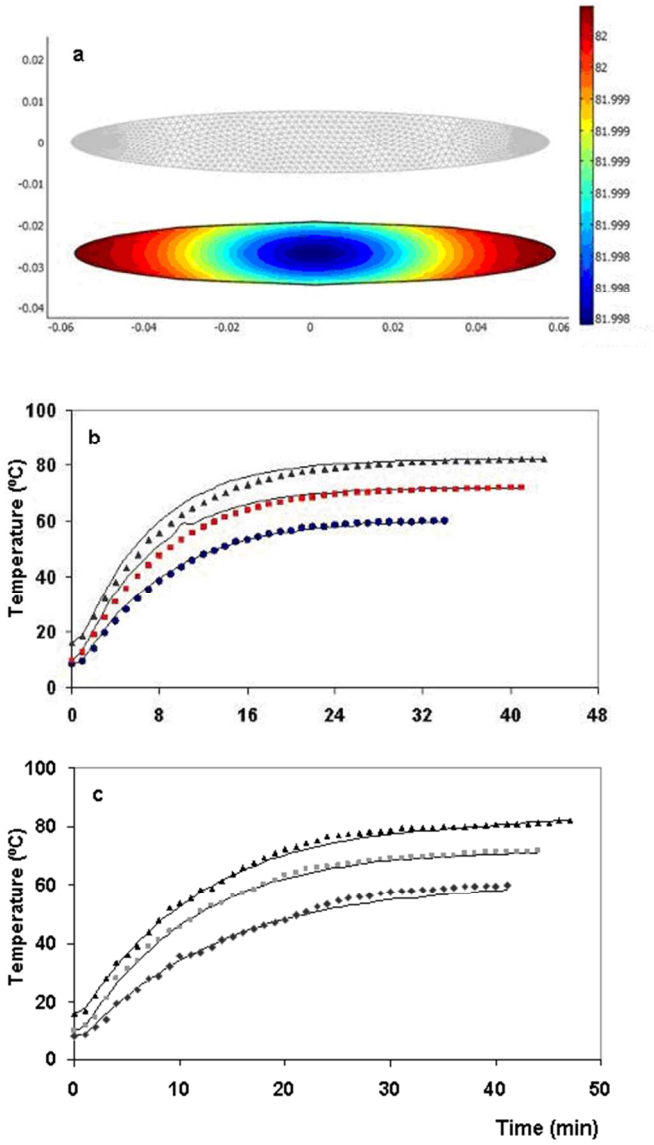

Figure 2. a) Elliptical cross section of the vacuum pouch of crab meat and the corresponding mesh. Model validation: comparison of the experimental data with the predicted thermal penetration curves in the pouch for different heating temperatures (■) 82ºC, (▵) 72ºC and (●) 60ºC: b) vacuum pouches, c) non-vacuum pouches
135x243mm (96 x 96 DPI)

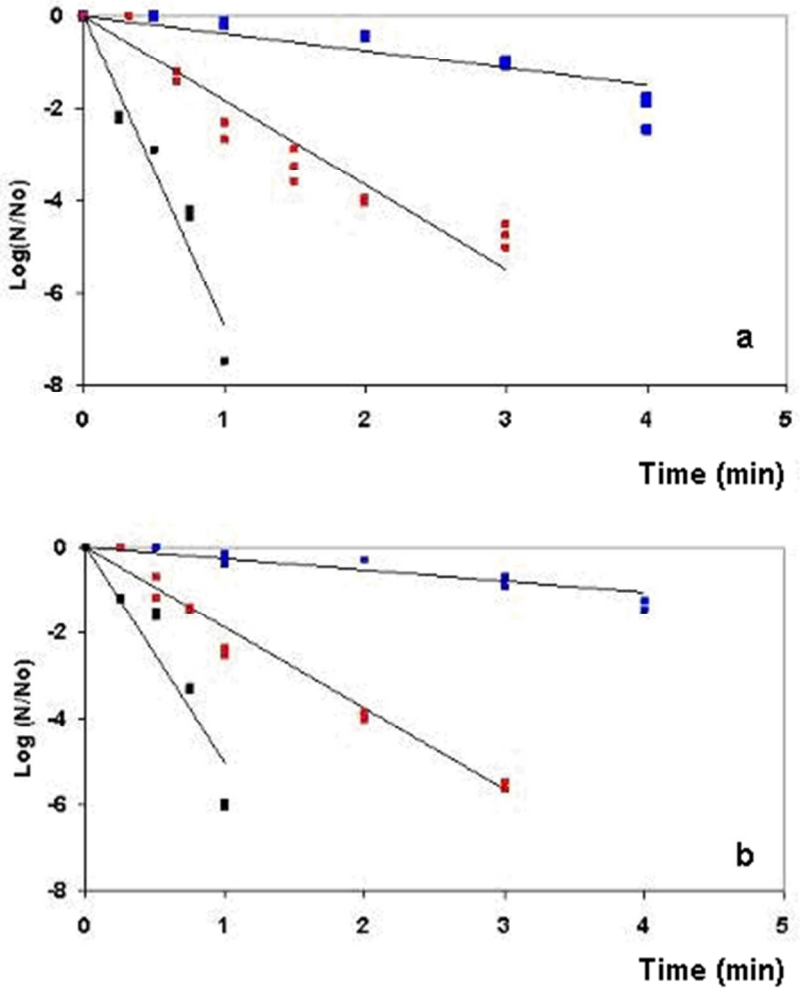

Figure 3. Microbial inactivation curves at the different tested pasteurization temperatures for: a) Listeria monocytogenes and b) Staphylococcus aureus.
Reciprocal of the slopes were used to determine D values.

116x146mm (96 x 96 DPI)

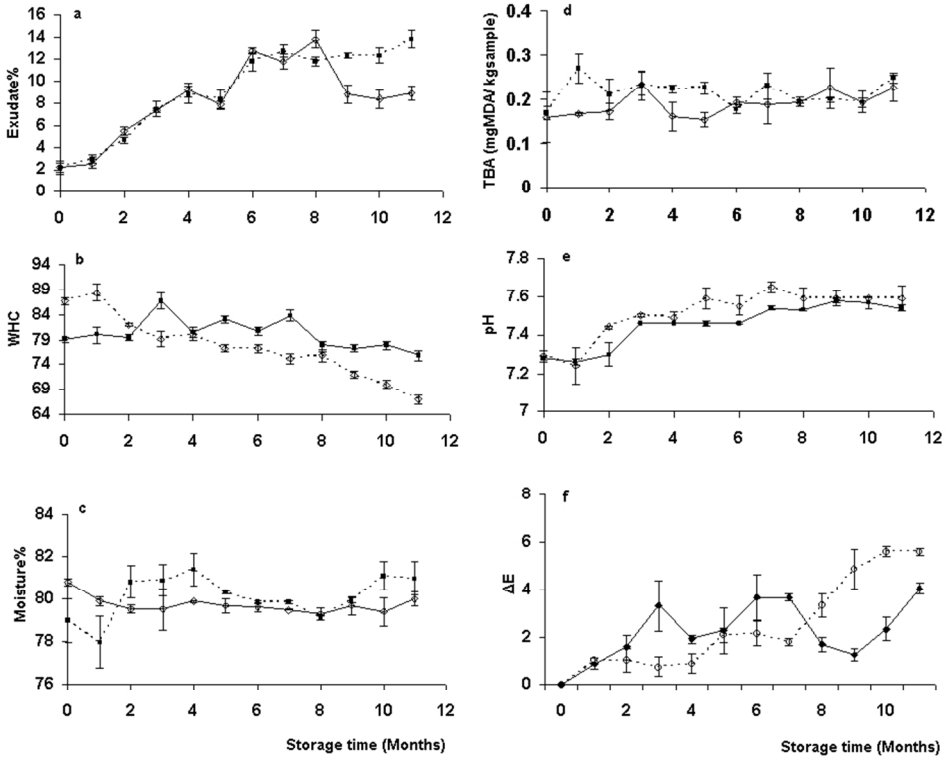

Figure 4. Changes in quality parameters of frozen crab meat vacuum packed (full line) and non-vacuum packed (dotted line) stored at -22ºC during 12 months. a) Exudate (%); b) Water Holding Capacity (HWC-%); c) Moisture (%); d) Thiobarbituric acid (TBA-mgMDA/Kg sample); e) pH ; and f) surface color (ΔE). 260x208mm (96 x 96 DPI)

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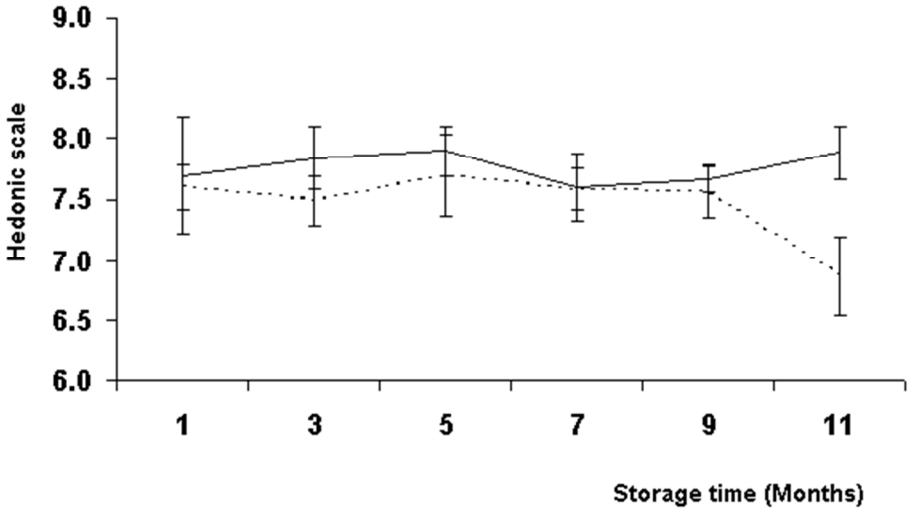

Figure 5. Variation of the average sensory score of all attributes of vacuum packed (full line) and non-vacuum packed (dotted line) crab meat stored at -22ºC during 12 months.
165x98mm (96 x 96 DPI)

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Table 1. Pasteurization times of vacuum and non vacuum pouches containing crab meat (Fp) at different thermal bath temperatures (60, 72, 82ºC)

Pasteurization time (Fp) min		
Temperature (ºC)	*Vacuum pouches*	*Non- vacuum pouches*
60	52	59.5
72	21.5	25
82	18	22

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