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Processing of chopped mussel meat in retort pouch

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Abstract

Chopped mussel meat packaged in retort pouches was processed in a laboratory-scale water immersion retort, adapted for processing under overpressure conditions. Retort temperature effects on product yield and on cook value were evaluated by setting the F_0 at 7 min. The effects of different pre-treatments (salting and marination) on the characteristics of mussels were evaluated after processing at retort temperature of 118 °C and during a whole year of storage at 25 °C. The salted samples showed better yield during storage, while no differences were found for the other physicochemical parameters.

Keywords: mussel meat; retort pouch; overpressure retort; sterilization; yield.

Practical Application: Mussel meat is a highly perishable food that requires the maintenance of the cold chain during distribution and commercialization. For this reason, the diffusion of this mollusk is limited, in Brazil, to the coastal area and to big metropolises. Moreover, a considerable part of the production is rejected because of damage occurring during harvest and processing. The retort pouch packaged, shelf-stable product presented in this work represents an interesting alternative to the valorization of mussel meat as it enables easier distribution and adds value to this product.

1 Introduction

Mussel meat is a highly perishable food that strongly depends on the cold chain during distribution and commercialization. For this reason, in Brazil and in other tropical countries, the diffusion of this mollusc is limited to the coastal area and big cities. Moreover, a considerable part of the production is rejected because of mechanical damage occurring during harvest and processing, or because of lack of commercial size.

Mussels are food with low fat and high protein content, very low caloric values and high mineral content (Tavares et al., 1998). Moreover, mussels are rich in vitamins as B12, thiamin and riboflavin, minerals as iron, phosphorus, zinc and selenium and polyunsaturated fatty acid such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (The U.S. Department of Agriculture, 2015; Almonacid et al., 2015). Moreover, food trend studies (Sloan, 2014, 2015) have confirmed that protein-rich products and ready to eat meals are actual food consumption trends. Although mussels have great commercial potential, these products are industrially unexplored in Brazil. In fact, most of the production is commercialized as fresh product, refrigerated or frozen. Thus, the development of processing alternatives that may enhance the shelf-life of this product at room temperature is important so that this good protein source can be offered to other regions of the country.

Thermal processing seems to be a good alternative to obtain a shelf-stable product with good nutritional value. Few data are available about the effects of thermal processing on the nutritional value of mussels. Almonacid et al. (2015) concluded that, after thermal processing, mussels present good nutritional value, because many nutrients are not significantly affected by such process (Technical University of Denmark, 2009). Biji et al. (2015) confirmed that thermal processed mussel meat was nutritionally balanced with respect to essential amino acids and fatty acids although some nutrient loss was detected.

The use of retort pouches (RPs) for thermal treatment of foods is a suitable alternative to traditional packaging materials, e.g., can and glass. *RPs* are flexible or semi-rigid pouches formed by assembling films with different materials that are laminated together, leading to good mechanical and heat transfer properties, high gas barrier and efficient sealing properties (Holdsworth & Simpson, 2007a).

RPs offer many advantages if compared to traditional containers: for example, reduced space is needed for storage of empty *RPs*; they are easy to transport and open, and they allow rapid heat penetration during thermal treatment, which reduces processing time, and results in foods with good nutritional and sensorial properties (Bindu et al., 2014). On the other hand, *RPs* require special care, such as mechanical protection during processing, storage and transportation. Moreover, *RPs* cannot be used for some products, particularly when food shape must be conserved, because they do not offer rigid support.

Many studies are reported in the literature about the thermal processing of seafood and fish in retort pouches (Byun et al., 2010; Kuda et al., 2008), e.g., mackerel (Gopal et al., 2001; Simpson et al., 2004), tuna (Bindu & Gopal, 2008), shrimp (Mohan et al., 2008; Mallick et al., 2010), anchovy (Bindu et al., 2010), Rohu fish (Majumdar et al., 2015), etc.

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However, only few studies were found about thermal processing of bivalve molluscs. Bindu et al. (2004) and Bindu et al. (2007) developed processing methods to prepare mussel meat and black clam products. In both studies, ready-to-eat mussel and clam products were packed in *RPs*. The heat-treated products were stored for 12 months, period in which their biochemical and sensorial properties were analyzed. In both cases, the mollusc meat products remained with the sensorial and biochemical properties preserved during storage. The authors concluded that retort pouch packaging represents an important tool for processing and adds value to these marine resources.

In this context, the objective of this study was to investigate the influence of thermal processing variables on the yield and water holding capacity of chopped mussel meat packaged in retort pouches. The cook value was used to estimate product quality degradation during processing. The present study also aimed to adapt a lab scale retort to operate with overpressure and determine important processing parameters, e.g., total lethality.

2 Materials and methods

2.1 Mussel meat

Pre-cooked mussel (*Perna perna*) meat was purchased from a local manufacturer (Palhoça, SC, Brazil). Mussels were previously steam-cooked at 100 °C for 5 minutes in an industrial plant, and chilled until 8 °C. Afterwards, mussel meat was manually separated from the shells and packaged in 5 kg bags, which were covered with ice and transported to the laboratory. All the experiments were performed within 24 h. Proximate composition of mussel meat was determined before processing. Protein content was calculated by converting the nitrogen content determined by Kjeldahl's method (6.25×N) (Association of Official Analytical Chemists, 1997). Fat content was determined by the Association of Official Analytical Chemists (1997) method, using a Soxhlet extractor system. Ash content was determined by ashing samples in a muffle furnace at 525 °C for 24 h (Association of Official Analytical Chemists, 1997). Microbiological and chemical analyses were performed in the mussel meat before processing to assess the quality of the samples. Total viable count was evaluated according to the method reported by Vanderzant & Splittstoesser (1992). Total volatile bases (TVB-N) and trimethylamine (TMA) were determined using the Conway micro-diffusion method (Conway & Byrne, 1933; Beatty & Gibbons, 1937). Samples (25 g) were homogenized in 25 mL of trichloroacetic acid (10% solution) for 1 min in an Ultra Turrax homogenizer (IKA, t25, Germany) at 12000 r.p.m. The homogenized samples were centrifuged (SIGMA Laborzentrifugen GmbH, SIGMA 4-16K, Germany) at 11000 g. Two millilitres of the supernatant of each sample were dropped on the outer ring of two Conway micro-diffusion plates and a boric acid solution (1% solution containing the Conway indicator) was pipetted into the inner ring. For TMA determination, a formaldehyde solution (0.5 mL –35%) was mixed with the extract and the mixture was left for 10 min. A saturated solution of K_2CO_3 was added to the outer chamber of all plates; the plates were covered, sealed with solid vaseline and mixed by gently swirling the plate. The Conway plates were placed in a convective oven for 2 h at 36 °C, allowing the complete diffusion of the volatile basis. Boric acid was titrated with a 0.01 M HCl solution using a micro-burette. The results were expressed as mg N/100 g of sample. All determinations were performed in triplicate for samples.

The samples were separated in three different batches: (i) control batch (B_0); (ii) salted batch with approximately 2 g/100 g NaCl (B_s); and (iii) marinated batch with 2 g/100 g NaCl and 0.2 g/100 g acetic acid (B_m). Salting and marination of mussels were performed following the method presented in Tribuzi et al. (2014), which helps establish the exact concentration of salt and acetic acid through appropriate operational diagrams. After pre-treatments, mussels were manually chopped with a knife (piece size < 10 mm) and vacuum packed in *RPs* (≈400 g each) using a vacuum sealer (Selovac 200b adapted with a bi-active sealing system, Brazil).

2.2 Characteristics and properties of retort pouches

Retort pouches used in this study were manufactured by KSP Co. Ltd. (Seoul, Korea), with a four layer configuration of 12 μm polyester, 9 μm aluminum, 15 μm biaxially oriented polypropylene and 80 μm cast polypropylene pouches measuring 190 mm × 240 mm.

Residual air content was verified following the method reported by Canadian Food Inspection Agency (2002). *RPs* were kept under water below a funnel attached to a graduated cylinder. Thus, the pouch was cut in one of its corners and the air was squeezed out into the funnel; the amount of residual air was corrected through Boyle's law. Tensile strength of the *RP* sealed area was measured using a texturometer (TA.XT Plus, Stable Micro System, UK), following the method described by Canadian Food Inspection Agency (2002). Three sections of the sealed area (2.5 cm in width and 7 cm in length) were taken from each RP. Samples were conditioned for 24 h at 25 °C and 50 ± 5% relative humidity before the tests. The force required to pull the seal apart at a loading rate of 25 mm/min was recorded in N/m.

2.3 Water immersion overpressure retort

A laboratory scale, vertical, 50 L capacity, water immersion retort (AV-50, Phoenix, Brazil) was adapted (Figure 1) for RP processing. Retort temperature was controlled with a temperature control system (10) (Climflex PLUS, Expectron, Brazil) connected to a thermocouple positioned at the bottom of the retort (7). Overpressure of 0.3 bar in relation to the water vapor pressure at the retort was maintained by a compressed air line (2) connected to the retort headspace. Cooling of RPs (50 ± 5 °C) was performed with a cooling water line (3) installed on the retort cover.

Stainless steel perforated trays (6) were used to support pouches during processing, which ensured good hot water circulation. Three complete cycles for testing heat distribution were carried out with the retort in fully loaded operational conditions (including tray and filled *RPs*). Thus, five thermocouples, T-type (copper-constantan thermocouple, TX-TF-TF-R-32AWG, Iope, Brazil), connected to a data logger (34970A, Agilent, Malaysia), were distributed in strategic positions. All thermocouples were

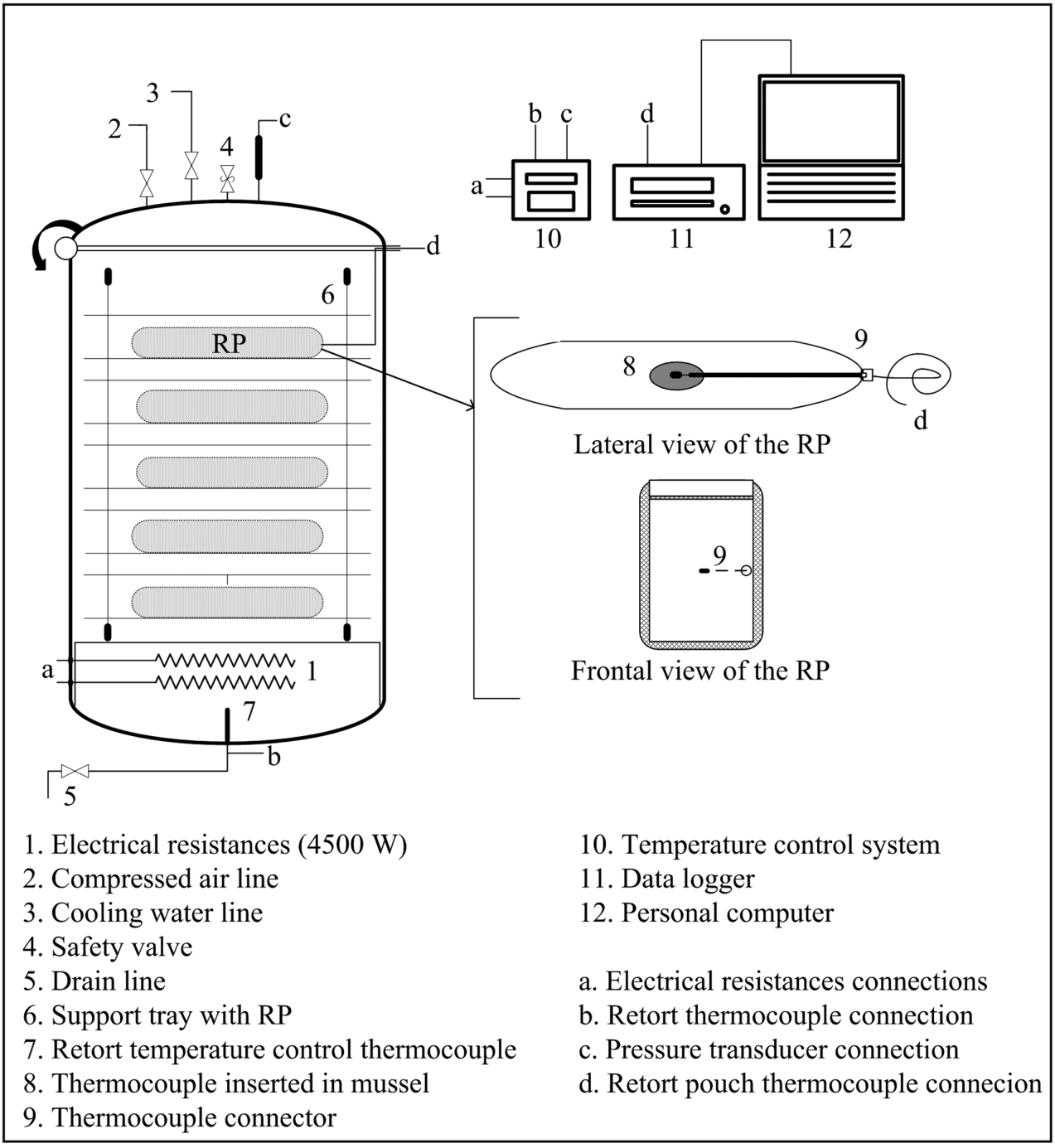

Figure 1. Water immersion overpressure retort and lateral and frontal view of the retort pouch with thermocouple.

calibrated against ASTM mercury-in-glass thermometers (Incoterm, Brazil).

2.4 Thermal process evaluation

Before treatment, pouches filled with mussel samples were conditioned for 30 min in water at room temperature to set a homogeneous product temperature at ≈20 °C.

The temperature at the slowest heating point of the *RPs* was monitored with a T-type thermocouple inserted into a needle (80 mm) and fixed into the RP with a Teflon stuffing box (Figure 1, item 9) sealed with heat resistant silicone. The thermocouple tip was inserted into an entire mussel positioned in the geometric centre of the pouch (Figure 1, item 8). Temperature outputs of thermocouples were recorded every 10 seconds.

Preliminary tests were performed with mussels of the batch B_0 to calculate the processing time needed to reach the required F_0 (lethality for *C. botulinum*, z = 10 °C and T_{ref} = 121.1 °C) of 7 min commonly used for retorted seafood (Mallick et al., 2010; Almonacid et al., 2015). The calculation was done with a classical

mathematical method (Stumbo, 1973). Three processing temperatures were assessed, i.e. 110 °C, 118 °C, and 121 °C. Lag factor for heating (j_h) and cooling (j_c), slope of the heating curve (f_h) were determined from the semi-log curve of the temperature deficit (retort temperature-slowest heating point temperature). Ball time (t_B) was calculated using the classical Ball formula and thus 42% of the come-up time (*CUT*) was subtracted to t_B to determine the process time (Stumbo, 1973). The thermal process experiments were performed in triplicate (three times for each processing temperature) with the calculated process times, and the general method was used to calculate (from the heat penetration data) the F_0 and the cook value (C_T) for thiamine (z_c = 33.1°C and T_{ref} = 100 °C) (Holdsworth & Simpson, 2007b) using the Simpson rule. The cook value is a measure of the thermal treatment effect on nutritional and sensorial properties of processed foods (Ansar Ali et al., 2006) and can be used to select times and temperatures for a specified microbial inactivation, without excessive loss of nutrients (Holdsworth & Simpson, 2007c).

The yield of chopped mussel of the batch B_0 processed at the temperatures of 110 °C, 118 °C and 121 °C was calculated as the rate between the drained sample mass and the total sample mass (Equation 1).

$$Yield\ \% = \left(\frac{DW}{IW}\right) \cdot 100 \quad (1)$$

where *IW* is the initial filling weight and *DW* is the weight of the drained product after processing.

Result analysis indicated the optimal temperature treatment, which was used for the product storage study. In this way, the three studied batches (B_0, B_s, and B_m) were processed under this condition. After processing, samples were labelled and stored at 25 °C for 52 weeks. During storage, two samples of each batch were periodically analysed on their yield, pH, and water holding capacity (WHC). The pH measurements were performed using a digital pH-meter (Q400A, Quimis, Brazil) after triturating and homogenizing the samples in distilled water (in the proportion 1:1, g:g). For WHC determination, 5 g of mussels were wrapped in filter paper, transferred to 50 mL Falcon tubes (containing absorbent cotton wool), and centrifuged at 465 g for 10 min, at 4 °C. Samples were weighted before and after centrifugation and the WHC was calculated according to Equation 2 (Ofstad et al., 1993).

$$WHC\ (g\ water\ /\ g\ dry\ matter) = \frac{(w_b * x_w) - (w_b - w_a)}{w_b(1 - x_w)} \quad (2)$$

where w_b and w_a are the weights of the samples before and after centrifugation, respectively, and x_w is the water mass fraction (g water/ g sample) before centrifugation. The mean value of six measurements, for each studied pouch, was calculated.

The effectiveness of the heat treatment, supposed to be equivalent to a commercial sterilization, was verified performing a sterility test. This was performed after one week of processing and at the end of the storage study (52 weeks), based on the methodology proposed by ANVISA (Brasil, 2001). Twelve *RPs* were randomly selected divided in two groups and incubated at 36 ± 1 °C for 10 days and at 55 ± 1 °C for 5 days. At the end of the incubation period, the *RPs* were visually examined to detect possible swelling and product loss. Then, the samples were conditioned at room temperature and opened to verify the presence of off-odours and alteration of the product aspect.

2.5 Statistical analysis

The variance analysis (one-way ANOVA) with probability of the 95% was performed using the software Statistica® (Statistica 8.0, StatSoft, USA). In case of significant differences ($p < 0.05$), the means were compared using Tukey's test.

3 Results and discussion

3.1 Characterization of the pre-cooked mussel meat

Table 1 shows the proximate composition (moisture, proteins, lipids, and ash content), WHC, pH, TVB-N, TMA, and total viable count and of the pre-cooked mussel meat used in this study. Samples presented proximate composition similar to those reported in the literature for the same mussel species (Parisenti et al., 2008; Tavares et al., 1998). The physicochemical and microbiological analysis showed that products used in this study had good freshness, confirmed by the high pH, low value of total viable count and by very low values of volatile amines (Shenderyuk & Bykowski, 1990; Dalgaard, 2000).

3.2 Retort pouch properties

According to the user manual of the vacuum sealer used in this work, sealing conditions of 30 seconds of vacuum application (35 mbar) coupled with 5 seconds of heat sealing time were used. Higher vacuum levels caused leakage of liquid from *RPs* contaminating the sealing area and causing sealing defects. For the established conditions, the residual air content was of 1.92 ± 0.35 mL, resulting in 0.5 mL air/100 g of sample. Bindu et al. (2007) reported that an amount of residual air lower than 2% shows effectiveness of vacuum, which avoids the blow up of the pouch during processing.

Table 1. Chopped mussel meat characterization (proximate composition, physico-chemical properties, and total viable count).

Proximate composition (g/100 g)	
Moisture	77.69 ± 0.98
Proteins	14.67 ± 0.805
Lipids	1.94 ± 0.09
Ash	1.99 ± 0.12
Physicochemical parameters	
WHC (g H_2O/g dry matter)	2.37 ± 0.06
pH	6.63 ± 0.05
TVB-N (mg N/100 g sample)	1.01 ± 0.12
TMA-N (mg N/100 g sample)	0.22 ± 0.03
Microbiological analysis	
Total viable count (Log CFU/g)	3.97 ± 0.12

Each value represents the mean ± standard deviation of triplicate.

Average heat seal strength, as determined in three sections, was 4069.03 ± 312.4 N/m of width. Lampi (1980) established that the value of 3000 N/m of width is the minimum requirement of heat seal strength. The *RPs* used in this study presented higher heat seal strength, which was satisfactory to overcome the heat treatment in overpressure retort.

3.3 Thermal processing

Preliminary tests, used to evaluate the performance of the modified retort, showed that with the retort at the operative temperature, the maximum difference between the thermocouples was 0.9 °C. The *CUT* for the temperatures of 110, 118, and 121 °C were 13, 19, and 22 min, respectively, and they presented repeatability ($p < 0.05$). Although the temperature distribution test did not show any slow heating areas in the retort, the retort pouch with thermocouple for heat penetration tests was placed in the highest position of the tray. It was done because the cooling period could be faster in this position.

The results of heat penetration into *RPs* filled with 400 g of chopped mussel meat, processed with the overpressure water immersion retort set at the temperatures of 110 °C, 118 °C and 121 °C, are shown in Table 2 and in Figure 2.

The lag factor for heating (j_h) and cooling (j_c) and the slope of the heating curve (f_h) were influenced by the retort temperature. As reported by Stumbo (1973), moderate changes in retort temperature greatly affect j_c and j_h, which is particularly affected by changes of the *CUT*. The process time was significantly affected by the retort temperature, the results showed that *CUT* was about 91.9, 23.5, and 17.9 min, respectively for retort set at 110 °C, 118 °C and 121 °C. Bindu et al. (2004) and Bindu et al. (2007) developed a fried mussel meat product and a clam based ready-to-eat product packaged in *RPs* processed at 121 °C. The values of f_h, j_h, and j_c calculated of processed chopped mussel meat found in the present study were similar to those presented by Bindu and co-workers, who reported values of f_h=21.5 min, j_h=0.50, and j_c=0.93 for the fried mussel product, and f_h=25 min, j_h=0.965, and j_c=1.1943 for the clam product.

When the retort temperature was 110 °C, C_T, which is a measurement of the heat treatment related to nutrient degradation and textural changes that occur during processing, was 194 min. On the other hand, when the retort temperature was 118 °C and 121 °C, C_T was respectively 84.3 min and 72.9 min. According to Awuah et al. (2007), a C_T value of 100 to 200 min can be considered a range beyond which food quality is impaired. Thus, the processes at 118 °C and at 121 °C presented adequate cook values (<100 min), while the process at 110 °C presented an excessively high C_T value. The yield of chopped mussel meat after processing confirmed the relation between quality and C_T. In effect, the yield of the process performed at 110 °C was approximately 6.5% lower than the yield of the processes performed at 118 °C and at 121 °C.

The product treated at 118 °C was chosen for the storage study for two reasons: i) yield and cook value were adequate, and ii) lower processing temperatures (between 118 and 121 °C) allowed easier process control in the adapted retort.

Table 2. Heat penetration data of mussel meat in retort pouch for different retort temperatures.

Parameters	Test		
	I	II	III
Retort temperature (°C)	110	118	121
CUT (min)	13	19	22
f_h (min)	16.1	12.5	18.35
j_h	0.38	0.22	0.51
j_c	1.03	1.20	1.64
t_B (min)	97.4	31.5	27.2
Process time (min)	91.9	23.5	17.9
F_0 (min)	7.07 ± 0.09	7.10 ± 0.04	7.04 ± 0.11
C_T (min)	194.5 ± 3.1	84.3 ± 0.9	72.9 ± 2.1
Product yield (%)	73.4 ± 1.5	73.4 ± 1.5	78.2 ± 0.9

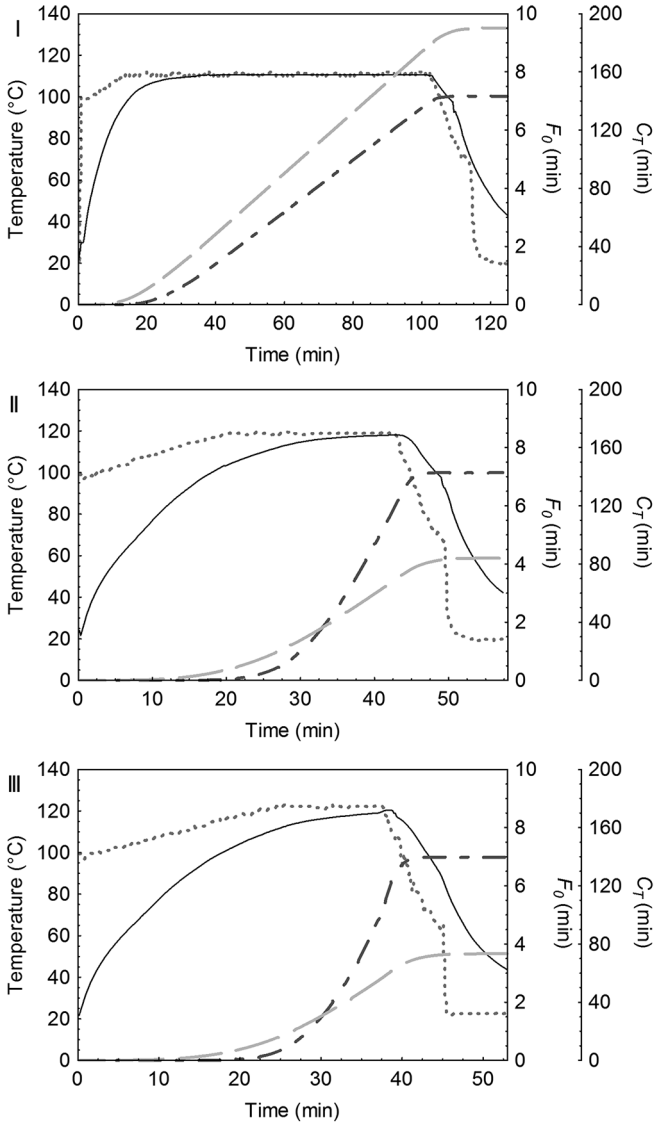

Figure 2. Heat penetration characteristics of chopped mussel meat in retort pouches at the retort temperature of 110 °C (I), 118 °C (II), and 121 °C (III). Slowest heating point temperature (——), Retort temperature (·····), F_0 (— - —) and C_T (— —).

3.4 Storage study

Changes in yield of chopped mussel meat of batches B_0, B_s and B_m stored at 25 °C are shown in Figure 3 (I). Product yield decreased about 20% after processing due to heat induced liquids exudation. Moreover, the yield observed for chopped mussels from the batch "B_s" was significantly ($p < 0.05$) higher than the yield from the batches B_0 (≈5.5%) and B_m (≈6.5%). This difference remained practically constant during the 52 weeks of storage. During retorting, chopped mussel meat loses water and other water-soluble components, because of heat-induced protein denaturation. The amount of liquid loss is influenced by pre-treatments, retorting conditions and salt concentration (Almonacid et al., 2012). Kong et al. (2008) reported that salt addition reduced cook loss in thermally processed salmon fillets. The authors assumed that salt solubilizes proteins, resulting in increased protein–protein and protein–water interactions. These interactions could lead to a gelation process after the retort processing. The positive effect of salt on the yield was limited when acetic acid was added to the product (B_m). Aidos et al. (1999) proved that salmon collagen is particularly soluble in dilute acids, which could explain the low yield of products pre-treated in brine with 0.5% acetic acid. Pricing of commercially sterilized seafood is based on drained weight (Codex Alimentarius, 1985). Thus, small increases of yield, as observed in batch B_s in the present study, may have a significant economic impact on the industry (Almonacid et al., 2012)

Figure 3 (II) shows the WHC of the chopped mussel meat before (time = zero) retort processing and during storage at 25 °C. The values of WHC before processing were influenced by the pre-treatment. The heat treatment caused reduction of approximately 9% of the WHC in samples from all the batches. During the storage period, the WHC of the chopped mussel meat remained practically constant and no significant differences were found among the three different batches. Only in weeks 36, 44, and 52, for the WHC of batch B_s there was a tendency of higher WHC values when compared to the other two batches.

Changes in mussel pH during storage at 25 °C are shown in Figure 4. Before processing, the pH of the batches B_0 and B_s were 6.72 and 6.63, respectively. The pH of batch B_m was 5.18, because of acidification caused by the pre-treatment. After the heat treatment, pH decreased slightly in the batches B_0 and B_s, while remaining constant in the chopped mussels of batch B_m. After the first week of storage, the pH values remained practically constant, with values of 6.39 ± 0.05 for batch B_0, 6.37 ± 0.09 for batch B_s and 5.22 ± 0.09 for batch B_m. The pH value of the marinated batch was 5.22, largely higher than the limit for *C. botulinum* ($pH < 4.6$), indicating that the heat treatment was necessary to allow a safe storage at room temperature.

Chopped mussels processed in retort pouches are a great market opportunity, because commercial sterilization adds value to this low cost seafood (Almonacid et al., 2012). However, a sterility test with the samples (*RPs*) produced in this study was carried out to establish if this product could be distributed in the market. From the visual analysis of retort pouches after the incubation period (36 ± 1 °C for 10 days and 55 ± 1 °C for 5 days), no swollen or product loss was found in the samples stored for 1 week and 52 weeks. The samples did not present any visual or olfactory sign of spoilage. The maximum pH variations of incubated and non-incubated samples (shown in Figure 4, week 1 and week 52) were 0.07, 0.02, and 0.09 for batches B_0, B_s, and B_m, respectively, for samples incubated at 36 °C. For samples

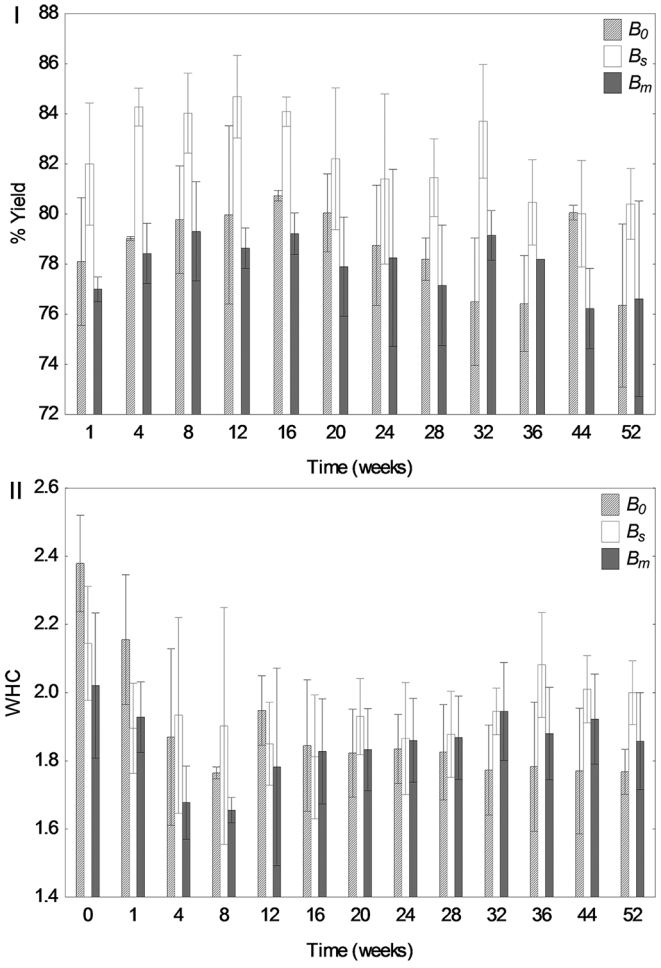

Figure 3. Changes in yield (%) (I) and WHC (II) of chopped mussel meat (b_0, b_s, and b_m) after processing and during storage at 25 °C.

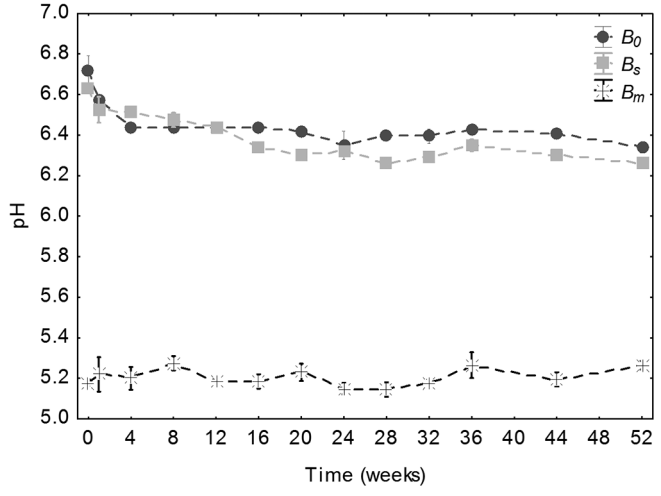

Figure 4. Changes in pH of chopped mussel meat (b_0, b_s, and b_m) after processing and during storage at 25 °C.

incubated at 55 °C, the pH changes observed after the first week of storage were 0.05, 0.06, and 0.08 for batches B_0, B_s, and B_m, respectively. Samples stored for 52 weeks at 36 °C presented differences of 0.03 (B_0) 0.08 (B_s) and 0.05 (B_m) and 0.06 (B_0), 0.07 (B_s), and 0.06 (B_m) when stored at 55 °C. According to the Brazilian legislation (Brasil, 2001), a product can be considered commercially sterile when pH does not present changes higher than 0.2, from the pH values of the samples before incubation; the product did not present any other sign of spoilage; thus, the chopped mussel meat processed in this study was commercially sterile.

4 Conclusions

The laboratory retort adapted to process retort pouches showed good performance and can be useful as alternative low cost equipment to perform heat penetration studies. The salting pre-treatment of mussel meat is important to increase product yield after processing. Chopped mussel meat packaged in retort pouches and processed in a water immersion retort with overpressure is a suitable alternative to the processing of shellfish for non-refrigerated storage and sales, allowing the diffusion of this product in regions where it is not distributed.

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