Title: An assessment of the application of ultrasound in the processing of ready-to-eat whole brown crab (*Cancer pagurus*)

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Abstract

This study assesses the potential of incorporating ultrasound as a processing aid in the production of whole cooked brown crab (*Cancer pagurus*). The FDA recommended heat treatment to reduce *Listeria monocytogenes* by 6 $\log_{10}$ cycles in this product is an $F_{70}^{7.5}$ of 2 min. An equivalent *F* value was applied at 75 °C in presence and absence of ultrasound in water alone or in water with 5% w/v NaCl added. Heat penetration, turbidity and conductivity of the cooking water and also salt and moisture content of the crab meat (white and brown) were determined. Ultrasound assisted cooking allowed a reduction of the cooking time by up to 15% while still maintaining an $F_{70}^{7.5}$ of 2 min. Ultrasound also enhanced the rate and total amount of compounds released from the crab, which suggests that crabs cooked in the presence of ultrasound would be expected to be cleaner. Ultrasound also proved to be effective in reducing the salt content but hardly affected the final moisture content of the crab meat.

Keywords: Ultrasound; Cooking; Brown crab; Ready-to-eat; Heat transfer; Mass transfer.

1. INTRODUCTION

Crustaceans are highly appreciated worldwide, and also most crustacean-based products are considered a healthy choice for consumers due to their high-quality protein, amino acid composition [1,2] and their low saturated fat content [3, 4]. Brown crab (*Cancer pagurus*) is one of the most consumed crustaceans in southern European countries and the market is expanding to the United States and Japan where consumption of processed ready-to-eat crab products is increasing [5]. Ireland is one of the top three producers of brown crab and brown crab products in the world [6]. Two-thirds of brown crab landings are exported with 42% of these exports in the form of live crabs though there can be significant associated losses during transportation (up to 50%) [7, 8, 9].

By contrast, exporting crabs in a ready-to-eat format avoids losses during transportation and also adds value to the final product. Ready-to-eat whole brown crab is exported mainly as a cooked, frozen product. The production of cooked-frozen crabs involves an initial cooking step followed by a washing/cooling step, packaging and a second heating step in-pack prior to freezing [5]. The processing of ready-to-eat crab has not evolved in line with current technological developments. Many producers still use traditional techniques and define their own cooking conditions in terms of time and temperature which leads to heterogeneity in the quality of marketable products (e.g. over or undercooking). The size of these companies is usually small and their investment in technology and process optimisation is generally low. However, novel processing technologies such as ultrasound have many benefits to offer them. For example, ultrasound technology is widely used in the food industry to enhance heat and mass transfer processes [10, 11] which could have great relevance and be easily adopted to improve traditional immersion cooking processes used in the production of ready-to-eat crab.

High-intensity ultrasound involves intensities greater than 1 W/cm^2 and is performed at frequencies ranging from 18 to 100 kHz. Cavitation is considered the main mechanism by which this form of ultrasound enhances heat and mass transfer phenomena [12] though other effects such as acoustic steaming are also involved [13]. The effects of ultrasound on heat transfer have been extensively studied since the 1990s in model systems such as water, metal tubes, metal balls, etc. [14, 15, 16] and its ability to enhance heat transfer in foods, mainly in processed meat products, during cooking processes has also been proven [17,18]. The potential for ultrasound to assist different processes such as extraction [19, 20, 21], cooking [22] and marinating [17, 23, 24, 25] have been assessed in vegetables tissues, meats and fish. However, its effects on the industrial heat processing of ready-to-eat crustaceans products has never been explored. Therefore, the aim of this study was to assess the potential of ultrasound for the cooking of brown crab by accelerating heat and mass transfer processes. The increase of heat penetration should lead to a reduction in cooking times which in turn should enhance product quality whilst ensuring adequate levels of safety. In addition, the production of ready-to-eat brown crab involves a cleaning/cooling step which is needed to remove crab dirt and cook exudate deposits before packing. This step takes 3-4 h and constitutes a microbiological risk due to a possible re-contamination of the product, hence requiring a subsequent pasteurization step with the sole purpose of eliminating microbial contamination [26, 27]. The ability of ultrasound to enhance mass transfer could also be used to remove dirt and exudate from crab shells during cooking thus eliminating or reducing the severity of the subsequent pasteurization ultimately resulting in greater yields, less energy input and a milder heat- treated higher quality product.

Therefore the objective of this research was to evaluate the potential improvements induced by the application of ultrasound in the cooking process of ready-to-eat whole brown crab, with particular reference to the benefits of associated heat and mass transfer phenomena.

2. MATERIALS AND METHODS

2.1. Raw material and cooking conditions

All experiments performed, during this research, were carried out with female crabs, landed in Ireland in the winter of 2014, with weights ranging from 375 to 732 g,. Those were obtained from a local fishmonger and maintained alive at 4 ºC in dry conditions for a maximum of 48 h. After storage, crabs were euthanized in a humane manner [28] while maintaining the integrity of the carapace. Before cooking each crab was characterised by measuring weight (grams) and dimensions (cm^2) (assuming that the crab shape was oval, the area of which was multiplied by two in order to account for both sides of the crab). After cooking the two kinds of crab meats were evaluated separately, namely white meat which was located in claws and legs and brown which was located inside the carapace. This separation were considered due to the different composition of the two kinds of meat, their location and respective market values.

Cooking experiments with and without ultrasound were performed immediately after euthanasia in an ultrasonic bath (Guyson mod. KS MK3 525, North Yorkshire, UK) with a tank capacity of 55 L, a maximum ultrasound power of 900 W and a heating power of 2000 W. All trials were carried out by using the maximum volume of water (55 L). Once the temperature of the water in the bath reached 75 ºC, eight crabs were submerged and cooked for 45 min. For those experiments applying ultrasound, the maximum ultrasonic power of the tank was used, 900 W (of ultrasonic energy consumption). In order to standardize the cooking conditions as much as possible, the total weight of all batches ranged from 4 to 4.3 kg. Preliminary experiments (data not shown) showed that 45 min was a sufficient cooking time to apply an equivalent $F_{70}^{7.5}$ of 2 min, which corresponds to the FDA recommended heat treatment for ready-to-eat seafood products. This heat treatment ensures the inactivation of at least 6 $\log_{10}$

cycles of *Listeria monocytogenes*, the target microorganism in pasteurised seafood products [29]. At least three replicates of these cooking experiments with and without ultrasound were performed on different days.

2.2. Heat transfer study

To assess the effect of ultrasound on the heat transfer phenomena in crabs two different comparisons, based on mathematical models, were carried out.

2.2.1. Heat penetration curves

Heat penetration curves were obtained by placing a K type thermocouple (Alhorn, Holzikirchen, Denmark) in the abdomen of the crab which corresponds to its cold spot which had been previously identified in preliminary experiments (Figure 1). The temperature was recorded using a data logger (Alhorn Type 2590-2) connected to a laptop with the data control software version 4.3 (32-bit). The heat penetration curves were subsequently fitted to the Ball & Olson equation [30] (Equation 1A and 1B):

$$\log \theta = \frac{1}{fh} \times \log j \qquad \text{(Equation 1A)}$$

$$\theta = \frac{T_{cook} - T_0}{T_{cook} - T_t} \qquad \text{(Equation 1B)}$$

Where *fh* is the maximum rate of heating up (dimensionless), *j* is the lag phase of the heat penetration curve (dimensionless), T_{cook} is the cooking temperature, i.e. 75 (°C), T_0 is the initial temperature in the crab's cold spot (°C) and T_t is the temperature reached in the crab's cold spot at specific times during the cooking process (°C).

2.2.2. Lethality and F value

From the temperatures recorded during cooking, the equivalent lethality (*L*) and cumulative *F* equivalent values at each temperature were calculated using Equations 2 and 3, respectively.

126 $L = 10^{\frac{T-Tref}{z}}$ (Equation 2)

127 $F = \sum_{x=0}^{xi} L \times \Delta t$ (Equation 3)

128 where *T* is the temperature (°C) reached in the crab's cold spot at specific times (x_i) during the
129 cooking process, T_{ref} is the reference temperature considered for the target microorganism, *L.*
130 *monocytogenes* (i.e. 70 °C), *z* is the *z* value for the target microorganism, *L. monocytogenes*
131 (i.e. 7.5 °C), and Δ*t* is the slot of time (min) during which the crab's cold spot is at the
132 temperature *T*.

133 2.3. Microbiological examination of fresh crab meat

134 To assess the microbial reduction during the cooking process, crabs of a similar size (± 20
135 g) were euthanized as described in Section 2.1 and stored at 4 ºC for 72 h to allow the growth
136 of the natural microflora in the crab tissues (initial microbial load in crab meat was ≈10^2
137 CFU/g). Following this, five crabs were cooked at 75 ºC with and without ultrasound in the
138 ultrasonic bath as described above. After 5, 10, 15, 30 and 45 min of cooking one crab was
139 removed from the tank and submerged immediately in ice water in order to cool it down as
140 quickly as possible. Following that, the white and brown meat from each crab was removed
141 under aseptic conditions in a laminar flow cabinet (Faster, Mod. Bio 48. Ferrera, Italy). To
142 assess the antimicrobial effect of sonication at temperatures below 30 ºC (at this temperature
143 the effect observed would be solely attributed to ultrasound and not to heat), the water from
144 the bath was recirculated through a heat exchanger with a coolant to avoid temperature
145 increases during treatment. Once the treatment conditions were stable (28 ± 2 ºC), two crabs
146 were submerged in the ultrasonic bath for 45 min and then their white and brown meat were
147 extracted. Each treatment was performed three times on different days.

148 Total bacterial counts (TBC) were quantified by diluting 5 g of each type of meat from each
149 crab in Maximum Recovery Diluent (MRD) (Oxoid, Hampshire, UK) and stomaching (400
150 circulator, Seward Stomacher, UK) for two min at 300 rpm. Then, 1 mL of the appropriate

151 serial dilution was pour-plated in Tryptone Soya Agar (Oxoid), supplemented with 0.6% Yeast
152 Extract (Oxoid), and incubated for 48 h at 30 ºC. Longer incubation times did not increase the
153 number of colonies observed on plates (data not shown). Microbiological assessment of raw
154 crab meat was performed using the same procedure.

155 2.4. Mass transfer study

156 Conductivity and turbidity of the cook water were measured to assess the effect of
157 ultrasound on mass transfer during the cooking of crabs (Method 1). In addition, the final salt
158 and moisture content of the crab meat were also assessed after cooking (Method 2).

159 *2.4.1. Method 1: Measurement of cook water turbidity and conductivity*

160 Cook water turbidity was used as an indicator of the degree of exudate deposits removal
161 during cooking. A volume of 10 mL of the cook water was taken at 5 min intervals during the
162 45 min cooking of each batch of eight crabs and the turbidity was measured using 1 cm of path
163 length cuvettes in a spectrophotometer (UVmini-1240, Shimadzu). Measurements were
164 performed at 515 nm, which was the wavelength at which the cook water showed the maximum
165 absorbance (data not shown). Results were expressed in absorbance units at 515 nm. Cook
166 water conductivity was also used as an indicator of particulate loss, which is likely to be
167 associated with the release of ionic compounds from the whole crab. Measurements were
168 performed every 5 min during the 45 min cooking process of crab batches using a conductivity-
169 meter (CyberScan mod. CON 400/410 & TDS 400). Each measurement was performed once
170 the water had been allowed to cool down below 30 ºC. Results were expressed in µS/cm.

171 *2.4.2. Method 2: Measurement of salt and moisture content of crab meat*

172 Salt content of white and brown crab meat was measured after cooking crabs in the presence
173 or absence of ultrasound in water with and without 5% NaCl added (w/v), following an
174 adaptation of the method described by [31]. In brief, 2 g meat samples were placed in glass
175 beakers (250 mL) to which 100 mL of a 0.1 N nitric acid solution (Fisher Scientific, Leicester,

176 UK) was added. The mixture was then homogenized with an ultraturrax (DI 25 basic, IKA-
177 WERKE, Germany) for 20 s at 10,000 rpm. After homogenization beakers were placed in a
178 water bath (Davidson & Hardy LTD, Dublin, Ireland) at 65 ºC for 15 min before cooling the
179 samples to room temperature (≈20 ºC). After cooling, samples were titrated against 0.1 N silver
180 nitrate solution ($AgNO_3$) (Fisher Scientific) using a magnetic stirrer. During titration, silver
181 concentrations were continually monitored using a coupled silver electrode with a reference
182 electrode (calomel) and the potential difference was measured in mV on a pH meter (Jenaway
183 3505, Bibby scientific Ltd., UK). The end of titration was determined when the pH-meter
184 reached +225 mV. The salt content in brown and white crab meat was then calculated using
185 Equation 4.

186 $$\% \text{ NaCl} = \frac{mL\ AgNO_3 \times 0.585}{weight\ of\ sample\ (g)} \qquad \text{(Equation 4)}$$

187 Crab meat moisture content was determined by oven drying following the AOAC (1995)
188 method. All analysis was carried out at least in triplicate.

189 2.5. Statistical analysis

190 *T*-tests (p=0.05) and ANOVA tests (p=0.05) followed by Tukey's test were used to define
191 statistical differences among samples. GraphPad PRISM 5.0 software (GraphPad Software,
192 Inc., San Diego, CA, USA) was used and differences were considered significant for $p \leq 0.05$.
193 Error bars in the figures correspond to the standard error of the mean.

194

195 **3. RESULTS AND DISCUSSION**

196 *3.1. Heat transfer*

197 To facilitate the study of the effect of ultrasound on heat penetration during the cooking of
198 crabs, heat penetration curves were fitted to the Ball & Olson equation due to its simplicity and
199 goodness of fit (Table 1). The parameters *fh* and *j* were calculated from the heat penetration
200 curves of crabs cooked in water at 75 °C without (n = 27) and with ultrasound (n = 10, 900 W).

For comparison purposes, crabs were grouped by weight into 3 categories, i.e. small (<450 g), medium (450-600 g) and large (>600 g). Table 1 also shows the R^2 and *RMSE* coefficients used as indicators of the goodness of fit of the model. In all cases the R^2 values were higher than 0.9, indicating a good goodness of fit and *RMSE* values ranged from 0.03 to 4.19. Regarding the *j* value, the ANOVA analysis revealed that there were no statistically significant differences between crab weight categories regardless of whether ultrasound was applied or not ($p>0.05$). The same was observed for the *j* values ($p>0.05$, Table 1). This result could be explained by variations on the morphology and integrity of the crab carapaces. For example, whilst every effort was made to maintain the integrity of the carapace during sample preparation, its integrity and morphology can vary depending on different factors such as the physiological status of the crab and the transportation [5, 32, 33]. This would result in the uptake of cook water which could directly affect the lag-phase. When compared with the heat-only cooking process, the application of ultrasound reduced the *fh* value by 5.4%, 14.9% and 29.2% for the 'small', 'medium' and 'large' crabs, respectively. These results indicate that the use of ultrasound not only increases the heat penetration in the crab's cold spot but also reduces the effect of crab weight (and size by extension) on the heating ratio of the cold spot. In addition, a linear relationship between *fh* values and crab weight was noted in each cooking process indicating that heat penetration in the crab cold spot is weight-dependent. Table 2 includes the first order equations which correlate the increase in *fh* values with the increase of the weight of the crab in both cooking processes. Significantly different slopes were observed between equations ($p\leq0.05$), indicating that the crab weight affected heat penetration to a differing extent depending on the cooking process. When ultrasound was used to assist the cooking the slope was 2.6-fold smaller, meaning that the weight of the crab had a much smaller effect on increases in *fh* value. Hence, the larger the crab the greater the impact of ultrasound in enhancing the heating rate as indicated in Table 2.

Much of the work published to date attributes the ultrasonic enhancement of heat transfer to the formation of cavitation bubbles [34], although other authors have also suggested that improvements in convection heat transfer may also be due to acoustic streaming [15, 35]. Either way, it is generally accepted that ultrasonically induced heating is a result of energy dissipation from the accumulation of cavitation bubbles at the interface of the submerged body [36, 37]. Additionally it is accepted that the number and density of cavitation bubbles can play an important role in the heat transfer caused by ultrasound and also that the cavitation of bubbles increases the micro-convection effect at the product surface [12].

In relation to the surface area, some researchers have reported a relationship between the weight and dimensions (width and length) of the carapace of a crab [38]. In the present study a linear relationship (represented by Equation 5) was found between the surface area (cm^2) of the carapace and the crab weight (g) (Figure 2).

$$Surface\ (cm^2) = 0.362 \times weight\ (g) + 54.83 \quad (R^2 = 0.85) \qquad \text{(Equation 5)}$$

This relationship indicates that heavier crabs had higher carapace surface areas (Figure 2). Therefore the greater impact of the ultrasonic field on the reduction of *fh* values in heavier crabs could be attributed to their larger surface areas and as a consequence a greater amount of cavitation bubbles around their surface.

The effectiveness of a heat treatment in terms of microbial inactivation is given by the applied *F* value. As indicated before, the cooking processes applied in the current study were designed following FDA recommendations [29] to ensure a 6 $\log_{10}$ reduction of *L. monocytogenes* in seafood products ($F_{70}^{7.5}$ = 2 min). A cooking process of 45 min in water at 75 °C was previously demonstrated to achieve this minimum recommended *F* value in all crabs irrespective of their weight (data not shown). For each cooking process, either with or without ultrasound, the actual equivalent $F_{70}^{7.5}$ value applied was calculated based on the corresponding heat penetration curves. Figures 3A, 3B and 3C show the $F_{70}^{7.5}$ values attained during the

cooking of crab at 75 °C without ultrasound (block line) and with ultrasound (dashed line) in small, medium and large crabs, respectively. Figure 3 also shows the threshold for the target $F_{70}^{7.5}$ of 2 min (horizontal dotted line).

The *F* value applied in the ultrasound-assisted cooking was 2.5-, 3.2- and 2.2-fold higher than the conventional heat-only cooking for the small, medium and large crabs, respectively. In other words, the cooking time was reduced by 15.5% (from 45 to 38 min), 16.9% (from 45 to 37.4 min) and 12.7% (from 45 to 39.3 min), respectively, while applying the same *F* value when ultrasound was used during cooking.

The efficacy of the two cooking processes was also evaluated by enumerating total bacterial levels in white (Fig. 4A) and brown crab meat (Fig. 4B). After 5 min of ultrasound-assisted cooking the microbial load in white meat was 0.6 $\log_{10}$ cycles lower than in samples which had undergone regular cooking (2.4 *vs* 1.8 $\log_{10}$ cycles), while brown meat needed a 10 min longer treatment to achieve similar reductions (2.3 *vs* 1.8 $\log_{10}$ cycles). These results indicate that the microbial reductions in crab are significantly higher in products exposed to an ultrasound assisted cooking. In addition, the effect of ultrasound alone was evaluated by measuring microbial loads in white and brown meat treated at temperatures below 30 ºC for 45 min. In this case no differences were detected in the microbial loads regardless of the meat type ($p>0.05$).

Some authors have suggested that the higher microbial inactivation levels often observed when ultrasound and heat are combined (i.e. thermo-sonication) is due to additive or synergistic effects between the two technologies [39, 40]. However, in the case of crab cooking, when ultrasound was applied at low temperatures the microbial loads did not decrease. Therefore, the greater microbial reduction observed could be attributed to the fact that ultrasound improved heat penetration in the crab rather than as a result of the effect of ultrasound itself. For example, as a consequence of more rapid heat penetration the cumulative *F* value increased

276 at a higher rate which would result in greater reduction in microbial loads during ultrasonic
277 cooking. Furthermore, ultrasound may have sub-lethally damaged bacterial cell envelopes thus
278 reducing heat tolerance and therefore resulting in greater microbial reduction values compared
279 to those samples that received the heat only treatment [41].

280 *3.2. Mass transfer*

281 Ultrasound technology is widely used in the food industry to improve processes involving
282 mass transfer phenomena such as cleaning, extraction, brining, pickling, marinating and curing
283 [11, 24]. In this section the effect of ultrasound on the mass transfer phenomena occurring
284 during the cooking of crabs is quantified using two different methods.

285 *3.2.1. Method 1: Turbidity and conductivity of cook water*

286 Figure 5A illustrates the mean cook water turbidity value after cooking with and without
287 ultrasound. It is very clear that the turbidity of the cook water increased more rapidly after 15
288 min when ultrasound was used to assist the process. After 40 min of cooking with ultrasound
289 the turbidity of the cook water reached a maximum of 1.04 absorbance units which constitute
290 a 113.7% increase compared to turbidity in conventional cook water. The conductivity of the
291 cook water (Fig. 5B) also increased in the presence of ultrasound after 10 min indicating a
292 faster rate of ionic compound release from the crab. The maximum increase in cook water
293 conductivity was reached after 35 min of cooking, being 55.7% higher than values observed in
294 water used for the cooking without ultrasound. Regular commercial practice in the Irish crab
295 industry involves a cleaning/cooling step with fresh water immediately after cooking. This step
296 is critical in terms of microbial safety as recontamination can potentially occur. As a result,
297 immediately after the cleaning/cooling step crabs are packed and pasteurized [5, 42]. If the
298 turbidity of the cook water is considered as an indicator of the removal of dirt from the crab's
299 surface, as Image 1 suggests, the results obtained with ultrasound indicate that the use of this

300 technology may have the potential for eliminating the cleaning step as this ultrasonically
301 induced cleaning would be done concurrenly with the cooking.

302 *3.2.2. Method 2: Salt content of the crab meat*

303 The salt content in both white and brown crab meat was measured to assess the potential
304 of ultrasound to transfer substances from the cook water into the crab meat. The initial salt
305 content for raw crab was 1.43% in the white meat, which is located mainly in claws and legs,
306 and 0.89% in the brown meat, which is located inside the carapace.

307 Salt content before and after cooking with and without ultrasound and in the presence or
308 absence of 5% NaCl in the cook water is shown in Table 3. When the crabs were cooked in
309 water without NaCl added the final salt content in white meat was reduced by 33.6% using
310 regular cooking and by 46.1% for the ultrasound-assisted cooking. When 5% NaCl was added
311 to the cook water, the salt content in white meat remained stable after regular cooking and was
312 reduced by 21.1% following ultrasound assisted cooking. For brown meat, the salt content
313 remained the same when crabs were cooked either with or without ultrasound in water without
314 5% NaCl added as opposed to white meat. However, when crabs were cooked in water with
315 5% NaCl added, the salt content in brown meat slightly increased from 0.89% (raw) up to
316 1.34% in the absence of ultrasound and remained unchanged with the application of ultrasound.
317 Our results suggest that ultrasound did not facilitate the uptake of salt during cooking in water
318 with 5% NaCl added. This effect may be due to the physical barrier of the crab carapace which
319 could act as a resonant box on which cavitation bubbles are produced on the internal surfaces.
320 These bubbles could create micro-currents which could aid in the release of the salt from the
321 meat to the cook water even against an osmotic gradient.

322 The effect on white meat when crabs were cooked in water without NaCl added suggests
323 that ultrasound enhances the release of salt from the meat. Some authors observed a similar
324 effect with enhanced extraction when vegetable tissues such as tomato peel were treated with

ultrasound [20,43]. Other studies assessing the effectiveness of ultrasound for accelerating the marinating of meats reported contrasting findings to those in the current study [22, 23, 24] showed that the content of salt in slices of pork tenderloin increased when they were soaked in a saturated salt solution for 45 min with increasing ultrasound intensity. Also Siró et al. [44] showed a significant improvement in salt difussion in pork loins when ultrasound was applied. A similar effect was observed by Turhan et al. [25] who reported enhanced rates of marinating in anchovies when ultrasound was used.

3.2.3. Moisture content

Table 3 shows the moisture content of both white and brown meat before and after cooking with and without ultrasound in water with or without 5% NaCl added. Raw white meat had a moisture content of 75% while that of raw brown meat was 49%.

Regarding white meat, the moisture content remained unchanged (70-75.8%) when cooked in water without NaCl added regardless of the presence/absence of ultrasound. When NaCl was added the moisture content decreased in both cooking processes though this reduction was less evident in the presence of ultrasound.

In the case of brown meat, the moisture content increased at the same ratio, from 49% up to 56%, when crabs were cooked in water without NaCl added regardless of the cooking process. However, when cooking in water with NaCl added, the moisture content remained unchanged with the regular cooking process but increased by up to 63.6% in the presence of ultrasound.

These results revealed that overall ultrasound did not reduce the moisture content in any type of crab meat and in most cases even increased it. Other authors also observed similar effects when ultrasound assisted cooking or brining processes in meat [17, 45, 46]. Also in the case of marinated anchovies Turhan et al. [25] observed that the lower ultrasound intensity tested did not affect the final moisture content of the anchovy marinades although it decreased

while ultrasonic intensity increased. Sánchez et al. [47] also reported that the use of ultrasound increased water losses during cheese brining. These authors attributed the reduction of the moisture to the cavitation phenomenon. This controversy could be attributed to the different matrixes and ultrasonic power used. In the case of the crab the carapace could act as a barrier, reducing the moisture losses. In addition, the low ultrasonic power used in this study (0.4 W/cm^2) could explain the small effect of ultrasonic cooking on the crab's moisture content.

The above literature has shown that ultrasound can either induce mass transfer in or mass transfer out of a matrix depending on the ultrasonic conditions and matrix characteristics. In our case, the cavitation of ultrasound that occurs in the interphase between the cook water and the carapace would create water jets which would clean the surface of the crab producing the increment of turbidity and conductivity. These implosions would limit the penetration of salt observed when 5% NaCl was added to the cook water and also would reduce the water movement from inside the crab to the cooking medium, as it has been described in other food matrices. It is clear that these mass transfer phenomena require more research and a deeper evaluation of each of them by studying the influence of different ultrasound parameters with adequate equipment. What it is clear from this study is that there are potential practical benefits for the crab cooking industry.

4. CONCLUSIONS

The main objective of this research was to evaluate the potential of ultrasound to enhance and optimize the cooking process of ready-to-eat brown crab (*Cancer pagurus*). From the results obtained it can be concluded that the application of ultrasound during the cooking process enhanced the heat transfer to the crab's cold spot (the abdomen) and also proved to be useful to reduce the effect of the crab weight on the heating rate. This effect would allow a reduction of the total cooking time while the same *F* value is applied or increase the total F value applied by more than 100 % applying the same treatment time. The application of ultrasound proved its efficiency to enhance the release of substances (dirt, cook loss deposits and ionic compounds) from the crab to the cook water which would also allow for the omission of the subsequent pasteurization routinely performed in the traditional procedure. Moreover, ultrasound prevented the uptake of NaCl in the brown meat when cooked in water with 5% NaCl which was in contrast with the conventional cooking. Despite the evident advantages in economic and quality terms, more research needs to be done in this field to optimize the ultrasonic conditions for the processing of crab.

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Table 1. Heat penetration parameters (*fh* and *j*, dimensionless) arising from the application of the Ball & Olson model to the heat penetration curves in the cold spot of brown crabs (*Cancer pagurus*) of different weights and sizes cooked with or without ultrasound in water at 75 °C.

Weight (g)	Cooking type	Length (cm)	Width (cm)	*fh*	*j*	R^2	*RMSE*
300.0	Conventional	13.0	8.0	18.45	1	0.95	4.19
348.0	Conventional	14.0	9.0	21.44	1.05	0.97	1.54
375.5	Conventional	14.0	9.0	31.48	1.21	0.99	1.15
388.0	Conventional	14.0	8.5	20.68	1	0.95	3.47
398.0	Conventional	14.5	9.0	29.09	1.11	0.99	0.31
404.0	Conventional	14.5	8.5	26.45	1.02	0.99	0.55
411.4	Conventional	15.0	9.5	22.79	1.07	0.99	0.59
428.0	Conventional	14.0	9.5	17.12	1	0.98	1.09
432.0	Conventional	14.5	9.0	24.15	1.47	0.99	1.01
455.4	Conventional	14.5	9.0	33.99	1.4	0.99	0.91
462.8	Conventional	15.0	10.5	27.68	1.48	0.99	0.69
472.0	Conventional	16.0	9.0	27.55	1.27	0.99	0.03
478.0	Conventional	14.5	9.0	30.12	1.36	0.99	0.21
484.0	Conventional	15.0	9.0	34.14	1.23	0.99	0.39
500.0	Conventional	16.5	10.5	35.45	1.58	0.99	0.47
538.0	Conventional	17.0	10.5	37.88	1.13	0.99	0.19
553.6	Conventional	16.0	10.0	40.62	1.11	0.99	0.31
586.9	Conventional	16.5	10.0	35	1.06	0.99	1.62
614.6	Conventional	16.0	10.0	39.91	1.47	0.99	0.48
630.5	Conventional	16.5	11.0	43.4	1.09	0.99	0.24
640.7	Conventional	18.8	11.0	43.67	1.5	0.99	0.47
714.0	Conventional	18.0	11.5	41.35	1.4	0.99	0.69
732.7	Conventional	17.5	11.0	42.99	1.05	0.99	0.30
799.4	Conventional	17.5	11.0	40.91	1.06	0.99	1.91
816.8	Conventional	19.0	12.5	42.65	1.28	0.98	1.60
829.7	Conventional	18.0	11.0	40.97	1.29	0.99	0.64
869.7	Conventional	19.0	13.5	64.32	1	0.90	0.06
394.5	Ultrasound-assisted	14.0	8.7	29.79	1.01	0.99	1.19
422.0	Ultrasound-assisted	14.3	8.9	28.85	1.45	0.99	0.63
432.0	Ultrasound-assisted	14.5	8.5	25.03	1.46	0.99	0.38
485.4	Ultrasound-assisted	15.1	9.5	36.07	1.52	0.99	0.58
624.9	Ultrasound-assisted	16.7	10.6	36.94	1.13	0.97	1.62
660.0	Ultrasound-assisted	16.0	9.5	32.51	1.12	0.99	0.82
693.3	Ultrasound-assisted	17.5	11.2	30.42	1.04	0.94	3.88
706.0	Ultrasound-assisted	17.0	10.5	28.42	1.0	0.99	1.24
822.0	Ultrasound-assisted	18.0	11.5	43.04	1.07	0.99	0.61
842.0	Ultrasound-assisted	19.3	12.5	37.39	1.59	0.99	0.46

Table 2. First order equations correlating *fh* values (dimensionless) with the weight of brown crabs (g) during conventional and ultrasound-assisted cooking.

Cooking type	Equation	*RMSE*	*Bf*	*Af*
Conventional	$fh = 0.053 \times weight\ (g) + 4.9$	5.57	1.01	1.14
Ultrasound-assisted	$fh = 0.020 \times weight\ (g) + 20.4$	4.23	1.01	1.12

Table 3. Salt content (%) and moisture content (%) in white and brown crab meat cooked with or without ultrasound in water and water with 5% NaCl. Values represent mean value ± standard error. Statistical analyses were performed by columns for white and brown meat separately. Different letters indicate significant differences ($p \leq 0.05$).

		White meat		Brown meat	
		Water	**Water with 5% NaCl**	**Water**	**Water with 5% NaCl**
Salt content (%)	**Raw**	1.43 (0.03)^{a}	1.43 (0.03)^{a}	0.89 (0.03)^{a}	0.89 (0.03)^{a}
	CC	1.06 (0.03)^{b}	1.33 (0.03)^{a}	0.96 (0.12)^{a}	1.34 (0.02)^{b}
	USC	0.84 (0.02)^{c}	1.17 (0.05)^{b}	0.98 (0.02)^{a}	0.81 (0.04)^{a}
Moisture content (%)	**Raw**	75.83 (3.85)^{a}	75.83 (3.85)^{a}	49.08 (0.54)^{a}	49.08 (0.54)^{a}
	CC	75.02 (1.65)^{a}	60.75 (0.92)^{b}	56.61 (1.44)^{b}	45.20 (0.20)^{a}
	USC	70.08 (0.89)^{ab}	69.98 (2.85)^{ab}	56.32 (1.70)^{b}	63.60 (1.51)^{c}

Raw: Raw meat, CC: Conventional cooking, USC: Ultrasound-assisted cooking. Superscript letters showed significant differences observed between treatments in presence or absence of salt, for salt and moisture content separately, in each quind of meat.

553 **FIGURE LEGEND**

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555 **Figure 1:** Time-temperature profiles over a cooking process at 75 °C in the claw (dashed line),
556 mandibula (dotted line) and abdomen (block line) of a 500 g crab in a conventional cooking
557 process without ultrasound.

558 **Figure 2:** Relationship between crab's weight (from 300 to 870 g) and the total surface of the
559 crab's carapace (cm^2).

560 **Figure 3:** $F_{70}^{7.5}$ value (min) applied during the cooking process in the crab's cold spot (i.e.
561 abdomen), with (dashed line) and without (block line) ultrasound for the (A) small, (B) medium
562 and (C) large crabs. The horizontal dotted line represents the target $F_{70}^{7.5}$ of two minutes.

563 **Figure 4:** Microbial load over conventional (white bars) and ultrasound assisted cooking
564 processes (black bars) in (A) white meat and (B) brown meat. Dotted line shows the detection
565 limit for the counts.

566 **Figure 5:** (A) Turbidity (OD_{515}) and (B) conductivity (µS/cm) values for the cook water during
567 the cooking of brown crabs in water at 75 °C with (black bars) and without (grey bars)
568 ultrasound.

569 **Image 1:** Picture of the crab exudate reached after a conventional cooking (A) and ultrasound-
570 assisted cooking (B).

571 **Figure 1**

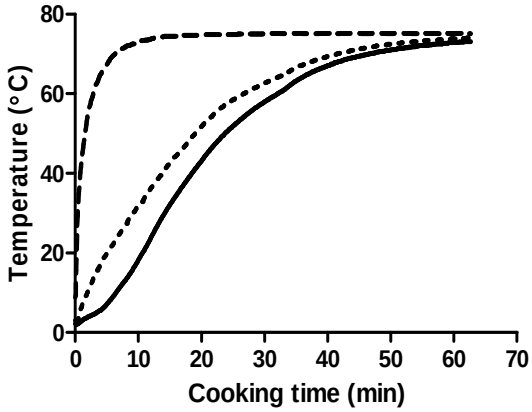

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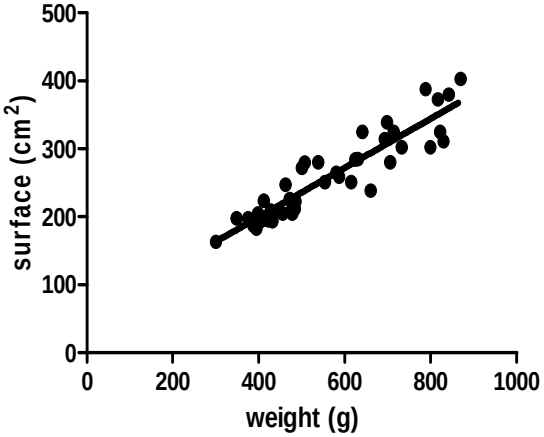
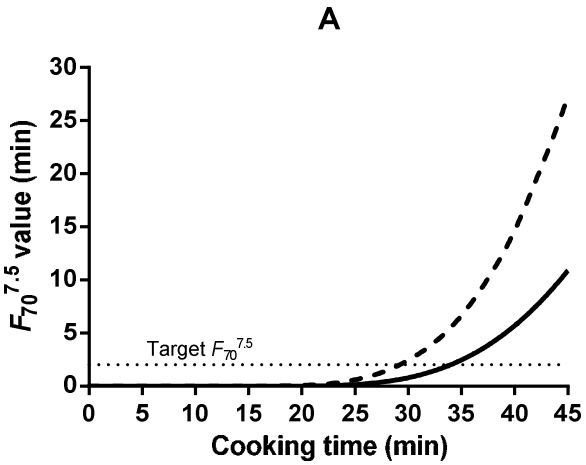
Figure 2


surface (cm2)
500
400
300
200
100
0
0
200
400
600
800
1000
weight (g)

609 **Figure 3**



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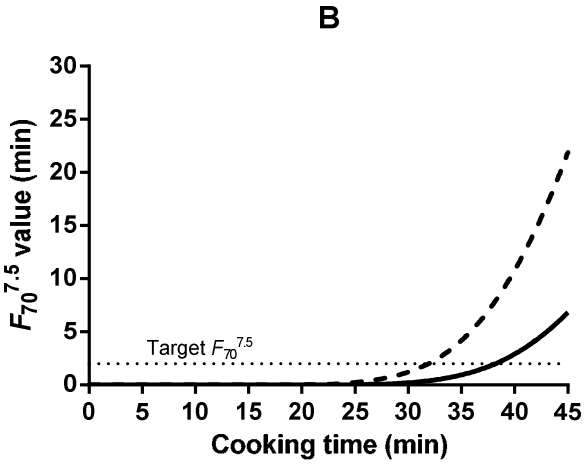

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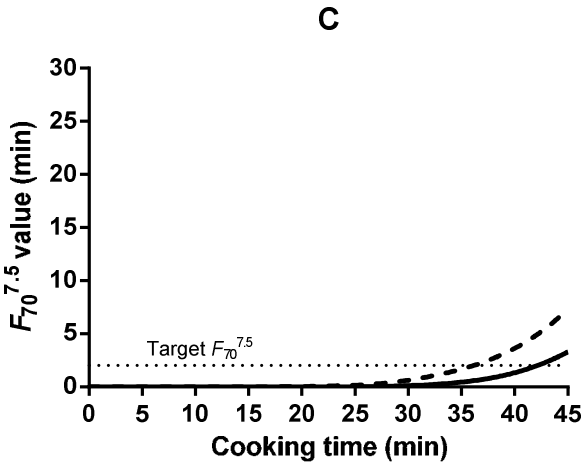

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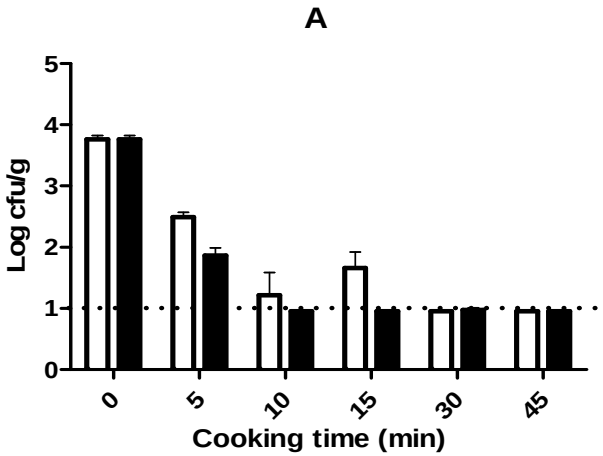
614 **Figure 4**



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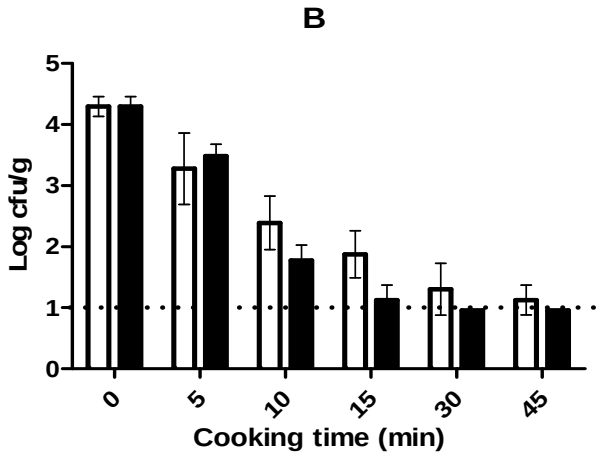

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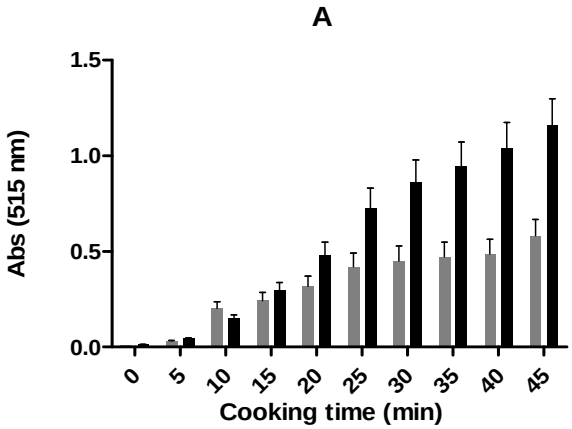
626 **Figure 5**



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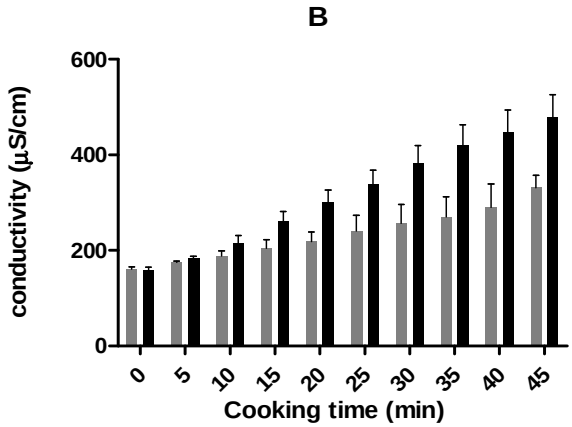

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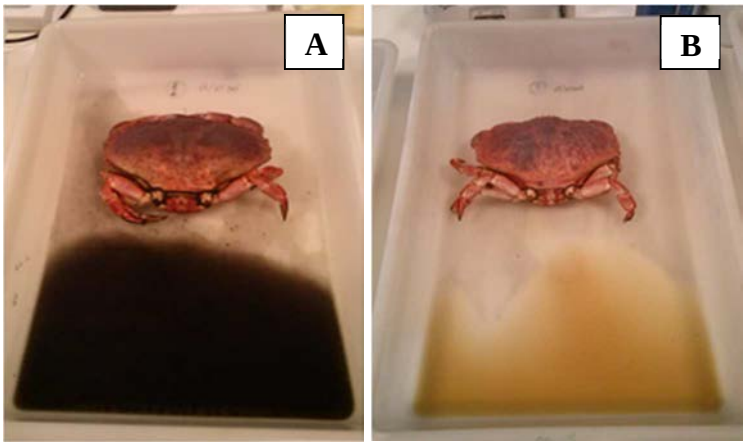
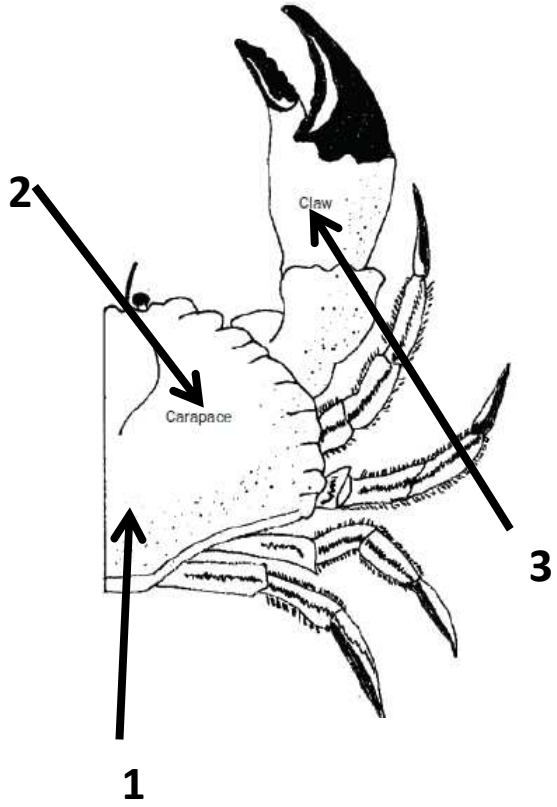
Image 1:



Complementary material

Figure 2.



Scheme of thermocouple locations during heat penetration experiments. Abdomen (1); mandibular (2) and claw (3). Image adapted from: Brown crab (Cancer pagurus). Handling and quality guide. Bord Iascaigh Mhara/Irish sea fisheries board (BIM). http://www.bim.ie/media/bim/content/publications/BIM%20Brown%20Crab%20Handling%20and%20Quality%20Guide.pdf