Facilitating shrimp (*Pandalus borealis*) peeling by power ultrasound and proteolytic enzyme

Thi Dang, Tem; Gringer, Nina; Jessen, Flemming; Olsen, Karsten; Bøknæs, Niels; Nielsen, Pia Louise; Orlie, Vibeke

Published in:
Innovative Food Science and Emerging Technologies

Link to article, DOI:
[10.1016/j.ifset.2018.04.019](https://doi.org/10.1016/j.ifset.2018.04.019)

Publication date:
2018

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Thi Dang, T., Gringer, N., Jessen, F., Olsen, K., Bøknæs, N., Nielsen, P. L., & Orlie, V. (2018). Facilitating shrimp (*Pandalus borealis*) peeling by power ultrasound and proteolytic enzyme. *Innovative Food Science and Emerging Technologies*, 47, 525-534. <https://doi.org/10.1016/j.ifset.2018.04.019>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Accepted Manuscript

Facilitating shrimp (*Pandalus borealis*) peeling by power ultrasound and proteolytic enzyme

Tem Thi Dang, Nina Gringer, Flemming Jessen, Karsten Olsen, Niels Bøknæs, Pia Louise Nielsen, Vibeke Orlie

PII: S1466-8564(18)30242-X
DOI: doi:[10.1016/j.ifset.2018.04.019](https://doi.org/10.1016/j.ifset.2018.04.019)
Reference: INNFOO 1980

To appear in: *Innovative Food Science and Emerging Technologies*

Received date: 21 March 2018
Revised date: 24 April 2018
Accepted date: 25 April 2018

Please cite this article as: Tem Thi Dang, Nina Gringer, Flemming Jessen, Karsten Olsen, Niels Bøknæs, Pia Louise Nielsen, Vibeke Orlie , Facilitating shrimp (*Pandalus borealis*) peeling by power ultrasound and proteolytic enzyme. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Innfoo(2017), doi:[10.1016/j.ifset.2018.04.019](https://doi.org/10.1016/j.ifset.2018.04.019)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Facilitating shrimp (*Pandalus borealis*) peeling by power ultrasound and proteolytic enzyme

Tem Thi Dang^a, Nina Gringer^b, Flemming Jessen^b, Karsten Olsen^a, Niels Bøknæs^c, Pia Louise Nielsen^c, Vibeke Orlie^{a,*}

^a Department of Food Science, Faculty of Science, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg C, Denmark

^b National Food Institute, Technical University of Denmark, Søtofts Plads, Building 221, DK-2800 Kgs. Lyngby, Denmark

^c Royal Greenland A/S, Hellebarden 7, DK-9230 Svenstrup J, Denmark

* Corresponding author: Department of Food Science, Faculty of Science, University of Copenhagen, Rolighedsvej 26, DK-1958 Frederiksberg C, Denmark.

E-mail: vor@food.ku.dk (V. Orlie).

Abstract

The potential of power ultrasound (24-kHz frequency) as an individual treatment and in combination with proteolytic enzyme to promote the shell-loosening of cold-water shrimp (*Pandalus borealis*) was investigated. Textural properties of shrimp were highly dependent on temperature control during the ultrasonic process (27.6- μ m amplitude, 120 min duration and 0.9-s pulse), while the peelability of shrimp monitored as peeling work, meat yield and proportion of completely peeled shrimp were less dependent on the temperature. Increasing amplitude (0-46 μ m) and time (0-45 min) of ultrasound prior to enzymatic maturation (0.5% Endocut-03L, 6 h, and 3 °C) increased the peelability of shrimp. The parallel combination of ultrasound and enzyme (18.4- μ m amplitude, 0.9-s pulse, 0.5% Endocut-3L, 3-h and 4-h duration, and $T \leq 5$ °C) considerably improved the shrimp peelability without detrimental effect on the texture and color of shrimp. Ultrasound was found to inactivate proteolytic enzyme in solution and to modify the structural properties of shrimp shells. From scanning electron micrographs (SEM), we proposed a mechanism for the ultrasound-enzyme-induced shell-loosening based on ultrasonic shell surface erosion and enzyme diffusion. Cavitation bubbles generated from sound waves pitted the surface of shrimp shell, generating pathways for enzyme diffusion into the muscle-shell attachment.

Keywords: ultrasound, enzyme, shrimp, peelability, shell-loosening.

Industrial relevance:

The shrimp industry is seeking green alternatives to the conventional shell-loosening methods *i.e.*, in brine/on ice maturations. The present study provides an investigation regarding the efficiency of power ultrasound with and without combination with proteolytic enzyme to enhance the shell-loosening and therefore improve the peelability. The findings show that ultrasound is a potential technique for shell-loosening and its efficiency is highest when simultaneously combined with enzyme. Ultrasound introduces spiral pathways in the shell and accelerates diffusion of enzyme through the pathways into the muscle-shell attachment. Thus, the use of simultaneous ultrasound-enzyme combination will shorten the shell-loosening time as compared to the conventional methods and increase the peelability without compromising the color and texture quality of shrimp.

1. Introduction

Peeling is a challenging step in the shrimp processing, especially to small sized shrimp like *Pandalus borealis*. The tight muscle-shell attachment makes it very difficult to peel freshly caught shrimp (Dang et al., 2018a). Therefore, shrimp are usually matured on ice or in brine up to several days to loosen the shell from the meat prior to peeling. Such a long maturation may cause degradation in quality and increase in microbial risk. The shrimp industry has been looking for a superior and sustainable method that promotes the shell-loosening while maintaining the organoleptic, physical and microbial quality.

Ultrasound refers to a sound wave with frequency above the range of human hearing (>20 kHz). In food industry, ultrasound can be divided into two main categories based on its application: low intensity (<1 W/cm²) with high frequency (>1 MHz) and high intensity ($10 - 1000$ W/cm²) with low frequency ($20 - 100$ kHz). Low intensity ultrasound is non-destructive and used for analytical purposes such as determination of composition, structure, physical and flow state. High intensity ultrasound (also known as power ultrasound) can cause physical disruption and is used for many purposes that require disruption such as emulsification, enzyme and microbial inactivation, extraction, foaming, bio-component separation, mass transfer enhancement, and cutting (Feng et al., 2008; Kentish & Feng, 2014; McClements, 1995). Acoustic cavitation, thermal and mechanical effects produced from ultrasound are responsible for the wide range of applications in the food industry. Acoustic cavitation is a phenomenon in which bubbles are formed, grown and collapsed. The collapse of cavitation bubbles generates physical effects such as shock wave, microjet, turbulence and shear force. Also implosion of the bubbles generates very high temperature (thousands of Kelvin degree) and pressure (hundreds of bars) for a short time (Ashokkumar, 2011). Due to its green nature, increased research and industrial

applications have focused on its use in many aspects, but few studies on the use of ultrasound for facilitating peeling exist (Dang et al., 2018a). The use of ultrasound on tomato peeling was reported to improve the peeling and yield of tomatoes without addition of any chemicals (Rock et al., 2010). Acoustic cavitation, thermal and mechanical effects produced from ultrasound were assumed to weaken the skin network of tomato, leading to the ease in skin separation.

Ultrasound has been found to either activate (Gaquere-Parker et al., 2018; Ma et al., 2011; Ma et al., 2015; Ovsianko et al., 2005; Yu et al., 2013) or deactivate (Gamboa-Santos et al., 2012; Islam et al., 2014; Kadkhodae & Povey, 2008) enzymes depending on the operating conditions and enzymes. The mechanism of the enzyme activation is explained by ultrasonic breaking of molecular aggregates, making enzymes more accessible for reaction and thus resulting in the increase of protease activity (Ma et al., 2011). Another activation mechanism is that ultrasound causes the conformational changes of the protein by forming a certain number of α -helix structure, losing β -sheet and decreasing random coil content of enzyme protein (Ma et al., 2011). Ultrasound increases the affinity between enzyme and substrate and accelerates the enzyme reaction (Jin et al., 2015). However, the excessive exposure of enzyme to ultrasound brings a counter effect, *i.e.*, the decrease of enzyme activity. The mechanism of enzyme inactivation by ultrasound is attributed mainly to the protein denaturation by shear force, temperature, pressure, and formation of free radicals during sonication (Islam et al., 2014).

The assistance of ultrasound has been reported to enhance enzymatic hydrolysis by several authors. Wang et al. (2017) found that ultrasound-glucoamylase combination increased potato starch hydrolysis due to enhancement in substrate-enzyme affinity, structural transformation of the enzyme exposing the catalytic center and binding sites, and degradation of starch granules by loosening their structure allowing water to reach the interior structure more easily. Similarly, Gaquere-Parker et al.

(2018) reported that hydrolysis of potato starch was dramatically increased by a sonication-amylase treatment regardless of various temperatures, explained by the accelerated de-polymerization of starch, the free radicals generated from sonication of water, intense mixing and diffusion in the solution. Also Li et al. (2016) found that the degree of hydrolysis of rice protein was increased upon sequentially ultrasonic and enzymatic (Alcalase) treatment, due to changes in molecular conformation as well as microstructure of substrate protein, promoting the degree of hydrolysis of protein by enzymes.

The objectives of this study were to (1) study the effects of three main treatments: ultrasound alone, sequential ultrasound-enzyme combination and parallel ultrasound enzyme combination at various conditions on shrimp peelability, (2) understand the mechanism of these treatments on shrimp shell-loosening in terms of morphological and structural properties of the shell, and (3) evaluate the color and textural properties of shrimp meat processed by these approaches.

2. Materials and methods

2.1. Shrimp

Cold water shrimp (*P. borealis*) were provided by Royal Greenland A/S (Svenstrup, Aalborg, Denmark). The shrimp were 160-200 pcs/kg in size, individually frozen, packed in carton packages and stored at -21°C . Fine table salt (NaCl) was obtained from a local supermarket (Copenhagen, Denmark). Endocut-03L (Endo3) enzyme preparation was obtained from Tailorzyme A/S (Søborg, Denmark) and stored at $4-5^{\circ}\text{C}$ until use. Endocut-03L (Endo3) is a food-grade endoprotease with 60 U/g activity and is produced from *Bacillus clausii*, as stated by the manufacturer.

2.2. Ultrasound treatments

An ultrasonic processor (UP400St, Hielscher Ultrasonics GmbH, Teltow, Germany) with a maximum power of 400 W and a fixed frequency of 24 kHz was used for sonication. A sonotrode with a tip diameter of 22 mm and a maximum immersion depth of 45 mm was coupled with the transducer. All experiments were conducted with 43-mm immersion depth in solution. The sonotrode amplitude at 100% was 46 μm (peak-to-peak) and maximum intensity 85 W/cm^2 . The pulse mode was operated for all experiments using a 0.9-s on and 0.1-s off duty cycle.

Non-temperature control and temperature control

Ultrasonic treatments were performed either without temperature control or with temperature control. Temperature control was conducted by a cooling system, and the setup is illustrated in **Figure 1**. A jacket beaker (1000 mL outer beaker, 500 mL inner beaker, Duran, Germany) containing 400 mL of 2% (v/v) pre-cooled (3 ± 2 °C) NaCl and 13 defrosted shrimp (~65 g) was connected to a cooling circulator (model Heto HMT 200, Heto-Holten A/S, Allerød, Denmark). Ethanol was used as the cooling medium (coolant) in the circulator. The coolant was kept at -10 °C and circulated to maintain shrimp temperature below 5 °C in the jacket beaker during sonication. Temperatures of shrimp and NaCl solution were monitored for each treatment, using a type-K thermocouple connected with TC-08 data logger (Pico Technology, Cambridgeshire, UK) and a thermocouple integrated with the ultrasonic processor (UP400St, Hielscher Ultrasonics GmbH, Teltow, Germany), respectively. Both non-temperature and temperature control treatments were implemented at 27.6- μm amplitude, 120-min duration and 0.9-s pulse.

Amplitude of ultrasound

In this experiment, the effect of ultrasound followed by enzymatic maturation (USE, sequential ultrasound-enzyme maturation) was studied with respect to the amplitude on the peelability of shrimp. A jacketed beaker containing 400 mL of 2% (v/v) pre-cooled (3 ± 2 °C) NaCl and 13 defrosted shrimp (~65 g) was kept cool by the cooling system (-10 °C coolant) as mentioned above during the ultrasonic process. The temperature of NaCl solution and shrimp ranged $3-10$ °C and $3-8$ °C respectively, depending on the amplitude used (temperature increased upon increasing amplitude). Ultrasound time and pulse mode was set constant at 15 min and 0.9 s respectively whereas the amplitude varied from 0 to 46 μm with a 9.2- μm interval. After sonication, the shrimp were transferred to a beaker containing 0.5% cooled Endo3 solution and matured for 6 h at 3 ± 2 °C.

Ultrasound time

The setup of this experiment was similar to the amplitude experiment, except for the ultrasound parameters. Amplitude and pulse mode were set constant at 36.8 μm and 0.9 s respectively, while the ultrasound time varied from 0 to 45 min with a 5-min interval.

Parallel ultrasound-enzyme combination

In this experiment, three different methods *i.e.*, enzymatic maturation (Endo3) alone, ultrasound alone (US), and parallel ultrasound-enzyme combination (US-Endo3) were conducted to compare their effects on shrimp peelability. Two durations of treatment, 3 h and 4 h, were investigated for all the three methods. For Endo3 maturation, the treatment conditions included an enzyme concentration of 0.5% in 2% NaCl solution, a temperature of 3 ± 2 °C and a magnetically stirring speed of 300 rpm (Dang et al., 2018b). For US alone, shrimp were submerged in a 2% NaCl solution and subjected to ultrasound (18.4- μm amplitude and 0.9-s pulse) with shrimp temperature below 5 °C during the ultrasonic process. For parallel US-Endo3 combination, shrimp were submerged in the enzyme solution

containing 0.5% Endo3 and 2% NaCl, and sonicated at 18.4- μ m amplitude, 0.9-s pulse with shrimp temperature below 5 °C.

2.3. Peelability

The shrimp peelability (proportion of completely and incompletely peeled shrimp, the peeling work of completely peeled shrimp, and the meat yield of completely peeled shrimp) was measured using the method reported in our earlier study (Dang et al., 2018b). Briefly, the three first abdominal segments of shrimp without legs were cut off, weighed (m_i) and horizontally hold by a pin attached to a customized fixture. The edge of the C-shaped shell of the three-segment portion was clamped by a clip attached to the texture analyzer (TA, 1000-g cell load, Brookfield AMETEK Inc., Middleboro, Massachusetts, USA) as the probe. The tension test was implemented to vertically pull off the shell from the meat (the peeling process). After this peeling process, the shrimp was classified as completely peeled or incompletely peeled, and peeling work and meat yield were calculated. The completely peeled proportion (%) was the percentage by count of the number of shrimp having no shells remaining on the meat relative to the total number of shrimp used for peeling (eq. (1)). The incompletely peeled proportion (%) was the percentage by count of the number of shrimp having any shells remaining on the meat relative to the total number of shrimp used for peeling (eq. (2)). The peeling work (mJ/g shrimp) was defined as the work required to pull off the shell from the three abdominal segments. The lower peeling work corresponded to the easier peeling. The work calculation was based on the force-distance curve by multiplying the force by the distance of shell pulling and dividing by the weight of the three segments (eq. (3)). Only the peeling work obtained from the completely peeled shrimp are presented and evaluated. The meat yield (%) was the amount of meat without shell obtained after peeling over the amount of the initial three-segment portion with shell on (eq. (4)).

$$\text{Completely peeled (\%)} = \frac{\text{Number of shrimp having all shell off}}{\text{Total number of shrimp used for TA peeling}} \times 100 \quad (1)$$

$$\text{Incompletely peeled (\%)} = \frac{\text{Number of shrimp having any shell attached}}{\text{Total number of shrimp used for TA peeling}} \times 100 \quad (2)$$

$$W = \frac{Fd}{m_i} \quad (3)$$

Where:

W (mJ/g) is the work.

F (N) is force applied to peeling.

d (mm) is distance pulling the shell off.

m_i (g) is initial weight of three segments with shell before peeling.

$$\text{Meat yield(\%)} = \frac{m_m}{m_i} \times 100 \quad (4)$$

Where:

m_m (g) is weight of three segments without shell after peeling.

2.4. Protease activity

The total proteolytic activity of Endo3 enzyme solution was determined using Cupp-Enyard's method (Cupp-Enyard, 2008) with some modifications. Accordingly, a 1-mL enzyme solution reacted with 5 mL of 0.65% casein in a 37 °C water bath for exactly 10 min. The reaction was stopped by adding 5 mL of 0.1 M trichloroacetic acid. A blank containing 5 mL of casein was also added 5 mL of trichloroacetic acid, followed by 1 mL of enzyme solution. All samples were then incubated in a 37 °C water bath for 30 min, followed by centrifugation at 4200 x g for 20 min. A 2-mL portion of the

supernatant was used to react with 1-mL Folin-Ciocalteu reagent in 5 mL of 0.5 M sodium carbonate environment in a 37 °C water bath for 30 min. The absorbance of samples was read at 660 nm. The calculation of proteolytic activity was as presented by Cupp-Enyard (2008).

2.5. Color and texture measurement

A raw shrimp sample (control) was used to evaluate the impact of the ultrasound and enzyme methods (3 h) on color and texture of shrimp, and an industrial reference shrimp sample (RG-Brine matured) was used to assess the practical application of the developed methods. The recipe for the industrial brine maturation was provided by Royal Greenland Seafood A/S (RG) and kept confidential in this study. Alternatively, the industrial reference shrimp could be purchased directly from many European supermarkets as it was identical to RG commercial ready-to-eat shrimp which are produced with the recipe. All untreated and treated shrimp were cooked and peeled before color and texture analyses. Cooking was performed by submerging the shrimp in 0.5% boiling salt water for 2 min. The cooked shrimp were immediately cooled in ice water for 30 s, followed by hand-peeling.

The color values of cooked and peeled shrimp were measured using a multispectral imaging system (VideometerLab 3, Videometer A/S, Hørsholm). The system illuminated the sample by a homogeneous diffuse illumination at 18 different wavelengths between 375 and 970 nm, and a CCD captures an image (2048 x 2048 pixels, and a resolution of 45 µm/pixel) of the resulting reflectance at each wavelength. The system was radiometrically calibrated according to the National Institute of Standards and Technology (NIST). Multispectral images of shrimp were acquired in the full spectral range. To build a calibration model, a solid color patch was used. Each of twenty different colors from the color patch was measured for CIELAB values by BYK colorimeter (BYK-Gardner GmbH, Geretsried, Germany), and was acquired for the multispectral image. The PLS regression models were

built based on the known CIELAB values of those colors and their corresponding averaged spectral intensity. These calibration models were used to predict the CIELAB values of shrimp from their acquired multispectral images. Twenty shrimp observations of lightness (L), redness-greenness (a), and yellowness-blueness (b) were recorded for each sample.

The texture profile of cooked and peeled shrimp was obtained by the texture profile analysis (TPA) test using a Texture Analyzer XT. plus (Stable Micro Systems Ltd., UK). A cylindrical probe of 5 cm diameter and a flat fixture were used. Trigger force was set at 0.067 N. Samples were compressed to 50% deformation with a speed of 0.1 mm/s and a pause between the two compressions of 5 s. Twenty observations of hardness, resilience and chewiness were recorded for each sample.

2.6. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of the shrimp shells were collected using a MB100 Fourier Transform Spectrometer (ABB Bomem, QC, Canada) equipped with an attenuated total reflectance (ATR) device. The spectra ranged within the region of 550 to 4000 cm^{-1} with a spectral resolution of 4 cm^{-1} , and obtained by accumulation of 64 scans and 6 replicates.

2.7. Scanning electron microscopy (SEM)

In order to understand the underlying mechanism of ultrasound-enzyme induced shell-loosening, the morphology of the surface of shrimp shell was studied by SEM. Air-dried shells were mounted, with the outer surface outward, on circular aluminum stubs with double-sided adhesive carbon tape. The sample-containing stubs were then coated with 20 nm of gold using a sputter coater (Polaron SC 7640, VG Microtech, UK). The samples were then examined and photographed in a Quanta 200

Scanning Electron Microscope (FEI Company, Hillsboro, OR, USA) at an accelerating voltage of 15 kV.

2.8. Statistical analysis

Statistical analysis was performed to the treatments through analysis of variance (ANOVA) and LSD test ($p < 0.05$), using the software SAS 9.4 (SAS ® Institute Inc., Cary, New York). The means and standard deviations were reported.

3. Results and discussion

3.1. Non-temperature and temperature control

Shrimp is highly perishable due to biochemical, microbiological, or physical changes, thus low temperature is usually required during processing. However, temperature is inevitably increasing during ultrasonic processing because of the absorption of ultrasonic energy by the solution and sample materials. The effects of ultrasound treatments with and without temperature control on the peelability and texture of shrimp are shown in **Table 1**. As can be seen, controlling temperature during US treatment resulted in significantly different textural values and meat yield, but insignificantly different peeling work. Non-temperature-controlled NTC-US-treated shrimp were firmer and chewier than temperature-control TC-US-treated shrimp. This textural behavior was attributed to the high temperature of shrimp generated during sonication (**Figure 2A**). Increased hardness of white shrimp (*Penaeus vannamei*) during NTC ultrasound treatment was previously reported by Li et al. (2011), and explained by ultrasound-induced dehydration and protein denaturation. The muscle protein denaturation by high-intensity ultrasound and long ultrasound time has been demonstrated by several researchers (McDonnell et al., 2014; Siró et al., 2009). Particularly for shrimp, the denaturation of myosin in *P. borealis* was around 37 °C and of sarcoplasmic and connective protein was around 72 °C

(Schubring, 2009). In the present study, the temperature of NTC-US-treated shrimp reached 72 °C after 42-min sonication (**Figure 2A**), thus resulting in protein denaturation, aggregation and, thereby, increased hardness. In contrast, due to the effective temperature control, the TC-US-treated shrimp maintained a temperature around 3 °C, therefore no thermal protein denaturation occurred in the shrimp, consequently, less effect on the textural attributes compared to the NTC-US-treated shrimp was observed (**Table 1**). However, the TC-US treatment consumed more ultrasonic energy than the NTC-US treatment (**Figure 2B**), probably due to higher density of the solution (NaCl) caused by low temperature and increased energy absorption rate of the solution (O'Brien, 2007).

The meat yield of NTC-US-treated shrimp (74%) was significantly lower as compared to TC-US-treated shrimp (89%) after peeling (**Table 1**). This yield difference (15%) was most likely an effect of moisture loss in the NTC-US-treated shrimp caused by a decrease in water holding capacity of proteins due to denaturation and by pressures from collagen shrinkage that expelled water from the shrimp, as presented by Erdogdu et al. (2004).

3.2. Amplitude of ultrasound

Amplitude is one of the indicators of ultrasound strength and is proportional to the power intensity of ultrasound. Increase of amplitude leads to an increase in the intensity of ultrasound, but the intended effects may not be obtained and will depend on the ultrasound application (Santos et al., 2009). The effect of amplitude on the peeling work and meat yield of shrimp treated by ultrasound followed by enzymatic maturation (USE) is presented in **Figure 3**. As can be seen, USE-treated shrimp had significantly lower peeling work and higher meat yield than the raw shrimp (at amplitude 0). The muscle-shell attachment was affected already at low amplitude, *i.e.*, the peeling work reduced by 19% at 9.2- μ m amplitude, followed by a slight reduction upon increasing amplitude levels. The same

relationship was also obtained for the meat yield. The results suggested that ultrasonic pretreatment facilitated the subsequent enzymatic maturation, resulting in easier peeling of shrimp probably due to the ultrasonically weakened muscle-shell attachment more vulnerable to the following enzyme attack. Increasing acoustic strength (amplitude) resulted in increasing weakening of the muscle-shell attachment. This could be attributed to increased cavitation and bubble dynamic upon increased amplitude. It was reported that increasing acoustic amplitude will result in a more violent cavitation bubble collapse (Adewuyi, 2001) and bubble size (Merouani et al., 2013).

3.3. Ultrasound time

The influence of different ultrasound time on peeling work and yield of USE-treated shrimp is shown in **Figure 4**. As can be seen, all the USE treatments with the ultrasound time from 5 to 45 min resulted in significantly lower peeling work and significantly higher ($p < 0.05$) meat yield than the control (raw shrimp). The muscle-shell attachment was affected already at 5 min of ultrasonic pretreatment, but without further reduction upon increasing treatment time. We suggest that the ultrasound introduced a high number of bubbles which grew and collapsed continuously resulting in mechanical effect that destructed the muscle-shell attachment to some extent. The bubbles could grow from the gas nuclei available in two sources: in the salt solution (Wu et al., 2013) and inside the shrimp tissue (Blatteau et al., 2006). Part of the bubbles derived from the salt solution would move to the surface of the solution and collapse there due to surface tension without contacting the shrimp, and part of the bubbles would collapse when contacting with the shrimp and thus affecting the muscle-shell attachment. The bubbles derived from the pre-existing gas nuclei inside the shrimp would also collapse inside the shrimp and damage the muscle-shell attachment to some extent. It is reported that bubbles were found dominantly in the joints, *e.g.*, leg joints, segment joint, head-abdomen joint, where the

shrimp mostly uses for swimming movement (Blatteau et al., 2006). After 10 min ultrasound exposure, the cavitation effect declined probably because the number of bubbles decreased.

The efficacy of USE on shrimp peelability was the result of both ultrasonic and enzymatic effects in a sequential manner impacting the muscle-shell attachment. To quantify the effect of enzymatic hydrolysis, the peeling work of Endo3-treated shrimp was measured. Accordingly, enzyme treatment alone (Endo3, 6 h and 3 °C) resulted in higher peeling work (6.44 ± 0.46 mJ/g) as compared to USE irrespective of ultrasound amplitude and time studied (**Figure 3** and **Figure 4**). It is plausible that the ultrasound degraded the shell and partial muscle-shell attachment, and the subsequent enzymatic hydrolysis took place easier, resulting in the efficient shell-loosening.

3.4. Parallel ultrasound - enzyme combination

Another approach was to combine the ultrasound and enzyme treatments simultaneously. The parallel combination of ultrasound and enzyme was investigated and the results are shown in **Figure 5**. As can be seen, the peelability of shrimp treated by the parallel US-Endo3 process was improved as compared to the individual treatments, *i.e.*, Endo3 alone or US alone, since higher proportion of completely peeled shrimp, lower peeling work and higher meat yield were obtained. US alone (either 3 h or 4 h) improved the peelability of shrimp as compared to raw shrimp, but was the least efficient treatment as a shell-loosening technique, as compared to Endo3 alone and US-Endo3, shown by the lowest proportion of completely peeled shrimp, the highest peeling work (6.8 mJ/g for 4-h treatment) and low meat yield. Although Endo3 was applied for only 3 h or 4 h, the peeling work, 6.2 and 5.8 mJ/g respectively, was much lower than the raw shrimp (7.8 mJ/g). US-Endo3 gave the highest peeling efficiency with the least peeling work of 3.9 mJ/g for 4-h treatment. Hence, the synergetic effect of ultrasound and enzyme was superior to the individual effect in shrimp peelability enhancement.

3.4.1. Proteolytic activity of enzyme solution

The activity of enzyme under ultrasonic process has been reported in two different directions: activation and inactivation, depending on types of enzyme and ultrasonic conditions. The activation effect will increase the enzymatic hydrolysis of muscle-shell attachment, consequently increasing the shell-loosening as well as peelability of shrimp. The activities of solutions containing shrimp under ultrasound and enzyme treatments individually and in combination are presented in **Figure 6**. As can be seen, the ultrasound showed inactivation effect on Endo3 activity, indicated by the constant decrease of proteolytic activity of Endo3 solution with the increase of ultrasound time. Particularly, Endo3 lost 20% (from 61 to 49 U/mL) and 30% (from 61 to 43 U/mL) of its activity after 3-h and 4-h sonication, respectively. Moreover, US did not release any intrinsic proteolytic enzymes from shrimp into salt solution, since no proteolytic activity was detected in the salt solution after US treatment alone. The findings of protease inactivation under sonications excluded the possibility that the improvement in shrimp peelability by parallel US-Endo3 treatment (**Figure 5**) resulted from ultrasound-induced activation of the enzyme.

3.4.2. Morphology of shrimp shell surface

A better understanding of the superior efficacy of the parallel US-Endo3 treatment over individual treatments can be obtained from SEM images in **Figure 7**. As can be seen, the raw shrimp shell surface exhibited a compact structure with white spots heterogeneously distributed and sized. The white spots were also observed in previous shrimp studies and were characterized as exclusive calcium carbonate (de Queiroz Antonino et al., 2017; Mikkelsen et al., 1997). The compact structure of the shell surface is attributed to dense epicuticle that composed of calcite islands surrounded by the tanned lipid-

protein matrix (Roer & Dillaman, 1984). The shell surfaces of raw shrimp and Endo3-treated shrimp were intact, rigid, smooth and non-porous, whereas the shell surfaces of US and US-Endo3-treated shrimp were rough and eroded with plenty of pits. The number of pits in the shell surface of US-Endo3-treated shrimp was substantially higher than in the shell surface of US-treated shrimp. Most of the pits had spiral shape, and their depth and width were heterogeneous. The erosion phenomenon on a solid surface (mostly metal) when treated by ultrasound has been already reported by several authors (Belova et al., 2011; Dotto et al., 2016; Pola et al., 2017). The density, shape and dimension of pits observed in those works and in the present work varied probably due to the different materials and ultrasound conditions. For instance, in the present work, pulse mode was applied in the ultrasonic process and the eroded material was a matrix made of many layers of protein-chitin-calcium; whereas in the other works continuous mode was applied and the materials were non-layered metal or chitin.

Based on SEM images, we propose a mechanism in which US-Endo3 acted to loosen the shrimp shell (**Figure 8**). In the parallel ultrasound-enzyme process, the enzyme solution played two roles: sound transmitting medium and biocatalyst. The bubbles generated from sound waves grew and collapsed continuously. The collapse of the cavitation bubbles generated shock waves, turbulence and microjets in the enzyme solution. The microjets moved at very high speed and bombarded the layers of shell, generating numerous spiral pits. The observed pits on the surface of shrimp shell functioned as micropathways or microchannels through which the enzyme passed to contact and cleave the muscle-shell attachment (see schematic **Figure 8**). Once in contact with the muscle-shell attachment, the enzyme met the substrate and started the hydrolysis along the muscle-shell attachment. When the muscle-shell attachment was hydrolyzed, the shell became loosened from the meat and thus the shell peeling became easier. It should be noted that the layers of the shell (epicuticle, exocuticle, and

endocuticle) consist of strong laminar chitin-binding proteins and calcium (Raabe et al., 2006), and therefore were not a prioritized substrate for the enzyme to cleave in the first place. On the other hand, the innermost part of the shell, termed the membranous layer, is comprised of thin and non-mineralized lamellae and is in direct contact with epidermis (Mrak et al., 2017) and would be more vulnerable to the enzyme attack.

3.4.3. Shell modification

The structural and functional properties of the shrimp shells untreated and treated by Endo3, ultrasound alone and parallel ultrasound-Endo3 combination were evaluated by FTIR spectra shown in **Figure 9**. The spectrum of Endo3-treated shell was similar to that of raw shell, suggesting that enzymatic maturation did not influence the chemical structure of the shell. Ultrasound-based treatments (US and US-Endo3) had more impacts on the structure of shrimp shell than enzyme. In particular, strong absorbance bands at 2930, 1535, 1313 and 1153 cm^{-1} were found in the spectra of US- and US-Endo3 treated shrimp shells while they were absent or very weak in raw and Endo3-treated shells. These bands are assigned to Amide B (Rahman & Halfar, 2014), Amide II (Rahman & Halfar, 2014), Amide III (Cardenas et al., 2004), and stretching C-O-C bridge respectively (de Queiroz Antonino et al., 2017). These strong peaks together are characteristic of α -chitin (Aklog et al., 2016; Lu et al., 2013), suggesting that ultrasound-based treatments were more likely to impact the chemical structure of chitin rather than other components in the shrimp shell. Especially, the peak at 2930 cm^{-1} is assigned for the $-\text{CH}_2$ stretching and $-\text{CH}_3$ vibration, not only present in chitin and protein molecule but may also be the result of a long hydrocarbon chain of lipids and carotenoids present in dried shell (Kjartansson et al., 2006). The ultrasonic erosion of shell surface might cause the exposure of carotenoids (mainly astaxanthin and its esters) and lipids which, in raw shrimp shell, are protected by

the outermost layer of the shell (epicuticle) made of wax, lipoprotein and calcites (Dall, 1965; Spearman, 1973). The stronger signals of Amide II and III in ultrasound-based treatments suggested that more protein and/or chitin were exposed during sonication. The exposure of more protein, chitin and carotenoids was probably associated to the dissociation to calcite, indicated by the less intensity in calcite peaks (1402 and 864 cm^{-1}).

There were no noticeable differences between the spectra of US- and US-Endo3-treated shell. This concluded that although the US-Endo3 treatment pitted the shell more intensely than US treatment (as shown in the SEM images) and generated the pathways for enzyme penetration into the muscle-shell attachment, the chemical structure of the shells resulted from both treatments were not affected. The superior efficiency of US-Endo3 on peelability, as compared to its counterpart US, was not necessarily associated with the chemical structure of the shell, but was apparently connected to some functional and structural changes as compared to raw and Endo3.

3.5. Color and texture quality

The CIELAB color parameters of cooked and peeled shrimp meats obtained from untreated (raw) shrimp, and shrimp treated with either Endo3, ultrasound (US), or parallel ultrasound-enzyme (US-Endo3) and an industrial reference (RG-Brine) shrimp are shown in **Figure 10A**. In general, the color (L^* , a^* , and b^*) of enzyme- and ultrasound-treated shrimp was insignificantly different from the color of raw shrimp but significantly different from the color of RG-Brine shrimp. The significant difference between the RG-Brine shrimp and the other shrimp was probably attributed to the difference in processing time (RG-Brine shrimp was matured for 24 h while the Endo3-, US- and US-Endo3-treated shrimp were processed for 3 h), in materials (*e.g.*, solutions in Endo3, US and US-Endo3 treatments did not contain any components able to discolor the shrimp), and in the cooking methods (*i.e.*, RG-Brine

shrimp was steamed for 90 s and the others were boiled for 120 s). The color results suggest that ultrasound and Endo3 enzyme either individually or in parallel combination at conditions studied did not influence the color attributes of shrimp (in comparison with raw shrimp). Although ultrasound has been reported to have astaxanthin extraction effect on shrimp byproducts (Zhang et al., 2014), the color of shrimp meat in the present study still remained unchanged after ultrasound treatment probably due to the shell barrier that protects the meat color.

The texture profile analysis (TPA) attributes of cooked shrimp meats are presented in **Figure 10B**. As can be seen, all texture attributes followed the same pattern as color parameters, *i.e.*, enzyme-treated and ultrasound-based-treated shrimp did not change the texture as compared to the untreated shrimp, but had higher values for all the texture attributes as compared to the industrial reference shrimp (RG-Brine).

The results of color and texture suggest that instrumental quality of shrimp meat was not compromised for the considerably improved peelability resulted from parallel ultrasound-enzyme combination. However, sensory analysis may be needed to make an overall evaluation regarding eating quality.

4. Conclusion

Individual and combined ultrasound and enzyme methods were used to facilitate the shrimp peeling by loosening the shell from the meat. Increasing ultrasonic amplitude and time enhanced the peelability of shrimp when the ultrasound was used as pretreatment to assist the subsequent enzymatic hydrolysis. The parallel ultrasound-enzyme combination substantially improved the peelability of shrimp due to erosion and diffusion effects. Cavitation bubbles generated from sound waves pitted the

surface of shrimp shell, generating pathways for enzyme diffusion into the muscle-shell attachment. The ultrasound either alone or in parallel combination with enzyme modified the shrimp shell not only the morphology but also the structural and functional properties *i.e.*, lower molecular weight, more carotenoids, lipid, protein and chitin exposed and loss of some calcite.

5. Acknowledgements

The authors are grateful to the Green Development and Demonstration Program (GUDP Denmark), The Danish Agrifish Agency, the Ministry of Food, Agriculture and Fisheries for the financial support to the project “Sustainable technologies for the optimization of shrimp production - TECHSHELL” (Grant number 4009-14-0870, 2015). Our thanks also go to Tailorzyme ApS (Søborg, Denmark) for kindly donating the enzymes. We thank Kirsten Sjøstrøm (Department of Food Science, University of Copenhagen) for her technical assistance with the color and texture analyses, and Franciscus Winfried J van der Berg (Department of Food Science, University of Copenhagen) for his guide in performing the color calibration from multispectral images.

References

- Adewuyi, Y. G. (2001). Sonochemistry: Environmental Science and Engineering Applications. *Industrial & Engineering Chemistry Research*, 40(22), 4681-4715.
- Aklog, Y. F., Egusa, M., Kaminaka, H., Izawa, H., Morimoto, M., Saimoto, H., & Ifuku, S. (2016). Protein/Caco(3)/Chitin Nanofiber Complex Prepared from Crab Shells by Simple Mechanical Treatment and Its Effect on Plant Growth. *International Journal of Molecular Sciences*, 17(10), 1600.
- Ashokkumar, M. (2011). The Characterization of Acoustic Cavitation Bubbles – an Overview. *Ultrasonics Sonochemistry*, 18(4), 864-872.
- Belova, V., Shchukin, D. G., Gorin, D. A., Kopyshev, A., & Mohwald, H. (2011). A New Approach to Nucleation of Cavitation Bubbles at Chemically Modified Surfaces. *Physical Chemistry Chemical Physics*, 13(17), 8015-8023.
- Blatteau, J. E., Souraud, J. B., Gemppe, E., & Boussuges, A. (2006). Gas Nuclei, Their Origin, and Their Role in Bubble Formation. *Aviation Space and Environmental Medicine*, 77(10), 1068-1076.
- Cardenas, G., Cabrera, G., Taboada, E., & Miranda, S. P. (2004). Chitin Characterization by Sem, Ftir, Xrd, and ¹³C Cross Polarization/Mass Angle Spinning Nmr. *Journal of Applied Polymer Science*, 93.
- Cupp-Enyard, C. (2008). Sigma's Non-Specific Protease Activity Assay - Casein as a Substrate. *Journal of Visualized Experiments : JoVE*(19), 899.

- Dall, W. (1965). Studies on the Physiology of a Shrimp, *Metapenaeus* Sp. (Crustacea: Decapoda: Penaeidae). Iii. Composition and Stucture of the Integument. *Australian Journal of Marine and Freshwater Research*, 16(1), 13-24.
- Dang, T. T., Gringer, N., Jessen, F., Olsen, K., Bøknæs, N., Nielsen, P. L., & Orlien, V. (2018a). Emerging and Potential Technologies for Facilitating Shrimp Peeling: A Review. *Innovative Food Science & Emerging Technologies*, 45, 228-240.
- Dang, T. T., Gringer, N., Jessen, F., Olsen, K., Bøknæs, N., Nielsen, P. L., & Orlien, V. (2018b). Enzyme-Assisted Peeling of Cold Water Shrimps (*Pandalus Borealis*). *Innovative Food Science & Emerging Technologies*, 47, 127-135.
- de Queiroz Antonino, R., Lia Fook, B. R. P., de Oliveira Lima, V. A., de Farias Rached, R. I., Lima, E. P. N., da Silva Lima, R. J., Peniche Covas, C. A., & Lia Fook, M. V. (2017). Preparation and Characterization of Chitosan Obtained from Shells of Shrimp (*Litopenaeus Vannamei* Boone). *Marine Drugs*, 15(5).
- Dotto, G. L., dos Santos, J. M. N., de Moura, J. M., & Pinto, L. A. D. (2016). Ultrasound-Assisted Treatment of Chitin: Evaluation of Physicochemical Characteristics and Dye Removal Potential. *E-Polymers*, 16(1), 49-56.
- Erdogdu, F., Balaban, M. O., Otwell, W. S., & Garrido, L. (2004). Cook-Related Yield Loss for Pacific White (*Penaeus Vannamei*) Shrimp Previously Treated with Phosphates: Effects of Shrimp Size and Internal Temperature Distribution. *Journal of Food Engineering*, 64(3), 297-300.
- Feng, H., Yang, W., & Hielscher, T. (2008). Power Ultrasound. *Food Science and Technology International*, 14(5), 433-436.

- Gamboa-Santos, J., Montilla, A., Soria, A., & Villamiel, M. (2012). Effects of Conventional and Ultrasound Blanching on Enzyme Inactivation and Carbohydrate Content of Carrots. *European Food Research and Technology*, 234(6), 1071-1079.
- Gaquere-Parker, A., Taylor, T., Hutson, R., Rizzo, A., Folds, A., Crittenden, S., Zahoor, N., Hussein, B., & Arruda, A. (2018). Low Frequency Ultrasonic-Assisted Hydrolysis of Starch in the Presence of α -Amylase. *Ultrasonics Sonochemistry*, 41(Supplement C), 404-409.
- Islam, M. N., Zhang, M., & Adhikari, B. (2014). The Inactivation of Enzymes by Ultrasound-A Review of Potential Mechanisms. *Food Reviews International*, 30(1), 1-21.
- Jin, J., Ma, H. L., Qu, W. J., Wang, K., Zhou, C. S., He, R. H., Luo, L., & Owusu, J. (2015). Effects of Multi-Frequency Power Ultrasound on the Enzymolysis of Corn Gluten Meal: Kinetics and Thermodynamics Study. *Ultrasonics Sonochemistry*, 27, 46-53.
- Kadkhodae, R., & Povey, M. J. W. (2008). Ultrasonic Inactivation of Bacillus Alpha-Amylase. I. Effect of Gas Content and Emitting Face of Probe. *Ultrasonics Sonochemistry*, 15(2), 133-142.
- Kentish, S., & Feng, H. (2014). Applications of Power Ultrasound in Food Processing. *Annual Review of Food Science and Technology*, Vol 5, 5, 263-284.
- Kjartansson, G. T., Zivanovic, S., Kristbergsson, K., & Weiss, J. (2006). Sonication-Assisted Extraction of Chitin from Shells of Fresh Water Prawns (*Macrobrachium Rosenbergii*). *Journal of Agricultural and Food Chemistry*, 54(9), 3317-3323.
- Li, S. Y., Yang, X., Zhang, Y. Y., Ma, H. L., Liang, Q. F., Qu, W. J., He, R. H., Zhou, C. S., & Mahunu, G. K. (2016). Effects of Ultrasound and Ultrasound Assisted Alkaline Pretreatments on the Enzymolysis and Structural Characteristics of Rice Protein. *Ultrasonics Sonochemistry*, 31, 20-28.

- Li, X. Y., Li, Z. X., Lin, H., & Samee, H. (2011). Effect of Power Ultrasound on the Immunoactivity and Texture Changes of Shrimp (*Penaeus Vannamei*). *Czech Journal of Food Sciences*, 29(5), 508-514.
- Lu, Y., Sun, Q., She, X., Xia, Y., Liu, Y., Li, J., & Yang, D. (2013). Fabrication and Characterisation of A-Chitin Nanofibers and Highly Transparent Chitin Films by Pulsed Ultrasonication. *Carbohydrate Polymers*, 98(2), 1497-1504.
- Ma, H. L., Huang, L. R., Jia, J. Q., He, R. H., Luo, L., & Zhu, W. X. (2011). Effect of Energy-Gathered Ultrasound on Alcalase. *Ultrasonics Sonochemistry*, 18(1), 419-424.
- Ma, X. B., Wang, W. J., Zou, M. M., Ding, T., Ye, X. Q., & Liu, D. H. (2015). Properties and Structures of Commercial Polygalacturonase with Ultrasound Treatment: Role of Ultrasound in Enzyme Activation. *Rsc Advances*, 5(130), 107591-107600.
- McClements, D. J. (1995). Advances in the Application of Ultrasound in Food Analysis and Processing. *Trends in Food Science & Technology*, 6(9), 293-299.
- McDonnell, C. K., Allen, P., Morin, C., & Lyng, J. G. (2014). The Effect of Ultrasonic Salting on Protein and Water-Protein Interactions in Meat. *Food Chemistry*, 147, 245-251.
- Merouani, S., Hamdaoui, O., Rezgui, Y., & Guemini, M. (2013). Effects of Ultrasound Frequency and Acoustic Amplitude on the Size of Sonochemically Active Bubbles – Theoretical Study. *Ultrasonics Sonochemistry*, 20(3), 815-819.
- Mikkelsen, A., Ronn, B., & Skibsted, L. H. (1997). Formation of White Spots in the Shell of Raw Shrimps During Frozen Storage. Seasonal Variation and Effects of Some Production Factors. *Journal of the Science of Food and Agriculture*, 75(4), 433-441.
- Mrak, P., Bogataj, U., Strus, J., & Znidarsic, N. (2017). Cuticle Morphogenesis in Crustacean Embryonic and Postembryonic Stages. *Arthropod Struct Dev*, 46(1), 77-95.

- O'Brien, W. D. (2007). Ultrasound - Biophysics Mechanisms. *Progress in biophysics and molecular biology*, 93(1-3), 212-255.
- Ovsianko, S. L., Chernyavsky, E. A., Minchenya, V. T., Adzerikho, I. E., & Shkumatov, V. M. (2005). Effect of Ultrasound on Activation of Serine Proteases Precursors. *Ultrasonics Sonochemistry*, 12(3), 219-223.
- Pola, A., Montesano, L., Tocci, M., & La Vecchia, G. M. (2017). Influence of Ultrasound Treatment on Cavitation Erosion Resistance of Alsi7 Alloy. *Materials*, 10(3), 256.
- Rahman, M. A., & Halfar, J. (2014). First Evidence of Chitin in Calcified Coralline Algae: New Insights into the Calcification Process of Clathromorphum Compactum. *Scientific Reports*, 4, 6162.
- Rock, C., Yang, W., Nooji, J., Teixeira, A., & Feng, H. (2010). Evaluation of Roma Tomato (*Solanum Lycopersicum*) Peeling Methods: Conventional Vs. Power Ultrasound. *Proceedings of the Florida State Horticultural Society*, 123, 245-245.
- Roer, R., & Dillaman, R. (1984). The Structure and Calcification of the Crustacean Cuticle. *American Zoologist*, 24(4), 893-909.
- Raabe, D., Romano, P., Sachs, C., Fabritius, H., Al-Sawalmih, A., Yi, S. B., Servos, G., & Hartwig, H. G. (2006). Microstructure and Crystallographic Texture of the Chitin-Protein Network in the Biological Composite Material of the Exoskeleton of the Lobster Homarus Americanus. *Materials Science and Engineering: A*, 421(1), 143-153.
- Santos, H. M., Lodeiro, C., & Capelo-Martínez, J.-L. (2009). The Power of Ultrasound *Ultrasound in Chemistry* (pp. 1-16): Wiley-VCH Verlag GmbH & Co. KGaA.
- Schubring, R. (2009). Comparative Study of Dsc Pattern, Colour and Texture of Shrimps During Heating. *Journal of Thermal Analysis and Calorimetry*, 95(3), 749.

- Siró, I., Vén, C., Balla, C., Jónás, G., Zeke, I., & Friedrich, L. (2009). Application of an Ultrasonic Assisted Curing Technique for Improving the Diffusion of Sodium Chloride in Porcine Meat. *Journal of Food Engineering*, 91(2), 353-362.
- Spearman, R. I. C. (1973). *The Integument: A Textbook of Skin Biology*. London: Cambridge University Press.
- Wang, D. L., Ma, X. B., Yan, L. F., Chantapakul, T., Wang, W. J., Ding, T., Ye, X. Q., & Liu, D. H. (2017). Ultrasound Assisted Enzymatic Hydrolysis of Starch Catalyzed by Glucoamylase: Investigation on Starch Properties and Degradation Kinetics. *Carbohydrate Polymers*, 175, 47-54.
- Wu, T. Y., Guo, N., Teh, C. Y., & Hay, J. X. W. (2013). Theory and Fundamentals of Ultrasound *Advances in Ultrasound Technology for Environmental Remediation* (pp. 5-12). Dordrecht: Springer Netherlands.
- Yu, Z.-L., Zeng, W.-C., & Lu, X.-L. (2013). Influence of Ultrasound to the Activity of Tyrosinase. *Ultrasonics Sonochemistry*, 20(3), 805-809.
- Zhang, H., Tang, B., & Row, K. H. (2014). A Green Deep Eutectic Solvent-Based Ultrasound-Assisted Method to Extract Astaxanthin from Shrimp Byproducts. *Analytical Letters*, 47(5), 742-749.

Table 1. Peelability and textural profile of shrimp after US treatment (27.6 μm , 0.9 s and 120 min) with and without temperature control.

Sample	Peelability				Texture		
	Complete (% count)	Incomplete (% count)	Work (mJ/g)	Yield (%)	Hardness (N)	Resilience (%)	Chewiness (N)
NTC-US	77	23	6.8 $\pm$ 0.3	74.2 $\pm$ 0.3	6.4 $\pm$ 0.1	40 $\pm$ 0.0	9.4 $\pm$ 0.2
TC-US	81	19	8.5 $\pm$ 0.9	89.0 $\pm$ 0.2	3.8 $\pm$ 0.2	40 $\pm$ 0.0	3.4 $\pm$ 0.2
<i>p-value</i>			0.116	<0.001	0.0059	<0.0001	0.0009

Values in the same column with $p < 0.05$ are significantly different ($n = 2 \times 13$). NTC is non-temperature control and TC is temperature control sonication.

Figure 1. Schematic setup of the temperature-control ultrasonic processing.

Figure 2. Temperature development and energy absorption during sonication. NTC is non-temperature control and TC is temperature control.

Figure 3. Effect of amplitudes on peeling work and meat yield of shrimp treated by sequential ultrasound-enzyme combination (USE). Data at 0 μm represent the mean work and yield of raw shrimp.

Figure 4. Effect of ultrasound time on peeling work and meat yield of shrimp treated by sequential ultrasound-enzyme combination (USE). Data at 0 μ m represent the mean work and yield of raw shrimp.

Figure 5. Effect of ultrasound-assisted proteolysis on shrimp peelability, (□) 3h and (■) 4h. Different letters, lower case for 3 h and upper case for 4 h, in the same parameter indicate significantly different means ($p < 0.05$, LSD test, $n = 2 \times 13$). Blue x marks on the Y-axis are data for raw shrimp.

Figure 6. Effect of ultrasound on the proteolytic activity of enzyme solution. Different letters indicate significantly different means among all treatments ($p < 0.05$, $n = 3$, nd = not detected).

Figure 7. SEM images of shrimp shell surfaces untreated (raw) and treated by endoprotease (Endo3), ultrasound (US), and parallel ultrasound-enzyme combination (US&Endo3) at 4h. Three rows display images of different magnification: LM (low magnification, 131x, scale bar = 1 mm), MM (medium magnification, 800x, scale bar = 100 μ m) and HM (high magnification, 1954x, scale bar = 50 μ m).

Figure 8. Proposed mechanism of ultrasound – enzyme combination on shell-loosening.

Figure 9. FTIR spectra of raw shrimp shell and shells treated by endoprotease (Endo3), ultrasound (US), and parallel ultrasound-enzyme combination (US&Endo3) at 4h. Each spectrum is the average of six replicates and the spectra are displaced along the Y-axis to prevent overlap.

Figure 10. Effect of ultrasound and enzyme on shrimp color (A) and texture (B). Different letters in the same parameter indicate significantly different means ($p < 0.05$, LSD test, $n = 19$).

Highlights

- Ultrasound and an endoprotease, individually and in combination, were used to loosen shell from meat of shrimp prior to peeling.
- Temperature control of ultrasound treatment significantly impacted texture but not peelability of shrimps.
- Increasing amplitude and time of ultrasound increased peelability of shrimps.
- Parallel combination of ultrasound and endoprotease considerably increased peelability of shrimps (decreased peeling work by 50%) without detrimental effects on texture and color of shrimps.
- Cavitation bubbles pitted the shrimp shell and generated pathways for enzyme diffusion into muscle-shell attachment.