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Enzyme-assisted peeling of cold water shrimps (*Pandalus borealis*)

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ABSTRACT

An enzymatic method to facilitate the peeling of cold water shrimps (*Pandalus borealis*) was developed. The protease solutions were used to mature the shrimps to promote shell-loosening prior to peeling. The efficiency of peeling enzyme-treated shrimps was evaluated by a new quantitative measurement based on the tensile force, presented as a peelability profile. It was found that enzymatic maturation efficiently improved the peelability of shrimps. The factors affecting the peelability of the enzyme-matured shrimps were the type of enzyme, enzyme concentration and maturation duration, while changes in pH had no impact. Maturation of shrimps in solutions of the endoproteases Endocut-01L (180 NU/g) and Endocut-03L (60 U/g) and the exoprotease Exocut-A0 (100 U/g) resulted in better peelability compared to shrimps matured in endoprotease Tail21 (65 U/mL) and 2% NaCl. A combination of 0.25% Endocut-03L and 0.25% Exocut-A0 for 20 h resulted in the best peeling of shrimps (100% completely peeled shrimps, 3 mJ/g work and 89% meat yield). Reuse of the enzyme solution was possible due to a 95% retention rate of proteolytic activity after two 20-h cycles of maturation. The studied enzymatic maturation offered a better shrimp product with respect to texture and color in comparison with an industrial brine-matured reference, *i.e.*, ~22% higher redness and ~31% higher hardness.

Industrial relevance: Enzymatic maturation is an attempt made as a pre-treatment to facilitate the removal of the shell from meat of shrimp. This approach would benefit the shrimp processing industry by 1) enhancing peeling efficiency that includes least efforts to remove the shell, high rate of completely peeled shrimps and high meat yield; 2) shortening the duration of maturation but still sufficiently loosening the shell for machine peeling; 3) performing as a chemical-free peeling aid, which may increase the preference of consumers over chemical compounds; and 4) being environmentally friendly since disposal of enzyme waste is harmless to the environment.

1. Introduction

Cold water shrimp, *Pandalus borealis*, is one of the most commercially important species of wild shrimps in the world (Myrset, Barletta, Di Felice, Egaas, & Dooper, 2013). The shrimp is diversely named as Northern shrimp (FAO), pink shrimp or deep-water prawn (UK, Canada and USA) and deep-sea prawn (Canada) (Holthuis, 1980). *P. borealis* is caught mainly in the North Atlantic, especially around Greenland, the Gulf of St. Lawrence, the Bay of Fundy, the Gulf of Maine and North Pacific (Holthuis, 1980). The global catch of *P. borealis* constantly decreased from around 446,909 tons in 2004 to around 260,488 tons in 2015 according to (FAO, 2017). Research in shrimp processing for improvements in efficiency, yield and quality is, therefore, important for economic sustainability.

Shell-loosening is an important step in processing of peeled shrimps. Structurally, the shell is tightly attached to the epidermis by attachment fibers (intracuticular fibers), and the epidermis is securely attached to the muscle by extensive interdigitation (Talbot, Clark Jr, & Lawrence, 1972). Thus, a pre-treatment is needed in order to loosen the muscle-shell attachment prior to peeling. Such pre-treatment is called maturation process. The current maturations of shrimps on ice and/or in brine (typically NaCl or NaCl with phosphates) solution for several days are the most common practices to facilitate the separation of the edible meat from the shell in the shrimp industry (Dang et al., 2018). Nevertheless, shrimp is highly perishable, and such long times of maturation may lead to diminish the meat quality *e.g.*, freshness, texture, color, flavor.

The intrinsic enzymes in the shrimp and enzymes from microorganisms during post-mortem storage are accountable for enhancing shell removal (Crawford, 1980). Addition of enzymes from external

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sources *e.g.*, microorganisms, plants and animals, to the maturation process may be a potential approach in accelerating shell-loosening. To the best of our knowledge, no publications or reports have documented an enzymatic maturation (also named as enzymatic peeling) of shrimps. However, technical knowledge can be found in two patents (Fehmerling, 1970; Gallant, Hong, & Ablett, 2001) for the application of an enzyme-assisted method to shell-loosening of crustaceans. Fehmerling (1970) applied a mixture of protease, carbohydrase, and cellulase to whole body crustaceans with support of vacuum, whereas Gallant et al. (2001) applied a strong concentration (~55%) of protease to the head of crustaceans. However, neither patents reported on the systematic investigation of the parameters tested in order to characterize and optimize the peeling process of shrimps. Though the enzyme-assisted method for peeling has been investigated for various types of food such as oranges (Pagan, Conde, Ibarz, & Pagan, 2006; Sanchez-Bel, Egea, Serrano, Romojaro, & Pretel, 2012; Soffer & Mannheim, 1994), pomelos (Soffer & Mannheim, 1994), grapefruits (Pagan, Conde, Ibarz, & Pagan, 2010; Rouhana & Mannheim, 1994), apricots (Toker & Bayindirli, 2003), nectarines (Toker & Bayindirli, 2003), peaches (Toker & Bayindirli, 2003), lemons (Pagan, Conde, Pagan, & Ibarz, 2011; Pagan, Ibarz, & Pagan, 2006), pears (Ibarz et al., 2013), mandarins (Pretel, Fernandez, Martinez, & Romojaro, 1998), vegetables (Suutarinen et al., 2003) and even egg-shell membranes (Stevenson, 1980). A detailed investigation of the effect of enzymatic maturation on peeling of shrimp is of industrial importance as well as adding scientific knowledge about the use of enzymes in seafood processing to the scarce literature. The aim of the present work was to provide further insight into the enzyme-induced shell-loosening, which leads to an easier peeling of the shell without affecting meat quality.

2. Materials and methods

2.1. Materials

Cold water shrimps (*P. borealis*) were provided by Royal Greenland A/S (Svenstrup, Denmark). The shrimps 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). Four proteolytic enzyme preparations were obtained from Tailorzyme A/S (Søborg, Denmark). The properties of the enzymes are shown in Table 1. All the enzymes used were food grade and stored at 4–5 °C until use.

2.2. Enzyme treatment

The setup of enzyme treatment is shown in Fig. 1A. Twelve frozen shrimps (~60 g) were thawed in tap water (~12 °C) for 20 min. Enzyme solutions were based on a standard brine solution prepared by dissolving sodium chloride (2% w/v) in 400 mL of distilled water. Appropriate amount of enzyme was added to obtain the enzyme concentration in final enzyme solution (see below). The enzyme solution was transferred into a Pyrex beaker covering the custom-made round holed rack (food-grade polyvinyl chloride (PVC)), ensuring that the enzyme solution was evenly distributed during the maturation. The thawed shrimps were horizontally aligned onto the rack, soaked completely into the enzyme solution, and magnetically stirred at 250 rpm in a fridge (5 ± 3 °C) until its peeling time.

The investigation of the efficiency of the different enzymes in shrimp shell-loosening during enzymatic maturation was carried out in seven different sets of experiments:

Experiment 1: The different enzymes were tested in a setup with all maturation parameters kept constant: enzyme concentration 0.5% v/v, maturation time 20 h, temperature 5 °C, and without pH adjustment.

Experiment 2: Buffer solutions of mixed sodium dihydrogen phosphate and disodium phosphate at pH 6, 7 and 8 were used to vary the pH of the enzyme solutions. Levels of pH were based on the enzyme data sheet from the manufacturer and the industrial preference having pH ≤ 8. Endo3 is an alkaline protease and was only adjusted to pH 7 and pH 8, whereas the Endo1 and Exo solutions were adjusted to pH 6, pH 7 and pH 8. Other maturation parameters were kept constant: concentration 0.5% v/v and for 20 h at 5 °C temperature.

Experiment 3: Each enzyme was investigated at four levels of concentration from 0.25 to 1% v/v with a 0.25% v/v interval. Other maturation parameters were kept constant: 20 h at 5 °C without pH adjustment.

Experiment 4: Three maturation times, 20, 48 and 72 h, were investigated. Other maturation parameters were kept constant: concentration 0.5% v/v and for 20 h at 5 °C temperature without pH adjustment.

Experiment 5: Pairwise combinations, 1:1, 2:1 and 1:2, of the enzymes, Endo1, Endo3 and Exo, were tested. Other maturation parameters were kept constant: total concentration 0.5% v/v and for 20 h at 5 °C temperature without pH adjustment.

Experiment 6: Optimization of the enzymatic maturation process was based on the results from experiments 1–5. Accordingly, the Endo3 solution and an Endo3 + Exo cocktail were selected. Other maturation parameters were based on the results of shortest time and lowest enzyme concentration.

Experiment 7: The efficiency of reusing the enzyme solution was investigated in a 2 × 20 h maturation cycle, thus, 1st cycle was maturation of shrimps followed by 2nd maturation of a new portion of shrimps. Maturation parameters were: Endo3 at 0.5% v/v, 5 °C and without pH adjustment.

Each experiment was conducted in two replicates with 12 shrimps for each replicate. Where relevant, the control maturation treatment is shrimp maturation in the 2% standard brine (NaCl) solution for 20 h at 5 °C.

2.3. Peelability

After enzymatic maturation, ten out of the twelve shrimps with intact shells were measured for their peelability using a force-based method. The measuring operation is demonstrated in Fig. 1B. The shrimp was wiped with absorbing paper to remove excessive water. The

Table 1
Properties of the enzymes used for shrimp maturation.

Enzyme	Code	Type of enzyme	Activity	pH range of activity	Effective temperature	Source of fermentation	Regulatory status
Endocut-01L	Endo1	Endo-protease	Min. 180 NU/g (Northrup units)	6–8	Up to 60 °C	*Bacillus subtilis*	Food grade and non-GMO
Endocut-03L	Endo3	Endo-protease	60 U/g	7–10	Up to 70 °C	*Bacillus clausii*	Food grade and non-GMO
Exocut-A0	Exo	Exo-protease	100 U/g	4–10	Up to 60 °C	*Aspergillus oryzae*	Food grade and non-GMO
Tail21	Tail	Endo-protease (narrow specificity)	65 U/mL	4–6.5	Up to 50 °C	*Rhizomucor miehei*	Food grade and non-GMO