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Kinetics of heat induced muscle protein denaturation of brown shrimp (*Crangon crangon*)

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ABSTRACT

To optimize the quality of cooked brown shrimp (*Crangon crangon*), quantitative data on the influence of all relevant process parameters (treatment time, temperature, NaCl concentration) on several quality attributes are required. Surprisingly, kinetic data and models on heat induced quality changes of brown shrimp are scarce. In this study, the denaturation of muscle proteins was studied by differential scanning calorimetry. The thermal denaturation kinetics of actin, the most heat resistant muscle protein, were determined and showed a first order decay with $k_{72\,^{\circ}C} = 0.038 \pm 0.001$ min^{-1} and $Ea = 388 \pm 7.3$ kJ/mol. Spiking experiments showed that NaCl (concentrations in the range from 1 to 5% NaCl) had a negligible influence on the actin thermal stability. The kinetic data obtained can be used to quantitatively evaluate the doneness of cooked shrimp.

1. Introduction

The brown shrimp (*Crangon crangon*) has a significant economic and social importance in the North Sea fisheries (Aviat et al., 2011). Brown shrimp are traditionally cooked and cooled directly after catch on board of the vessel by means of seawater (±3 min, 80–100 °C) (Schubring, 2009a). As fishermen do not apply a standard cooking process (in terms of cooking time, temperature and NaCl concentration of the brine), major quality differences and high yield losses can occur. Surprisingly, reports on optimizing the cooking process of brown shrimp are scarce. Schubring (2009a) measured the changes in texture, color and protein denaturation as a result of heating and Verhaeghe et al. (2016) made a kinetic study of the thermal induced inactivation of the major spoilage enzymes (proteases and polyphenoloxidase). When a cooking process is optimized, the doneness of the product is an important quality parameter. Doneness of cooked shrimp can be defined by the surface color change and denaturation of the muscle proteins at the product center (Brookmire et al., 2013).

Thermal denaturation of muscle proteins causes shrinkage of myofibrils, which leads to a loss of water binding capacity and expulsion of water (Niamnuy et al., 2008; Ofstad et al., 1995; Skipnes, 2014). On the other hand, Skipnes et al. (2008) showed that when heating cod, the proteins are denatured before major cook losses occur and the fish will appear to be cooked without major cook losses. High salt contents in the water can also induce protein conformational changes, influencing their stability and the rate of cook loss (Arason et al., 2014; Hastings et al., 1985; Thorarinsdottir et al., 2002). Therefore, a study on the influence of the process time, and temperature and the salt content of the brine on the protein denaturation can reveal mechanisms behind these quality changes. Differential scanning calorimetry (DSC) represents a method that can be used for measurement of thermal protein denaturation. Conversion of a protein from its native to a denaturated state by heat is a cooperative phenomenon, accompanied by a significant uptake of heat, resulting in an endothermic peak in the DSC curve (Schubring, 2009b).

To the best of our knowledge, no data on thermal protein denaturation kinetics of *Crangon crangon* muscle tissue has been reported so far. Therefore, the aim of this study was to quantify the *C. crangon* muscle protein thermal denaturation kinetics and the influence of the brine concentration on these kinetics. The protein

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Nomenclature

β	Beta-value
c	Concentration of standard volumetric silver nitrate solution, N
D	Decimal reduction time, min
D_{ref}	Decimal reduction time at T_{ref}, min
DSC	Differential Scanning Calorimetry
Ea	Activation energy, kJ/mol
f	Factor transforming Cl content in NaCl content
H	Residual denaturation enthalpy, J/g
H_0	Residual denaturation enthalpy at t_0, J/g
HF-DSC	Heat Flux DSC
HPLC	High Performance Liquid Chromatography
ISO	International Organization for Standardization
k	Denaturation rate constant, min^{-1}
k_{ref}	Denaturation rate constant at T_{ref}, min^{-1}
m	Mass, g
MHC	Myosin Heavy Chain
PC-DSC	Power Compensation DSC
r^2	Pearson correlation
R	Universal gas constant, J/K.mol
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
t	Time, min
t_0	Time t = 0 min
T	Temperature, °C, K
ΔT	Temperature difference, °C
T_{max}	Peak maximum temperature in thermogram, °C
T_{ref}	Reference temperature, °C
TDT	Thermal Death Time
TTT	Temperature Time Tolerance
V_0	Volume of standard volumetric silver nitrate solution in blank test, ml
V_1	Volume of standard volumetric silver nitrate solution in measurement, ml
w_{Cl}	NaCl concentration, %
z	z-value, °C

denaturation kinetics contribute to an important step in the optimization of the heating process and can give more insight in mechanisms leading to cook losses.

2. Materials and methods

2.1. Sample collection and preparation

Live brown shrimp with a minimal carapax width of 6.8 mm were bought from a local fisherman in Nieuwpoort, Belgium. The shrimp were transported at room temperature to the Institute for Agricultural and Fisheries Research within one hour. On arrival, the live shrimp were selected and decapitated. The tails were peeled and the muscle tissue was immediately frozen in liquid nitrogen. 50 frozen tails were milled under liquid nitrogen by means of a centrifugal mill, equipped with a 0.75 mm sieve (Retsch Grindomix ZM 100, Retsch, Haan, Germany). The homogeneous powder was stored at −80 °C until further analysis.

2.2. Characterization of raw material

Total protein content of the muscle tissue was analyzed by determination of the total nitrogen content, based on the Kjeldahl method according to ISO 937–1978 (ISO, 1978). The total nitrogen content determined was multiplied with 6.25 to calculate the total protein content. The water content was determined gravimetrically following drying at 103 °C as described in ISO 1442:1997 (ISO, 1997).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on the muscle tissue according to the method of Laemmli (1970) with slight modifications (Lakshmanan et al., 2005). Muscle tissue (0.5 g) was mixed with 10 ml 0.125 M Tris buffer (pH 7.5) containing 8 M urea (Merck, Darmstadt, Germany), 2% SDS (Sigma-Aldrich, St. Louis, MO, USA), and 2% 2-mercaptoethanol (Sigma-Aldrich) and homogenized (Ultra Turrax T25, IKA, Staufen, Germany) and stirred overnight at 4 °C. Samples were centrifuged (12,000 g, 20 min, 4 °C) and an aliquot of the supernatant was mixed with an equal volume of denaturing buffer (0.125 M Tris, 10% SDS, 0.002% 2-mercaptoethanol and 0.002% bromophenol blue, pH 6.7). The mixture was heated at 100 °C for 5 min and cooled in ice water. 18 µl of the mixture was loaded on a 8–18% polyacrylamide gradient gel (GE Healthcare, Buckinghamshire, UK) at 600 V and 50 mA. Gels were stained with Coomassie brilliant blue and the unknown bands were compared with those of two molecular weight markers: 14.4–97 kDa (GE Healthcare) and 10–250 kDa (Bio-Rad, Hercules, CA, USA).

2.3. Determination of salt concentration

To make a relevant assumption of salt concentrations in the shrimp during a heat treatment in salted water, shrimp were heated in a 2–10% NaCl (VWR Chemicals, Leuven, Belgium) solution for 7.5 min at 94 °C. Therefore, whole shrimp were heated in a circulated water bath at constant temperature (Grant TX150, Grant Instruments, Cambridgeshire, UK) using a high water:product ratio (25 l of water for 50 g product (30 shrimp)). After heating, shrimp were cooled for 2 min at 0 °C in a NaCl solution with the same concentration as the heating solution. The shrimp were treated in wired cages (10 × 10 × 20 cm) to avoid floating. These heating experiments were executed in duplicate.

Salt concentrations in treated and raw shrimp were quantified by determination of the chloride content according the potentiometric titration method described in ISO 1841-2:1996 (ISO, 1996) with slight modifications. Whole shrimp (2.5 g) were homogenized (Ultra Turrax) in 10 volumes of heated HPLC grade water (55 °C). 3 ml of nitric acid (Aristar BDH Laboratory Supplies, Poole, UK) was added and the mixture was titrated with a standard volumetric silver nitrate solution (0.1 N, Titripur, Merck) using a digital titrator (Brand Bürette Digital III, Brand, Wertheim Germany). The added volume of standard volumetric silver nitrate solution to reach the maximum potential difference (= titration endpoint), measured with a potentiometer (PHM83, Radiometer, Copenhagen, Denmark), was used to calculate the NaCl-content (w_{Cl} in %):

$$w_{Cl} = \frac{(V_1 - V_0)\ .\ c\ .\ f}{m}\ .\ 100\% \quad (1)$$

With V_0 the volume of standard volumetric silver nitrate solution used in a blank test (HPLC grade water with 3 ml nitric acid), V_1 the volume of silver nitrate solution used for the determination, m the mass (in grams) of the test portion, c the concentration of the standard volumetric silver nitrate solution (= 0.1 N) and f the factor expressing the result as a percentage of NaCl (= 5.84).