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NEW HORIZONS IN FOOD RESEARCH

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Gastrophysical and chemical characterization of structural changes in cooked squid mantle

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Abstract: Squid (*Loligo forbesii* and *Loligo vulgaris*) mantles were cooked by sous vide cooking using different temperatures (46°C, 55°C, 77°C) and times (30 s, 2 min, 15 min, 1 h, 5 h, 24 h), including samples of raw tissue. Macroscopic textural properties were characterized by texture analysis (TA) conducted with Meullenet-Owens razor shear blade and compared to analysis results from differential scanning calorimetry. The collagen content of raw tissues of squid was quantified as amount of total hydroxyproline using ultra-high-performance liquid chromatography. Structural changes were monitored by Raman spectroscopy and small-angle X-ray scattering and visualized by second harmonic generation microscopy. Collagen in the squid tissue was found to be highest in arms (4.3% of total protein), then fins (3.0%), and lowest in the mantle (1.5%), the content of the mantle being very low compared to that of other species of squid. Collagen was found to be the major protein responsible for cooking loss, whereas both collagen and actin were found to be key to mechanical textural changes. A significant decreased amount of cooking loss was obtained using a lower cooking temperature of 55°C compared to 77°C, without yielding significant textural changes in most TA parameters, except for TA hardness which was significantly less reduced. An optimized sous vide cooking time and temperature around 55–77°C and 0.5–5 h deserves further investigation, preferably coupled to sensory consumer evaluation.

Practical Application: The study provides knowledge about structural changes during sous vide cooking of squid mantle. The results may be translated into gastronomic use, promoting the use of an underutilized resource of delicious and nutritious protein (*Loligo vulgaris* and *Loligo forbesii*).

1 | INTRODUCTION

In many countries there are examples of unexploited sources of food. In Scandinavia, such an example is cephalopods, a group within the molluscs (e.g., squid, octopus, and cuttlefish). There is no tradition of eating cephalopods here although many species are common in the North Atlantic Ocean and the North Sea (Jensen, 2005), the most abundant being *Loligo forbesii* and *Loligo vulgaris*. Cephalopods have a high processing yield, which for squid is reported to be 60%–80% of the full body weight compared to 40%–70% in teleosts (Ampola, 1974; Raman

& Mathew, 2015). While many fish species are in decline (Food & Agriculture Organization of the United Nations, 2018), cephalopod populations have been increasing globally in the last six decades (Doubleday et al., 2016). This is conceivably due to their superior ability to adapt to changing habitats and climatic conditions, possibly caused by their relatively short lifecycle of 1–2 years (Wangvoralak & Hastie et al., 2011). Squid can therefore be an important future source of animal protein for an increasing global human population. However, cephalopods including squid are prone to have a rubbery texture, which may be a major reason for lack of choice and acceptance among Scandinavian consumers. The rubbery nature of squid is caused by their biology, their muscles being muscular hydrostats. A muscular hydrostatic mechanism is highly reliant on fibrous collagenous connective tissues and muscle fiber structures (Kier & Thompson, 2003). This may explain the high content of collagen normally reported for squid, ranging from 3% to 11% (of total protein) in the mantle of *Illex* and *Loligo*, respectively (Sikorski & Kolodziejska, 1986), to as high as 15.0% in the mantle of *Dosidicus gigas* (Uriarte-Montoya et al., 2010). Assuming a content of 12% collagen in squid, this makes its content 2.5–5 times as high compared to scallops (Findlay & Stanley, 1984) and 3 times as high compared to beef *latissimus dorsi* muscle (Lawrie, 1966; Stanley & Hultin, 1982).

Collagen is the major protein in connective tissues in all higher animals. All collagens contain a relatively large amount of hydroxyproline (Hyp), generally assumed to be 14% by weight (Bailey & Light, 1989; Vázquez-Ortíz et al., 2004) and is considered a unique identifying amino acid for collagen (Bailey & Light, 1989). Native collagen is tough in perceived texture but is softened when cooked and converted into gelatin, yielding the perceived texture of the meat more tender. Tenderness, together with juiciness and flavor, are the most important sensory aspects of meat quality (Maltin et al., 2003; O'Quinn et al., 2018). It is in general considered a paradox obtaining a tender eating quality without high amounts of cooking loss when preparing meat, as found in various studies investigating cooking and meat quality of terrestrial animals and cephalopods (Mortensen et al., 2012; 2015; Schmidt et al., 2021). This challenge can be met by applying increasingly popular low-temperature-long-time cooking methods such as sous vide cooking.

Sous vide cooking is a method of cooking in vacuumed plastic pouches at a precisely controlled temperature, yielding more choice over doneness and texture than traditional cooking methods to enhance taste and meat tenderness (Baldwin, 2012). Specifically, the physical restraint of the meat, provided by the vacuumed pouches in sous vide cooking, may increase the shrinkage temperature of collagen (Bailey & Light, 1989). In a recent review article of sous vide cooking of vegetables and seafood, Zavadlav et al. (2020) suggested sous vide cooking to be a good technique for preparing cephalopods, also highlighting a decreased cooking loss as a potential benefit. Thus, a gastrophysical approach, being the combination of scientific and culinary investigations to obtain science-based practical knowledge (Mouritsen, 2012), is applied in the present study to reveal optimized sous vide cooking treatment of squid applying multiple characterization techniques looking into the complex structure of squid mantle. A well-known physico-chemical method to obtain information about the thermodynamics of proteins in meat is differential scanning calorimetry (DSC). Total amino acid (TAA) analysis, quantifying the amount of Hyp by ultra-high pressure liquid chromatography (UHPLC) can be applied to estimate the collagen content in tissue. To complement the thermal analysis and amino acid quantification, small-angle X-ray scattering (SAXS) and Raman spectroscopy (RS) techniques are applied in the present study to provide structural information at the nanoscale, while the microscopic structure is further investigated with second harmonic generation microscopy (SHGM). Results from SHGM are further linked to mechanical texture measurements using texture analysis (TA) in order to characterize the macroscopic properties of meat structure. To the extent of the authors' knowledge, a similar comprehensive combination of the wide range of experimental techniques used in the present study has not been applied before to a single study of food. The study complements a previous physico-chemical investigation linked to sensory descriptive and hedonic analysis of squid by Danish consumers (Schmidt et al., 2021).

2 | MATERIALS AND METHODS

2.1 | Sample preparation

2.1.1 | Raw materials

Freshly caught *L. forbesii* and *L. vulgaris* were purchased from a Danish retail seafood supplier (Fiskerikajen Engros, Rungsted Havn 48, DK-2960 Rungsted Kyst, Denmark). The squids were caught by trawlers in the North Sea (Jammerbugten) in November-December 2018. Only mid-sized males were included, with full body weight in the range of 0.6–1.2 kg. Both species were included in equal amounts, as preliminary results from DSC analysis had shown no significant effect on the nature and denaturation temperature of primary proteins between the different species (results not shown). Handling and storage procedure followed previous work described in Faxholm et al. (2018) and Schmidt et al. (2020, 2021).

2.1.2 | Sous vide cooking

The cooking procedure followed the one described in Schmidt et al. (2021). Initial DSC analysis revealed three endothermic peaks at 45, 53, and 72°C, respectively. Therefore, squid mantles were sous vide cooked at 46, 55, and 77°C selected 1–5°C above the denaturation temperatures by preliminary trial and error to target denaturation of the specific protein structures. Various time points were additionally selected in addition to the temperatures. Frozen samples of squid (−40°C for >48 h and <3 months) were thawed 10 min in a water bath at room temperature (20–22°C), weighted, and repacked under vacuum. Then the samples were placed into preheated water baths (46, 55, or 77°C) and heated for 30 s, 2 min, 15 min, 1 h, 5 h, and 24 h. The samples were placed in an ice bath (0°C) for 10 min and then in a water bath at room temperature for 5 min immediately after cooking. One sample (raw squid = 0 s) went through all steps except for the cooking.

For samples for TA and FAA (free amino acid) analysis of cooking loss, the cooking treatments were performed in triplicate (true replicates), and the order of the cooking treatments and the use of squids (species and individual specimens) were from the same batch and were blindly randomized, using both *L. forbesii* and *L. vulgaris*. Samples for SAXS were also from the same sample preparation batch and were limited to one measurement per treatment ($n = 1$) with results used strictly for indicative interpretations to evaluate effectiveness of the method for future applications. Samples for collagen quantification, DSC, RS, SHGM, and cooking loss volume determination were taken from another batch of specimens selected according to the same criteria, however, only including *L. forbesii*. The cooking treatments were performed individually for each analytical method and performed in analytical triplicate. Samples for DSC and cooking loss used a randomized block design for DSC, with each block as one biological specimen, and each subject to 7 treatments with randomized treatment order.

2.2 | Texture analysis

TA was performed using a TA.XT*plus* texture analyzer (Stable Micro Systems, Vienna Court, Lammas Rd, Godalming, United Kingdom). The samples were analyzed using a Meullenet-Owens razor shear blade (MORS) (Stable Micro Systems) with a 5-kg load cell. The cooked samples were analyzed within 30 min after the sous vide cooking where they were then additionally cut into rectangular pieces (2 cm length, parallel to the long body axis, and 2.5 cm width). For each sample, five repeated measurements were performed on the same sample. The texture analyzer sheared the samples parallel to the circumflexial muscle fibers, from the outer to the inner tunic. The test was performed with a pre-test speed of 0.5 mm/s, test speed of 1 mm/s, post-test speed of 10 mm/s, distance 20 mm, and penetration distance 6 mm. The razor blade was removed and replaced regularly throughout the whole experiment to ensure sharpness. Parameters were calculated with the Texture Exponent software (version 8.1, Stable Micro Systems). TA MORS parameters included were: first peak force (the max force at the first peak), first peak distance (the distance traveled at the point of the first peak), max force (the maximum force endured across the total shearing), max force distance (the distance traveled at the point of the max force), total work of shear (the integral area of the total shearing), peak count (count of multiple peaks [fractures] across shearing), and linear distance (the linear distance of all peaks within the shearing). See Supplemental information, Appendix Figure 1 for graphical output.

2.3 | Differential scanning calorimetry

DSC analysis followed the procedure described in Schmidt et al. (2021). DSC was carried out using a micro DSC III (Setaram Inc., Caluire, France). Approximately 400 mg of sample was used for each measurement, and the water content (the differences in sample weight before and after drying at 105°C for 24 h in a drying oven) of the samples was quantified to adjust a water reference sample accordingly. The temperature scanning range was 25 to 90°C and the heating rate 1°C/min. The obtained heat flow curves were analyzed with the processing software Calisto (version 1.38, AKTS, Valais, Switzerland) calculating the integral of each endothermic peak, that is, the enthalpy change of protein denaturation. Further, the temperature at peak max (midpoint between peak onset and peak offset, i.e., protein denaturation temperature) was recorded.

2.4 | Collagen determination and amino acid content by UHPLC

2.4.1 | Amino acid analysis

UHPLC was applied to analyze the TAA content of squid tissue, in particular, the content of hydroxyproline, proline, and glycine, as well as the FAA content of sous vide cooking loss liquid. The procedure of analysis, as well as sample preparation procedures for FAA analysis, were performed as described in Schmidt et al. (2020), adapted from Hildebrand et al. (2020) and Poojary et al. (2017). All solvents used were HPLC grade, and solvents and amino acid

standards were purchased from Sigma-Aldrich, Copenhagen, Denmark. Ultrapure Milli-Q water (Milli-Q system, Millipore, Bedford, MA, USA) was used throughout the amino acid analysis procedure.

For collagen quantification, solid samples of squid tissue from the mantle, arms, and fins were subjected to TAA analysis to quantify amount of hydroxyproline using microwave hydrolysis (Biotage Initiator, Biotage, Stockholm, Sweden) before chromatographic quantification. Freeze-dried homogenized samples as described above (10 mg) were mixed with 600 µL 4 M methane sulfonic acid in a 0.5–2.0 mL Biotage glass vial added a star magnet (Biotage). The samples were flushed for 30 s with nitrogen before capping and then hydrolyzed at 165°C for 12 min and 35 s in total, using settings power = 60–80 W and abs = "very high." The sample hydrolysate was then neutralized with 4 M sodium hydroxide, diluted with Milli-Q water, and added with an internal standard, filtered, and stored in UHPLC glass vials at −20°C until analysis.

The chromatographic quantification was carried out according to the method by Hildebrand et al. (2020), using a UHPLC (UltiMate 3000 UHPLC, Thermo Fischer Scientific, CA, USA) with a reversed-phase UHPLC column (Advanced Bio AAA column, Agilent, Glostrup, Denmark 3.0 × 100 mm, 2.7 µm particle size, with a guard cartridge) and fluorescence detector (FLD). Primary amino acids were derivatized with a solution of 7.5 mM o-phthaldialdehyde (OPA) and 225 mM 3-mercapto-propionic acid (MPA) prepared in 0.1 M borate buffer (pH 8.2). OPA-MPA solution was prepared freshly and used within 8 h. Secondary amino acids (Hyp and Pro) were derivatized with 9.7 mM 9-fluorenyl-methyl chloroformate solution prepared in acetonitrile, prepared fresh every 12 h. A binary gradient system was applied with a mobile phase consisting of 10 mM Na_2HPO_4, 10 mM $Na_2B_4O_7$, and 0.5 mM sodium azide (pH 8.2, mobile phase A), and acetonitrile mixed with methanol and Milli-Q water in ratio 45:45:10 v/v/v (mobile phase B). All samples were analyzed in triplicate and amino acid concentrations were calculated into milligrams of amino acid per 100 g of hydrated squid meat (mg/100 g) or per 100 mL cooking liquid (mg/100 mL). The content of total collagen was calculated from the content of Hyp multiplied with 7.14 (Vázquez-Ortíz et al., 2004), based on the assumption that collagen contains 14% Hyp (Bailey & Light, 1989).

2.5 | Cooking loss volume quantification

The weight of each sample was recorded before and after sous vide cooking to determine the amount of cooking loss, calculated as percent lost water (cooking loss) of the sample (w/w)

$$\%\ \text{cooking loss} = \frac{(m_1 - m_2)}{m_1} \times 100 \quad (1)$$

where m_1 is the sample weight before sous vide cooking, m_2 is the sample weight after sous vide cooking, and the difference between m_1 and m_2 is assumed to be the cooking loss mass left in the sous vide bag.

2.6 | Small-angle X-ray scattering

SAXS was applied using a SAXS-lab instrument (JJ-X-ray, Copenhagen, Denmark) equipped with a 100 XL+ microfocus sealed X-ray tube (Cu-K_α radiation, Rigaku, The Woodlands Texas, USA) and a 2D 300 K Pilatus detector (Dectris Ltd, Baden, Switzerland). The samples included selected sous vide treatments (2 min at 77°C; 15 min at 77°C; 15 min at 55°C; 1 h at 55°C; and 1 h at 46°C) compared to the raw sample. A thin slice (app. 0.5–1.0 mm) was cut from a frozen piece of mantle, using the middle part between the inner and outer tunic. The samples were loaded into a vacuum-tight sample container with the circumflexial muscle fibers oriented in the vertical direction. The samples were sealed in between 5–7-µm thick mica windows (final sample thickness of 1 mm). The samples were analyzed at room temperature within a q-range of 0.01−0.93 $Å^{-1}$. The scattering vector q was defined by $q = (4\pi/\lambda) \sin\theta$, where $\lambda = 1.54$ Å is the wavelength of the incoming beam, and θ is half of the scattering angle. Each measurement was conducted under vacuum with an acquisition time of 60 or 120 min. This data collection only included one sample replicate ($n = 1$) per treatment, and the results should consequently be interpreted with caution.

2.7 | Raman spectroscopy

The fiber-optic Raman system used consisted of a near-infrared (NIR) multimode diode laser (B&W TEK Inc.) emitting at a wavelength of 785 nm into a fiber coupled into the excitation terminal of a Raman filtered fiber-optic probe (EmVision LLC). The probe consisted of eight 300-µm core, low OH 0.22 numerical aperture (NA) optical fibers with a single excitation fiber centered in a ring of seven collection fibers. The probe tip contained a doughnut-shaped long-pass filter for suppression of Rayleigh scattered light, and a circular laser clean-up filter was applied for removal of unwanted spectral emissions from the laser and fibers. The probe tip was terminated by a glass window, and in-line laser clean-up and

long-pass filters (Semrock) were utilized between laser to probe and probe to spectrometer. Raman scattered light collected from the probe was directed to the 0.3 NA, transmission grating-based Eagle Raman-S spectrometer (Ibsen Photonics) integrated with an iVAC 316, Peltier cooled (−70°C), back-illuminated, deep depletion charge-coupled device (CCD) (Andor, Oxford Instruments). All data analysis was performed in costumer written algorithms in MATLAB R2017a environment (MathWorks Inc.). Spectra were subject to baseline correction using a Whittaker filter-based, asymmetric least squares fitting (Eilers, 2003; Felten et al., 2015) to remove unwanted auto-fluorescent background and were also subject to vector normalization for removal of any instrument effects. The analysis was carried out for the interior side, the exterior side, and the middle of the squid mantle. Each spectrum was measured three times, using 30 s acquisition time and 60 mW laser power.

2.8 | Second harmonic generation microscopy

SHGM was performed on a Leica SP8 microscope (Leica Microsystems GmbH, Mannheim, Germany) equipped with an optical parametric oscillator and tunable picoEMERALD multiphoton laser (APE, Berlin, Germany) using a combination of a fixed beam set to 1064.4 nm and a second tunable beam at 970 nm. Imaging of squid mantle samples was done using a water immersion objective (Leica, 40x HC PL IRAPO 1.10), and the signal was collected in backscatter mode through a filter cube with a dichroic mirror at 560 nm and a broad band pass filter at 465/170 on a photomultiplier tube (PMT) detector. Imaging was performed at room temperature (22°C). The output-data were collected as image stacks at different levels in the z-direction from the outer and inner tunic (0–100 μm). The samples included selected sous vide treatments (0 s at 0 min [raw sample]; 30 s at 77°C; 15 min at 77°C; 24 h at 77°C; 15 min at 55°C; and 1 h at 46°C).

2.9 | Statistical analysis

All instrumental data was obtained in triplicate unless otherwise stated, and the values were expressed as mean ± standard deviation or standard error mean. Statistical comparisons among different sous vide cooking treatments were made using 1-way ANOVA and Kruskal–Wallis test for parametric and non-parametric data, respectively. A multiway comparison was conducted using Tukey's HSD test (parametric data) and correlations were evaluated using Spearman's correlation (ρ_S) coefficient (non-parametric data). A p-value <0.05 was considered statistically significant. All statistical analyses were performed using SAS-JMP software (version 14.0.0, SAS Institute, Cary, NC, USA). Principal component analysis (PCA) was conducted in Latentix (version 2.12, Frederiksberg, Denmark) of mean values (TA, DSC, cooking loss) with all data auto-scaled before analysis.

3 | RESULTS AND DISCUSSION

3.1 | Texture analysis

The TA parameters calculated from the force versus distance curve include: first peak force, first peak distance, max force, max force distance, peak count, linear distance, and total work of shear. All TA parameters are found to differentiate sample treatments significantly ($p < 0.05$).

A PCA bi-plot (Appendix Figure 2) of the TA data reveals a clear different textural pattern for samples cooked at 46°C compared to samples cooked at 55 and 77°C with principal component (PC) #1 accounting for 71.3% of the variance and PC#2 accounting for 23.9%. The variation between samples cooked at 46°C at different cooking times are only described by PC#2, being a small portion of the variation described by TA, whereas samples cooked at 55 and 77°C are described by PC#1 as well, PC#1 accounting for most of the variation displayed by mechanical texture. This indicates that samples cooked for 46°C do not undergo the same textural changes as at the higher cooking temperatures of 55 and 77°C, indicating that 46°C is a too low cooking temperature to obtain a desirable tenderization, a fact that is also confirmed by statistical analysis where no significant mechanical textural changes across all TA parameters compared to raw squid are obtained for samples cooked at 46°C. Samples cooked at 46°C are dominated by TA peak count, whereas samples cooked at 55 and 77°C are dominated by TA linear distance, TA total work of shear, TA max force, and TA first peak force, seen for the less-cooked samples, and with TA first peak distance and TA max force distance seen to describe the samples cooked for a longer time, meaning that the resistance of the surface layers are eliminated by these cooking treatments (55°C: 5 h; 77°C: 15 min, 5 h, 24 h). Multivariate correlation analysis show significant positive and negative Spearman's correlation values between TA total work of shear and TA max force ($\rho_S = 0.95$, $p < 0.0001$), TA max force and TA first peak force ($\rho_S = 0.95$, $p < 0.0001$), TA linear distance and TA total work of shear ($\rho_S = 0.90$, $p < 0.0001$), TA linear distance and TA max force ($\rho_S = 0.90$, $p < 0.0001$), TA total work of shear and TA first peak force ($\rho_S = 0.90$, $p < 0.0001$), and TA linear distance and TA first peak force ($\rho_S = 0.88$, $p < 0.0001$). This implies that fewer TA

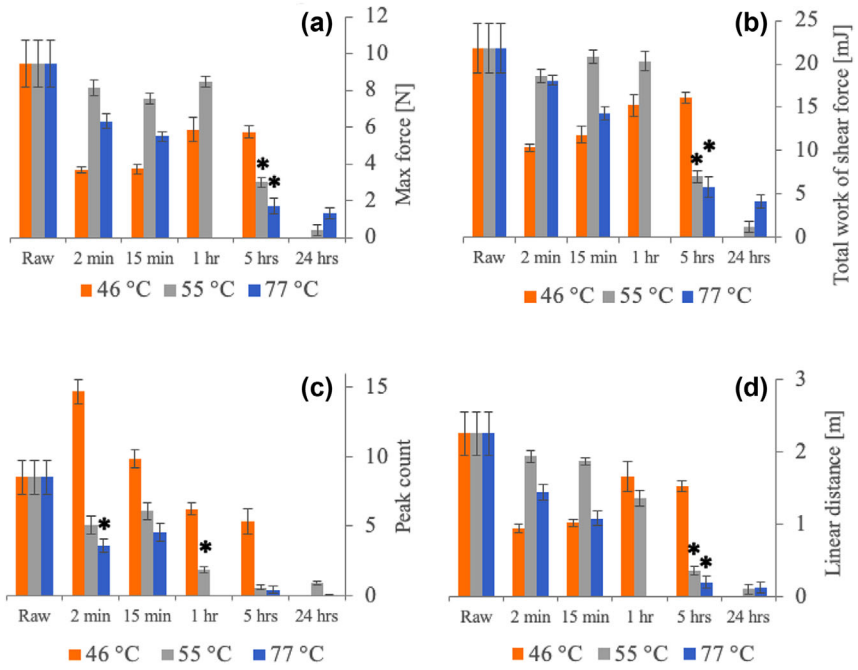

FIGURE 1 Texture analysis by Meullenet-Owens razor shear blade (MORS) of sous vide cooked squid mantle displaying (a) TA max force, (b) TA total work of shear, (c) TA peak count, and (d) TA linear distance. Mean values ($\bar{x}$) of force in [N], distance in [m], and work in [mJ]. Error bars display standard error mean of cooking treatment ($n = 3$) with a blocked effect from analytical replicate ($n = 4$). *Note*: Missing values for cooking treatment at 77 °C for 1 hr; and cooking treatment at 46 °C for 24 hrs, that is, do not denote zero force

parameters can possibly be included in future studies. For example, in addition to TA peak count, TA linear distance may be applied instead of TA first peak force, TA max force, and/or TA total work of force. This will be an advantage since the mechanical output for very cooked samples does not typically display very clear peaks (fractures). Nonetheless, for surface-hard foods and foods that consist of different structural layers such as squid, additional information can be revealed by including both TA first peak and TA max peak in addition to TA linear distance, by noticing when TA first peak force is no longer equal to TA max force. This provides an indication of time of denaturation across the different structural layers across the mantle thickness. This is the case after 5 h of cooking at 55 and 77°C for squid mantle, meaning that the hard surface structures of raw squid are eliminated and the maximum force endured by the shear blade occurs at a later stage of the shearing, deeper inside the mantle layers. TA by MORS was previously introduced to analyze chicken breast quality (Cavitt et al., 2001) and has frequently since been applied for meat quality testing (Chatterjee et al., 2016) where peak count and linear distance or max force are the most often used parameters. The present results show that TA by MORS may be a useful method for analyzing squid mantle, especially when measuring raw tissue quality using TA peak count, and for cooked squid using TA linear distance while including additional parameters of TA first peak force and max force.

Selected TA parameters are presented in Figure 1, displaying a bar plot of TA max force [N], TA total work of shear [mJ], TA peak count, and TA linear distance [m] with stars of significance indicating significant difference ($p < 0.05$) to raw squid in mechanical texture (Figure 1). No significant mechanical textural changes compared to raw squid are obtained when cooking at 46°C, whereas cooking at 55 and 77°C yields a significant decrease in TA max force, TA total work of shear, and TA linear distance from 5 h as well as a significant decrease in TA peak count from 2 min and 1 h, respectively (Figure 1). Comparing 55 to 77°C treatments (i.e., leaving out 46°C treatments in the statistical analysis) the two treatments differed significantly only at selected cooking times and parameters, with TA max force being significantly different between the two cooking temperatures for all cooking times ≥15 min ($p < 0.05$). The mechanical force of raw squid for TA max force was 21% and 18% less decreased by cooking at 55°C compared to 77°C for 15 min and 5 h, respectively. In case of TA total work of shear force, the corresponding results were 30% and 6%, respectively, for TA peak count 18% and 2%, and for TA linear distance 35% and 8%.

An increase in the mechanical texture of TA max force, TA total work of shear, and TA linear distance (Figure 1a,b,d) for samples cooked at 46°C is noticed, despite a decrease in TA peak count (Figure 1c). The increase in TA max force, TA total work of shear, and TA linear distance with cooking time points to the possibility that enzymatic activity may occur in the tissue when cooking at 46°C, denaturing myofibrillar structures (decrease in peak count) with simultaneous enforcement of collagen structures by enzyme-catalyzed protein crosslinking. This is consistent with results from cooking loss volume and FAA content (see Section 3.2).

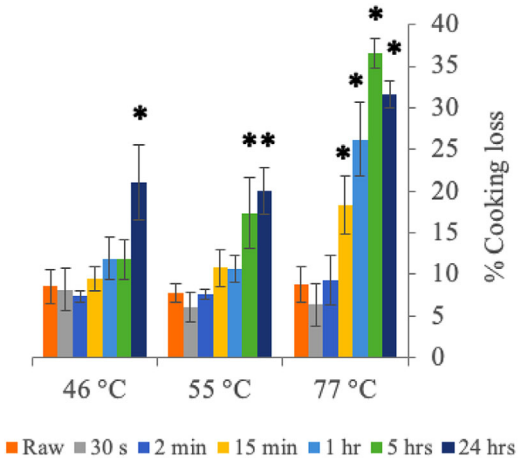

FIGURE 2 Cooking loss amount (%) (w/w) mean ($n = 3$) and error bars of the standard deviation of different sous vide cooking treatments of squid. The bars are colored according to cooking time. Star of significance (*, $p < 0.05$) indicates a significant difference from raw squid at the particular cooking temperature (i.e., cooking loss onset), based on a 1-way ANOVA of comparison within group

3.2 | Cooking loss

Cooking loss volumes are presented in Figure 2, assigning a star of significance to given cooking time that leads to a significant increase in cooking loss compared to raw squid for each cooking temperature (i.e., comparison within group) (Figure 2). A noticeable increase in cooking loss is seen after a shorter time of cooking when applying a higher cooking temperature (77°C), implying that a lower cooking temperature (46, 55°C) results in a lower amount of cooking loss, except when the cooking time is extended to very long time (24 h). By excluding the 46°C treatments from statistical comparison (yielding insufficient textural changes as per results from TA [Section 3.1]), it is found that the amount of cooking loss is significantly decreased by lowering the temperature from 77 to 55°C, at cooking for 15 min ($p = 0.0247$) by 41%, 1 h ($p = 0.0020$) by 60%, 5 h ($p = 0.0005$) by 53%, and 24 h ($p = 0.0102$) by 35%, as previously reported in Schmidt et al. (2021). Yet, applying a lower temperature is not without compromising the textural outcome (see Sections 3.1 and 3.3).

In addition to records of the amount of cooking loss, the FAA content in the cooking loss liquid was analyzed to assess the degree of protein hydrolysis of the tissue into released FAA in the cooking loss, in particular, those derived from collagen, being Hyp, Pro, and Gly. The contents of selected FAA in the cooking liquids from all sous vide cooking treatments are presented in Appendix Table 1 (Supplemental Information). A substantial sum of collagen-specific amino acids Hyp, Pro, and Gly is noticed in the 46°C at 24 h treatment, being two times larger compared to the corresponding content found in the cooking liquid for treatments using a higher cooking temperature (55 and 77°C). This may be caused by proteolytic activity, which is known to be high in cephalopods and higher than in most fish species (Kolodziejska et al., 1987; Stanley & Hultin, 1985). Proteolytic peak activity has previously been reported for *Loligo* squid to occur at 40 and 60–65°C at a neutral pH (Ayensa et al., 1999; Rodger et al., 1984) which may be a possible explanation of the different behavior of the samples cooked at cooking temperature of 46°C, signaled in terms of results from FAA content of the cooking loss and TA parameters (Section 3.1). However, mechanical textural measurements were not performed for samples cooked at 46°C for 24 h, as these samples were too dissolved, which, however, also points to enzymatic activity, since this was not observed for samples cooked at a higher temperature for 24 h.

Quantification of collagen in terms of total Hyp is displayed in Table 1, presenting the content of total Hyp found in the tissue of squid mantle as well as for arms and fins for comparison. From Table 1, it is seen that the arms (4.3% collagen of total protein) and fins (3.0%), respectively, contain three and two times as much collagen compared to the raw mantle (1.5%). It is further seen that the free amount of Hyp out of total Hyp is greater in the cooked mantle (25%) than in raw (18%), but it is noticed that the free Hyp consists of a substantially greater percentage of total Hyp in the mantle than in the arms (3.5%) and fins (4.9%) which may be caused by high protease activity in squid mantle (Ayensa et al., 1999; Kolodziejsk et al., 1987; Rodger et al., 1984; Stanley & Hultin, 1985). Overall, the collagen content of the mantle for Nordic squid, *L. forbesii*, is substantially lower than the content reported in the literature for other species of squid, being 3%–15% of total protein (cf. Raman & Mathew, 2014; Sikorski & Kolodziejska, 1986; Uriarte-Montoya et al., 2010).

3.3 | Differential scanning calorimetry

A typical DSC thermogram for a sample of raw squid is presented in Figure 3. As described in a previous work (Schmidt et al., 2021) DSC of squid show three endothermic denaturation peaks in the overall heat capacity as a function of temperature, suggesting thermal denaturation of three different structural proteins, identified from their respective peak max temperature. The three proteins are found to denature in the following order: myosin ($peak_{max}$ temperature = 45–46°C), collagen ($peak_{max}$ temperature = 53–58°C), and actin ($peak_{max}$ temperature = 72–74°C), which is consistent with findings by other authors, observing three endothermic peaks at 46–50, 57, and 74–79°C for other species of squid (Mochizuki et al., 1995;

TABLE 1 The free and total content of hydroxyproline (mg/100 g) and calculated collagen content in squid tissue (hydrated weight). Collagen is based on a 14% content of Hyp (Bailey & Light, 1989) in collagen and 16.2% and 18.5% total protein in squid arm and mantle, respectively (Atayeter & Eroskun, 2011) from *Loligo vulgaris*. Cooked mantle refers to sous vide cooking at 55°C for 30 min

Squid tissue	Free Hyp		Total Hyp		Free / Total Hyp	Collagen	
	$\bar{x}$	σ	$\bar{x}$	σ	%	g collagen/100 g tissue	% collagenin total protein
Arms	3.4	0.2	97.0	10.7	3.5	0.69	4.28
Fins	3.4	1.1	68.8	15.2	4.9	0.49	3.03
Mantle, raw	6.6	0.1	37.6	7.9	18	0.27	1.45
Mantle, cooked	8.9	1.9	35.3	6.2	25	0.25	1.36

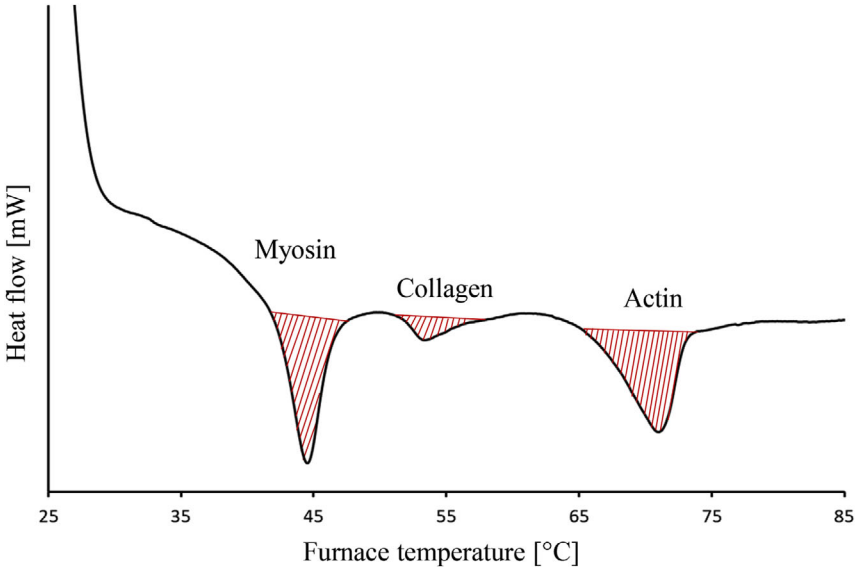

FIGURE 3 Thermogram from DSC analysis of raw squid mantle. Three protein denaturation events identified are pertaining to myosin, collagen, and actin

Paredi et al., 1996). Yet, exact protein identification for each endothermic peak can be difficult, since different proteins may denature at the same $peak_{max}$ temperature. Still, in the following we will assign that the endothermic peaks to myosin, collagen, and actin.

Table 2 displays percent native protein of myosin, collagen, and actin over time at each cooking temperature. Protein denaturation to a degree ≤10% native protein is assumed to comprise a fairly denatured protein with a negligible structural effect. Myosin seems to be largely degraded (i.e., ≤10% native protein) after only 30 s at 77°C, 2–15 min at 55°C, and 1 h at 46°C. Collagen seems to be largely denatured to a similar degree after 1–5 h at 77°C, 5 h at 55°C, and 24 h at 46°C. Actin, on the other hand, seems to be largely degraded after 1 h at 77°C, while is not degraded at all by cooking at 55°C and 46°C. The chosen cooking treatments successfully consist of samples that can be differentiated based on remaining native protein, enabling the ascription of different macroscopic effects (texture and cooking loss amount) to different proteins.

It should be noted that large standard deviations for DSC measurements are present, in particular for collagen. The high standard deviation from collagen denaturation analyzed by DSC may be caused by sample position origin (variation across the mantle). In particular, differences in the degree of hydroxylation (i.e., increased Hyp content) may increase the collagen denaturation temperature due to stabilization of the helix structure of collagen (Ikoma et al., 2003; Bailey & Light, 1989) which was also suggested to be a significant cause of variation by other authors (Otwell & Hamann, 1979). This may be supported by the varying degree of denaturation temperature for collagen ($peak_{max}$ = 53–58°C), which varied between samples to a much greater extent compared to denaturation temperatures of myosin ($peak_{max}$ = 45–46°C) and actin ($peak_{max}$ = 72–74°C). Yet, the cause of variation for denaturation temperature of collagen can also be due to the presence of other proteins around the same denaturation temperature, such as paramyosin. Paramyosin is also known to be present in high amounts in invertebrates (Kantha et al., 1990) and especially high amounts in molluscs (Belitz et al., 2009) and has previously been identified in squid by SDS-PAGE (Ketnawa & Rawdkunen, 2011). Paramyosin may potentially be present at around 55°C as found for whole scallop muscle (Paredi et al., 2003), which can explain the wider range of $peak_{max}$ temperature of collagen in the present study (Figure 3).

TABLE 2 Differential scanning calorimetry analysis of *Loligo forbesii* mantle cooked by sous vide (46, 55, 77°C) for different times (raw, 30 s, 2 min, 15 min, 1 h, 5 h, 24 h). Identified proteins are myosin, collagen, and actin. Values display mean ($n = 3$) in percent native protein after sous vide cooking ± standard deviation

Cooking treatment		Myosin		Collagen		Actin	
Time	Temperature	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ
30 s	46°C	107	12	128	89	126	12
2 min		86	16	118	78	113	24
15 min		23	27	80	37	128	40
1 h		9	2	118	28	117	38
5 h		5	5	101	65	90	24
24 h		0	0	0	0	96	15
30 s	55°C	42	48	119	77	86	14
2 min		17	11	107	110	104	8
15 min		6	6	52	61	94	25
1 h		1	2	41	71	120	5
5 h		0	0	0	0	96	7
24 h		0	0	0	0	100	11
30 s	77°C	11	2	146	153	111	29
2 min		7	1	36	46	65	56
15 min		8	13	69	98	30	46
1 h		3	6	16	27	3	6
5 h		0	0	3	6	1	1
24 h		0	0	0	0	0	0

3.4 | Small-angle X-ray scattering

Results from SAXS are presented in Figure 4 showing the 2D pattern of SAXS for a selection of sample treatments. The measurement of the middle part of the muscle layer in raw squid showed an anisotropic 2D-scattering pattern with a clear structural feature (Figure 4a) that disappears gradually with prolonged cooking (Figure 4b–f). The anisotropy partly remains after 2 min of cooking at 77°C (Figure 4e), while further cooking at 77°C for 15 min results in a complete isotropic scattering pattern (Figure 4f). The anisotropy also disappears after 1 h cooking at 46°C and 15 min at 55°C (Figure 4b,c), which affect the anisotropy of the scattering pattern less compared to cooking at 77°C for 2 and 15 min. Finally, cooking for 1 h at 55°C results in a nearly isotropic pattern, which possibly indicates complete collagen denaturation (Figure 4d). Similar scattering patterns from collagen have been reported for human tissue (Suhonen et al., 2005) and beef (Wells et al., 2013).

Besides the loss of anisotropy qualitatively inferred from the 2D data in Figure 4, structural information can also be derived based on the 1D data output. Bragg peaks, signaling structural features, were identified at $q = 0.095$ nm^{-1} and $q = 0.2$ nm^{-1} in the azimuthally averaged 1D data of raw squid in the present study (results not shown) indicating a layered periodicity. The first Bragg peak ($q = 0.095$ nm^{-1}) can be converted to a real space dimension via

$$d = 2\pi/q \tag{2}$$

where d denotes the length of the so-called D-gap and q is the position of the first Bragg peak. From this relation, the exact length of the D-gap of the structural feature was calculated to be 66 nm. The loss of anisotropy during cooking compared to raw squid (Figure 4) can thereby be correlated with a loss of the 66 nm periodicity, which, according to the literature, is ascribed to changes of both perimysia and endomysia collagen fibers and overall collagen shrinkage (Bailey & Light, 1989). The D-gap, generally reported to be 60–70 nm, is caused by a highly ordered periodicity in the electron density along the long axis of the collagen fibers. Consequently, collagen denaturation is likely to be responsible for the Bragg peaks observed here for squid mantle as well as the loss of anisotropy by cooking displayed by SAXS. The presented SAXS measurements therefore presumably illustrate structural changes in collagen fibers during cooking, which is here for the first time applied to investigate thermo-induced changes in squid.

3.5 | Second harmonic generation microscopy

The data obtained by second harmonic generating microscopy (SHGM) is presented in Figure 5, displaying the second harmonic generation structures in the squid tissue, identified as myofibrillar structures and collagen (Brüggemann et al., 2009). The displayed SHGM images from raw squid (Figure 5a) are from different depths in the tissue from both the interior and exterior sides of the mantle (i.e., inner and outer tunics). A clear change in tissue structure is seen from both sides. From the interior side, 1–2 μm-thin collagen fibers arranged in multiple directions dominate at low depths (z: 5 μm); at increasing depths (z: 15–45 μm) the fibers become gradually thicker to about 10–20 μm while still being arranged in multiple directions; at further depths (z: 60–75 μm), the observed SHG structures become dominated by myofibrillar structures aligned along the same direction with an occasional crossing of fibers on a longer length scale (images not shown). From the exterior side (i.e., outer tunic), a layer of thin collagen fibers is also observed (z: 5–15 μm), but this layer is thinner and quickly changes into aligned myofibrillar structures (z: 20 μm). These findings are similar to those previously described for *Loligo pealei* using electron microscopy (Ketnawa & Rawdkunen, 2011; Otwell & Giddings, 1980).

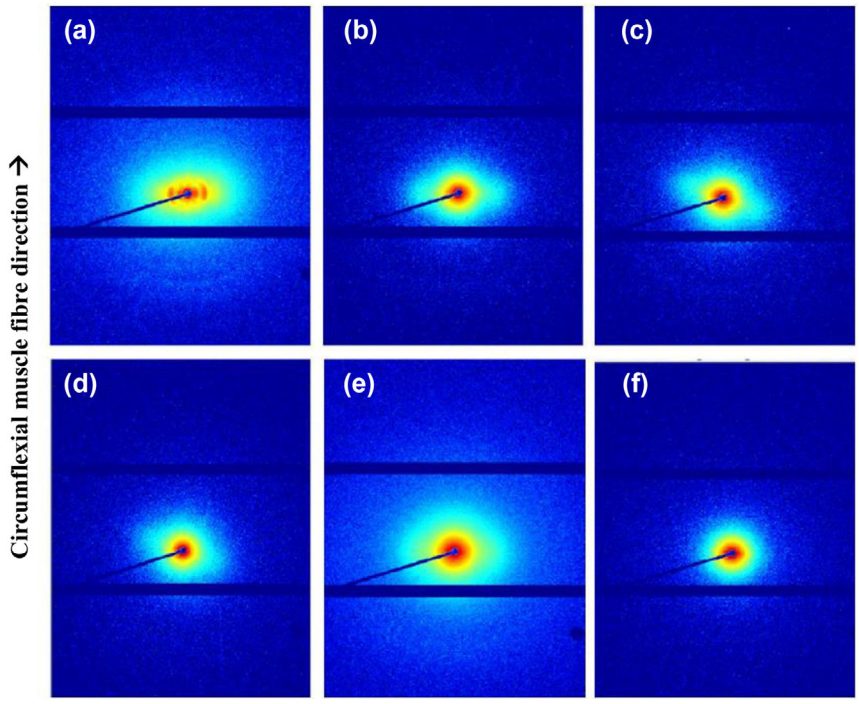

FIGURE 4 Small-angle X-ray scattering 2D patterns. The samples are oriented with circumflexial muscle fibers aligned with the vertical axis. (a) Raw squid mantle; (b) Squid mantle cooked at 46°C for 1 h; (c) Squid mantle cooked at 55°C for 15 min; (d) Squid mantle cooked at 55°C for 1 h; (e) Squid mantle cooked at 77°C for 2 min; (f) Squid mantle cooked at 77°C for 15 min

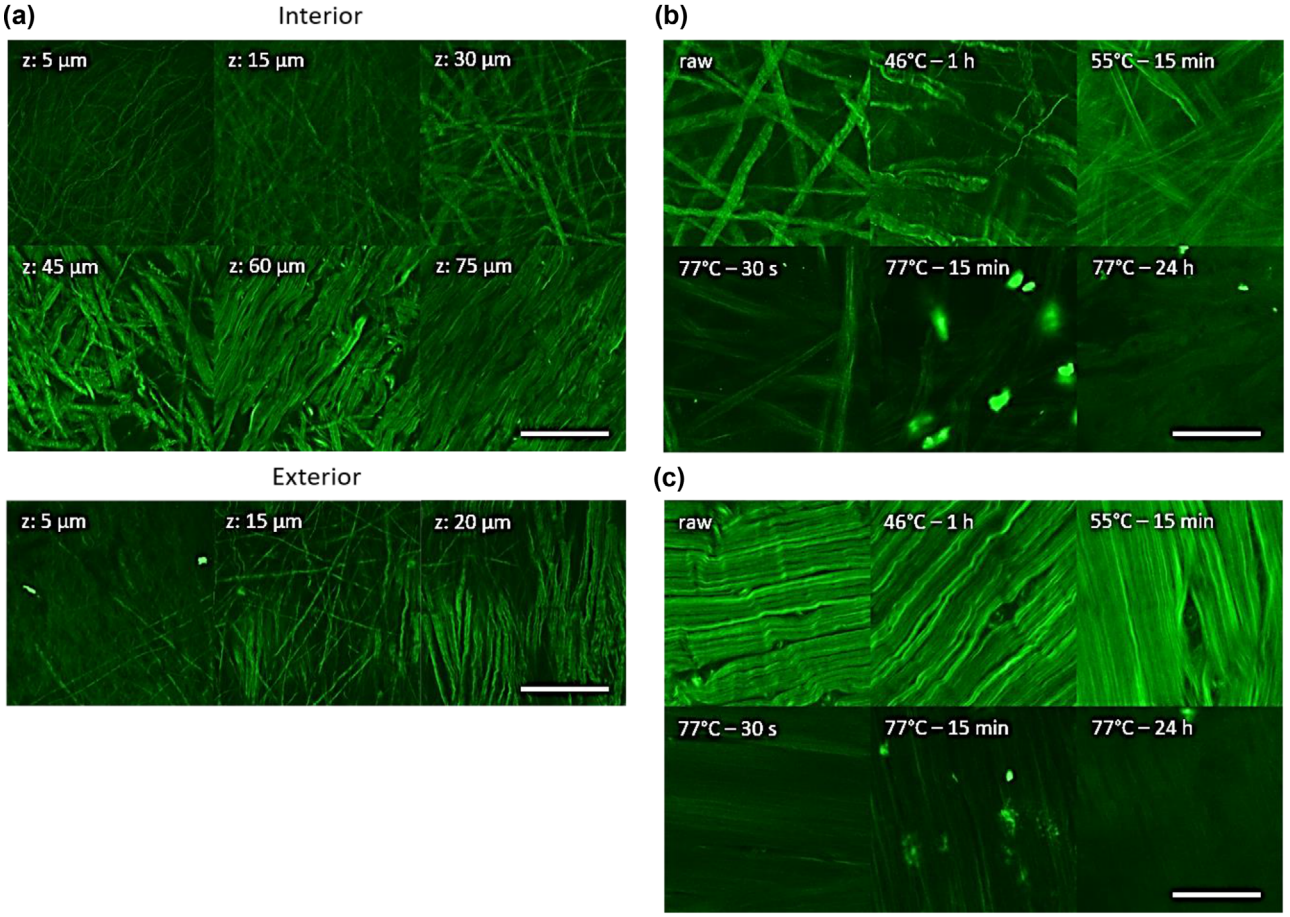

FIGURE 5 SHG microscopy images of *Loligo forbesii* mantle. (a) Raw squid imaged at different depths from both the interior side (top) and exterior side (bottom) of the mantle showing different collagen and myofibrillar structures. (b) Visualization of collagen structures for different time–temperature sous vide preparations. (c) Visualization of myofibrillar structures for different time–temperature sous vide preparations. Scale bar in (a) is 100 µm and in (b, c) is 50 µm

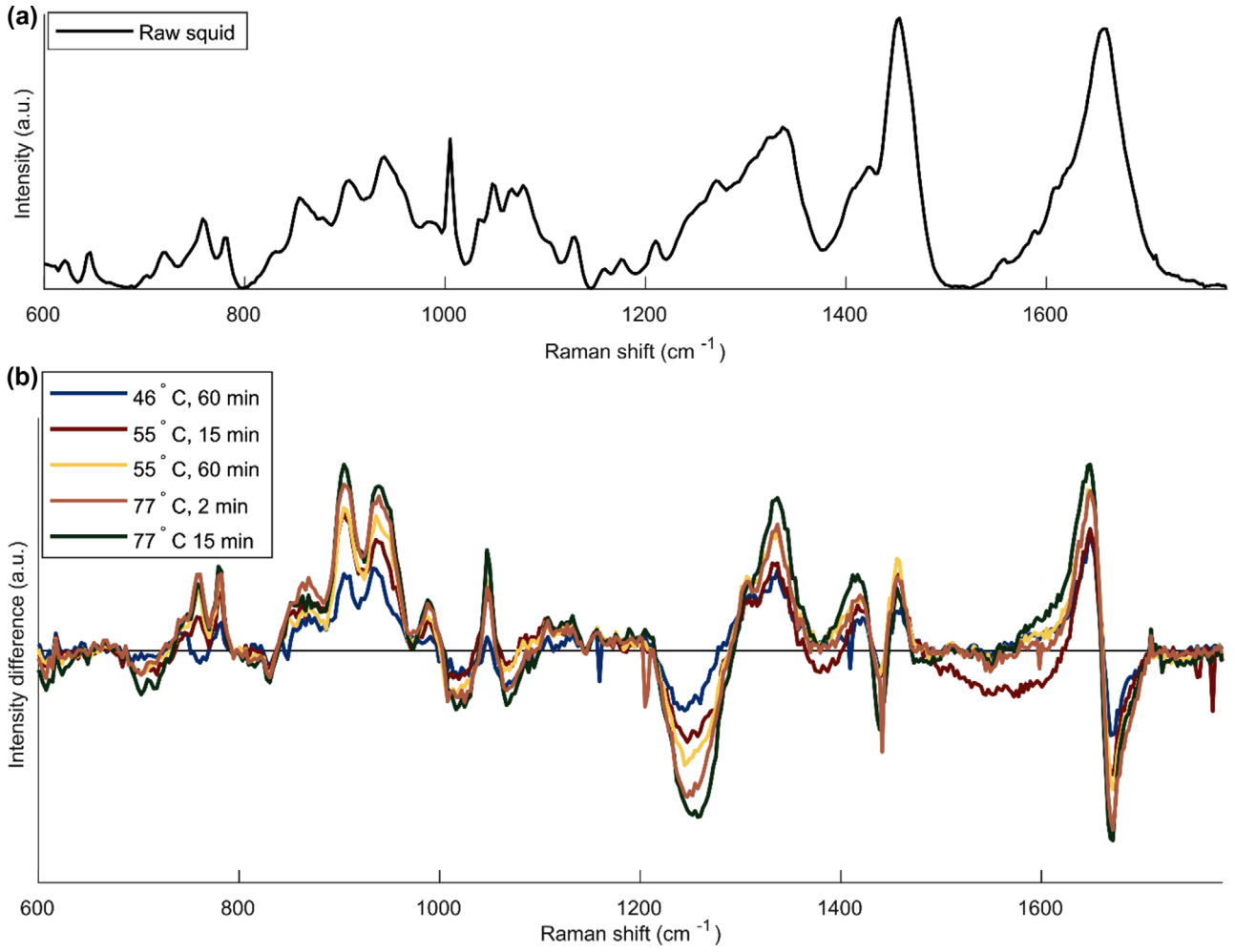

FIGURE 6 Fingerprint region (600–1800 cm^{-1}) (a) Raman spectrum of raw squid and (b) Raman difference spectra for different sous vide preparations. The difference spectra correspond to spectra of prepared squid subtracted from the spectrum of raw squid

Figure 5b, c displays the effect of sous vide cooking at different time–temperature combinations on the tissue dominated by the collagen structures (Figure 5b) and myofibrillar structures (Figure 5c), respectively. The collagen structure largely remains unchanged at the low cooking temperature (46°C, 1 h), while the structure starts to loosen slightly at 77°C, 30 s, and a bit more at intermediate preparations (55°C, 15 min; and 77°C, 15 min), while keeping its overall organization. Further, for the highest temperature applied with the longest cooking time (77°C, 24 h), the collagen loses most of the signal intensity as a result of denaturation (Figure 5b). The myofibrillar structures are for all presented time and temperature combinations different from the raw preparation, showing a substantial loss in the signal intensity, thereby indicating a loss of structural integrity (Figure 5c).

3.6 | Raman spectroscopy

Figure 6 displays the baseline-corrected fingerprint region of the Raman spectrum for raw squid (Figure 6a) and the Raman spectra for different sous vide preparations subtracted from the Raman spectrum of raw squid (Figure 6b), that is, positive values mean that higher intensity is found in the raw squid, highlighting changes due to the cooking treatments. The spectra contain information from the entire tissue measured and is thus dominated by the myofibrillar region due to the lack of sectioning.

RS is highly applicable for the study of structural changes in muscle tissue, with typical changes in the amide I (1650–1680 cm^{-1}) and amide III (1200–1300 cm^{-1}), and C—C stretching (940 cm^{-1}) bands due to changes in connective and myofibrillar tissue (Herrero, 2008) as seen

in Figure 6. Upon sous vide cooking, clear changes in the protein structures are seen with the relative change being consistently larger in the order (from highest to least difference compared to raw squid): 77°C at 15 min, 77°C at 2 min, 55°C at 1 h, 55°C at 15 min, and 46°C at 1 h, showing that temperature is a more dominant factor as compared to cooking time, though time also is a factor. Specifically, changes are seen in the amide I region with decreasing signal intensity around 1650 cm^{-1} upon sous vide cooking associated with loss of protein α-helical structure, and an increased signal intensity upon sous vide cooking around 1665 cm^{-1} associated with random coil protein structures. The same pattern is seen in the amide III region with a relatively decreased signal around 1260–1300 cm^{-1} (α-helical) for sous vide cooking and relatively higher intensity around 1240–50 cm^{-1} (random coil) (Herrero, 2008). Loss of ordered structure is further seen in the C—C stretch region with a gradual decrease in intensity in the α-helical region of 890–945 cm^{-1} as a function of increased sous vide cooking (Herrero, 2008). The observed changes in intensity at 759 cm^{-1} are due to changes in protein tertiary structure resulting in exposure of buried tryptophan residue (Herrero, 2008). The observed changes around 1450 cm^{-1} are typically assigned to CH_2- and CH_3-bending vibrations and have, in the context of proteins, been interpreted as a result of hydrophobic interactions of aliphatic residues (Lippert et al., 1976). Further, it is seen that α-helices are present at 1650, 1260–1300, and 890–945 cm^{-1}, which are characteristic for collagen and have previously been used to track structural changes of collagen from α-helix to random coil during heating of burnt skin (Ye et al., 2019). Nonetheless, myosin also contains α-helixes (Batchelor et al., 2015) as well as other protein structures. Consequently, the different events displayed by RS of squid meat are as opposed to purified samples difficult to ascribe to specific proteins, based on the included sample treatments for RS.

The structural identifications based on a full Raman difference plot are summarized in Appendix Table 2 in Supplemental information with interpretations based on the comprehensive review article of RS of biological tissues by Movasaghi et al. (2007).

3.7 | Instrumental data compared

A comprehensive overview of all collected data and corresponding PCA loading plot of data from TA, DSC, and cooking loss is presented in Table 3. Table 3 is also intended to provide an overview and ease comparison of cooking treatment effects across characterization methods that range over quantitative as well as qualitative results. The data in Table 3 is presented with values relative to raw squid tissue (TA, RS) and by degree protein denaturation (DSC) as indicated by +/− representing more or less than 10% native protein relative to raw squid. The chosen limit of 10% is based on the assumption that a presence of less than 10% native protein will yield a minimal effect on both texture and cooking loss. Analytical methods yielding qualitative results (RS, SHGM, and SAXS) are subjectively divided into relative levels to ease interpretation across the different types of analysis.

By PCA of the results derived from TA, DSC, and cooking loss, with PC#1 and PC#2 accounting for respectively 70% and 14% of the variation, it is seen that TA peak count is associated to DSC myosin and actin, that is, low values of peak count are seemingly linked to myosin and actin denaturation. Further, PCA suggests TA max force, TA total work of shear energy, and TA linear distance to be associated to DSC collagen, that is, low values of these TA parameters are associated with collagen denaturation (confer Supplemental Information, Appendix Figure 3). Besides, TA max force distance is associated with an increasing amount of Hyp and TFAA in the cooking liquid, with DSC myosin and actin being oppositely positioned in the PCA diagram, explained by PC#1. This indicates that denaturation of action and myosin facilitate more FAA components in the cooking liquid than collagen denaturation. Further, cooking loss volume seems to be oppositely positioned to both myosin, collagen, and actin denaturation, with collagen denaturation being positioned across PC#1 as the most dominant factor for cooking loss volume.

Cooking loss volume is affected by both cooking time and temperature. Cooking loss onset, defined as the cooking time for which a significant cooking loss volume occurs compared to that of raw squid, is first present after 24 h cooking at 46°C ($p = 0.002$), 5 h cooking at 55°C ($p = 0.0009$), and 15 min cooking at 77°C ($p = 0.005$). By comparison with results of protein denaturation by DSC, significant cooking loss onset generally results once myosin and collagen are fully denatured (0% native protein), with actin somewhat preserved (96% native protein) for 46–55°C while completely denatured at 77°C (cf. Tables 2 and 3). Further, by the DSC results of sample treatments 77°C for 30 s and 55°C for 2 min, cooking loss volume does not increase significantly, with myosin being fairly denatured to 11% and 17% native protein, while collagen is approximately not denatured at all (cf. Figures 2 and 3), indicating that collagen denaturation is the primary contributor to cooking loss.

Decreasing the cooking temperature from 77 to 55°C significantly decreases cooking loss volume by 35%–60% when cooking for ≥15 min ($p < 0.05$). Nonetheless, decreasing the temperature without increasing the cooking time affects the mechanical texture significantly in some textural parameters. Comparing cooking treatments of 55 to

TABLE 3 Overview of results of sous vide cooking treatments (time: 30 s, 2 min, 15 min, 1 h, 5 h, 24 h; temperature: 46, 55, 77°C) relative to raw squid mantle: TA (texture analysis), DSC (differential scanning calorimetry) (M = myosin, C = collagen, A = actin), cooking loss per cent volume, cooking loss hydroxyproline (Hyp) and total free amino acids (TFAA) by UHPLC (ultra-high-performance liquid chromatography), Raman spectroscopy (RS) rank by difference spectra, SHGM (second harmonic generated microscopy) of collagen, and SAXS (small-angle X-ray scattering). NA denotes values that are not available for the given method

Treatment		Results TA							DSC			Cooking loss			RS	SHGM	SAXS
		First peak force	First peak distance	Max force	Max force distance	Linear distance	Total shear energy	Peak count	Native protein (>10%)			Volume	Free Hyp	Total FAA ($n = 18$)			2D
Time	Temp.	[N]	[mm]	[N]	[mm]	[m]	[mJ]		M	C	A	% (w/w)	mg/100 mL	mg/100 mL		Collagen	
Raw		7.4	3.0	9.5	3.6	2.3	21.8	8.5	+	+	+	8.4			1	Ordered	Anisotropy
46°C	**30 s**	NA	NA	NA	NA	NA	NA	NA	+	+	+	8.2	NA	NA	NA	NA	NA
	2 min	3.6	2.5	3.7	2.5	0.9	10.4	14.7	+	+	+	7.4	0.2	86	NA	NA	NA
	15 min	3.4	2.3	3.7	3.3	1.0	11.9	9.8	+	+	+	9.5	0.2	93	NA	NA	NA
	1 h	5.1	2.9	5.9	4.5	1.7	15.2	6.3	+/−	+	+	11.9	NA	NA	2	Ordered	Part anisotropy
	5 h	5.7	4.1	5.7	4.5	1.5	16.1	5.3	−	+	+	11.8	4.1	1296	NA	NA	NA
	24 h	NA	NA	NA	NA	NA	NA	NA	−	−	+	21.1	14.0	4388	NA	NA	NA
55°C	**30 s**	NA	NA	NA	NA	NA	NA	NA	+	+	+	6.0	0.1	36	NA	NA	NA
	2 min	7.1	3.6	8.1	3.9	1.9	18.6	5.1	+	+	+	7.6	0.4	207	NA	NA	NA
	15 min	5.9	3.0	7.5	3.7	1.9	20.9	6.1	−	+	+	10.8	2.8	398	3	Loosening/ ordered	Part anisotropy
	1 h	8.1	5.0	8.5	5.3	1.4	20.4	1.9	−	+	+	10.7	NA	NA	4	NA	Near isotropy
	5 h	3.0	5.6	3.0	5.6	0.4	7.1	0.6	−	−	+	17.3	3.1	1287	NA	NA	NA
	24 h	0.4	5.1	0.4	4.9	0.1	1.2	0.9	−	−	+	20.0	4.8	1237	NA	NA	NA
77°C	**30 s**	5.2	3.2	6.9	4.0	2.0	18.0	7.4	+/−	+	+	6.3	0.3	127	NA	Semi-ordered	NA
	2 min	4.7	3.0	6.3	4.6	1.4	18.1	3.6	−	+	+	9.4	0.4	120	5	NA	Near isotropy
	15 min	3.6	3.6	5.5	5.4	1.1	14.3	4.6	−	+	+	18.3	3.5	1749	6	Loosening/ ordered	Isotropy
	1 h	NA	NA	NA	NA	NA	NA	NA	−	+	−	26.6	NA	NA	NA	NA	NA
	5 h	1.7	5.6	1.7	5.6	0.2	5.9	0.4	−	−	−	36.6	4.7	1830	NA	NA	NA
	24 h	1.3	6.1	1.3	6.0	0.1	4.1	0.0	−	−	−	31.6	6.1	1891	NA	No signal/ denaturation	NA

77°C for cooking times 15 min to 24 h (1 h excluded due to missing data by TA), it is found that decreasing the temperature increases the mechanical force for all TA parameters, but not significantly for all parameters. TA max force between 55 and 77°C for all cooking times ≥ 15 min is significantly affected ($p < 0.05$). Specifically, cooking at 55°C instead of 77°C for 15 min decreases TA max force (i.e., hardness) with 21% instead of 42% compared to raw squid, and cooking for 5 h, the corresponding numbers are 68% against 82%. For TA total work of shear (i.e., toughness) the corresponding numbers are 4% against 34% cooking for 15 min, and 67% against 73% cooking for 5 h. The overall conclusion in terms of decreasing the cooking temperature from 77 to 55°C and cooking for the same amount of time, is that cooking loss is significantly decreased while mechanical hardness suffers the most, and mechanical toughness suffers more for less cooked samples, possibly ascribed to lack in denaturation of actin. The mechanical texture may however be improved by increased cooking times being less extreme than in the present study. Further, the differences in mechanical force measured by TA by MORS, are not necessarily differences that can be detected by humans; for this, sensory consumer evaluation must be performed, cf. Schmidt et al. (2021). Concerning the findings of cooking loss and mechanical texture, it may be advised to use a cooking temperature of 55°C instead of 77°C to secure juicy, but still tender meat. From hedonic ratings in a previous study (Schmidt et al., 2021), the sample treatment at 77°C for 5 h was found to yield the highest hedonic rating, which was rated well above neutral. Based on a similarity in overall mechanical texture (with the exception of TA hardness) to this sample treatment, it can be argued that the cooking treatment 55°C for 5 h will be equally liked, or possibly even more, due to presumed increased juiciness. Nonetheless, this deserves further sensory investigations.

A fairly low temperature of 46°C was included in the present study. Even though this temperature may not be regarded as microbiologically safe, it was included for the mere purpose of studying the structural changes. DSC revealed that a cooking temperature of 46–55°C was not sufficient to denature actin, even though prolonged cooking time was applied (up to 24 h). Besides, a cooking temperature of 46°C was not sufficient to properly denature collagen, possibly explaining why TA first peak force, TA max force, TA linear distance, and TA total shear energy did not decrease for this treatment temperature. Only when a prolonged cooking time was applied (24 h), collagen was denatured (Table 2). However, this was most likely facilitated by proteolytic activity rather than the actual cooking, supported by a very high amount of FAA (Table 3, Appendix Table 1) in the cooking liquid, as well as the fact that it did not yield a palatable outcome (judged based on smell when handling these samples), which is in alignment with the fact that squid mantle contains a high concentration of proteolytic enzymes (Stanley & Hultin, 1985) with peak activity reported at 40 and 60°C (Ayensa et al., 1999; Rodger et al., 1984).

SHGM observations are in accordance with results from DSC and TA. By SHGM visualization, collagen denaturation characterized by DSC displays no collagen denaturation for treatment 46°C cooked at 1 h and 77°C cooked for 30 s, visualized by an ordered and semi-ordered structure by SHGM (cf. Figure 5b). The semi-ordering by SHGM visualizing is likely due to the sampling including only the outer layer of the tissue that for the 30 s treatment exhibits a stronger effect of heat treatment. A gradual loosening of collagen structures, while still being highly ordered, is displayed for sample treatments at 55 or 77°C cooked for 15 min in which collagen was gradually denatured.

Results from SAXS displayed a loss of anisotropy in the 2D scattering associated with the loss of a 66 nm D-gap by cooking. This observation is somewhat in agreement with results from SHGM but displays changes at an earlier stage and as well as for samples where collagen is not yet fully denatured (Table 3). Collagen denaturation includes the breaking of hydrogen bonds between the three amino acid α-chains, thereby loosening of the fiber structure. This affects the staggering arrangement of the collagen molecules and changes the electron density along the fibers, which may cause the observed changes in 2D scattering when squid is cooked being visible by SAXS up to 1 h when cooking at 46 or 55°C. This can partly be confirmed by SHGM (Figure 5a) where it can be seen that collagen structures largely remain unchanged after 1 h at 46°C, also consistent with results from RS (Table 3). For the present study, SAXS was included as a try-out for a new technique applied to study squid meat quality. SAXS did not seem to provide more insight than SHGM and RS, but provided a direct insight into structural changes that could not be obtained by using the macroscopic and thermodynamic techniques TA and DSC. RS is in accordance with SAXS as well as SHGM (Table 3). However, RS output also includes signals from possible changes in myofibrillar structures. Nonetheless, some specific features of the Raman spectrum may, in light of the results obtained from DSC and SHGM, be ascribed to collagen, specifically the structural changes observed at 1650, 1260–1300, and 890–945 cm^{-1} (Figure 6, Appendix Table 2, and Table 3). Thus, structural changes analyzed by SAXS, SHGM, and RS gave in combination, a direct and indirect visualization of the structural changes that could be linked to quantitative results derived from DSC and TA.

Texture is the sensory and functional expression of the structural, mechanical, and surface properties of foods detected by the human basic senses (Szczesniak, 2002).

The comprehensive set of methods applied to investigate squid structure in the present study cannot be directly related to what changes human tasters may perceive, but can draw on previous work presenting such information (Schmidt et al., 2021). By the results in the present study, collagen and to some degree also myosin, are both found to be responsible for cooking loss volume (i.e., juiciness), and collagen and actin are found to be major drivers for textural changes (i.e., tenderness). Consequently, obtaining a tender while juicy piece of squid mantle is a paradox, since the denaturation of actin, seemingly responsible for mechanical hardness, occurs at higher temperatures than the denaturation of collagen, being responsible for textural changes as well as cooking loss. Nonetheless, collagen denaturation should be the main focus for designing an optimal cooking treatment of squid, although it contains a relatively low amount of collagen compared to other species, indicating that it may be the quality (degree of hydroxylation) of the collagen rather than the quantity that is affecting the textural characteristic of squid.

Applying a cooking temperature around the denaturation temperature of collagen (55°C) may prove advantageous in terms of texture, but should be further investigated in terms of microbiological food safety, if the final product is not consumed directly after sous vide cooking. All samples for the present study were frozen fresh at −40 °C for at least 48 hrs, which reliably inactivates higher organisms such as pathogenic protozoa and parasitic worms (Adams & Moss, 2008). Freezing and thawing procedures were controlled using blast freezing and thawing in water baths to avoid long hold times at temperatures above 0 °C minimizing the risk of bacterial spoilage on the surface of the samples. For small-scale and immediate consumption, the presented results may provide a safe food product; however, if the procedure is applied for large scale preparations and/or used for products that require a shelf-life (e.g., ready-to-eat product for retail), then the applied sous vide treatment should eliminate anaerobic bacteria prone to seafood. Thus, applying low-temperature sous vide cooking for purposes other than immediate consumption deserves further investigation in terms of shelf-life and food safety.

4 | CONCLUSIONS

Low-temperature sous vide cooking treatment above 46°C, preferably around 55°C, is an optimal cooking temperature in terms of sustaining a minimum of cooking loss while facilitating tenderizing textural changes. The optimal sous vide cooking temperature of around 55°C proves to denature myosin and collagen, obtaining a decrease in the mechanical force of shearing, while preserving native actin. Lowering the cooking temperature from 77 to 55°C significantly decreases the amount of cooking loss, when cooking for more than 15 min up to 24 h, while obtaining a decrease in mechanical shear force. However, this presents significant differences in some textural parameters related to native actin. Thus, cooking loss was linked to denaturation of collagen (and to some degree myosin) whereas mechanical texture was in general linked to collagen and actin, suggesting that controlling the denaturation of collagen is key for obtaining both tenderness and juiciness of squid mantle. Further studies in improving the cooking time around 55°C to decrease mechanical force might be rewarding and should preferably be coupled to investigations in microbiological food safety.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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