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Tracking hydrophobicity state, aggregation behaviour and structural modifications of pork proteins under the influence of assorted heat treatments

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ABSTRACT

Structural modifications of pork proteins under an assortment of industrial heat treatments were studied. With raw as control, assorted heat treatments involved were 58, 80, 98 and 160 °C for 72 min, 118 °C for 8 min and 58 °C for 17 h, resembling most common processing procedures. Protein denaturation, surface protein hydrophobicity state and protein aggregation behaviour were investigated. Modifications and molecular chemistry in protein structures were tracked by Fourier Transform Infrared Spectroscopy in order to extract relative proportions of β-sheet, α-helix and residual conformations. In comparison to uncooked samples, cooked ones showed more than two-fold increase in hydrophobicity and larger particles. Thermograms from differential scanning calorimetry showed endothermic transitions (positive enthalpy) indicating a different pattern of protein denaturation as a result of varied cooking temperatures and cooking times. Deconvolution and curve fitting procedures (R^2 = 0.99) provided information on rise of the β-sheet to α-helix ratio that further confirmed aggregation with thermal rise and longer cooking time.

1. Introduction

Meat processing has always been a topic of interest due to its potential for offering highly nutritious and palatable products for humans. This includes processes with a variety of cooking temperatures and times, from conventional cooking methods like grilling, stewing, pan-frying or roasting, to the more recent sous vide cooking at low temperature for long time (LTLT) (Boles, 2010). As a response to the different heat treatments, proteins undergo varied modifications that could alter their secondary, tertiary and quaternary structure, leading to changes in their hydrophobicity state or aggregation behaviour, which in turn has a potential effect on their nutritional and technological properties, including digestibility, water holding ability, gel formation, light scattering, solubility or extractability (Christensen, Ertbjerg, Dall, & Christensen, 2011b; Lund, Heinonen, Baron, & Estévez, 2011; Traore et al., 2012). On top of the obvious impact on the quality of the products, these changes might also have a remarkable importance for those consumers in which protein intake should be optimized, as it happens to elderly, who are frequently not capable of reaching the dietary protein recommendations, and thus impaired nutritional quality of proteins could worsen this situation (Bauer et al., 2013).

There is indeed a lack of understanding about the significance of protein structure modifications in influencing protein functionality and quality and nutritional outcomes. Over recent years, vibrational spectroscopy methods like FTIR, have proved to be a rapid and non-destructive tool to track secondary structural changes and hydration affinities in meat proteins (Perisic, Afseth, Ofstad, Narum, & Kohler, 2013). This may in turn be used to identify their potential final influence on protein functionality or nutritional value. For example, a relatively high proportion of the β-sheet structure may induce a lower accessibility to the digestive enzymes, which might result in lower protein availability (Yu, 2005). Heating leads to an increase in the dipole coupling that indicates presence of high content of aggregated β-sheet structures (Calabrò & Magazù, 2012; Herald & Smith, 1992; Yu, 2005). With escalating temperatures, heat denaturation occurs with a conformational transition and geometrical alteration at around 65 °C (Zhang, Yamamoto, Ishikawa, & Carpentier, 1999). However, not much work has been carried out in the field of vibrational spectroscopy to understand how modifications in structural elements of meat could influence nutritional bioavailability or quality changes.

In this project, our objective was to elucidate the effect of different common heat processing methods on the structural modification of pork proteins. We also shed some light on the intricate chemistry

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behind changes in protein structures and how the folding patterns are affected by thermal processing after Fourier Self-Deconvolution (FSD) and Gaussian multicomponent peak fitting. Alongside, authors would like to put emphasis on effect of temperature-time combinations that could possibly alter the physicochemical status of pork proteins leading to either enhancement or deterioration in functionality.

2. Materials and methods

2.1. Chemical reagents

List of chemicals includes: Sodium dodecyl sulphate (SDS Ultrapure, AppliChem GmbH, Darmstadt, Germany), Bromo Phenol Blue (BPB, AppliChem GmbH, Darmstadt, Germany), Urea (Merck KGaA, Darmstadt, Germany), Cleland's reagent (DTT, 1,4-Dithio-DL-Threit (ol), AppliChem GmbH, Darmstadt, Germany), Trizma (Tris) Hydrochloride (Sigma Aldrich, Missouri, USA), Phosphate buffered saline (PBS, Sigma Aldrich, Missouri, USA), Potassium Chloride (KCl, Sigma Aldrich, Missouri, USA), Potassium Dihydrogen Phosphate (KH_2PO_4, Chemika, Girraween, New South Wales, Australia), Di-Potassium Hydrogen Phosphate (K_2HPO_4, Merck, Darmstadt, Germany), Magnesium Chloride ($MgCl_2$, Sigma Aldrich, Missouri, USA) and Ethylene glycol-Bis (β-amino ethyl ether)-*N*,*N*,*N*′,*N*′-tetra acetic acid (EGTA, Sigma Aldrich, Missouri, USA). For dissolving chemicals, water was prepared using a Millipore-Milli-Q purification system (Milli-Q Plus Corporation, Bedford, MA). All reagents used were of analytical grade.

2.2. Collection of meat samples

Seven female pigs from the same vendor (Supplier No. 77752, Danish Crown) were selected with a slaughter weight of 83–86 kg. The animals were stunned with CO_2 and dressing activities were performed within 60 min post-mortem at the abattoir (DC-Herning, Denmark). The lean percentage of the carcass varied between 59 and 63%, with a final pH ranging from 5.5 to 5.6. Carcasses were chilled-stored at 4 °C for 24 h PM, and subsequently cut up. Loins were transported to the meat pilot plant (Danish Meat Research Institute, Taastrup, Denmark), where they were trimmed to obtain the *Longissimus dorsi* (LD) muscle. Steaks (thickness = 2 cm) were chopped from the oyster end (hip) of the right and left LD. First 10 steaks were obtained from the right loin and the next 11 steaks were from the left loin. Steaks were then coded, weighed, packed in vacuum bags (LogiCon EM-628824 - Vacuumpose 200 × 270 × 0,090 mm^3, Kolding, Denmark) transported to University of Copenhagen and kept at − 80 °C.

2.3. Cooking treatments

One hundred and forty-seven sample samples (7 Animals × 7 Treatments × 3 Steaks) were chosen for the following study. Before the samples were subjected to cooking, steaks were thawed 24 h at 4 °C. Steaks were subjected to 7 different treatments with variation in temperature and time profiles, trying to mimic commonly used heating treatments for producing different types of meat products. The 7 treatments included: Control raw samples (RAW); *sous vide* at 58 °C for 72 min (SV5872, as used for LT-LT cooking meat recipes at caterings), *sous vide* at 80 °C for 72 min (SV8072, as used in numerous cooked meat products), braising at 98 °C for 72 min (B9872, as used for production of stews), autoclave operations at 118 °C for 8 mins holding time (AC1188, as used for canned meat products), oven roasting at 160 °C for 72 min (OV16072, mimicking grilled meat products) and *sous vide* at 58 °C for 17 h (SV5817, LT-LT cooking, frequently used in restaurants and gastronomic playgrounds). In all the cooking treatments, probes (Testo 176T4, 4-channel temperature data logger with TC Type T) were used to record and assess core and surface temperature. Before cooking, steaks were coded and vacuum-packed in Cryovac CN 300 bags (Sealed Air Corporation, Charlotte, North Carolina, USA) for cooking temperatures below 100 °C and in LogiCon vacuum bags (EM-62890, Kolding, Denmark) for the autoclave operations at 118 °C, while oven roasted samples were cooked unpacked. As soon as the heating processes were completed, all sample replicates were immediately submerged into ice cold water (4 °C). Pouches were then opened and samples were weighed, and subsequently repackaged and frozen at − 80 °C for further analysis. Cooking loss was calculated by difference in weights before and after the treatments.

2.4. Protein content

Two gram of meat sample were homogenized in 20 mL of 0.01 M phosphate saline buffer, pH 7.4, in a 50 mL centrifuge tube using an Ultra Turrax T25 equipped with a S25 N-18 G dispersing element (IKA Labortechnik, Staufen, Germany) for 30 s with a speed of 20,500 rpm. Separate aliquots of 30 μL were taken in Eppendorf tubes, and 5% (w/v) SDS (1148 μL) in 50 mM Tris HCl (pH 8), Urea (8 M, 20 μL) and DTT (1 M, 2 μL) were added to achieve a dilution of 40 times. Eppendorfs were then vortexed and incubated into a microplate incubator (Provocell, Model No. PV-PVC-1, Order No. BLC-1, Esco technologies Pty Ltd., Centurion, South Africa) at 80 °C for 30 min. The absorbance of this solution at 280 nm was measured (SpectraMax i3x Multi-Mode Detection Platform, Molecular Devices, Inc., Silicon Valley, USA) and used to estimate protein concentration.

2.5. Protein surface hydrophobicity (H_0)

The hydrophobicity (H_0) pattern of total protein pool was assessed by binding of hydrophobic chromophore bromophenol blue (BPB) as previously described by Chelh & Gatellier (2006) with minor modifications. Frozen meat samples (2 g) were homogenized with an Ultra Turrax T25 (30 s × 20,500 rpm) in 20 mL of 20 mM PBS (pH 6). Total protein concentration was then adjusted to 5 mg/mL with PBS. To 1 mL of total sample suspension, 200 μL of 1 mg/mL BPB (solubilized in Millipore water) was added and vortexed. For the control, 200 μL of 1 mg/mL BPB was added to 1 mL of 20 mM PBS. Both the samples and the control were continuously agitated at room temperature in a microplate incubator and then centrifuged at 2000*g* for 15 min at 4 °C. The supernatant was removed, diluted 10 times with PBS, and the absorbance at 595 nm was measured. The amount of bound BPB (hydrophobicity index) was calculated by the formula:

BPB bound (μg) = 200 μg × (OD Control − OD Sample) / OD Control.

Determinations were performed in duplicate.

2.6. Differential scanning calorimetry (DSC)

Samples were thawed at 4 °C for 4 h and minced using a coffee grinder. Meat samples (30 mg) were placed in a sample cell of a differential scanning calorimeter (DSC 1, STARe System, Mettler Toledo, Schwerzenbach, Switzerland). An empty sealed aluminium crucible (capacity 40 μL) was positioned into the reference cell. The sample was scanned from 30 °C to 90 °C with a heating rate of 2 °C/min to track major endothermic transitions that implicates protein denaturation. Three samples per treatment group were analysed.

2.7. Particle aggregation and size distribution

Particle aggregation and size distribution profile of total protein pool was measured by static light scattering, using a Malvern Mastersizer Micro Plus (Malvern Instruments, Worcestershire, UK) instrument, according to Liu et al. (2016) with some modifications. Meat samples (2.5 g) were solubilized in 29 mL of cold homogenization buffer, pH 7, (0.1 M KCl, 0.01 M KH_2PO_4, 0.01 M K_2HPO_4, 0.001 M EGTA and 0.001 M $MgCl_2$) in a 50 mL centrifuge tube (Lametsch,