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Effects of electrospun chitosan wrapping for dry-ageing of beef, as studied by microbiological, physicochemical and low-field nuclear magnetic resonance analysis

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ABSTRACT

The effects of using electrospun chitosan fibres as a wrapping material for dry-ageing beef was studied and compared to traditional dry-ageing and wet-ageing of beef for up to 21 days. The chitosan treatment showed improved results in terms of yield, reduction of microbial counts, yeasts and moulds, and lighter appearance compared to traditional dry-ageing. Weight and trimming losses were minimal in the wet-ageing beef. However, significant growth of lactic acid bacteria was observed in this group. Transverse relaxation times indicated a lower degree of muscle denaturation during ageing in the chitosan dry-ageing beef compared to the traditional dry-ageing meat. A principal component analysis furthermore indicated that 60.6% of the variation between samples and ageing treatments could be described by differences in the water content and distribution in the muscle. The study showed that electrospun chitosan fibre mats have potential as a wrapping material for improved quality during dry-ageing of beef.

1. Introduction

Ageing is a process within the conversion of muscle to meat, involving biochemical and physicochemical processes, which enhance the palatability of the product, as demonstrated by increased tenderness and development of unique flavours over time (Campbell, Hunt, Levis, & Chambers, 2001; Sitz, Calkins, Feuz, Umberger, & Eskridge, 2006; Warren & Kastner, 1992). Amongst these are endogenous proteolysis of the muscle fibres, changes in water permeability of membranes and weakening of connective tissues (Damez & Clerjon, 2008).

Two ageing techniques have been practiced in post-mortem handling operations of meat; firstly wet-ageing where the meat is sealed in highly moisture-impermeable vacuum bags held at a refrigerating temperature during the ageing procedure. This approach was developed during 1960s and has gained wide recognition in the meat industry as the most practical due to its convenience during storage and transport, and for having very low ageing losses (DeGeer et al., 2009; Warren & Kastner, 1992). The second technique is dry-ageing, where the meat is left unpackaged and exposed to the refrigerating conditions, with strict monitoring of temperature, humidity, and air-flow (Ahnstöm, Seyfert, Hunt, & Johnson, 2006). Most primal cuts are wet-aged, especially during shipping and storage, while entire carcasses or individual sub-primal cuts can be dry-aged (Sitz et al., 2006). To produce dry-aged meat is a costly procedure because of the significant proportion lost due to shrinkage and trimmings (Oreskovich, McKeith, Carr, Novakofski, & Bechtel, 1988; Parrish, Boles, Rust, & Olson, 1991; Warren & Kastner, 1992). To overcome this weight loss issue, meat scientists have recently developed novel bags which are used as an alternative to traditional dry-ageing of meat. The new material used for these bags are designed to have permeability for both moisture and air, thus simulating the dry-ageing technique, while limiting the access of microorganisms. Studies have been performed on the effectiveness of using these novel bags in terms of reducing weight loss, trim loss, and cooking loss, microbial contamination, improving tenderness, and other sensory attributes compared to other ageing methods (Ahnstöm et al., 2006; DeGeer et al., 2009; Li, Babol, Bredie, Wallby, & Lundström, 2013; Li et al., 2014). The majority of studies using these innovative bags came up to a common understanding that these can provide benefits in terms of meat quality attributes, without compromising the distinctive flavour found in dry-ageing beef. Convincing results of

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this innovation in dry-ageing of meat have been recognized by the meat industry, and these commercially available bags have penetrated the market.

Electrospinning produces fibres from polymer solutions that are of nano- to microscale sizes. Amongst promising applications of nanofibres in the biomedical field of research includes development of tissue templates, scaffolds, medical prostheses, wound dressing, artificial organs, drug delivery systems, pharmaceutical composition, and many others (Frenot & Chronakis, 2003; Gibson, Gibson, & Rivin, 2001; Huang, Zhang, Kotaki, & Ramakrishna, 2003). However, in food industry, the interest of extending electrospinning advances has just started to grow. Encapsulation of food ingredients, enzymes and many bioactive compounds beneficial to the food industry has been targeted by electrospinning of various biopolymers, but recently also in the development of active packaging materials that aim to preserve nutritive value of foods. Chitosan was chosen in this study since it is known to be abundant in nature, non-toxic, biodegradable, biocompatible, and having anti-microbial properties (Angelova, Rashkov, Maximova, Bogdanova, & Domard, 1995; Jiang, Fang, Hsiao, Chu, & Chen, 2004; Son, Youk, & Park, 2004), as well as shown to possess potential as a biodegradable polymer for food packing materials to prolong shelf life (Dutta, Tripathi, Mehrotra, & Dutta, 2009). Furthermore, the successful use of chitosan polymer in various electrospinning studies is well-documented.

The acceptance of innovative packaging used during dry-ageing of meat is one of the main motivations for the present study, which aim was to assess the effectiveness of electrospun chitosan fibre mats as a wrapping material to modify the dry-ageing processing of beef, compared to traditional dry-ageing and wet-ageing, considering meat quality attributes, microbial counts and water distribution by the use of low-field nuclear magnetic resonance (LF-NMR) measurements in the ageing beef.

2. Materials and methods

2.1. Experimental setup

2.1.1. Production of chitosan fibre mat

For the electrospinning, a low molecular weight (LMW) commercial chitosan was purchased from Sigma–Aldrich (St. Louis, MO, USA) and was used without further purification. Pre-experiments indicated that dissolving 5% (m/v) chitosan in a 70:30% (v/v) triflouroacetic acid (TFA) vs. dichloromethane (DCM) solvents produced the most homogenous network of fibres. Both solvents were obtained from Sigma–Aldrich (Sigma–Aldrich, Copenhagen, Denmark). The solutions were homogenized under constant magnetic stirring at room temperature overnight. The homogenized solution was carefully withdrawn to avoid air bubbles with a disposable 5 mL syringe (Soft-Ject®Luer, Germany), which was fitted with a 26 gauge needle with a 12 mm tip length and a 0.53 mm inner diameter. The syringe was installed in a single syringe pump machine (NE-1000, New Era Pump Systems, Inc., Farmingdale, NY, USA). A 10 × 10 cm copper plate, which had previously been wrapped with aluminium foil, was placed 10 cm from the needle tip, thus serving as a collector of the electrospun polymer. The electrospinning conditions were set to an applied voltage of 25 kV with a high voltage power supply (ES50P-10 W, Gamma High Voltage Research, Inc., Ormond Beach, FL, USA), and the feed rate was 0.02 mL/min. The anode of the power supply was connected with the needle tip, while the cathode was attached to the collector copper plate. The electrospinning procedure took place inside a fume hood chamber at a steady temperature of 21 °C, and relative humidity ranging from 20% to 40%. The collected fibre mats were left to dry for several days to remove further residual solvent, before they were peeled from the collector plates, using a surgical scalpel. The as-spun fibre mats were then neutralized, by soaking the as-spun chitosan fibre mats in a 5 M sodium carbonate (Na_2CO_3) solvent for up to 3 h, to make the fibres more stable during storage. The solvent was obtained from Sigma–Aldrich (Sigma–Aldrich, Copenhagen, Denmark) and was used without further purification. The chitosan fibre mats were finally hydrated for 1 h in distilled water prior to wrapping the material to the raw beefs, as described in Section 2.2.1.

2.1.2. Steak preparation and treatments

Since the ageing process can vary significantly between individual animals (Damez & Clerjon, 2008; Lepetit & Hamel, 1998), uniformity in the raw material was obtained by using only meat from one animal. This supported that the experimental design indicated how the ageing methods affected the meat, rather than showing the individual differences in the raw material. Two pieces of beef *M. longissimus dorsi* muscle (representing both the left and right strip loin) from a 259 kg dairy cow were purchased a day after slaughter from a commercial slaughter house. Based on the EUROP carcass classification system, the beef had a conformation of –O, a fatness score of 3.0, and category D score, meaning beef of a mature cow. Upon acquiring the raw meat material, it was trimmed and sliced in approximate uniformity with dimensions of 7.5 cm length × 7.5 cm width × 2.5 cm thick steaks. A total of 24 steaks were used in this study, 12 from each loin side, and they were randomly classified into three ageing treatments described as (i) *dry-ageing beef wrapped with chitosan* (referred to as DC in tables and figures), (ii) *traditional dry-ageing* (DT) without chitosan, and (iii) *wet-ageing* beef (W) in vacuum bags. Sampling took place of the *raw material* (RM) on day 0, as well as from the different ageing treated beef after 7, 14 and 21 days of ageing. Two steaks were sampled from each treatment at each sampling point.

For the chitosan treated beef, each steak was carefully wrapped with the neutralized chitosan that had been freshly hydrated with distilled water for an hour, to facilitate handling and adherence of the fibre mat into the meat. Then, to aid further its contact to the meat, the steaks were kept in a plastic net with approx. 1 cm^2 mesh size. These nets were tied at each end and hung up in random positions in a refrigerator at 4 °C. Similarly, for the traditional dry-ageing beef (DT), the same procedure was applied, except that no chitosan fibre mat was applied as a wrapping material. Finally, the wet-ageing beef (W) was vacuum packed into highly impermeable vacuum plastic bags using a PLUSVAC 24 vacuum packaging machine (KOMET Maschinenfabrik GmbH, Plochingen, Germany), before storing in the cooler for the ageing process. The steaks were randomly arranged in the cooler trays and each day the location of the steaks was rotated to minimize location effects.

At each sampling occasion the steaks were weighed and then trimmed and cut in two halves for the various analytical quality assessments. The first half of the steak was used for assessment of cooking yield and tenderness using the Warner–Bratzler shear force (WBSF) method. The second half was used for analysis of muscle pH, meat colour, water content and water distribution in the muscle by low-field NMR. Microbial counts were performed on the trimmings. These methods are described in further detail in chapter 2.3.

2.2. Analytical methods

2.2.1. Morphological characterization of electrospun chitosan wrapping

A small piece of as-spun chitosan (about 1 cm × 1 cm) was cut from the aluminium foil and attached to a pin for scanning electron microscopy (SEM) for fibre morphology analysis. To prevent charging of the samples they were sputtered with gold for 10 s prior to