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Relationship between hardness and myowater properties in Wooden Breast affected chicken meat: A nuclear magnetic resonance study

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ABSTRACT

The role of myowater-holding on the development of the hardness of Wooden Breast (WB) affected chicken breasts was investigated. Transverse (T_2) relaxation times and proportions of myowater populations (T_{2B}, T_{21} and T_{22}) were assessed using low-field nuclear magnetic resonance (NMR) relaxometry and integrated with meat compression measurements. Two muscle conditions (M: Normal (N) vs WB), four sampling locations (L), four sampling times (T) and interactions (M x L and M x T) were considered. Compared to N, WB was harder, the extramyofibrillar myowater population (T_{22}) was increased and the relaxation time of the water trapped into the myofibrillar matrix (T_{21}) was also increased. A link between the T_{21} relaxation time of water trapped into the myofibrillar matrix and hardness was suggested for the WB muscles. During storage, a redistribution of water occurred over time, as revealed by an increasing trend of the T_{21} population, but a concomitant texture evolution did not reflect this change. The cranial/superficial part of the breasts exhibited the highest amount of the extramyofibrillar water population (T_{22}), and the texture of this muscle part was harder than the deep layers. However, the role of myowater on muscle hardness was not fully clarified by this study.

1. Introduction

An emergent myopathy in fast growing, meat-type broiler chickens has been described and named Wooden Breast (WB) (Sihvo, Immonen, & Puolanne, 2014). Macroscopically, the affected *Pectoralis major* muscle is hard, pale, outbulging and sometimes superficially covered with small haemorrhages, exudate and occasionally White Striping; extended areas with poor cohesion of the muscle bundles are also visible beneath the lesioned areas (Dalle Zotte et al., 2017). Histologically, the condition was defined as a moderate or severe polyphasic myodegeneration with regeneration of the muscle tissue; therefore, inflammation and necrosis are detected and are followed by accumulation of a variable amount of interstitial connective tissue (fibrosis) as reparative response (Sihvo et al., 2014). Starting from the first surveys on WB and until more recent studies, the fibrotic response has been considered as the primary factor for the typical hardness of the affected tissue (Clark & Velleman, 2016; Sihvo et al., 2014), as extensive hard consistency of raw meat and increased intramuscular collagen often occur together (Chatterjee, Zhuang, Bowker, Rincon, & Sanchez-Brambila, 2016; Petracci et al., 2015; Sihvo et al., 2014; Soglia et al., 2016a). Interestingly, it was demonstrated that WB muscle does not possess a homogeneous structure, as fibrosis was found to affect the anterior portions of the fillets, whereas the middle-ventral and postero-ventral locations were less or not at all affected (Clark & Velleman, 2016). In addition, contrary to normal breast muscles, also superficial and deep layers in raw affected *Pectoralis major* muscle has been found to differ in terms of textural properties, which probably reflects a variation in the muscle architecture between layers. Indeed, according to Gao (2015), hard consistency of the muscle is mainly present at the surface level at the early *post mortem* stage and becomes as soft as the normal condition during a chilled storage. As previously mentioned, fibrosis has been considered the major reason for the hard texture of affected chicken breasts so far. However, some cases of hard breasts without a significant accumulation of collagen were detected in broiler chickens both in very young birds (Sihvo, Lindén, Airas, Immonen,

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& Puolanne, 2017a; Sihvo et al., 2017b) and at commercial slaughter age (Dalle Zotte et al., 2017; Sihvo et al., 2014), thus opening new hypotheses on the reasons for muscle hardness development. One hypothesis may involve the role of myowater. Indeed, changes in muscle microstructure directly affect water distribution among the three water populations defined by nuclear magnetic resonance (NMR) T_2 relaxation studies: T_{2B} (H_2O closely associated to macromolecules/proteins), T_{21} (H_2O trapped into myofibrillar matrix) and T_{22} (extramyofibrillar H_2O), with each water compartment exhibiting its typical relaxation time (Bertram, Purslow, & Andersen, 2002; Bertram et al., 2001). In a previous study of Soglia, Laghi, Canonico, Cavani, and Petracci (2016b), the WB condition resulted in a remarkable decrease in the intramyofibrillar fraction and a concomitant increase in the extramyofibrillar water fraction. Consequently, the present study aimed to investigate the role of the chemical-physical state of myowater on hardness in WB affected chicken meat, integrating low-field NMR relaxometry with texture analysis (compression test). NMR properties and hardness were evaluated not only according to muscle condition but also considering a 72 h *post mortem* chilled storage and four different sampling locations, as these factors have been shown to affect meat textural properties (Soglia et al., 2017).

2. Material and methods

2.1. Samples collection and preparation

During two different sampling times, a total of 96 breast muscles from 34-day-old broiler chickens were collected at a commercial Danish slaughterhouse (Danpo A/S, Aars, Denmark). Each time, 48 fillets were selected according to the presence or the absence of severe Wooden Breast lesions, thus obtaining 24 macroscopically unaffected or normal (N) and 24 Wooden Breast (WB) samples. The selection based on the visual and palpatory inspection of *Pectoralis major* muscles; breasts exhibiting diffused hardened areas were scored as WB. The presence of bulges, pale colour and the surface covered with exudate, haemorrhages and white striping were also detected in the selected WB breasts (Dalle Zotte et al., 2017; Sihvo et al., 2014). On the contrary, fillets with soft and elastic tissue with uniform colour were scored as N. After selection, breasts were immediately packed into polyethylene bags, kept cool and transported to the NMR laboratory of the Department of Food Science (Aarhus University, Årslev), where further analyses took place. At the laboratory, fillets were kept at 4 °C for four different storage times: 10, 24, 48 and 72 h *post mortem* (*pm*); 6 WB-affected (WB) and 6 normal (N) fillets were used for each time. Four stripes per breast (1 cm × 1 cm x 4 cm, 5 ± 0.5 g) were excised parallel to the fibre direction; two of them were obtained from the cranial end of the fillet (CRA), whereas the other two were cut from the medial portion (MED). Within each portion, one stripe was snipped from the superficial layer (S: 0.2–1.2 cm deep under the muscle surface) and the other was snipped from the deep layer (D: 1.5–2.5 cm deep under the muscle surface) (Gao, 2015; Soglia et al., 2017). Locations were named as follows: CRA/S = cranial/surface; CRA/D = cranial/depth; MED/S = medial/surface; MED/D = medial/depth (Fig. 1). Accordingly, 24 + 24 meat samples were prepared and analysed per each of the eight measurement days.

2.2. NMR measurements

Meat stripes (1 cm × 1 cm x 4 cm) were placed in glass test tubes, which were sealed with paraffin film and thermostated at 25 °C for 20 min in a waterbath. Thereafter, transverse relaxation time (T_2) measurements were performed on a Maran Benchtop Pulsed NMR Analyser (Resonance Instruments Ltd, Witney, UK)

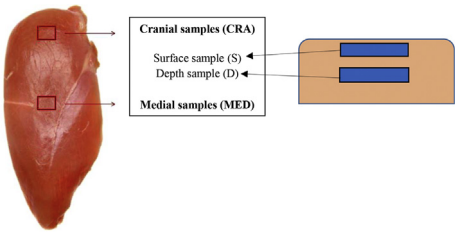

Fig. 1. Sampling locations.

equipped with a 18 mm probe head operating at a magnetic field strength of 0.47 T and a corresponding resonance frequency for protons of 23.2 MHz. Transverse relaxation time (T_2) was measured using the Carr-Purcell-Meiboom-Gill (CPMG) spin-echo sequence (Carr & Purcell, 1954; Meiboom, Gill, Huxley, & Niedergerke, 1958) and a τ-value (time between 90 and 180° pulse) of 150 μs Data from 4096 echoes were acquired as 16 scan repetitions. The obtained T_2 data were analyzed using distributed exponential fitting analysis according to the regularization algorithm by Butler, Reeds, and Dawson (1981) and carried out in MatLab (The Mathworks Inc., Natick, MA, USA) using in-house scripts. Plots of relaxation amplitudes for individual relaxation processes vs relaxation times revealed the presence of three relaxation populations. For each of the three water populations T_{2B}, T_{21} and T_{22}, relaxation times were calculated from the peak position and proportions of protons exhibiting those relaxation times were calculated from the corresponding area under each peak, using an in-house programme written in Mathlab (The Mathworks Inc., Natick, MA, USA).

2.3. Hardness measurements

After the NMR analyses, the same meat samples were then subjected to a single compression test in order to determine their hardness. A Brookfield CT3 texturometer (Texture Technologies Co, Hamilton, MA, USA) equipped with a 250 N load cell was used, setting the trigger load at 0.3 N and the test speed of at 50 mm/min (0.83 mm/s). One sample from each muscle (48) was taken at each storage time (10, 24, 48 and 72 h *pm*). Strips were cut before analysis (1 cm × 1 cm x 3 cm) in order to fit to the compression probe (1 cm^2) and to the measuring cell (1 cm × 1 cm x 3 cm), which were modified according to Lepetit and Culioli (1994) and Campo, Sañudo, Panea, Alberti, and Santolaria (1999). Strips were compressed to 80% of their initial height (Lyon & Lyon, 2001) perpendicularly to the fibre direction, which, therefore, could extend only longitudinally. From each measurement a curve was obtained, and the peak force represented the maximum hardness (expressed in newtons).

2.4. Statistical analysis

The “Location” (L) effect was created combining breast portion (CRA/MED) and layer (S/D); four locations resulted from the combinations (CRA/S; CRA/D; MED/S; MED/D). Then, data were analysed using a SAS 9.1.3 statistical software package for Windows (SAS, 2004). Variables were evaluated by ANOVA, choosing a mixed model (PROC MIXED) which considered muscle condition (M: N; WB), location (L), hour *pm* (T: 10 h, 24 h, 48 h, 72 h) and sampling time (1; 2) as fixed effects, whereas sample (breast) was considered as random repeated effect. The interactions M x T and M x L were also studied. Post-hoc pairwise comparisons were evaluated by Bonferroni adjustments. Pearson correlations between hardness and NMR variables were performed with the two muscle conditions separately considered. Two significance levels were assigned: