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Application of quantitative magnetization transfer magnetic resonance imaging for characterization of dry-cured hams

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ABSTRACT

Quantitative magnetization transfer magnetic resonance imaging (qMT-MRI) was employed to characterize dry-cured ham tissues differing in anatomical positions and processing protocols. Experimentally obtained MR images of dry-cured ham sections were analyzed by the well-established binary-spin-bath (BSB) model. The model enabled an efficient discrimination between a free-water proton pool and a restricted-macromolecular proton pool. Significant differences in restricted pool sizes were found among different ham sections. Values of the restricted pool size obtained by the model were in a good agreement with chemically determined protein content. The study confirmed the feasibility of the applied qMT-MRI as a nondestructive tool for characterization of dry-cured ham tissues.

1. Introduction

Traditional dry-cured ham is a high-quality product with characteristic texture and flavor, which gradually develop during continuous processing steps of salting, post-salting and ripening (Pugliese et al., 2015). During the processing, controlled tissue dehydration (Fantazzini, Gombia, Schembri, Simoncini, & Virgili, 2009) at the meat-air interface progresses along with salt diffusion in a direction from superficial meat layers to meat interior (Vestergaard, Risum, & Adler-Nissen, 2005). Simultaneously with the processes, tissue enzymes initiate intensive lipolytic and proteolytic reactions (Toldra & Flores, 1998), of which kinetics significantly depend on salt and water content (Toldra, 2006). The hydrolytic reactions and other chemical reactions, such as Maillard reactions, Strecker degradations and oxidative reactions, contribute to the development of characteristic product flavor and hence to consumer acceptance (Pham et al., 2008). In dry-cured ham products with long processing time (longer than 10 months) proteolysis can result into an extensive breakdown of major myofibril proteins into a high number of small peptides and finally in a large amount of free amino acids (Mora, Fraser, & Toldra, 2013).

Proteolytic action in dry cured-hams can be conventionally determined by various well-established proteomic techniques, such as two dimensional gel electrophoresis (Di Luccia et al., 2005), high performance liquid chromatography (Rodríguez-Nuñez, Aristoy, & Toldra, 1995) and mass spectrometry (Mora, Sentandreu, & Toldra, 2010). These techniques have high sensitivity for protein identification, however, they are destructive and time consuming as they are based on fractionation and isolation of the generated peptides and their subsequent physical separation. Another complementary technique for proteomic tissue characterization is spectroscopic high-resolution magic angle spinning nuclear magnetic resonance (HRMAS NMR) (Castejon et al., 2010), in which different spectral peaks are assigned to different protein components.

Magnetic-resonance based techniques were in general recognized as powerful biophysical tools for assessment of meat structure (Damez & Clerjon, 2008). The imaging modality of high-resolution NMR, i.e., magnetic resonance imaging (MRI) that provides spatial distribution of water protons, was efficiently applied for dynamical monitoring of longitudinal (T_1) and transversal (T_2) relaxation times in dry-cured ham samples during different processing steps (Fantazzini et al., 2009). In another study, water apparent diffusion coefficient (ADC) and diffusion-tensor anisotropy was used to analyze fresh bovine meat (Renou, Foucat, & Bonny, 2003). However, in these MRI studies only free water protons of meat tissue with sufficiently slow transversal relaxation (long T_2 time) were considered, while the macromolecular protons were not detected by conventional MRI pulse sequences due to their

Abbreviations: ADC, apparent diffusion coefficient; AFI, actual flip-angle imaging; BF, *biceps femoris*; BSB, binary spin bath; CPMG, Carr-Purcell-Meiboom-Gill pulse sequence; CWPE, continuous-wave power equivalent; DD, dual delay; HRMAS, high-resolution magic angle spinning; MR, magnetic resonance; MRI, magnetic resonance imaging; MT, magnetization transfer; MTR, magnetization transfer ratio; NMR, nuclear magnetic resonance; PFG, pulse-field gradient spin echo; qMT, quantitative magnetization transfer; SM, *semimembranosus*; SNR, signal-to-noise ratio; SPGR, spoiled gradient echo; T_1, longitudinal relaxation time; T_2, transversal relaxation time; VFA, variable flip angle.

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fast transversal relaxation. Spatial distribution of these protons can be obtained by magnetization transfer (MT) MRI that accounts for interaction of tissue-water protons residing in two biochemically different environments (Wolff & Balaban, 1989). In a widely accepted two-pool picture (Henkelman et al., 1993), water protons (a free magnetization pool) contribute to conventionally visible MRI signal, while the macromolecular protons attached to proteins and macromolecules (a restricted magnetization pool) exhibit too fast transversal relaxation to enable their signal detection by conventional MRI. Nevertheless, an indirect detection of the restricted pool is still possible due to coupling between the two pools through proton exchange and cross-relaxation. The MT effect is established by applying an off-resonance radiofrequency preparation pulse that selectively saturates the restricted pool exhibiting much broader absorption line as the free pool. Biochemically important components of the restricted pool are hydroxyl, amine and possibly carboxyl groups, although the other macromolecular mechanisms including hydration layer state and mobility of hydroxyl groups at the macromolecular surface might be also important (Cercignani & Barker, 2008).

In medical science and clinical practice, the MT effect was recognized as a valuable intrinsic biomarker to distinguish between healthy and diseased tissue. Therefore, MT-MRI was successfully introduced as a minimally invasive and nondestructive method for soft-tissue suppression in MR angiography (Lin, Tkach, Haacke, & Masaryk, 1993), delineation of white matter lesions in multiple sclerosis (Natt, Watanabe, Boretius, Frahm, & Michaelis, 2003), tracking of protein depletion in articular cartilage (Regatte, Akella, & Reddy, 2005), and recently also for identification of proton exchanging macromolecules in collagen- and fibrin-rich tissue regions of human atherosclerotic plaques (Qiao, Hallock, & Hamilton, 2011). A prominent MT effect was found also in a human muscle tissue (Sinclair et al., 2010). The MT approach was also translated to the field of meat sciences, where mostly the effect of freezing/thawing on lamb, bovine and pork meat quality was analyzed (Evans, Nott, Kshirsagar, & Hall, 1998), while the MT effect in dry-cured meat products undergoing structural changes by proteolytic action and tissue dehydration was not yet fully addressed.

The MT effect can be analyzed by two different approaches. The first approach includes semiquantitative interpretation of the acquired MT data on the basis of magnetization transfer ratio (MTR). MTR is calculated as a relative difference between the MRI signal with the applied preparation off-resonance pulse, yielding a significant MT effect, and the MRI signal with no preparation pulse applied. Since the MT effect reduces MRI signal intensity in high protein density regions, these regions could be discriminated from the surrounding tissue in the MTR maps (Qiao et al., 2011). More advanced approach is quantitative MT (qMT) that provides quantitative determination of the underlying physical MT tissue properties. The qMT method is performed by applying fitting analysis of the presumed MT models (with different numbers of water compartments) to the experimental MT datasets. While multi-compartment tissue models aim to account for tissue complexity (Ceckler, Maneval, & Melkowits, 2001), the binary spin bath (BSB) model, that considers tissue as an interacting two-pool system, was found as the best compromise between the tissue and model complexity (Natt et al., 2003; Ramani, Dalton, Miller, Tofts, & Barker, 2002).

In our study, we demonstrate feasibility of qMT-MRI approach for discrimination among different dry-cured ham tissues. The tissues differed in processing protocols (high vs. low salt) and anatomical positions (outer *semimembranosus* (SM) muscle, inner *biceps femoris* (BF) muscle and combined SM-BF position). Our hypothesis was that tissue dehydration and proteolytic action in different dry-cured ham tissues can result into a significantly altered proportion between free and restricted magnetization pools. Experimentally obtained MT datasets of different ham sample groups were quantitatively analyzed by using the BSB model, yielding a restricted magnetization pool size that was compared to conventional MR parameters (T_1, T_2 and ADC) and to chemically determined protein content.

2. Theory

Dry-cured ham tissue was modeled as a two-compartment system by using the well-established BSB model (Henkelman et al., 1993). The MT effect, arising due to selective off-resonance saturation of the restricted pool and the transfer of magnetization to the free magnetization pool, can be mathematically described by a set of coupled Bloch equations that include both magnetization exchange terms and RF absorption rate terms (Henkelman et al., 1993). By assuming a steady-state condition, which is experimentally met either by very long MT saturation pulse (of several seconds) or by shorter MT pulse (of several milliseconds) followed by a signal acquisition sequence with a short repetition time, magnetization time derivatives in Bloch equations can be set to zero and the BSB signal equation (or the so called *z-spectrum*) can be derived in a form of (Ramani et al., 2002)

$$S(\Delta,\omega_1,R)=gM_0^A\frac{R_B\left[\frac{RM_0^B}{R_A}\right]+R_{RFB}+R_B+RM_0^A}{\left[\frac{RM_0^B}{R_A}\right](R_B+R_{RFB})+\left(1+\left[\frac{\omega_1}{2\pi\Delta}\right]^2\left[\frac{1}{R_AT_2^A}\right]\right)\left(R_{RFB}+R+RM_0^A\right)}. \quad (1)$$

Here, $S(\Delta,\omega_1;R)$ denotes MRI signal intensity, Δ and ω_1 correspond to MT frequency offset (with respect to the resonant Larmor frequency ω_0) and MT amplitude frequency, respectively, while the indexes A and B denote the free and restricted magnetization pool, respectively. Parameters M_0^A (M_0^B) and R_A (R_B) denote equilibrium magnetization and relaxation rate of the free (restricted) pool, respectively, whereas T_2^A (T_2^B) denotes the transversal relaxation time of the free (restricted) pool. The parameter R is the rate coupling constant between the pools and the parameters g accounts for the spectrometer characteristics such as receiver gain. Since the free pool undergoes motional narrowing regime, the respective RF absorption rate in the MT signal equation commonly includes a Lorentzian lineshape, $R_{RFA}\approx\left[\frac{\omega_1}{2\pi\Delta}\right]^2\frac{1}{T_2^A}$. The restricted pool, however, cannot be consistently described neither by a Lorentzian lineshape (that is common for liquids) nor by a Gaussian lineshape (that is common for well-ordered solid materials). However, an RF absorption rate including a super-Lorentzian lineshape, that averages dipolar interactions over all possible orientations, was found most adequate for a description of partially ordered materials such as biological tissues (Henkelman et al., 1993; Natt et al., 2003; Sinclair et al., 2010)

$$R_{RFB}=\sqrt{2\pi}\,\omega_1^2\,T_2^B\int_0^1\frac{du}{|3u^2-1|}\,exp\left(-2\left(\frac{2\pi\Delta T_2^B}{3u^2-1}\right)^2\right). \quad (2)$$

By introducing the restricted pool fraction (Ramani et al., 2002), $f=(1+M_0^A/M_0^B)^{-1}$, the signal Eq. (1) contains eight fundamental MT parameters, however, only six combinations of these parameters can be uniquely determined due to their interdependence (Ramani et al., 2002; Sinclair et al., 2010): R_B, RM_0^A, $f/R_A(1-f)$, T_2^B, $1/R_AT_2^A$ and gM_0^A. The last parameter is often omitted from analysis as it depends mostly on spectrometer characteristics rather than sample properties. Among these combinations, transversal relaxation time of the restricted pool T_2^B and the restricted pool fraction f were found characteristic for healthy/diseased tissue characterization and due to discriminating potential also received highest attention (Mallik, Samson, Wheeler-Kingshott, & Miller, 2014; Yarnykh et al., 2015). While T_2^B can be obtained directly as a fitting parameter, an additional independent measurement of $T_{1^A}=1/R_{A,obs}$ is needed to extract the restricted pool fraction. As described in Henkelman et al. (1993), from the expression

$$R_A=\frac{R_{A.obs}}{1+\frac{B\cdot C(1-R_{A.obs})}{1+B-R_{A.obs}}}, \quad (3)$$