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# Use of multiparametric magnetic resonance microscopy for discrimination among different processing protocols and anatomical positions of Slovenian dry-cured hams

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ABSTRACT

A novel multiparametric magnetic resonance microscopy (MRM) approach was applied to the Slovenian *Kraški pršut* dry-cured ham samples in order to evaluate its potential for discrimination among *biceps femoris* and *semimembranosus* muscle from two hams, differing in processing (salting duration) and thus in water and salt content. The approach is based on apparent diffusion coefficient (ADC) mapping as well as on longitudinal ($T_1$) and transversal ($T_2$) nuclear magnetic resonance relaxation time mapping. Three-dimensional maps were acquired and analyzed by one dimensional (1D) ADC, $T_1$, and $T_2$ distributions as well as by paired two-dimensional ADC-$T_1$, ADC-$T_2$ and $T_1$–$T_2$ distributions. The discriminating potential of the applied MRM approach was confirmed by differences among both 1D and 2D distributions of different ham samples. In addition, distribution peak positions highly correlated with the conventionally determined moisture content.



## 1. Introduction

*Kraški pršut* is a traditional Slovenian dry-cured ham of Mediterranean type characterized by dry salting, absence of smoking and long ripening time. Being a dry-cured meat product, most recognized by Slovenian consumers, an ongoing research interest exists (Andronikov, Gašperlin, Polak, & Žlender, 2013; Pugliese et al., 2015) in order to keep quality control during its processing and hence providing its optimal textural, volatile and sensorial traits relevant for customer acceptability. Among the most important factors influencing final properties of dry-cured ham products are moisture and salt distribution, which also determine enzymatic activity during proteolysis and lipolysis (Čandek-Potokar & Škrlep, 2012). Conventional methods for assessment of moisture and salt contents are based on chemical and gravimetric analyses that are usually destructive and time-consuming. Although these methods provide detailed quantitative information on meat product composition, they cannot be used for a prompt composition assessment and possible optimization of processing conditions (Fantazzini, Gombia, Schembri, Simoncini, & Virgili, 2009).

Magnetic resonance imaging (MRI) is an established non-invasive and non-destructive method for determination of structural and functional tissue properties and is thus widely used in medicine as well as in material (Song, 2013) and food science (Bajd & Serša, 2011; Mohorič et al., 2009; Van As & van Duynhoven, 2013). In last decades, MRI became recognized as an efficient method in the field of quantitative quality control during processing of meat products. In dry-cured hams, the method can provide quantitative information on lean meat and fat proportions (Collewet et al., 2005; Monziols et al., 2006) as well as moisture content (Renou, Foucat, & Bonny, 2003). Moreover, $^{1}$H-MRI was efficiently applied for dynamical follow-up of a moisture loss (Antequera, Caro, Rodriguez, & Perez, 2007), while $^{23}$Na-MRI was

*Abbreviations:* ADC, apparent diffusion coefficient; AUC, area under (the ROC) curve; BF, *biceps femoris* muscle; CPMG, Carr–Purcell–Meiboom–Gill; DWI, diffusion weighted imaging; FLASH, fast low-angle shot; IMF, intramuscular fat; IR, inversion recovery; MR, magnetic resonance; MRI, magnetic resonance imaging; MRM, magnetic resonance microscopy; NMR, nuclear magnetic resonance; PGSE, pulse-field gradient spin echo; ROC, receiver operating characteristic; SM, *semimembranosus* muscle; SNR, signal to noise ratio; STIR, short $T_1$ inversion recovery; $T_1$, longitudinal relaxation time; $T_2$, transversal relaxation time; TCN, total count number.

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exploited for a dynamical monitoring of salt uptake into the ham interior (Vestergaard, Risum, & Adler-Nissen, 2005) and determination of sodium mobility in dry-cured hams subjected to high-pressure processing (Picouet et al., 2012). Recently, a low-field $^1$H-MRI study showed that both nuclear magnetic resonance (NMR) relaxation times, longitudinal ($T_1$) and transversal ($T_2$), can serve as characteristic markers for monitoring of *Parma* dry-cured ham processing during salting, resting, maturing and ageing periods (Fantazzini et al., 2009). Moisture loss during the processing steps is accompanied with a decrease of the relaxation times, which could be very significant at the exposed meat-air interface. Strong dehydration at the interface could in turn prevent further drying of the ham interior, which implies severe impairment of the ham quality (Fantazzini et al., 2009).

Some of recent $^1$H-MRI studies of dry-cured hams were focused on individual ham types such as *Iberico* (Antequera et al., 2007), *Parma* (Fantazzini et al., 2009) and *San Daniele* (Manzocco et al., 2013). In the studies, standard clinical 1.5 T and low-field 0.2 T MRI scanners were used. Therefore, the obtained spatial resolution was relatively low and slices were up 5 mm thick, which could result in the partial-volume effect, i.e., MR signal averaging over different ham structures, such as lean meat and intramuscular fat (IMF) inclusions. In addition, set of applied MRI methods was limited to the most common ones ($T_1$-weighted, $T_2$-weighted, $T_1$ mapping and $T_2$ mapping). However, diffusion properties of water in dry-cured ham were rarely investigated despite its obvious advantages in meat tissue characterization. It was shown that diffusion tensor and apparent diffusion coefficient (ADC) of water molecules provide a valuable additional information on water mobility within anisotropic tissue microenvironment (Renou et al., 2003).

The study presented here is an upgrade of previous $^1$H-MRI studies on dry-cured ham and employs high-field three-dimensional (3D) proton-based magnetic resonance microscopy ($^1$H-MRM) with a comparatively larger spatial resolution. In addition, the applied MRM protocol consists of MR pulse sequences for morphological assessment of ham tissue structure as well as of multiparametric $^1$H-MRM (Vidmar, Kralj, Bajd, & Serša, 2014) for determination of intrinsic MR parameters ($T_1$, $T_2$ and ADC) of the examined *Kraški pršut* ham samples. The parameters were measured independent of each other and then analyzed by one-dimensional (1D) ADC, $T_1$ and $T_2$ distributions as well as by paired two-dimensional (2D) ADC-$T_1$, ADC-$T_2$ and $T_1$–$T_2$ distributions that were calculated by combing the corresponding parameter maps on a pixel basis. These results were also correlated with the results of conventional chemical and textural analyses.

## 2. Material and methods

### 2.1. Dry-cured ham processing and sample preparation

Ham samples for the present study originated from the experiment described in (Škrlep et al., 2016) that was designed to study the effect of different salting duration on various ham chemical, rheological and sensory traits. Shortly, after applying different salting durations (6 vs. 18 days) the hams were processed according to the rules of *Kraški pršut* consortium. After the end of processing (460 days in total) ham sections with a size of $5 \times 5 \times 2$ cm$^3$ were taken from the central portions of *biceps femoris* (BF) and *semimembranosus* (SM) muscles and subdivided into two parallel ham section groups for chemical analyses and for MRM examination. All the sections were vacuum-packed and then frozen to −20 °C for approximately 6 weeks until chemical and imaging analyses began. For each analysis (chemical or imaging), a sample with a size of $1 \times 1 \times 2$ cm$^3$ was cut from a selected ham section and then allowed to spontaneously thaw at room temperature. Analyses of each section took no longer than a week. According to the processing protocol, i.e., high-salt (HS) vs. low-salt (LS), and anatomical position (BF vs. SM), *Kraški pršut* ham samples were grouped into four ham sample groups, denoted as HS-BF, LS-BF, HS-SM, LS-SM.

### 2.2. Chemical analyses of dry-cured ham samples

Dry-cured ham samples were analyzed by chemical analyses for determination of dry matter, NaCl, and intramuscular fat (IMF) contents, as described in (Škrlep et al., 2016). Briefly, NaCl concentration was determined by using a titration-based technique (Škrlep et al., 2012), while dry-matter and IMF contents were determined according to the ISO international standards 6496 and 1443 (ISO, 1973, 1999), respectively. Each ham sample group was examined by chemical analyses in duplicates.

### 2.3. $^1$H-MRM of dry-cured ham samples

Prior to MRM examination, each sample was tightly wrapped into a Parafilm foil (Bemis Company, Inc., WI, USA) to avoid from desiccation and then inserted into an MRM probe. The foil had a short transversal NMR relaxation time ($T_2 < 1$ ms) that prevented NMR signal detection of the foil by MR imaging. During the examination time, samples were kept at a constant temperature of 20 ± 1 °C, provided by the MRM system. MR microscopy experiments were performed on a MR scanner consisting of a 2.35 T (corresponding to the proton frequency of 100 MHz) horizontal bore super-conducting magnet (Oxford Instruments, Abingdon, United Kingdom) equipped with a Bruker micro-imaging system (Bruker, Ettlingen, Germany) with a maximum imaging gradient of 250 mT/m and controlled by a TecMag Apollo spectrometer (Houston, TX, USA). Each dry-cured ham sample was inserted into a 25 mm diameter micro-imaging probe and consecutively examined by 3D morphological and multiparametric $^1$H-MRM protocol. Morphological MRM was performed by employing spin-echo-based $T_1$-weighted MR imaging, gradient-echo-based fast low-angle shot MR imaging pulse (FLASH) with the excitation angle $\alpha = 11°$ and short $T_1$ inversion recovery (STIR) pulse sequence with the inversion recovery time $\tau_{IR} = T_1^{fat} \ln(2)$ set to 210 ms for fat signal suppression. Multiparametric MRM was performed by using diffusion-weighted imaging (DWI) based on a pulsed gradient spin-echo (PGSE) sequence for ADC mapping, spin-echo-based inversion-recovery (IR) pulse sequence for $T_1$ mapping and multi-spin-echo imaging sequence based on the Carr–Purcell–Meiboom–Gill (CPMG) multi-echo train for $T_2$ mapping (Foucat, Benderbous, Bielicki, Zanca, & Renou, 1995). With all MR pulse sequences, MR signal was acquired with a dwell time of 20 μs. Each sample group was examined by MRM in two replicates. The experimental setup is schematically shown in Fig. S-1 and the imaging parameters of the applied MR sequences are given in Table S-1. The applied multiparametric MR pulse sequences are schematically shown in Fig. S-2.

### 2.4. Image analysis

The obtained MRM data were analyzed retrospectively using in-house written image-analysis software, developed within the Matlab programming environment (MathWorks, Inc., Natick MA, USA), and partially visualized using the POV-Ray program (Persistence of Vision Raytracer, Williamstown, Victoria, Australia). In the software, fully automated MR image segmentation was performed as follows. Firstly, multi-dimensional raw MRM data were consistently reshaped and 3D-Fourier transformed to obtain MR images of dry-cured ham samples. For each ham sample, a 3D set of DWI images with $b = 0$ was used to create a mask for automatic