Dry-cured ham tissue characterization by fast field cycling NMR relaxometry and quantitative magnetization transfer

Franci Bajd, Anton Gradišek, Tomaž Apih and Igor Serša

Fast field cycling (FFC) and quantitative magnetization transfer (qMT) NMR methods are two powerful tools in NMR analysis of biological tissues. The qMT method is well established in biomedical NMR applications, while the FFC method is often used in investigations of molecular dynamics on which longitudinal NMR relaxation times of the investigated material critically depend. Despite their proven analytical potential, these two methods were rarely used in NMR studies of food, especially when combined together. In our study, we demonstrate the feasibility of a combined FFC/qMT-NMR approach for the fast and nondestructive characterization of dry-curing ham tissues differing by protein content. The characterization is based on quantifying the pure quadrupolar peak area (area under the quadrupolar contribution of dispersion curve obtained by FFC-NMR) and the restricted magnetization pool size (obtained by qMT-NMR). Both quantities correlate well with concentration of partially immobilized, nitrogen-containing and proton magnetization exchanging muscle proteins. Therefore, these two quantities could serve as potential markers for dry-curing process monitoring. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: dry-cured ham; protein concentration; dehydration; quadrupolar peaks; NMR relaxometry; magnetization-transfer NMR

Introduction

Dry-cured hams are high-quality meat products that are featured by dry salting, absence of smoking and long ripening time. Efficient dry-curing is typically achieved by applying special processing protocols with predefined optimized parameters, such as salting time, ripening temperature and relative humidity. Dry-cured hams consist of muscles from different anatomical positions, i.e. a semimembranosus (SM) muscle that is directly exposed to ambient conditions, and a biceps femoris (BF) muscle that is protected against the influence of the ambient conditions by a hypodermic adipose tissue layer. The final quality of dry-cured hams and with it associated consumer demand is highly dependent on the level of proteolysis that is catalyzed by endogenous ham-tissue enzymes. Proteolytic action of these enzymes is, apart from the origin and quality of native fresh meat, determined by two principal dry-curing processes, i.e. salt uptake during the salting period and tissue dehydration during the ripening period. Although the dry-curing process is accompanied by salt diffusion from the exposed SM muscle to the interior BF muscle and by moisture migration in the opposite direction, salt and moisture contents do not reach uniform distribution even at the final (equilibration) stages of the dry-curing process. Therefore, dynamic quality control of dry-curing meat tissue during the demanding and extensive dry-curing process necessitates an application of on-line analytical methods enabling a prompt and exact determination of moisture and salt contents.

Composition of dry-curing hams is usually monitored by standard chemical and gravimetric quantitative analytical methods. However, these methods are usually destructive as well as time consuming and are therefore not convenient for prompt monitoring of external parameters that control the dry-curing process. Unlike these methods is proton nuclear magnetic resonance ($^1$H-NMR), which is a well-established noninvasive technique in clinical diagnostics. Proton NMR is capable of distinguishing subtle muscle tissue variations that emerged as a consequence of various diseases, as for example muscle inflammation or muscle dystrophy. The method is sensitive to the water content and water mobility in different tissue compartments. As such, it is also promising for quantitative analysis of dry-cured ham tissue. The tissue is characterized by a broad range of water-to-protein content, spanning from high water content in fresh meat to low water content in dry-cured hams after a completed dry-curing process. Recently, magnetic resonance imaging (MRI) and NMR relaxometry techniques were efficiently employed for a nondestructive follow-up of local tissue variations during processing of Parma dry-cured hams. In the study, spatial distributions of moisture and marbling fat contents in dry-cured tissue sections, originating from different anatomical positions and from different stages of the dry-curing process, were characterized by spatial variations of intrinsic NMR signatures, which was enabled by combined fast-field cycling (FFC) and quantitative magnetization transfer (qMT) NMR methods. The potential of these techniques was further demonstrated by quantifying quadrupolar peak areas in dispersion curves obtained by FFC-NMR and by quantifying magnetization pool sizes in dispersion curves obtained by qMT-NMR. Both quantities are highly related to the concentration of partially immobilized, nitrogen-containing and proton magnetization exchanging muscle proteins. Therefore, these two quantities could serve as potential markers for dry-curing process monitoring. Copyright © 2016 John Wiley & Sons, Ltd.

**Abbreviations:** ADC, apparent diffusion coefficient; BF, biceps femoris; FFC, fast field cycling; MRI, magnetic resonance imaging; MT, magnetization transfer; NMR, nuclear magnetic resonance; NP, non-polarized; Q-peak, quadrupolar peak in a dispersion curve; qMT, quantitative magnetization transfer; PP, pre-polarized; RF, radiofrequency; SM, semimembranosus; VFA, variable flip angle

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parameters, i.e. longitudinal ($T_1$) and transversal ($T_2$) relaxation times. In addition, Renou et al. applied diffusion tensor imaging for mapping of apparent diffusion coefficient (ADC) and diffusion fractional anisotropy to noninvasively determine water mobility within anisotropic fresh-meat tissue microenvironment. In one of our previous studies, discrimination between differently processed SM and BF muscle sections was further improved by combining $T_1$, $T_2$, and ADC mapping followed by a two-dimensional $T_1-T_2$, ADC-$T_1$, and ADC-$T_2$ histogram analysis.

Fast field cycling (FFC) NMR relaxometry and quantitative magnetization transfer (qMT) NMR are two advanced $^1$H-NMR-based techniques that could also be exploited for a nondestructive determination of muscle protein concentration. Partially immobilized muscle proteins contain also nitrogen atoms (e.g. in the peptide bonds) with nuclear electric quadrupole moments. Because of the quadrupole moments nitrogen atoms act as a relaxation sink for proton magnetization and remarkably promote proton longitudinal relaxation whenever the Zeeman energy is equal to one of the three quadrupole energy levels. In the FFC-NMR dispersion curves, i.e. $T_1$ as a function of magnetic field strength, this effect is manifested by an emergence of quadrupole dips that are superimposed on the dipolar power-law background. In different model systems of partially immobilized proteins, such as cross-linked bovine serum albumin, cross-linked fibrin, partially hydrated collagen and glycosaminoglycan, a positive correlation was found between the Q-peak (peaks in the $R_1$ dispersion curve) area and the concentration of the proteins. The other technique used in the study, i.e. qMT-NMR, was developed as a method for an indirect detection of tissue compartments with short $T_2$ relaxation times. A typical tissue can be modeled as a two compartment system. The first compartment is the restricted proton pool ($T_{2r} \approx 10 \mu s$) that consists of protons attached to muscle proteins and of protons in the hydration layer around the proteins, while the second compartment is the free proton pool ($T_{2f} > 10$ ms) that consists of extracellular-water protons. In qMT-NMR, the sample is RF irradiated in a narrow frequency band that delivers most energy to the restricted pool and almost no energy to the free pool. As the pools are coupled via cross-relaxation and/or proton chemical exchange, some of the energy is then transferred from the restricted pool to the free pool. This is manifested in a partial saturation of the free pool magnetization and therefore by a reduction of the detectable free-pool NMR signal, from which a relative proportion of proton magnetization exchanging macromolecules can be calculated.

The aim of this study was to demonstrate the feasibility of FFC-NMR and qMT-NMR techniques for a prompt and nondestructive determination of protein concentration in dry-cured as well as fresh-meat ham samples, originating from different anatomical positions (SM vs BF). Based on the experimental evidence, the potential of these techniques for a possible industrial application is also discussed.

### Materials and Methods

#### Ham sample preparation

A commercially available vacuum-packed Primorski pršut dry-cured ham (Pršutarna Lokev na Krasu, Slovenia) with a total weight of 1.5 kg was purchased at a local vendor. Samples intended for FFC-NMR and qMT-NMR analysis were cut from the representative regions of SM/BF muscles and of adipose tissue of the dry-cured and fresh-meat tissues. The size of samples was approximately 0.4 × 0.4 × 1.2 cm, in order to fit in the FFC-NMR tube. FFC-NMR and qMT-NMR experiments were performed successively in less than 12 h. Therefore there was no need for deep freezing of the samples as it could affect fresh meat tissue texture. Instead, the bulk tissues and the samples were stored at 8 °C up to either two weeks (dry-cured ham) or one day (fresh-meat). The experimental setup and meat tissue microenvironment are schematically shown in Fig. 1.

#### Fast-field cycling NMR experiments

Proton longitudinal (spin-lattice) relaxation times $T_1$ were measured using a Stelar Spinmaster 2000 fast field-cycling relaxometer (Stelar s.r.l., Pavia, Italy). The relaxometer employed an electromagnet with a fast switching time (on the millisecond timescale), thus enabling relaxation measurements over a broad range of Larmor frequencies, with $\nu_L = \gamma B/2\pi$ and $\gamma = 2.675 \times 10^8$ rad/s/T being the proton gyromagnetic ratio. Relaxation times were measured at room temperature in the proton NMR frequency range from 20 MHz to 5 kHz (corresponding to magnetic field from 470 mT to 0.12 mT). For frequencies above 6 MHz, a non-polarized (NP) sequence was used.
used to measure $T_1$ relaxation times. The sample was initially kept in
the zero magnetic field, and the magnetization build up was then
measured at a selected field. At frequencies below 6 MHz, a pre-
polarized (PP) sequence was used. In this case, the sample was ini-
tially magnetized in a polarizing magnetic field $B_{1\text{pol}}$ of 420 mT for a
polarization time of $4 \times T_1 (v_{\text{max}} = 20 \text{ MHz})$. The time was sufficiently
long for magnetization to reach the equilibrium value and then
let to relax at a selected field. Data acquisition was performed on
a single FID following a 90° excitation radiofrequency pulse at a de-
tection frequency $v_{\text{det}}$ of 9.25 MHz using a dwell time of 0.1 ms and
256 data points. For each measured frequency point, the character-
istic $T_1$ relaxation time was determined by regression analysis of a
magnetization time course measured at 12 different delay times
with four accumulated scans. The measurement took 1 min per
NMR frequency point.

From frequency-dependent longitudinal relaxation time curves,
the corresponding longitudinal relaxation rate dispersion curves
were calculated as $R_1 (v) = 1 / T_1 (v)$. A dipolar background
contribution in each $R_1 (v)$ curve was phenomenologically using a
power-law function $R_1^d (v) = R_{1\text{d}0} v^{-\beta}$ [18] where $R_{1\text{d}0}$ and $\beta$ were the
unknown model parameters. For each sample, the dipolar back-
ground was determined from the two frequency intervals of 0.02–
0.3 MHz and 3.7–6.0 MHz. Pure quadrupolar peaks (Q-peaks) $R_1^Q (v)$
were obtained by subtracting the background from the dispersion
curves $R_1^O (v) = R_1 (v) - R_1^d (v)$. Distinction between the different fre-
quency intervals was performed based on visual inspection that
yielded optimal background subtraction. For the samples, three quadrupolar peaks are expected.[15,17] The peaks were modelled
by a sum of three Lorentzian functions

$$R_1^O (v) = \sum_{k=0}^{3} \frac{\tau_k}{1 + 4\pi^2 (v - v_k)^2 / \tau_k^2},$$  

(1)

where $R_1^O$, $v_k$, and $\tau_k$ were unknown model parameters. The three qua-
drupolar resonance peaks are expected at frequencies approximately
equal to $v_k = 2Kq / h$ and $v_k = K (3 \pm \eta) / h$, with $K = e^2 qQ / 4h$; here $e$
is the elementary charge, $q$ is the electric field gradient along the $z$
axis of the principal axes system, $Q$ is the quadrupolar moment and $\eta$
is the asymmetry parameter (approximately equal to 0.4 for amide
nitrogen).[18] The measured curves of pure quadrupolar peaks were
then analyzed by the model in Eqn (1) to obtain best fit model parameters.
These were then used to determine the Q-peak area, i.e. the
area under the quadrupolar dispersion contribution $R_1^Q (v)$ that corre-
sponds to the integral

$$k = \int_{v_{\text{min}}}^{v_{\text{max}}} R_1^Q (v) \, dv.$$  

(2)

To avoid divergence of the integral in Eqn (2)

$$\lim_{v_{\text{min}} \to \infty} \int_{v_{\text{min}}}^{v_{\text{max}}} R_1^Q (v) \, dv = \infty,$$

the integral range was set to $v_{\text{min}} = 0.1 \text{ MHz}$ and $v_{\text{max}} = 3.7 \text{ MHz}$. The range was wide enough to contain all the expected peaks of the examined samples.

Quantitative magnetization-transfer NMR experiments

The same meat/ham samples were analyzed also by qMT-NMR. The
experiments were performed on a NMR system consisting of a
2.35 T (100 MHz proton NMR frequency) horizontal bore super-
conducting magnet (Oxford Instruments, Abingdon, United King-
dom) and controlled by a TecMag Apollo spectrometer (Houston,
TX, USA). A 25-mm i.d. NMR probe with high $B_1$ field homogeneity
was used. Each sample was examined spectroscopically by
magnetization-transfer (MT)[22] and variable flip angle (VFA)[23]
pulse sequences with the following common parameters: excita-
tion angle 9°, repetition time 70 ms, dwell time 10 µs and number
of averages 4. The z-spectra were measured by the MT pulse se-
quence, in which a 12-ms Fermi saturation radiofrequency (RF)
pulse preceded low flip angle excitation followed by signal
acquisition.[24] The saturation pulse was performed with four differ-
et amplitudes corresponding to effective frequencies $ω_i$ of 40, 79,
140 and 185 Hz and with 50 different frequency-offsets $Δ$ that were
logarithmically distributed in the frequency range from 0.3 kHz to
100 kHz. For the determination of the MT parameters the corre-
sponding longitudinal relaxation time $T_1$ was needed.[14,25] This
was measured by the VFA pulse sequence with 12 different excita-
tion angles of 3.1, 4.1, 4.7, 5.8, 7.5, 9.1, 10.9, 13.3, 15.6, 18.0, 20.5 and
23.9°. From the acquired VFA signal, the corresponding $T_1$ value was calculated using

$$S_{\text{VFA}} (ω) = M_0 \sin (ω) \left[ 1 - e^{-T_1 / T_1} \right],$$

(3)

where $M_0$ is the equilibrium sample magnetization. In the MT pulse
sequence, magnetization steady state was established after a 20-s
dummy scan period prior to data acquisition. Experimentally ob-
tained z-spectra, i.e. MT datasets normalized to the signal value
exhibiting no MT effect (with largest frequency offset value and
with smallest MT frequency amplitude), were analyzed by fitting
analysis employing the BS8 signal equation[14,25]

$$S_{\text{MT}} (Δ, ω) = \frac{R_0}{R_0 + R_{\text{RB}} + R_{\text{RB}F\text{B}}} \left[ \frac{R_{\text{RB}F\text{B}}}{R_0 + R_{\text{RB}F\text{B}}} \right] + \frac{R_{\text{RB}}}{R_0 + R_{\text{RB}}} \left[ \frac{R_{\text{RB}}}{R_0 + R_{\text{RB}}} \right] \left[ 1 + \frac{ω^2}{\omega_0^2} \right],$$

(4)

in the continuous-wave power equivalent approximation.[26] The
fitting analysis yielded best-fit MT model parameters, i.e. $R_0$ (usually
set to unity[26]), $R_{\text{RB}} / R_{\text{RB}F\text{B}} (1 - f) T_{2B}$, $1 / R_{\text{RB}} T_{2B}$ and $g_{\text{MB}}$. In the signal
equation, super-Lorentzian and Lorentzian absorption lineshapes
were assumed for the restricted and for free magnetization pool,
respectively.[14] Using these MT parameters and the corresponding
high-field $T_1$ value, the size of the restricted pool size $f$ was cal-
culated as described in.[14]

Data analysis

Fitting analysis of the acquired FFC-NMR and qMT-NMR datasets as
well as the corresponding statistical analysis (based on a two-
sample t-test) was performed using customized software that
was developed within the Matlab programming environment
(MathWorks, Inc., Natick MA, USA). Visualization for the presented
figures was in part performed by the POV-Ray program (Persistence
of Vision Raytracer, Williamstown, Victoria, Australia).
Results

Figure 2 shows magnetic field strength-dependent longitudinal relaxation times $T_1(\nu)$ (Fig. 2a) and the corresponding relaxation rates $R_1(\nu)$ (Fig. 2b) averaged over the corresponding sample-group: three dry-cured SM samples (large red squares), three dry-cured BF samples (large dark green circles) and one dry-cured adipose sample (large blue diamonds). In addition three sample groups of fresh meat samples were also analyzed: two fresh SM samples (small orange squares), two fresh BF samples (small green circles) and one fresh adipose sample (small light blue diamonds). The plots are displayed in the log–log format in the frequency range between 5 kHz and 20 MHz. As can be seen from Fig. 2, the dispersion curves span over nearly four decades in frequency and allow for determination of several different frequency intervals (A–E), i.e. a low-frequency interval below 0.02 MHz where $T_1$ values are frequency-independent (interval A), two frequency intervals of 0.02–0.3 MHz and 3.7–6 MHz both exhibiting dipolar power-law behavior (intervals B and D), the quadrupolar dip/peak region in the frequency range between 0.1 and 3.7 MHz (interval C) that is followed by a high-frequency interval in the range 6–20 MHz (interval E), again featured by a power-law behavior. The solid curves correspond to best fit of the power-law dependence to the experimental data. The power-law behavior in frequency ranges from 0.02 to 0.3 MHz (interval B) and 3.7 to 6 MHz (interval D) was considered also as the quadrupolar peak region background. The background was subtracted from the $R_1$ dispersion curves in order to obtain pure quadrupolar peaks $R^Q_1(\nu)$. The measured differences among the dispersion curves corresponding to different dry-cured and fresh sample groups are significant. While adipose tissue exhibits no quadrupolar peaks because of a lack of quadrupole nitrogen nuclei, all three quadrupolar peaks can be well resolved in dry-cured and fresh SM/BF muscle tissues. These peaks are most pronounced with the dry-cured SM muscle, which shows comparatively higher $R_1$ values also in the other frequency intervals. In comparison to the dry-cured ham samples, $R_1$ values of the fresh samples are low and the corresponding peaks are smaller. The best fit power-law parameters obtained for frequency ranges 0.02–0.3 MHz and 3.7–6 MHz are for all the examined samples given in Table 1.

Pure quadrupolar peaks $R^Q_1(\nu)$ (solid symbols) of representative dry-cured and fresh samples from SM, BF and adipose tissues, along with the best fit of a function identical to a sum of three Lorentzian shapes to the measured Q-peak data (solid curves, Eqn (1)) and the corresponding Q-peak area (gray area, Eqn (2)) are shown in Fig. 3. Quadrupolar peaks emerge at 0.7, 2.1 and 2.8 MHz (corresponding to 16, 49 and 65 mT) with both dry-cured and fresh muscles, while no distinctive quadrupolar peaks are observed with adipose tissue samples. The dry-cured SM ham sample exhibits approximately a twofold larger Q-peak area compared to the corresponding BF ham sample. No significant difference in the Q-peak area can be observed between fresh SM and BF samples. In adipose tissue samples, a non-zero Q-peak area can be explained by scattered $T_1$ values resulting also in an incomplete background subtraction. Q-peak areas corresponding to the Q-peak data are given in Table 1.

Figure 4 shows experimentally obtained $z$-spectra (symbols) for all examined dry-cured and fresh samples from SM, BF and adipose tissue along with the best fit BSB model (Eqn (4), solid curves). The $z$-spectra (normalized NMR signal as a function of the MT frequency offset $\Delta$) were measured with four different MT pulse amplitudes $\nu_0$. Inserts in the $z$-spectra plots show the corresponding experimental $S_{\nu FA}(\alpha)$ curves (symbols), from which high-field $T_1$ values were calculated using Eqn (3) (solid curves). The dry-cured SM/BF muscle samples exhibit significantly different $S_{\nu FA}(\alpha)$ curves and hence also the $T_1$ values, however, relatively similar $z$-spectra. The $z$-spectra have the highest signal variation in the frequency offset range of 1–10 kHz. The high-field $T_1$ values of adipose tissue are similar to the values of dry-cured BF tissue; however, the $z$-spectra of adipose tissue are very different from ham muscle tissues only in the offset frequency region below 1 kHz. The displayed BSB model curves are in a good agreement with the experimentally obtained adipose $z$-spectra; however, large errors of the best fit MT parameters indicate that MT properties of adipose tissue cannot be well described by the BSB model. However, relative errors of the BSB model parameters were below 20% with muscle tissue samples. The best fit BSB model parameters of the examined dry-cured and fresh samples along with the corresponding high-field $T_1$ values are given in Table 1.

A correlation between the restricted pool size $f$ obtained by high-field qMT-NMR, and Q-peak area $l_Q$ obtained by FFC-NMR, is...
Table 1. Best fit parameters of the FFC-NMR and qMT-NMR results, corresponding to Fig. 2 (FFC-NMR best fit parameters) and to Fig. 4 (qMT-NMR and VFA-NMR best fit parameters).

<table>
<thead>
<tr>
<th>NMR Parameter</th>
<th>Dry-cured tissue</th>
<th>Fresh tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SM</td>
<td>BF</td>
</tr>
<tr>
<td>FFC $R_{1,0}^{1}$ [s$^{-1}$]</td>
<td>28.7</td>
<td>15.6</td>
</tr>
<tr>
<td>β [1]</td>
<td>0.45</td>
<td>0.41</td>
</tr>
<tr>
<td>$l_0$ [MHz s$^{-1}$]</td>
<td>12.0 ± 0.3</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td>qMT $R_{1,0}^{A}$ [s$^{-1}$]</td>
<td>17.27(1 ± 0.09)</td>
<td>11.48(1 ± 0.11)</td>
</tr>
<tr>
<td>f/R0(1-f) [s$^{-1}$]</td>
<td>0.19(1 ± 0.04)</td>
<td>0.23(1 ± 0.06)</td>
</tr>
<tr>
<td>$T_{2B}$ [μs]</td>
<td>5.92(1 ± 0.02)</td>
<td>5.89(1 ± 0.03)</td>
</tr>
<tr>
<td>$1/R_{2A}$ [1]</td>
<td>48.84(1 ± 0.04)</td>
<td>81.08(1 ± 0.04)</td>
</tr>
<tr>
<td>$gM_0$ [1]</td>
<td>1.01(1 ± 0.00)</td>
<td>1.01(1 ± 0.00)</td>
</tr>
<tr>
<td>f [%]</td>
<td>86</td>
<td>51</td>
</tr>
<tr>
<td>VFA $T_1$ [ms]</td>
<td>220 ± 10</td>
<td>380 ± 40</td>
</tr>
</tbody>
</table>

Figure 3. Pure quadrupolar peaks $R_1(v)$ of representative dry-cured and fresh-meat samples, obtained by subtracting a power-law background form the corresponding $R_2$ curves. The peaks differ by height and thus also by their area.
shown in Fig. 5. As can be seen from the figure, four different dry-cured tissue groups can be identified based on \((f, IL)\) values, i.e. the dry-cured SM muscle group with \((f, IL) \approx (85\%, 12 \text{ MHz s}^{-1})\), the dry-cured BF muscle group with \((f, IL) \approx (50\%, 5.5 \text{ MHz s}^{-1})\), both SM and BF fresh muscle groups with \((f, IL) \approx (20\%, 1.7 \text{ MHz s}^{-1})\) and the adipose tissue group with \(IL \approx 1.5 \text{ MHz s}^{-1}\) \((f\) value obtained by the BSB model is unreliable). The dependency of the Q-peak area on the restricted pool size \(f\) can be modeled by a linear function \(IL = kf\) with \(k = 0.127 \text{ MHz s}^{-1}/\%\) (solid line). In the fitting analysis, the adipose tissue samples were not considered because of indefinite restricted pool size values.

**Discussion**

The aim of the study was to demonstrate the feasibility of a new approach combining FFC-NMR and qMT-NMR methods for a relatively fast and nondestructive characterization of different fresh and dry-cured ham tissue sections. The tissue sections differed by anatomical location (SM vs BF) and thus by dry-matter/water content. The approach, which relies on proton magnetization exchanging macromolecules in nitrogen-containing abundant dry-matter, enables determination of tissue dry-matter concentration. Principal findings of the study are that (1) all three quadrupolar peaks in dispersion curves of dry-cured and fresh muscle tissues can be well resolved; (2) dry-cured ham tissue exhibits a pronounced dehydration-dependent MT effect; (3) the Q-peak area correlates positively with the restricted magnetization pool size; and (4) the FFC/qMT NMR approach is a powerful tool in analysis of meat tissue dehydration and is therefore appropriate for meat dry-curing process monitoring.

Biochemically, native meat tissue consists of various proteins (one fifth of the total mass) that are involved functionally into energy-conversion processes (water soluble sarcoplasmic proteins), into muscle fibrous architecture (myofibrillar proteins, such as myosin and actin) or are involved as a connective tissue (collagen...
and elastin). In the dry-curing process, gradual tissue dehydration results in an increase of dry-matter content, which is altered additionally because of progressive proteolytic action of endogenous enzymes. With respect to water microenvironment, muscle tissue can be considered as a two-compartment structure, consisting of restricted water molecules attached to or close to proteins, and of relatively free water molecules residing in the interstitial tissue voids. Because the ratio of the two compartments is changing during a progressive tissue desiccation, protein concentration itself could serve as a biomarker for a monitoring of the dry-curing processes.

In our study, two advanced $^1$H-NMR methods, i.e. FFC-NMR and qMT-NMR, enabled determination of protein content in the examined dry-cured and fresh-meat tissue samples. While the FFC-NMR method enabled determination of only relative protein content of the examined samples by means of $Q$-peak area ($I_{Q}$) and of the corresponding absolute relaxation rate $R_{1,Q}$, the qMT-NMR method along with the VFA method enabled absolute determination of the protein content by means of the size of the proton magnetization exchanging macromolecular magnetization pool $I_{L}$. In our study, a good correlation between the restricted pool size $f$ and the $Q$-peak area $I_{Q}$ was obtained (Fig. 5). The measured restricted-pool size values of fresh muscle tissue were in a range of 20–25%, while $f$ values in the dry-cured BF muscle samples were in the range of 40–55%, which is in an agreement with the corresponding average dry-matter content in dry-cured hams measured by conventional chemical analyses. In dry-cured SM tissue, the measured restricted-pool size was surprisingly large ($f = 87\%$). The value could be to some extent overestimated because of the effect of $B_1$ inhomogeneity on qMT. In addition, for extensively dried meat samples, which resemble properties of solids, the qMT analysis could be further improved by using other types of absorption lineshapes for the solid pool instead of the applied super-Lorentzian lineshape. Such shapes include Gaussian or Abragam lineshapes. A possible explanation for the high protein content is an intense dehydration of the superficial SM layers, which could be confirmed by gravimetric analysis or NIR-based chemical analyses. The SM samples from these layers were also dark-red colored and visibly differed from deeper SM layers as well as from BF tissue that was of red color. In addition, the twofold increase in the restricted pool size is in agreement with the corresponding twofold increase of the $Q$-peak area. A broad range of dry-matter content in fresh meat and dry-cured ham samples (from 25% to 65%) were obtained also in a study employing X-ray computed tomography technique.

In our study, the $Q$-peak area $I_{Q}$ and the restricted pool size $f$ were found as two parameters with the highest correlation. The two parameters are also most suitable for determination of dry-matter content in dry-cured meat tissue. Other tissue parameters provided by the FFC/qMT-NMR techniques (listed in Table 1) could also be used for tissue characterization; however, not all of them are related to the protein/water content. For example, the parameter $R_{1,0}$ decreases with an increasing water content only in lean meat tissue, while it is relatively large also in adipose tissue, which contains almost no water. Variations of the parameter $T_{2H}$ could be exploited as an intrinsic marker for disease progression. In our study, however, $T_{2H}$ variations among the examined tissue groups were not as significant as the corresponding restricted pool size variations.

Quadrupolar peaks have already been observed in in vitro systems of partially immobilized proteins, such as cross-linked bovine serum albumin, cross-linked fibrin clot, partially hydrated collagen and glycosaminoglycan. In these studies, a proportionality between the $Q$-peak area and the protein concentration was confirmed. Recently, this finding was efficiently exploited for an early-stage detection of osteoarthritis in articular cartilage in vivo, of which initialization is associated with a depletion of glycosaminoglycan from the tissue extracellular matrix. In dry-cured hams, water molecules are depleted from the tissue, which affects the water/protein content. In our study, interestingly, all three expected quadrupolar peaks were well resolved, while in hydrated-collagen systems in vitro the first peak was not sufficiently resolved.

For a reliable determination of quadrupolar peaks and a precise dipolar background subtraction, determination of $T_1$ values over the range of several dozens of different Larmor frequencies is needed. Thus, the total examination time of FFC-NMR is roughly in a range of 1–2 h per sample. In qMT-NMR, z-spectra along with the corresponding high-field $T_1$ value can be determined in less than 5 min per sample. Because the total examination time of the FFC/qMT NMR approach is much shorter than the examination time of conventional analyses and is negligible in comparison to the dry-curing processing time that can extend also beyond 15 months, the applied NMR methods have a potential also for industrial applications, specifically with an advent of cost-efficient benchtop NMR devices.

In our study the samples were not analyzed by conventional chemical analysis. Instead, the FFC/qMT-NMR results were descriptively compared with chemical analysis results of identical meat tissue regions. Another limitation of the study is that the determined qMT parameters are susceptible to errors associated with excitation-field inhomogeneity. This effect should be considered, especially when the sample size is comparable to the NMR probe size. Neglecting excitation-field inhomogeneity in the study was a sound assumption because the sizes of the examined samples were much smaller than the probe size and they were positioned precisely into the probe’s isocenter. Nevertheless, minor sample size variations and a possible mismatch between the isocenter and the sample’s position could give rise to an unwanted field inhomogeneity contribution, which could also bias the restricted pool size determination. It is also likely that this effect...
contributed to intragroup variations in the restricted pool size and in high-field $T_1$ value.

Conclusions

We demonstrated the feasibility of a combined FFC/qMT-NMR approach for a fast and nondestructive characterization of dry-curing ham tissues differing by protein content. The characterization is based on quantifying the pure quadrupolar peak area (obtained by FFC-NMR) and the restricted magnetization pool size (obtained based on quantifying the pure quadrupolar peak area (obtained by qMT-NMR). Both quantities correlate well with concentration of partially immobilized, nitrogen-containing and proton magnetization exchanging muscle proteins so that they could serve as markers for a dry-curing process monitoring.

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Conflict of Interest

Authors declare no conflicts of interest.

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