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Magnetic resonance imaging studies of water interactions in meat

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Abstract

The use of MRI shows spatial resolution of water content, and NMR parameters including relaxation times (T_1 and T_2), and diffusion coefficients (D) define the state of water interactions with other molecules. These parameters are potentially sensitive to local variations of water mobility and result from modification of water–macromolecule interactions and changes in tissue structure. MRI gives a unique opportunity to better-understand the dynamic phenomena that occur during processing and storage of food. By measuring apparent diffusion coefficient, both axially and radially in meat, it is possible to probe the influence of intracellular diffusional barriers or post-mortem structural changes. The effects of different freezing methods on trout muscle were investigated using MRI. The variations of the relaxation time, T_2, and the radial diffusion coefficient, characterize the structural changes of tissue produced by the freezing process.

Keywords: Magnetic Resonance Imaging; Water; WHC; Muscle/meat diffusion; NMR relaxation times; Freezing

1. Introduction

Nuclear Magnetic Resonance (NMR) has proved an irreplaceable research tool in both analytical chemistry and biology. However, NMR has not been widely used in meat science, even though a broad range of applications is feasible. The high cost of NMR spectrometers may be an obstacle. Magnetic resonance spectroscopy (MRS) provides non-invasive means of monitoring small molecules in solution. Magnetic resonance imaging (MRI) gives a spatial resolution that allows characterisation of the tissue morphology by ^{1}H-MRI. The main advantage of the MRI technique is that internal images in two or three dimensions are obtained non-invasively and non-destructively, without the need for any preliminary sample preparation. The use of MRI permits spatial resolution of water content, and NMR parameters, including relaxation times (T_1 and T_2), and diffusion coefficients (D) define the state of water interaction with other molecules. These parameters are potentially sensitive to local variations of water mobility, resulting from modification of water-macromolecule interactions and changes in tissue structure.

2. Meat quality

The main constituents and their amounts in mammalian skeletal muscle are: water 65–80%, protein 16–22%, carbohydrate 1–2%, fat 1–13% and other soluble material 1%. As water is the major constituent, the interactions between water and macromolecules determine the water holding capacity (WHC) of meat. Skeletal muscle is highly organized (Fig. 1) and made up of groups of muscle fibres surrounded and supported by connective tissue. The epimysium comprises the heavy sheath surrounding the entire muscle. Fibre bundles are surrounded by the perimysium, while the endomysium envelopes each individual muscle fibre. The muscle fibre is long (1–40 mm) and cylindrical (10–100 μm diameter). Bundles of muscle fibres may run parallel to the main muscle axis or have a certain pennation angle between muscle and fibre directions.

Water holding capacity of meat depends primarily on the extent of the post mortem myofibrillar shrinkage and the correlative changes in the extracellular water compartments (Offer & Knight, 1988a, 1988b). NMR relaxation measurement of water protons gives information about dynamics of water. The general features of proton relaxation in muscle are characterised by longitudinal relaxation time (T_1) and transverse relaxation time (T_2). In rigor muscle, a multicomponent T_2-relaxation behaviour is observed. T_{2s} and T_{2l} correspond to the T_2 with the shortest and the longest times,

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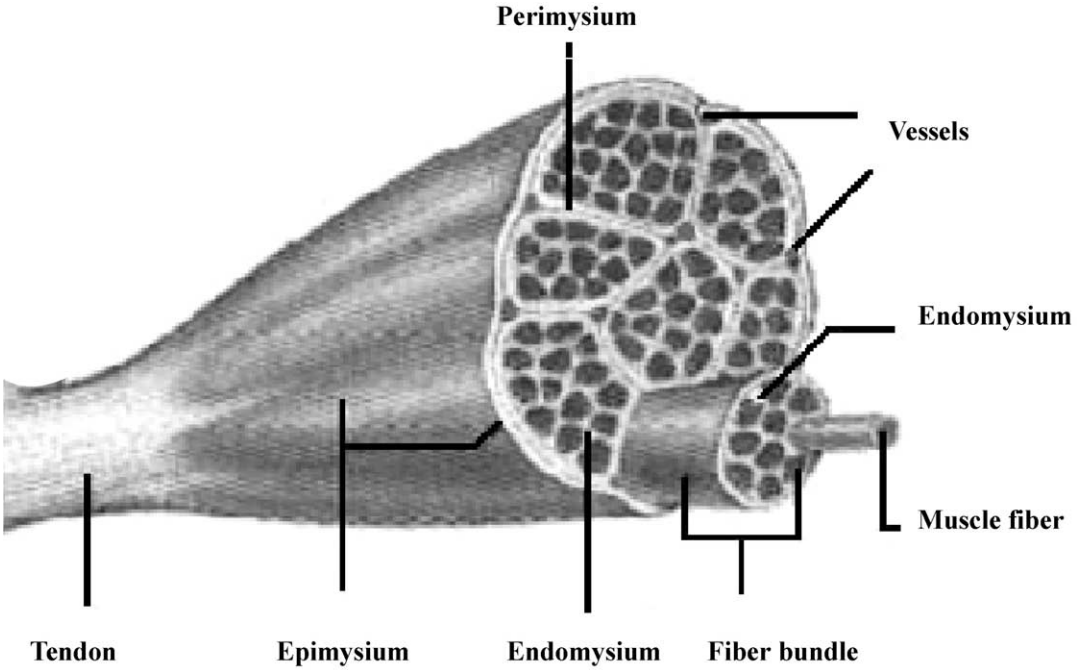

Fig. 1. Diagram of a muscle showing the arrangement of muscle fibres and the supporting connective tissues.

respectively, and P_{2s} to population relative to T_{2s}. Highly significant relationships were found (Table 1) between P_{2s}, T_{2l} and some other characteristics, such as pH measured 30 min post mortem (pH_{30}), reflectance and cooking yield, while T_{2s} was only correlated with pH_{30} (Renou, Monin, & Sellier, 1985).

Working on pig muscles, Fjelkner-Modig and Tornberg (1986) and Tornberg, Andersson, Göransson, and Von Seth (1993) considered that three water compartments could be identified. These components were assigned as extracellular water, water in myofibrils and reticulum, and water in interaction with macromolecules. The longest T_2 was regarded as corresponding to "free" or "expelled" water. The major (80%) fraction of water was considered as being mainly held by the myofibrils. This histological picture of a compartmentation of water between intra- and extra-cellular domains is attractive but there is little evidence to support this concept, structural microheterogeneity being sufficient to explain the non-exponential relaxation. The same behaviour is observed for hydrated collagen fibre (Traoré, Foucat, & Renou, 2000). Moreover, the population associated with the different relaxation times depends on the exchange rates between the different water compartments (Hills, 1992a, 1992b). If the NMR relaxation studies allow an assessment of meat characteristics, this method cannot determine the location within the tissue. MRI provides maps of relaxation times in the tissue on a supramolecular scale. Diffusion MRI is a well-established tool (Le Bihan et al., 2001) for non-invasive investigation of structure in muscle. The success of diffusion magnetic resonance imaging is deeply rooted in the powerful concept that, during their random, diffusion-driven displacements, molecules probe tissue structure on a microscopic scale, well below the usual image resolution. As diffusion is truly a three-dimensional process, molecular mobility in tissues may be anisotropic. With diffusion tensor imaging (DTI), diffusion anisotropic effects can be fully extracted, characterised, and exploited, providing even more accurate details on tissue microstructure.

Table 1
Correlations between NMR measurements and meat quality traits[a]

	T_{2s}	T_{2l}	P_{2s}
pH_{30}	NS	***	***
Reflectance	NS	**	***
Cooking yield	**	*	***

[a] Number of samples: 98; NS $P>0.10$; *$P<0.05$; **$P<0.01$; ***$P<0.001$ (Renou et al., 1985).

In an isotropic system, diffusion is fully described using a single parameter, the diffusion coefficient, D. However, in the presence of anisotropy, diffusion can no longer be characterized by a single scalar coefficient, but requires a tensor, D, which fully describes molecular mobility along each direction and correlation between these directions.

$$\underline{D} = \begin{matrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{matrix}$$

To fully determine the diffusion tensor, one must first collect diffusion-weighted images along several gradient

directions, using diffusion pulse sequences. A second step is to estimate the coefficients from the set of diffusion/orientation-weighted images. From the diffusion tensor components, one may calculate three types of parameter: the intra-voxel and inter-voxel anisotropic indices and two angles giving the fibre orientation. The last family of parameters that can be extracted from the DTI concept relates to the mapping of the orientation, in space, of tissue structure. The assumption is that the direction of the fibres is colinear with the main diffusion directions. This approach opens a completely new way to gain direct and in vivo information on the organization in space of oriented tissues. Direction orientation can be derived directly by DTI, from diffusion/orientation-weighted images or through the calculation of the diffusion tensor.

Anatomy indicates that the bovine *Semitendinosus* muscle has a parallel muscle fibre arrangement; the angle of pennation was 0 degree with respect of the long axis of the muscle-tendon unit. In this MRI experiment, the muscle was cut into two pieces and placed, one on top of the other, but so that the fibre directions were perpendicular between them. For one piece, the fibre axis is parallel to the main magnetic field direction (z) while, for the other, the fibre axis is parallel to x direction. Fig. 2A is a morphologic image while Fig. 2B–D are parametric images of diffusion parameters along the x, y and z axes. Note that the diffusion coefficient is always greater (hypersignal) for the main fibre direction. The diffusion coefficient, perpendicular to the fibre axis, is the same for both muscle pieces.

By measuring apparent diffusion coefficient axially ($D_{//}$) and radially ($D_{\perp}$), it is possible to probe the influence of intracellular diffusional barriers (Kinsey, Locke, Penke, & Moerland, 1999) during the post-mortem structural changes (Foucat, Benderbous, Bielicki, Zanca, & Renou, 1995). When the $D_{//}$ and $D_{\perp}$ values increase, the increase is larger for $D_{//}$ (~20%) than for $D_{\perp}$ (~10%). The diffusion anisotropy, defined by the ratio $D_{//} / D_{\perp}$, increases from 1.6 to 1.9 between 1 and 11 h post mortem. These results agree with the presence of extracellular space during the rigor mortis onset, which allows the diffusion of water molecules along the fibre axis. Free-water accumulation, which diffuses more freely and isotropically than that in the rest of the muscle, was detected and localized in *Semitendinosus* (Fig. 3). This image was obtained by suppression of fat (Laurent, Bonny, & Renou, 2000). The fat shows as black while the water with the highest mobility appears in hypersignal. These results agree with the work of Offer and Cousins (1992) which showed, by optical microscopy, that an interstitial space appears, post-mortem, between fascicles of muscle fibres. Such voxels correspond to free water exuded into extracellular gaps.

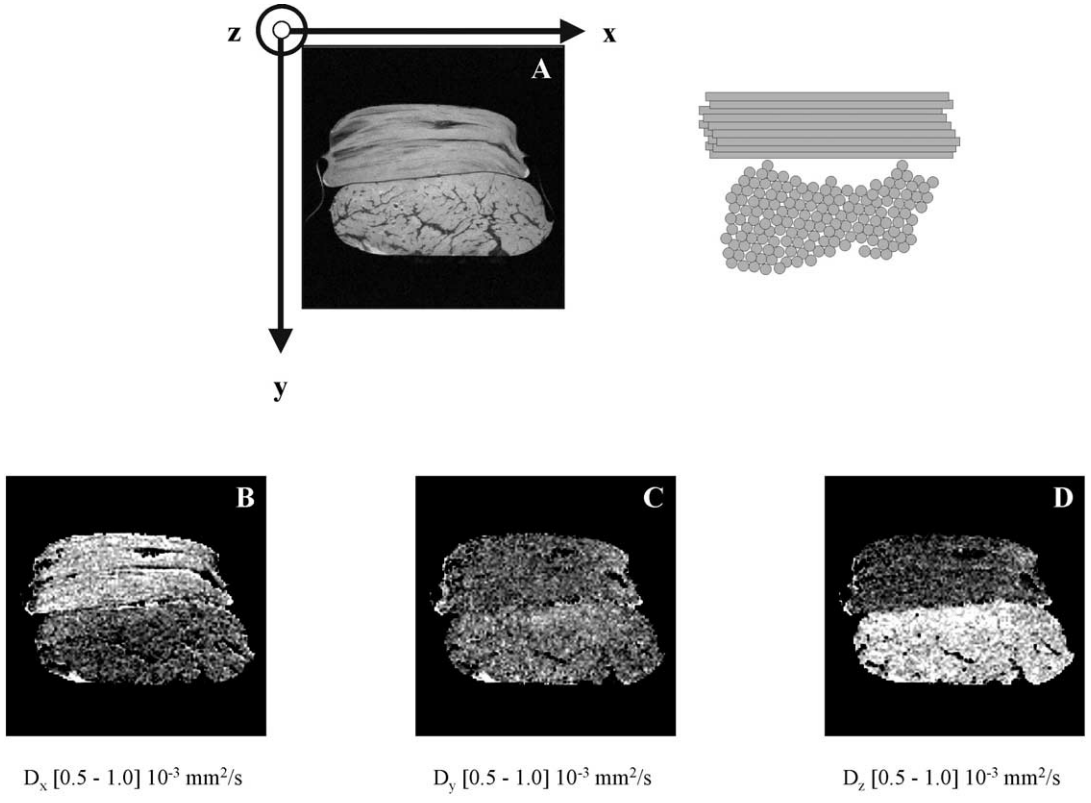

Fig. 2. Nuclear magnetic resonance images of *Semitendinosus* bovine muscles: (A) anatomical and (B, C, D) diffusion parametric images along the three directions x, y, z.

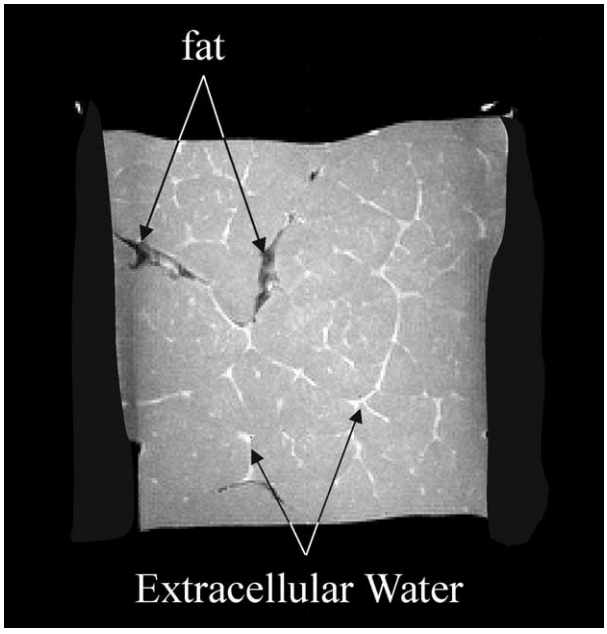

Fig. 3. Fat-suppressed magnetic resonance image of a *Semitendinosus* bovine muscle.

These results stress the importance of diffusion tensor measurements for characterizing muscle structure and understanding the mechanisms of *post-mortem* water exudation (Bonny & Renou, submitted for publication). Moreover, voxels showing free-water are not homogeneously distributed in the muscle. This corroborates the large dispersions of the sizes of the extracellular water compartments reported by Offer and Cousins (1992).

3. Technological process: freezing

The meat industry uses different technological processes for food preservation. The oldest are salting and drying. Freezing is now currently used for extending shelf-life. All these technologies induce modification of water content or water interaction with the macromolecules in the muscle structure. Physical and chemical changes of proteins occurring during frozen storage may result in texture deterioration, which decreases the functional, nutritional and sensory properties of the product. MRI allows a unique opportunity to better-understand dynamic phenomena that occur during processing and storage of food. The effect of different freezing methods on whole trout muscle was investigated using MRI (Foucat, Taylor, Labas, & Renou, 2001). After evisceration, Rainbow trout was stored in evacuated plastic bags at −20 °C for 0 (unfrozen), 1, 7, 21, 29 and 41 days. Thawing (and fresh trout storage) was at 4 °C for 24 h, immediately prior to analyses. Six trout, each subjected to one of the different frozen storage periods, were placed simultaneously in the magnet. Anatomical features, such as bones and backbone, are clearly visible in Fig. 4A because they have low signal intensities compared to the muscle tissue. These images give only "morphological" information. They do not allow any objective discrimination between the six trout while the $D_{\perp}$ values varied according to the fresh or frozen trout. Table 2 summarizes the data extracted from the three classes of NMR parametric images for trout muscle. In comparison with fresh trout, neither the freeze-thaw process, nor the frozen storage duration within the time range investigated (from 1 to 41 days), seem to have an effect on $D_{//}$ parameters. On the other hand, a statistically significant difference of about 15% of the T_2 mean value is observed between fresh and frozen-thawed trout. The longer T_2 in frozen-thawed trout is consistent with more protein denaturation. It results from a redistribution of the water within the muscle due to partial extrusion of water from the intracellular compartment (Belton & Ratcliffe, 1985). The ice crystal formation that occurs during frozen storage

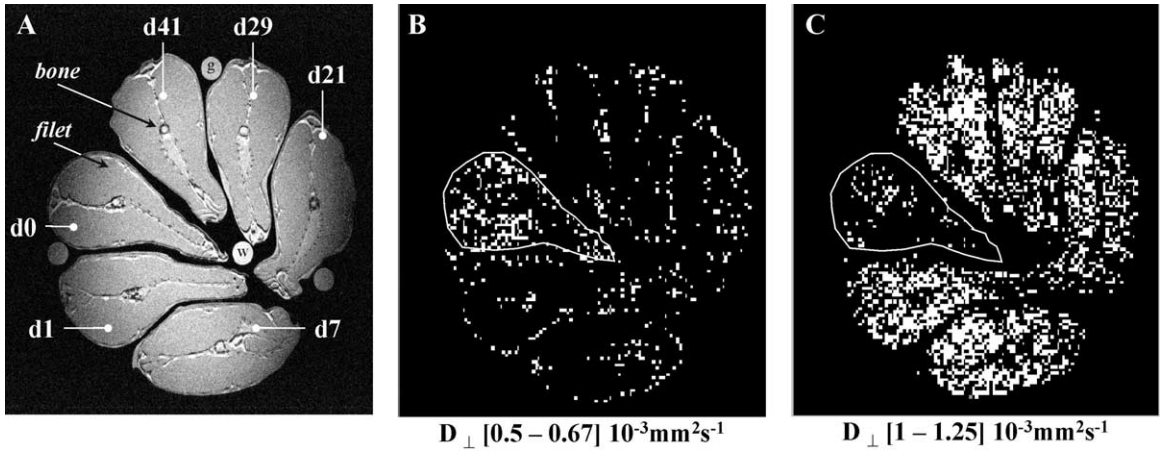

Fig. 4. Morphological nuclear magnetic resonance images of whole trout: (A) anatomical and (B, C) diffusion parametric ($D_{\perp}$) images for the same set of six trout; (g) and (w) are reference tubes of gelatin gel and water, respectively.

Table 2
The NMR parameters for water in unfrozen and frozen/thawed trout

	Fresh	Frozen storage (day)				
	0	1	7	21	29	41
$D_{//}(10^{-3}mm^2s^{-1})$	1.15					
$D_{\perp}(10^{-3}mm^2s^{-1})$	0.85	1.08				1.15
T_2 (ms)	42	48				

induces an increase of the extracellular volume, and gaps between fibres appear. These results were confirmed by histological images. The fibre separation occurs as early as the first day of frozen storage; at day 41 the sample had changed more extensively, with separation between fibres, torsion of the fibres and breakage of the connective tissue, which all became more pronounced.

This method allowed distinction of frozen-thawed trout from fresh or whole intact fish. The variations of NMR parameters, especially the relaxation time, T_2, and the radial diffusion coefficient, with the frozen storage duration are consistent with histological observations and permit characterisation of the structural changes of tissue induced by the freezing process.

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