

● *Invited Lecture***MEASUREMENT OF TEXTURAL CHANGES OF FOOD BY
MRI RELAXOMETRY**

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This article summarises the current status of, and future prospects for, the use of magnetic resonance imaging (MRI) to evaluate the texture of a range of foods, and changes therein which accompany pathogen infection, natural ageing, damage, ripening and processing. The basic concept is that the magnetic resonance parameters of water are sufficiently sensitive to the texture of the food matrix, that magnetic resonance images of the spatial distribution of those parameters are effectively maps of the structural status of the foodstuff. This is illustrated in the context of the effects of pathogen infection in cucumbers, internal necrosis of melons, bruising in peaches, ripening of pineapples, and the effect of freeze-thawing on meat and fish. © 1998 Elsevier Science Inc.

Key Words: Ripening; Disease; Authentication; Bruising.

INTRODUCTION

There is a growing awareness that magnetic resonance imaging (MRI) of water can provide useful insight into many structural aspects of raw and processed foods. The main thrust of the present studies is based on the hypothesis that the MR parameters of water in foodstuffs are dependent on the cellular architecture of the food; therefore, changes which affect the structure of the food and, hence, quality can be detected with MRI. Specifically, we speculate that textural changes associated either with the natural ageing of the food, or with its processing, should be reflected by changes in both the spatial distribution of the water and the values of its MR parameters. As a result of the latter dependencies, MRI can be used to measure the molecular environment of the water and hence could be used to optimise the quality of fruit ripening, or to detect changes in the molecular dynamics of water associated with inappropriate or illicit treatment of meat, for example, selling as fresh, meat which has been previously frozen and thawed.

There was already sufficient work in the literature to encourage us to pursue such studies; the bruising of apples¹ had been clearly detected, and Guiheneuf et al.²

had demonstrated the effects of freeze-thawing on fresh pork. The first study in this report investigated the suitability of MRI to detect textural changes in fruits caused by pathogen infection, ripening, internal necrosis and bruising. These processes are increasingly relevant to the fruit distribution business, where the shipping and storage of huge quantities of produce necessitates careful management to ensure optimum quality for the consumer. The second, relevant to authentication of fresh meat, fish and poultry, stemmed from the substantial seasonal variation of the cost of producing meat, fish and poultry, which makes it profitable to freeze-store tissue produced during the low cost season and then to sell it as “fresh” after thawing.

MATERIALS AND METHODS

All measurements were made at 2 Tesla using MRI protocols implemented via modified Bruker BMT (Bruker Medzintechnik Biospec II, Karlsruhe, Germany) imaging consoles, each controlled by an Aspect 3000 computer. Small samples (meat) were studied using a 31-cm horizontal bore superconducting magnet and the larger ones (fruit) in a 100-cm bore magnet. In both cases

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the gradient coils and the quadrature birdcage radio frequency resonator coils were constructed in the Herchel Smith Laboratory. All data were transferred to computers running under either UNIX or LINUX, and were processed using software written in-house.

Fruit Pathogen Infection, Bruising and Ripening

Cucumbers naturally infected with *Mycosphaerella* sp. were supplied by a local cucumber grower. *Mycosphaerella* is a fungus that infects cucumbers during growth and causes them to become deformed during development.³ A local supermarket supplied ripe and unripe pineapples, ripe and over-ripe yellow melons, and healthy and bruised peaches. The T_2 value was measured in pineapples by varying the echo time (TE) in the multi-echo Carr–Purcell–Meiboom–Gill (CPMG) MRI sequence.⁴ Eight images, inter-echo time 30 ms, repetition time (TR) 5 s were acquired and the T_2 value calculated with Eq. (1):

$$M_{xy} = M_0 e^{\left(-\frac{TE}{T_2}\right)} + C \quad (1)$$

where M_{xy} is the magnetisation at a particular pixel; C is the estimate of average noise in the images; M_0 and T_2 are the estimates required for starting the iterative curve-fitting process based on the Levenberg–Marquardt method.⁵

Gradient-echo and spin-echo images were acquired from melons and peaches using a TE of 7.5 and 30 ms, respectively. Spin-echo images were acquired from cucumbers with a TE of 20 ms.

Meat Samples

Authenticated fresh samples of *Longissimus dorsi* muscle with precisely known history, were obtained locally from 25 animals per species which comprised a variety of cross-bred cattle and sheep, and 2-year-old White Landrace pigs. Authenticated fresh *Pectoralis major* and thigh muscles were also obtained locally from twenty chickens and twenty turkeys. Studies of the effects of freeze-thawing of fish were complicated by the difficulty in obtaining fish whose previous “thermal” history could be precisely guaranteed. Consequently, the main development work used eighteen rainbow trout, caught from a local trout farm. The diversity of samples and randomization of experimental errors was assured by conducting the MRI measurements on different animals per species per week, over a 5-week period.

Each meat sample, weighing approximately 250 g was divided into two portions; one half was frozen at -18°C in a domestic freezer for 12 days, and the other half used for the analysis of the fresh meat. Samples were thawed to room temperature between two aluminium

plates for 4 h prior to being analysed. Each sample of fresh and frozen/thawed meat was analysed for moisture content using the protocol detailed by Kirk.⁶ The pH was measured using a pH meter (Model 220, Corning Science Products, Corning, NY, USA).

For the quantitation of MR parameters, four samples of meat were excised from each muscle sample using a modified “cork borer” (inner diameter 7 mm) held in an electric drill rotating at 60 rpm which was mounted in a drill stand. By “drilling” the “cork borer” into the meat samples, up to four samples could be removed at one time and the integrity of each sample was maintained. The “cork borer” was then used to insert each sample into Perspex multi-well counter plates (commonly used for tissue culture; Sterilin Ltd., Staffordshire, UK). Each multi-well plate was modified from 96 wells per plate (6.4 mm diameter per well) to a 6 by 7 array using a band saw. By stacking four such plates in the probe, it was possible to acquire MR data from up to 168 samples at one time. Sample preparation time took ca. 20 min to fill four multi-well sample plates with about 168 samples. Unoccupied wells on each plate were filled with 2% (w/w) agar gel to as an M_0 reference, and magnetic susceptibility effects caused by air gaps between wells were eliminated by filling the space with 2% (w/w) agar gel doped with 2 mM MnCl_2 ; this improved the performance of the automatic computerized analysis of the images.⁷

Determination of T_2 , T_1 and Magnetisation-Transfer (MT) Parameters

The quantitation of MR parameters was achieved by varying the TR, TE and saturation pulse delay SP of the multi-echo Carr–Purcell–Meiboom–Gill MRI sequence.⁴ T_1 and T_2 values were calculated from images obtained without a saturation pulse, with different T_R (0.12, 0.375, 0.75, 1.5, 3 and 5 s) or T_E values (eight images, inter-echo time 20 ms, T_R 5 s), respectively, using Eqs. (1) and (2):

$$M_{xy} = M_0 \rho \left(1 - e^{\left(-\frac{T_R}{T_1}\right)}\right), \quad (2)$$

where M_{xy} is the magnetisation at a particular pixel; ρ is the estimate of average noise in the images; M_0 and T_1 are the estimates required for starting the iterative curve-fitting process based on the Levenberg–Marquardt method.⁵ The actual M_0 value was quantified by back-projection of the T_2 decay curve to where it intersected the y-axis at time 0. The M_0 values were standardised with respect to the M_0 values from the 2% (w/w) agar gel references using Eq. (3):

$$M_0\% = 98 \cdot \frac{M_{0\text{meat}}}{M_{0\text{agar}}} \quad (3)$$

where M_{0meat} is the average M_0 value of the meat sample; M_{0agar} is the average M_0 value of the 2% (w/w) agar gel references in each plate; and 98 is the percentage water in the agar gel reference⁸; this gives the liquid proton density ratio ($M_0\%$).

MT experiments were also carried out to determine the proportion of protons “bound” to macromolecules compared to those which are “free” in water. MT is a consequence of chemical exchange between protons attached to slowly tumbling macromolecules and the protons of free water, which tumble freely; due to this interchange, dipolar relaxation of the macromolecular population will influence the relaxation of the water protons (cross-relaxation). The current models for this phenomenon⁹ consider the water and macromolecular protons to be two systems, and that equilibrium is established between them through molecular exchange and self-diffusion. In practice, the MT experiment depends on the fact that the water has a narrow range of resonant frequencies, while the protons of macromolecules have a much broader range. Consequently, it is possible to apply a low amplitude pulse of radio-frequency energy, 10 kHz offset from the water resonance, the signal intensity of which is determined by the spin echo pulse sequence. This pulse has the effect of saturating the magnetisation of all the protons of the macromolecules, which reduces the signal intensity when their exchangeable protons migrate to the “free pool.” The extent of this reduction is proportional to the relative amounts of protons in the two pools, their relative mobility, the efficiency at which chemical interchange occurs between the two pools, and the efficiency of the saturation pulse. There is also a reduction in the T_1 value (T_{1sat}) due to the magnetisation transfer between the macromolecules and water pools.⁹

For quantification of T_{1sat} , the length of the saturation pulse (10 kHz off resonance and 10% of the radio-frequency power needed to achieve a 90° pulse) was 0, 0.12, 0.5, 1.25, 2.5 and 5 s, with T_R 5 s and T_E 20 ms. The magnetisation measured after the saturation pulse is given by Eq. (4):

$$M_{xy} = M_{sat} + (M_0 - M_{sat})e^{\frac{S_p}{T_{1sat}}} \quad (4)$$

where M_{xy} is the longitudinal magnetisation at a particular pixel; M_0 is the longitudinal magnetisation when $S_p = 0$; M_{sat} is the longitudinal magnetisation when saturation reaches a steady state ($S_p = 5T_{1sat}$); and T_{1sat} is the longitudinal relaxation time measured during saturation. The T_{1sat} and M_{sat} values were then derived from the fitting of the saturation weighted images to a single exponential using the Levenberg–Marquardt method.⁵ Assuming that the protons of the macromolecules are

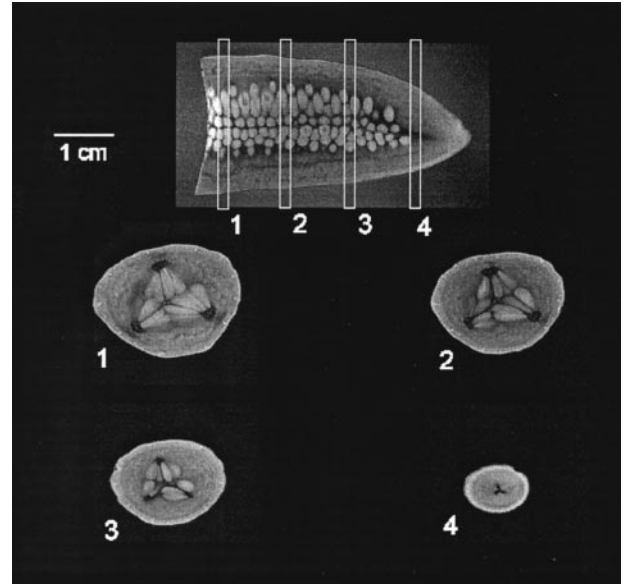


Fig. 1. Multi-slice images from a healthy cucumber. Field of view 7 cm, slice 1 mm, T_R 2500 ms, T_E 20 ms, 256 by 256 pixels.

saturated during the application of the saturation pulse, the apparent proton exchange rate (k) is given by Eq. (5):

$$k = \frac{1}{T_{1sat}} \cdot \left(1 - \frac{M_{sat}}{M_0} \right). \quad (5)$$

The apparent MT-rate (k) image was calculated using Eq.⁵ from the images previously fitted to M_{sat} , M_0 and T_{1sat} ^{10–13} Eq. (5) is based on the assumption that all of the macromolecular protons are kept in complete saturation during the application of the saturation pulse.

The mean MR parameters for each well was estimated by using fully automated computerised analysis.⁷ Statistical analysis of the data involved two-tailed t -tests assuming unequal variances at 1% confidence to determine whether freezing and thawing, or chilling regime, had a significant effect on the gravimetric and MR parameters. All statistical analyses were conducted with SPSS software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Fruit Bruising and Ripening

Figure 1 shows multi-slice echo images from healthy cucumbers; there is very good contrast between the different tissues. When those images are compared to those of the cucumber infected with *Mycosphaerella* sp. it can be seen (Fig. 2) that the development of that cucumber has been severely altered due to the infection by this pathogen. This example shows that MRI could be used to

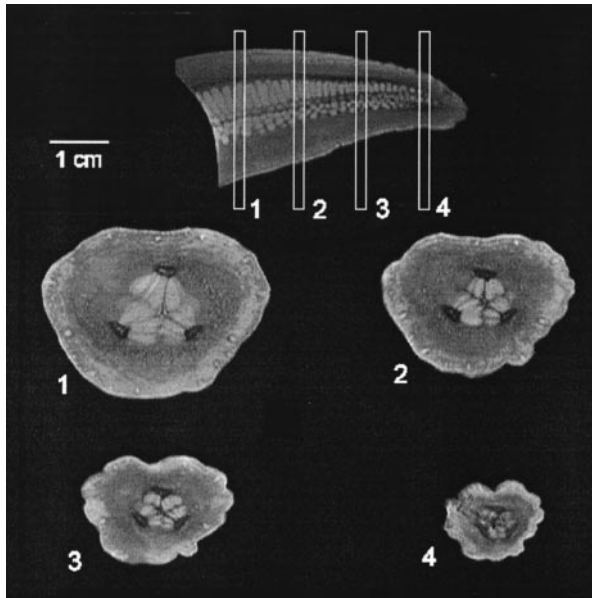


Fig. 2. Multi-slice images taken from a cucumber infected with *Mycosphaerella* sp. Field of view 7 cm, slice 1 mm, T_R 2500 ms, T_E 20 ms, 256 by 256 pixels.

track the infection of this pathogen into cucumbers and monitor environmental factors that may affect its susceptibility to disease.

Figure 3 shows the spin-echo images from normal and

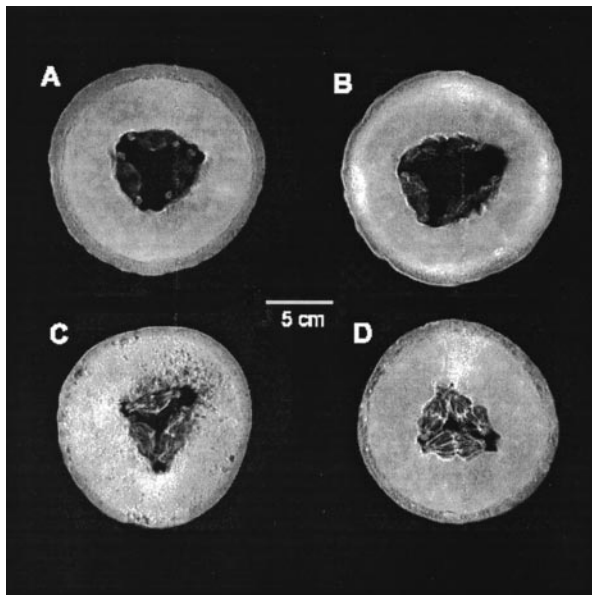


Fig. 3. Spin-echo image from healthy melons (images A and B) and melons with internal necrosis (images C and D). Field of view 20 cm, slice 3 mm, T_R 3000 ms, T_E 30 ms, 256 by 256 pixels.

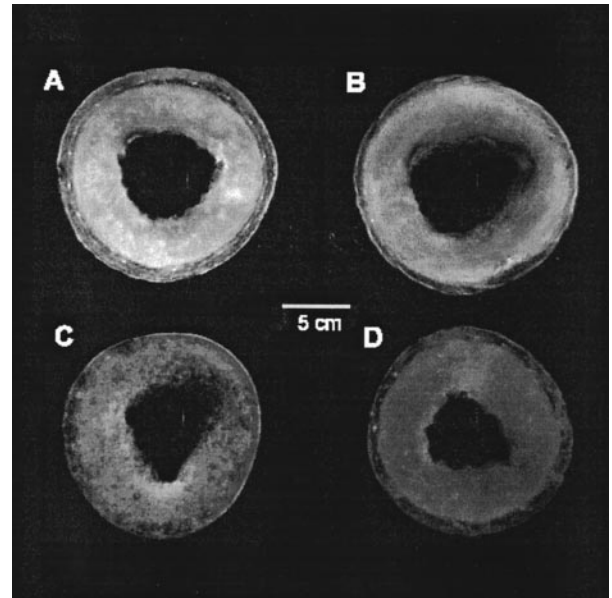


Fig. 4. Gradient-echo image from healthy yellow melons (images A and B) and melons with internal necrosis (images C and D). Field of view 20 cm, slice 3 mm, T_R 100 ms, T_E 10 ms, 256 by 256 pixels.

over-ripe melons; although there is no obvious difference between these melons the gradient-echo images clearly demonstrate the effects of internal necrosis, with a decrease in signal intensity in melons labelled C and D (Fig. 4). The reason for that decrease in signal is the presence of more air in the damaged tissues, which increases the magnetic susceptibility of those tissues.¹ Similarly, gradient-echo images of bruised and unbruised peaches show enhanced contrast between damaged and healthy tissue when compared to the corresponding spin-echo images (Fig. 5 and 6). The mechanism for enhancing the contrast between healthy and damaged tissue is

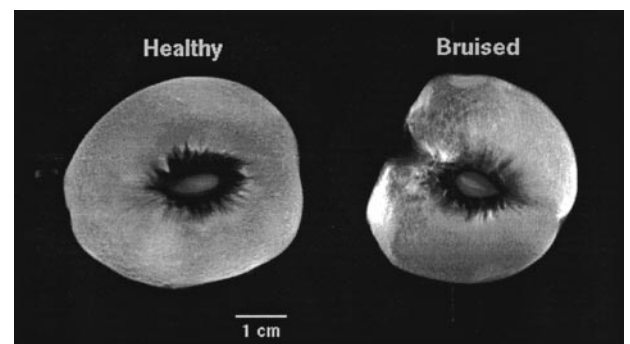


Fig. 5. Spin-echo image from healthy and bruised peaches. Field of view 10 cm, slice 2 mm, T_R 2500 ms, T_E 30 ms, 256 by 256 pixels.

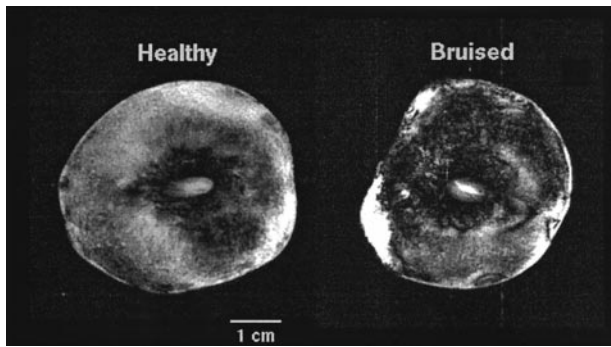


Fig. 6. Gradient-echo image from healthy and bruised peaches. Field of view 10 cm, slice 2 mm, T_R 100 ms, T_E 10 ms, 256 by 256 pixels.

believed to be identical to the internal necrosis of melons; bruising results in local changes in magnetic susceptibility because the regions of bruised tissue now contain more air than in the undamaged fruit.

Detection of tissue changes associated with fruit ripening necessitated a more quantitative approach, as is illustrated by the data obtained from pineapples. Figure 7 clearly shows that ripening increases the T_2 value (81 ± 30 ms) of ripe, compared to unripe pineapples (45 ± 12 ms). The increase in T_2 value is thought to be due to an increase in sugar concentration and to the breakdown of the cellular matrix upon ripening. While MR imaging is impractical for on-line analysis of fruit, due to the time needed to acquire data (ca. 5 min per fruit), the time for data acquisition could be reduced to a few milliseconds if "bulk" MR parameters were measured.

Meat, Poultry and Fish

The images shown in Fig. 8 and 9 illustrate the excellent "anatomical" resolution of fish tissues which

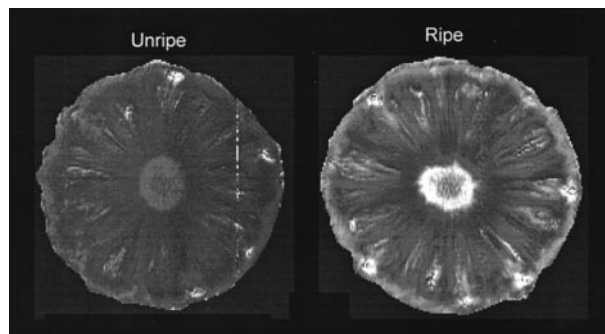


Fig. 7. T_2 maps from unripe and ripe pineapples. Mean T_2 value of unripe pineapple was 45 ± 12 ms (SD), mean T_2 value of ripe pineapple was 81 ± 30 ms (SD). Field of view 15 cm, slice 3 mm, T_R 5000 ms, T_E 30 ms, 256 by 256 pixels.

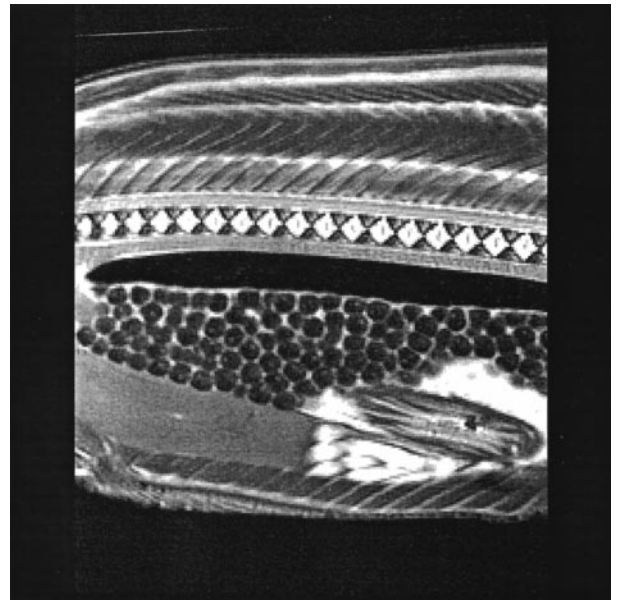


Fig. 8. MT-weighted sagittal image of the body of a rainbow trout. Field of view 10 cm, Slice 1.5 mm, T_R 7500, T_E 20 ms, SP 750 ms, 256 by 256 pixels.

can be achieved by MRI. However, the effect of freeze-thawing on the structure of trout muscle cannot be detected visually from such MRI scans, so quantitative MRI had to be used instead. The most significant effects of freeze-thawing on the MR parameters of water in trout



Fig. 9. MT-weighted sagittal image of the head of a rainbow trout. Field of view 10 cm, Slice 1.5 mm, T_R 7500, T_E 20 ms, SP 750 ms, 256 by 256 pixels.

Table 1. Effect of freeze-thawing on T_1 , T_{1sat} and MT rate in meat, poultry and fish

	T_1 (sec)		T_{1sat} (sec)		MT (sec)	
	mean	SE	mean	SE	mean	SE
Beef						
Fresh	1.09 ± 0.01		0.27 ± 0.006		3.01 ± 0.09	
Frozen/thawed	0.98 ± 0.01		0.24 ± 0.005		3.53 ± 0.08	
Lamb						
Fresh	1.15 ± 0.01		0.29 ± 0.006		2.81 ± 0.08	
Frozen/thawed	1.05 ± 0.01		0.26 ± 0.004		3.17 ± 0.06	
Pork						
Fresh	1.12 ± 0.01		0.28 ± 0.008		2.90 ± 0.08	
Frozen/thawed	1.02 ± 0.01		0.25 ± 0.004		3.41 ± 0.07	
Chicken						
Breast fresh	1.10 ± 0.01		0.29 ± 0.005		2.90 ± 0.06	
Breast thawed	1.05 ± 0.01		0.24 ± 0.003		3.59 ± 0.05	
Thigh fresh	1.18 ± 0.02		0.30 ± 0.006		2.71 ± 0.08	
Thigh thawed	1.12 ± 0.01		0.29 ± 0.008		3.05 ± 0.10	
Turkey						
Breast fresh	1.06 ± 0.02		0.176 ± 0.003		4.86 ± 0.09	
Breast thawed	0.94 ± 0.01		0.176 ± 0.002		4.73 ± 0.07	
Trout						
Fresh	1.35 ± 0.01		0.31 ± 0.002		2.70 ± 0.02	
Frozen/thawed	1.18 ± 0.01		0.20 ± 0.002		4.19 ± 0.04	

can be seen in Table 1 along with the results for beef, pork, lamb, turkey and chicken. Gravimetric measurements (% water content and pH) did not show a significant change upon freeze-thawing; therefore, those data have not been reported here. It was found that T_1 and MT rate are particularly sensitive to freeze-thawing for all species, with a significant ($p < 0.01$) decrease in T_1 value for all species, and a significant increase ($p < 0.01$) in MT value for all species except turkey.

It is suggested that the observed increase in MT rate could be due to protein denaturation and aggregation which occurs upon freezing and thawing. Virta et al.¹⁴ demonstrated in vitro that MT rate is decreased by protein denaturation, while protein aggregation and cross-

linking increased the MT rate. Enzymatic studies of frozen/thawed bovine muscle have also shown that myosin, a protein contained in muscle fibers, partially unfolds when it is frozen¹⁵. The unfolding of the myosin then results in the exposure of hydrophobic groups which then cause protein aggregation and cross-linking during frozen storage.¹⁶ The denaturation of myosin and protein aggregation has also been demonstrated in fish during freezing and frozen storage.¹⁷⁻¹⁹

Additional MRI evidence for protein denaturation and aggregation in frozen/thawed meat is demonstrated by the consistent and statistically significant decrease of about 100 ms in the T_1 value for all species. A possible hypothesis for the decrease in T_1 value is that protein denaturation and aggregation in frozen/thawed meat will increase the effective molecular weight of the proteins; this will reduce the mobility of the proteins and enhance cross-relaxation (spin exchange), thereby reducing the T_1 value²⁰ of the water protons. The total quantity of exchangeable protons in the macromolecular pool is also probably reduced by the loss of soluble sarcoplasmic proteins contained in the drip when the meat is frozen.²¹⁻²³ In light of the above, the increase in MT rate in frozen/thawed meat is probably due to a combination of protein denaturation followed by protein aggregation and drip loss.

The effects of freeze-thawing on the MR properties of water in pork confirm the earlier findings of Guiheneuf et al.² However, the present study which includes a larger number of species and animals demonstrates that inter-animal variations are significant; therefore, specific values for a particular MR or gravimetric parameter cannot be relied upon as being indicative of the freshness of a meat sample. Clearly this poses huge problems to the routine use of this MR approach by any regulatory agency. Consequently, we evaluated the differential effects of a deliberately induced freeze-thaw cycle; the hypothesis was that the induced change in MR parameters will be greatest for the first freeze-thaw cycle, and all subsequent freeze-thaw cycles would all be smaller. The experimental findings confirm this for trout and pork,

Table 2. Effect of repeat freeze-thawing on ratio of MR parameters in pork and trout

	$M_0 \pm SD$	$T_2 \pm SD$	$T_1 \pm SD$	$T_{1sat} \pm SD$	MT $\pm SD$
Pork					
Fresh vs. FT	0.51 ± 0.07	1.18 ± 0.14			
FT vs. FTFT	1.42 ± 0.62	0.99 ± 0.12			
Trout					
Fresh vs. FT			0.81 ± 0.01	0.66 ± 0.01	1.50 ± 0.01
FT vs. FTFT			0.92 ± 0.01	1.03 ± 0.01	0.97 ± 0.02

Note: FT is one freeze/thaw cycle; FTFT is two freeze/thaw cycles.

in so far that the ratio of MR parameters between fresh and one freeze-thaw cycle are significantly greater ($p < 0.01$) than the ratio of MR parameters from the first freeze-thaw cycle compared to multiple freeze-thaw cycles. Those encouraging results suggest that the ratio method may offer the best use of MRI to authenticate meat.

CONCLUSIONS

Although the main thrust of this article has been directed to the characterisation of natural food tissues, the ever-increasing sophistication of pre-prepared and pre-cooked convenience foods represents a substantial array of opportunities for MRI. Thus, there is precedence in the NMR literature to suggest that MRI can be used to study the molecular interactions that are responsible for the mechanical and organoleptic properties of food, and thereby help to fabricate specifically structured products. Such activities should also be aided by the ability of MRI to map temperature distribution^{24–26} and to study the rheological properties of complex fluids.^{27,28}

In addition to providing aesthetically attractive pictures of fruits, studies of, the results produced during this study amply vindicate the original hypotheses which were that the MRI parameters of meats, fish, fruit and vegetables should be sensitive to the “freshness” or “ripeness” of the produce and infer that MRI will have a diverse array of applications in food processing and authentication. However, the present data also indicate that there is considerable biologic diversity that will have to be allowed for, if MRI is to be used on a routine basis.

Although that seems possible, it will necessitate substantial additional research. Before that work is undertaken it is appropriate to consider how the cost of suitable hardware could be reduced so that the optimisations achieved can be realistically implemented. There are at least two encouraging prospects in this regard. First, the development of low cost “benchtop” nuclear MR spectrometers which use low field, permanent magnets; whilst not suitable for measurements of large samples, there is absolutely no reason why such existing systems should not be used for individual “biopsy” samples along the lines of those used here in the multi-well sample plates; indeed, the routine automation of such a system would be relatively straightforward. For those future applications which necessitate inspection of large intact food samples, such as a whole water melon, it may be economically more attractive to adopt the “inside-out” approach favoured by those who have developed MR systems for downhole logging of oil-wells,²⁹ and more recently by Blümich³⁰ on a smaller scale. Clearly each of those hardware configurations will bring such different, specific technical problems in terms

of data-acquisition, that it seems prudent to couple any more detailed studies of biologic diversity with each individual hardware implementation.

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