Journal of Food Engineering **16** (1992) 165–172

Nuclear Magnetic Resonance Imaging of Fresh and Frozen Courgettes

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(Received 5 March 1991; accepted 29 April 1991)

ABSTRACT

The internal structure of courgette and the effect of freezing the courgette was investigated by nuclear magnetic resonance imaging. The skin, vascular tissue, cortex, seed-bed and seeds were clearly delineated in the fresh vegetable; however, the image of frozen/thawed courgette showed very little contrast between the different tissues. Freezing ruptures the cell walls and alters the tissue morphology; this affects the transverse relaxation of water in the tissue, which in turn changes the image contrast. This report demonstrates that a correct theory to explain the mechanisms that cause proton relaxation in tissues is vital for correlating image contrast with the physicochemical properties of water in plant tissue.

INTRODUCTION

The development of new analytical methods for evaluating the effects on food of freezing and subsequent thawing would be very advantageous for the expanding frozen-food industry. Nuclear Magnetic Resonance (NMR) imaging (MRI) is a relatively new technique and it is gradually proving to be a very versatile method for studying food (Chen, *et al.*, 1989; German & McCarthy, 1989; Ishida, *et al.*, 1989; Duce, *et al.*, 1990*a*,*b*). This paper shows that it can be used to detect the changes that occur to the sample morphology and the state of water in food, when it is frozen and subsequently thawed.

NMR images can be used to investigate the internal structure of large, heterogeneous samples, and serial studies are feasible since the technique is non-invasive. The NMR signal normally originates from protons in the fat and water molecules. Two- or three-dimensional images can be

Journal of Food Engineering 0260-8774/92/$05.00 —

acquired, which map the spatial distribution of 'liquid-like' protons in the sample. The image contrast between various regions occurs because of differences in either the concentration of the protons or their relaxation times. The relaxation times of the protons are dependent upon the dynamics of the domains in which the nuclei are located and on the morphology of the different compartments in the sample (Luz & Meiboom, 1963; Robertson, 1966; Walters & Hope, 1971; Packer, 1977; Brownstein & Tarr, 1979; Mathur-De Vre, 1979; Belton & Ratcliffe, 1985; Belton & Hills, 1987; Hills, *et al.*, 1989); hence, changes in the physical or chemical state of the water protons will alter the proton relaxation times and may affect the appearance of the image. In this paper we present NMR images of fresh and frozen/thawed courgettes. The images not only contain impressive 'anatomical' information about the internal structure of the courgette, but they also give an insight into the way freezing disrupts the cells and changes the morphology of the plant tissues.

MATERIALS AND METHODS

The NMR imaging experiments were carried out using an Oxford Research Systems Biospec I spectrometer, operating at 84·7 MHz for protons, connected to an Oxford Instruments 31 cm horizontal bore, 2T superconducting magnet. Home-built coils with 20-cm diameter were used to produce the linear magnetic field gradients of 8 kHz/cm (Carpenter, *et al.*, 1989).

The images were acquired with a 90° slice-selective spin-echo pulse sequence (Edelstein, *et al.*, 1980). The non-selective 180° pulse length was 100 μs and the slice-selective 90° pulse excited a horizontal plane 2.5 mm thick through the sample. The echo time (TE) was 40 ms and the recycle time (TR) was 6 s. The signal was digitised every 32 μs and 256 complex points were acquired. The sequence was repeated using 256 phase encode steps and no signal averaging was required. All experiments were performed at ambient temperature (295 K). A 6 cm slice taken from a 5 cm diameter fresh courgette was imaged. Two 4 cm slices were cut from a 3 cm diameter courgette and one was rapidly frozen in liquid nitrogen, then removed and left to thaw. When its temperature had risen to 296 K it was imaged beside the slice that had not been frozen.

RESULTS AND DISCUSSION

The 40 ms echo time image of fresh courgette displays fine 'anatomical' detail (Fig. 1). The different regions such as the skin, vascular tissue,

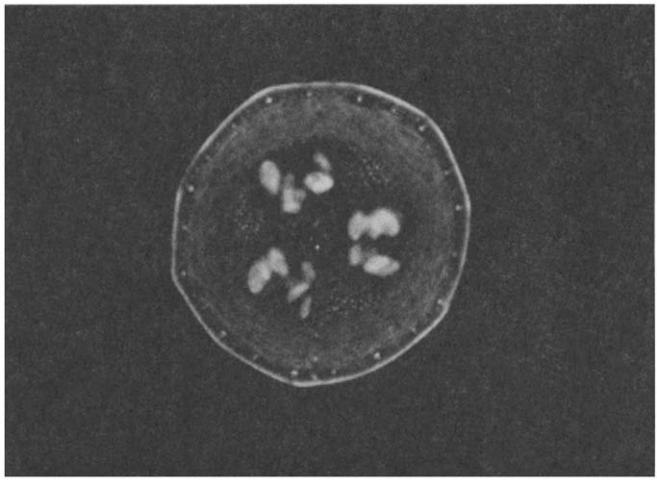

Fig. 1. 256 × 256 NMR spin-echo image of 5-cm diameter fresh courgette. TE = 40 ms; TR = 6 s.

cortex, seeds and seed bed are all well resolved. The signal originating from the skin, vascular tissue and seeds has a higher intensity than that associated with the cortex and the seed bed. The internal structure of the vegetable is clearly delineated. In the cortex region the image contrast reflects the orientation of the cells; the cells in the outer cortex are aligned tangentially whilst those of the inner cortex lie axially along the length of the courgette.

The intensity of signal in a spin-echo image depends upon a number of parameters including the density of protons in the region (ρ), their longitudinal (T_1) and transverse (T_2) relaxation times, the echo time (TE) between the excitation and detection of the transverse magnetisation and the recycle time (TR) between successive pulse sequences. The signal intensity 'M' from a pixel in the spin-echo image can be expressed as:

$$M = k.\rho.\exp(-\mathrm{TE}/T_2).[1-\exp(-\mathrm{TR}/T_1)] \qquad (1)$$

assuming TR ≫ TE, and that there is no mass transport such as diffusion or flow. For the two imaging experiments described in this paper, the recycle time (TR) was at least five times longer than the longitudinal relaxation time of the water protons in the tissue; thus, the term in square brackets in the equation is effectively unity. It has been demonstrated that the density of water protons in the different tissues of courgette are very similar (Attard *et al.*, 1990). Therefore, the distinct contrast between the different tissues in the image of fresh courgette (Fig. 1) cannot be due to differences either in the density of protons in the various tissues or their longitudinal relaxation times. Rather, it is the dif-

ferences in the water proton transverse relaxation times of the various tissues that is responsible for the appearance of the image of fresh courgette (Fig. 1); the image of tissue with a short T_2 relaxation will be darker than that with a long T_2 relaxation.

Although the T_2-weighted image of fresh courgette reflects the anatomical structure of the vegetable, only recently has a theory become available that provides an understanding of the mechanisms that cause water proton relaxation in such tissues. A phenomenological theory has recently been developed that can predict the transverse relaxation time of water protons in plant tissue (Hills & Duce, 1990). The theory demonstrates that chemical exchange and diffusion are dominant transverse relaxation mechanisms of water protons; it has proven to be successful in predicting the transverse relaxation characteristics of water protons in a wide range of aqueous samples (Belton & Hills, 1987; Hills, *et al.*, 1989, Hills & Duce, 1990, and references cited therein). The two important transverse relaxation mechanisms for water in courgette tissue are proton exchange between water and solute protons, and diffusion of water through internally generated magnetic field gradients. The local magnetic field gradients are generated at the boundaries between cellular water and air-cavities since there is an abrupt change in the magnetic susceptibility (Packer, 1973; Gasel & Lee, 1974; Callaghan, 1990). Diffusion of water through these gradients is a particularly potent relaxation process when the interpulse spacing between the radiofrequency pulses is longer than a millisecond; since the spacing between pulses in the spin-echo imaging sequence used in this study is 20 ms, there is plenty of opportunity for this diffusion-driven relaxation process to occur. This relaxation pathway has the greatest influence on the transverse magnetisation of water protons in the cortex and seed-bed tissues since those tissues contain a high proportion of air cavities; that is the reason why the image signal intensity arising from these tissues is so low. The transverse relaxation of water protons in the skin, vascular tissue and seeds is less affected by diffusion-driven relaxation mechanisms, as they are more spatially homogeneous; as a result, the signal intensity in the spin-echo image from those tissues is relatively high. Thus, the image contrast of the fresh courgette is strongly dependent upon the morphology of the tissue, and especially on the presence of air cavities.

Samples of fresh and frozen/thawed courgettes were imaged simultaneously and the results are displayed in Fig. 2; clearly, freezing and thawing the sample has a dramatic effect on the appearance of the image. The image of the fresh (Fig. 2A) and thawed (Fig. 2B) samples differ from each other in two important respects. First, the overall intensity of the image of the thawed vegetable is greater than that of the fresh

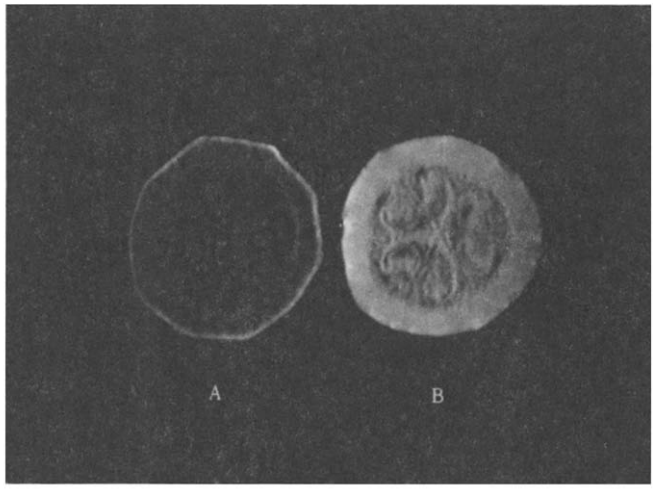

Fig. 2. 256 × 256 NMR spin-echo image of fresh and frozen 3-cm diameter courgette. TE = 40 ms; TR = 6 s. A: fresh sample; B: frozen/thawed sample.

courgette; second, the relative image contrast between different types of tissue is less distinct in the thawed sample compared to the fresh courgette. The timing of the pulse sequence cannot be responsible for changes in image intensity as the fresh- and thawed-courgettes were imaged simultaneously. Furthermore, the longitudinal relaxation time of water in the tissue will not affect the image contrast since the recycle time of the experiments was set to be at least five times longer than the longest longitudinal relaxation time. Neither can an increase in the overall water concentration be responsible for the observed changes in the appearance of the image, since the total mass of the courgettes was unaffected by freezing and thawing the sample, although water in the tissues has more freedom after thawing and can redistribute itself through the sample.

Rather, the observed changes in the image contrast arise because freezing and thawing the courgette alters the morphology of the tissues, which in turn increases the transverse relaxation time of the water protons in the tissue. The ice crystals formed on freezing rupture the cell walls and destroy the cell's turgor pressure, and on thawing the courgette's texture becomes very flaccid. The intracellular fluid is then free to drain into the air cavities in the tissues. The displaced air forms bubbles, which have a higher symmetry and have a lower surface area compared to the air cavities. They will, therefore, generate much weaker internal magnetic field gradients (Packer, 1973; Gasel & Lee, 1974; Callaghan, 1990); consequently, the diffusion-driven transverse relaxation mechanisms

become less efficient, and the transverse relaxation of water protons in thawed tissue measured when the pulse spacing is 20 ms will be longer than that of the fresh tissue. Thus, less transverse magnetisation irreversibly dephases during the TE delay period and, as a result, the overall image intensity in the image of the frozen/thawed courgette is higher than that of the fresh courgette. The transverse relaxation time of frozen/thawed tissue is also longer than the echo time and thus it will have less influence on image contrast. Therefore, whilst the contrast in the image of fresh courgette reflects the distribution of the transverse relaxation time of the water protons, the contrast in the image of thawed courgette will reflect the water distribution in the sample.

CONCLUSIONS

The image of fresh courgette clearly delineates between the skin, vascular tissue, cortex, seed-bed and seeds. The contrast in the image is sensitive to the morphology of the plant tissue, which results in a dramatic change in the appearance of the image of the courgette after the cells have been ruptured by freezing and thawing. Although it is intuitively obvious that those changes in the appearance of the image result from changes in tissue morphology, detailed interpretation cannot be made by inspection of a single image. Instead, it is necessary to investigate the transverse relaxation processes of water protons in the sample. Such studies can either be qualitative, as in this study of courgette, or quantitative; however, the latter involves considerably more effort. Undoubtedly, a precise theory of the mechanisms that cause water proton relaxation in tissues is essential for an understanding of their contribution to image contrast. From the standpoint of food technology, the significance of this work is that it demonstrates new opportunities for monitoring an industrially important food process. Clearly further work will be required to fully investigate other applications of NMR imaging to food processing. This technique appears to have huge potential and promising results have already been obtained from studies of vegetables, pies, sweets, meat and dairy produce.

ACKNOWLEDGEMENTS

We thank Dr Herchel Smith for his endowment to this Laboratory, and the Agriculture and Food Research Council for a studentship (SLD).

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