Appl Magn Reson
DOI 10.1007/s00723-009-0004-8

Applied
Magnetic Resonance

Characterisation of Ripening and Pressure-Induced Changes in Tomato Pericarp Using NMR Relaxometry

N. A. Marigheto · G. K. Moates · M. E. Furfaro · K. W. Waldron · Brian P. Hills

Received: 30 November 2008
© Springer 2009

Abstract A combination of two-dimensional nuclear magnetic resonance (NMR) relaxometry and optical microscopy is used to investigate the effects of ripening and high-pressure treatment on tomato pericarp tissue. It is shown that the relaxation times of the vacuolar water increase during the early stages of ripening even when there is no visible change and that this could be the basis of a useful on-line sensor for sorting tomatoes. The membrane rupturing and biopolymer denaturation associated with high-pressure treatments are also reflected in the NMR relaxation spectra.

1 Introduction

From a commercial perspective tomato fruit suffers from several related problems. Tomatoes destined for international export are usually picked in the mature green (MG) stage of ripening and transported in boxes gassed with ethylene. However, on arrival the tomatoes display the entire range of ripeness up to red-ripe and require a second costly (and damaging) hand-sorting stage. If the tomatoes, when picked, could be classified as to their exact degree of ripeness, the ripening would be more uniform and the second sorting stage would be rendered unnecessary. One aim of this paper is therefore to use nuclear magnetic resonance (NMR) as a non-invasive probe of the sub-cellular changes associated with tomato ripening with a view to using these changes as the basis for an on-line NMR grader of ripeness. As an independent measure of ripeness, the NMR changes were correlated with pectin methylesterase and polygalacturonase activity, texture analysis and observations using light microscopy. In this paper we therefore investigate whether low field NMR can be developed as a low-cost, non-invasive sensor of the degree of tomato ripening. Six

N. A. Marigheto · G. K. Moates · M. E. Furfaro · K. W. Waldron · B. P. Hills (✉)
Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK
e-mail: brian.hills@bbsrc.ac.uk

Published online: 12 August 2009

industry standard ripening stages have been defined from green to red, namely 'green', 'breaker', 'turning', 'pink', 'light red' and 'red' [1]. Four additional sub-stages of the first 'green' stage have been identified on the basis of the internal colour and tissue consistency. These substages are M1, characterised by firm green locular tissue and soft seeds that cut easily; M2, characterised by soft green locular tissue and hard seeds that do not cut easily; M3, characterised by some gel in the locule but no internal red colour; and M4 (or mature green, MG) where locular tissue is predominantly gel-like with some red visible. The locular tissue referred to here is indicated in Fig. 1, which shows the main tissue types. Of special interest are the early stages of ripening M1–M4 where the tomatoes are still visibly green, but where changes are occurring inside the pericarp and locular regions of the tissue. Obviously, once external colour changes begin, optical sensors are the method of choice for sorting degrees of ripeness. For export purposes, such optical methods are already too 'late stage'. If we can show that non-invasive NMR relaxometry can distinguish the M1–M4 stages then this might be the basis of an on-line sorting sensor.

A second problem is related to the thermal processing of tomatoes which is associated with creation of off-flavours and the destruction of natural ingredients such as vitamins. For this reason the last decade has seen a growing interest both from industry and the research community in the commercialisation of high pressure food processing as an alternative to thermal techniques. High pressure processing (HPP) not only inactivates undesirable food-borne microbial pathogens but also helps preserve natural ingredients, such as vitamins and flavour compounds. In addition, the operating costs of HPP equipment are lower than conventional thermal techniques. HPP treatment also affects quality-determining enzymes in different ways and offers possibilities for engineering novel quality characteristics in many food materials. One important unknown is the effect of differing degrees of ripeness on the effectiveness of the high pressure treatment. In this paper we therefore also subject tomatoes with differing levels of ripeness (as determined by increasing polygalacturonase activity) to high pressure treatments. The microstructural changes induced by the HPP treatment will then be assessed with a combination of NMR relaxometry, and optical microscopy. Of particular interest is the effect of the HPP on tomato texture. Accordingly, the HPP of the pericarp was undertaken for pressures of 200, 400 and 600 MPa at room temperature and the samples were compared with the non-pressure treated tomato pericarp.

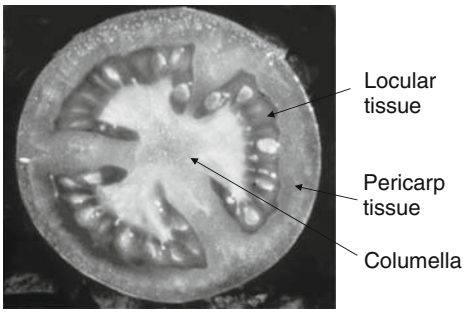

Fig. 1 Main tissue types in a tomato fruit

2 Materials and Methods

2.1 Materials

Tomatoes (*Lycopersicon esculentum* var. *Moneymaker*) were grown from seed at the Institute of Food Research. Five seedlings were selected and grown until they were mature. Tomato fruits were harvested at the six industry standard ripening stages from green to red, namely 'green', 'breaker', 'turning', 'pink', 'light red' and 'red' [1]. After instrument studies the locular tissue of the green tomatoes was examined and the tomato ripening stage further classified as M1, M2, M3 or M4 (mature green) according to the classification of Kader and Morris [1].

The tomato pericarp is the mature ovary wall of the fruit. It consists of the peripheral pericarp, radial arms and columella. The peripheral pericarp of the fruit is divided into three different parts: the exocarp or skin, mesocarp and endocarp. The exocarp represents the outermost layer which is composed of a thin layer of epidermal cells and two or more layers of relatively small, flattened cells. The main portion of pericarp is the mesocarp which is composed largely of parenchymatous cells as well as vascular bundles. The endocarp is a layer of thin-walled cells lining the locules [2]. In the following experiments, the pericarp tissue used was the mesocarp and endocarp.

2.2 High Pressure Processing

The samples were heat sealed under vacuum in plastic bags before pressure treatment on a Stansted 900 MPa pressure-generating system (Stansted Fluid Power, Stansted, Essex, UK) with a cylindrical pressure vessel (i.d. 20 mm, ca. 95 ml). The pressure transmitting fluid was an aqueous mixture containing 30% propylene glycol and the treatment time was 20 minutes for all samples. The rate of pressure increase was 3 MPa/s and the decompression rate was 5 MPa/s.

2.3 Texture Analysis

Samples of pericarp tissue (10 mm × 10 mm, 6 mm thick) were excised from the tomato fruit and tested using a Texture Analyser TAXT2 (Stable Microsystems, Godalming, Surrey, UK) fitted with a 5 kg load cell.

The system was fitted with a 6 mm wide probe with rounded end travelling at a constant speed of 1 mm/s from an initial height of 2 cm above the platform. The peak force was recorded to a penetration depth of 3 mm and the average of five samples calculated for each treatment.

2.4 Pectin Methylesterase Activity Assay

PME activity is determined by measuring the release of acid per time unit at constant pH and temperature (pH 7 and 22°C). The reaction mixture consists of 250 µl enzyme sample and 30 ml of a 3.5 g/l apple pectin solution (DM 70–75%, Fluka) containing 0.117 M NaCl. During pectin hydrolysis, the pH is maintained

constant by addition of 0.01 M NaOH using an automatic pH-stat titrator (Titrino 718, Metrohm, Buckingham, UK). The PME activity is proportional to the rate of base consumption. One unit (U) of PME activity is defined as the amount of enzyme capable of catalyzing the hydrolysis of 1 μmol methyl–ester bonds per minute at constant pH and temperature (pH 7 and 22°C).

2.5 Polygalacturonase Activity Assay

For the extraction procedure (undertaken at 4°C), 10 g of tomato pericarp was homogenized using a Ystral homogeniser (Ystral, Ballrechten-Dottingen, Germany) with 30 ml of cold distilled water, adjusted to pH 3 with concentrated HCl and stirred for 15 min.

The mixture was centrifuged at 20,000*g* for 30 min using a Beckman J20 Centrifuge equipped with a JA-14 rotor before resuspending the pellet in 30 ml of cold distilled water at pH 3 and stirring for a further 15 min. The mixture was centrifuged again at 20,000*g* for 30 min and resuspended in 20 ml of cold 1 M NaCl. The pH was adjusted by addition of 1 N NaOH and maintained at pH 6 for 30 min before stirring overnight.

The resulting mixture was centrifuged at 20,000*g* for 30 min and the recovered supernatant adjusted to pH 4.4 and assayed for the polygalacturonase activity measurement.

For the enzyme assay, standard solutions of galacturonic acid of citrus origin in sodium acetate buffer, pH 4.4 were prepared at concentrations of 0–180 μg/ml. Samples were prepared using a mixture of 20 μl of the recovered supernatant (or an appropriate dilution) and 180 μl of substrate (citrus pectin solution (0.5% w/v in 0.1 M NaCl) and incubate for 20 min at 37°C. 300 μl of DNS (1% w/v, 30% potassium sodium tartrate) was added to the standards, samples and controls before boiling for 5 min in a water bath and centrifuging at 13,000*g* for 3 min. 200 μl of the standards, samples and control was transferred to a microtiter plate and measured spectrophotometrically at 580 nm and room temperature. The enzyme blank was prepared using 180 μl of substrate, 300 μl of DNS and 20 μl of sample in an eppendorf tube which was then boiled for 5 min, centrifuged and measured at 580 nm. The plate reader was a Molecular Devices Softmax microplate reader (Molecular Devices Corporation, Sunnyvale, USA).

2.6 Nuclear Magnetic Resonance of Tomato Tissue

NMR relaxation measurements were carried out on Resonance Instruments DRX23 and DRX100 spectrometers (Resonance Instruments, Witney, Oxfordshire, UK) operating at proton frequencies of 23.4 and 100 MHz respectively. High power, thermostated probes were used with 90° pulse lengths of 5–10 μs. The measurements were carried out at room temperature (300 K). T_1–T_2 correlation measurements were carried out using the inversion recovery-CPMG pulse sequence, where the inversion recovery step $[180° - t_1 -]$ is inserted in front of the CPMG sequence. The two independent variables are the recovery time, t_1 and the CPMG acquisition time t_2, which is $2n\tau$ with $n > 1$ and τ the CPMG 90–180° pulse spacing.

The T_1 dimension was acquired with 150 steps with the inversion recovery time (t_1) being logarithmically varied between 5 ms and 20 s. This long t_1 was necessary in order to reach equilibrium. The T_2 dimension was acquired with a τ spacing of 300 μs. The probability distribution for the longitudinal and transverse relaxation times was obtained by 2D-inverse Laplace transformation. The fast algorithm for performing this two-dimensional inversion allows 'zooming' into regions of interest, peak area integration and identification of the position of peak maxima.

Measurements of tomato pericarp were done on slices of dimensions of $5 \times 5 \times 10$ mm^3 and $2 \times 2 \times 5$ mm^3 for the DRX23 and DRX100 respectively. The slices were cut after the skin was removed.

2.7 Light Microscopy

Sections of tomato pericarp were taken and examined using an Olympus BX60 microscope (Olympus UK Ltd, Southall, Middlesex, UK). The device works by passing visible light through a condenser and an objective lens. The Brightfield adjustment was used to analyse the pictures in a 4× or 10× magnification. The data capturing software was AcQuis software provided by Syncroscopy (Syncroscopy Europe, Cambridge, UK). The images of each section were saved and assessed.

3 Results and Discussion

3.1 Ripening-Induced Changes

3.1.1 Texture Analysis

As expected, the process of ripening is accompanied by a large decrease in the firmness of the tomato pericarp as measured by the peak force in the force-deformation curve. The firmness declined to ca. 10% of initial value for green (unripe) fruit (Fig. 2a).

3.1.2 Enzyme Activity

The action of cell wall degrading enzymes in fruits and vegetables has a negative influence of the functional properties of the food. The two enzymes pectinmethylesterase (PME) and polygalacturonase (PG) are counted among the pectinases and their joint action is responsible for the viscosity loss in tomato based products [3]. During ripening, PME is responsible for the de-esterification of the highly methyl-esterified polygalacturonase in the cell wall [4]. The resulting de-esterified pectin is the substrate for the depolymerising enzyme PG [5]. In other words PME potentiates the PG to be active. Pectinmethylesterase is mainly present in the middle lamella and the cell junctions of tomato tissues as different isoenzymes that change with the tomato variety and the degree of maturity [6].

Koch and Nevins [7] studied the degree of esterification (DE) of pectins during the tomato fruit maturation. A significant decrease in degree of esterification (DE)

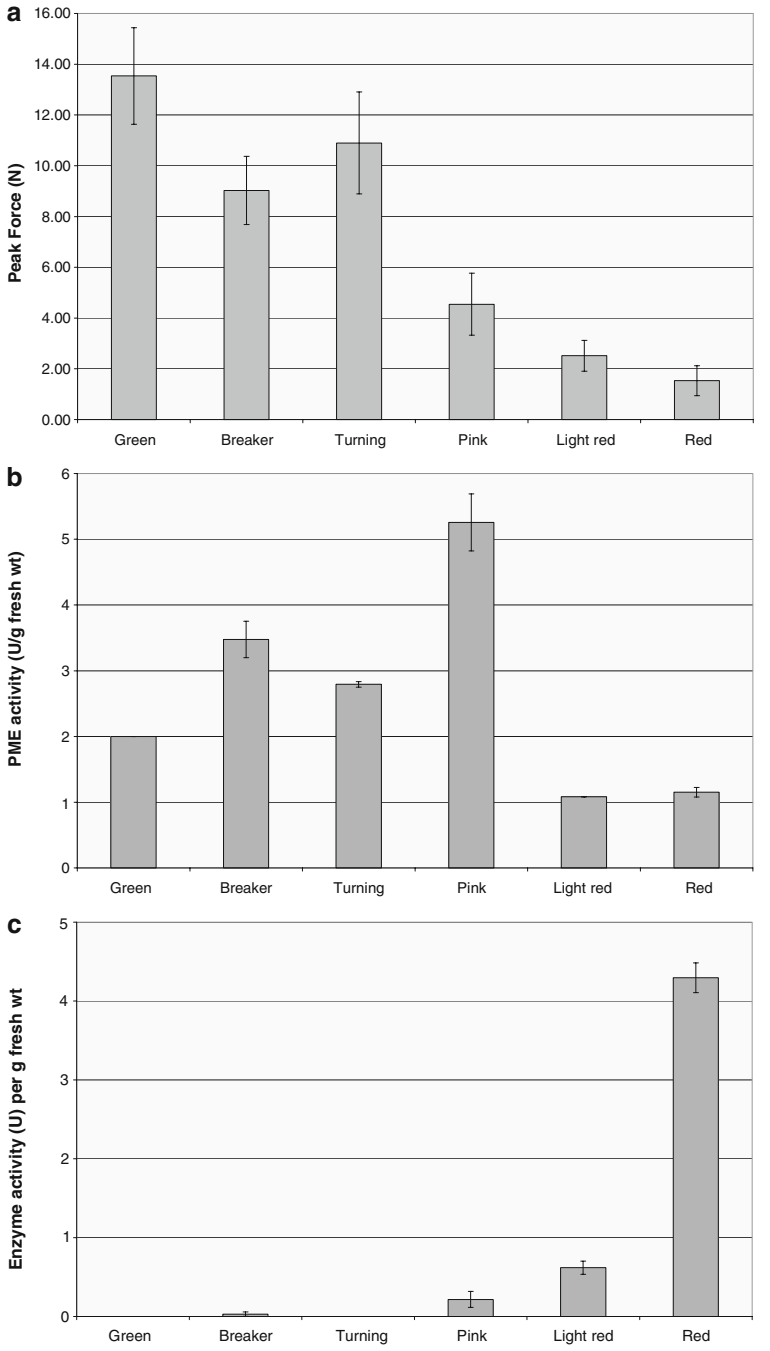

Fig. 2 **a** Graph of peak compression force versus ripening stage of tomato fruit. **b** Graph of pectinmethylesterase activity in ripening tomato fruit. **c** Graph of polygalacturonase activity in ripening tomato fruit

was observed during the ripening process from 90% DE in the green fruit to 35% DE at the ripe stage was identifiable. This is confirmed in our own work. Figure 2b shows the dependence of PME activity on the ripening stage. Activity is observed at all stages of ripening with an apparent peak at the 'pink' stage declining thereafter. Our data also confirms that PME is being expressed before PG and that the action of PME enhances PG activity. Indeed Fig. 2c shows that PG activity is either very low or not apparent in the 'green', 'breaker' or 'turning' stages of ripening tomato fruit, but its activity rapidly increases during the later stages of ripening. A very low polygalacturonase activity is initially observed at the 'pink' stage of ripening before rising rapidly between the 'light red' and 'red' stages. In other words the onset of polygalacturonase activity coincides with the peak activity of pectinmethylesterase. A similar phenomenon has been observed for peaches, pears and avocados [8].

3.1.3 NMR Relaxometry and Optical Microscopy

The T_1–T_2 correlation spectrum of green pericarp at a proton frequency of 23.4 MHz is shown in Fig. 3a and the peaks associated with water in the vacuole (peak 1), extra-cellular water (peak 2), cytoplasm (peak 3) and the more rigid components of the cell wall (peak 4) can be observed. As the fruit ripens, the optical micrographs show that the cell wall swells, which, together with the breakdown of the pectins and cell wall biopolymers causes the water proton relaxation times to increase (Fig. 3b). What is of particular interest is whether this increase can be detected while the fruit is still green, during the M1–M4 ripening stages. Figure 4 strongly suggests that it can. It must, however, be remembered that a different tomato was used for each of the M1–M4 stages, so there is a need to repeat this correlation on a much larger sample. It is surprising to note that no such trend in relaxation times was observed for the dissected locular tissue during its gelation in the M1–M4 stages.

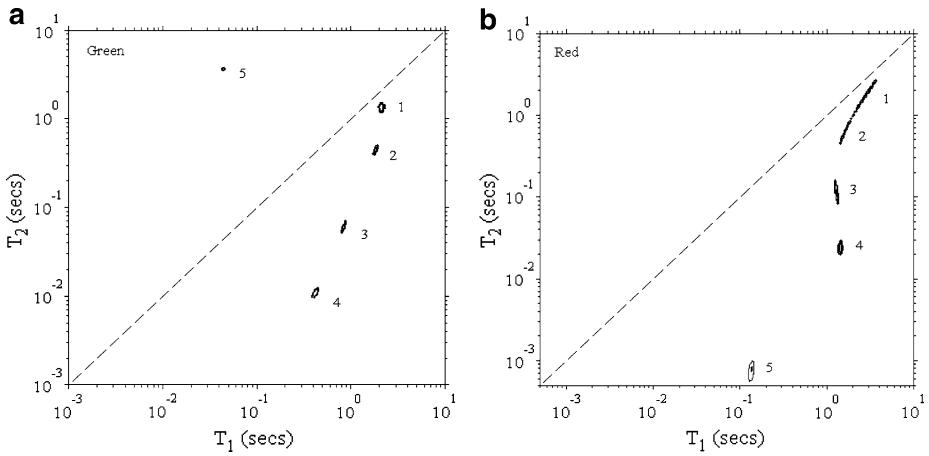

Fig. 3 Experimental T_1–T_2 correlation spectra of tomato pericarp at two different stages of ripening acquired at spectrometer frequency of 23.4 MHz with a CPMG pulse spacing of 300 μs. **a** Green, **b** red

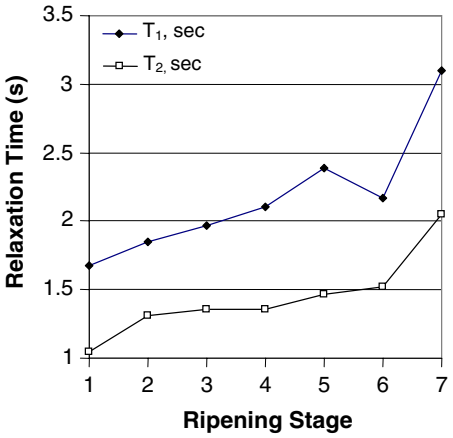

Fig. 4 Dependence of the T_1 and T_2 values of the main vacuolar water peak 1 on degree of ripening. Ripening stages correspond to *1* M1, *2* M2, *3* M3, *4* M4, *5* pink, *6* light red, *7* red

3.2 High Pressure Treatment of Tomato Pericarp Tissue

3.2.1 Texture Analysis

In the case of the unripe fruits, the average peak force was slightly lower for the 200 and 400 MPa pressure-treated samples compared to the fresh and 600 MPa samples (Fig. 5a). However, there was no significant difference between the samples. The ripe fruits were, as expected, much softer than the unripe fruits. A further large decrease in the peak force was observed upon pressure treatment at 200 MPa with the 400 MPa sample being so soft that a value could not be recorded (Fig. 5b). The 600 MPa pressure-treated sample showed a similar value to the 200 MPa sample suggesting a recovery in firmness. Similar results have been observed previously by Tangwongchai et al. [9] during a study of cherry tomato. It is suspected that this is due to pressure-induced gelation caused by denaturation and cross-linking of intracellular proteins and the gelation of the starch granules resulting in a firmer gel-like structure.

3.2.2 Enzyme Activity

Figure 5c shows that the pressure treated red samples appear to have higher PME activity than the equivalent fresh sample, which could originate from increased accessibility of the enzyme to the cell wall pectins following membrane rupture during pressure treatment. There is however no reduction in enzyme activity (inactivation) with increasing pressure to 600 MPa, i.e. it is barotolerant. This finding is in agreement with Tangwongchai et al. [9] and also Shook et al. [10] who undertook studies to 800 MPa.

Figure 5d shows the effect of pressure treatment on polygalacturonase activity. As discussed, this enzyme is absent in green (unripe) tomatoes but at the red-ripened stage pressure treatment at 200 MPa causes an apparent increase in PG activity compared to the untreated fresh red-ripe sample. As with PME activity it is suspected that this is due to increased accessibility of the enzyme to the cell wall

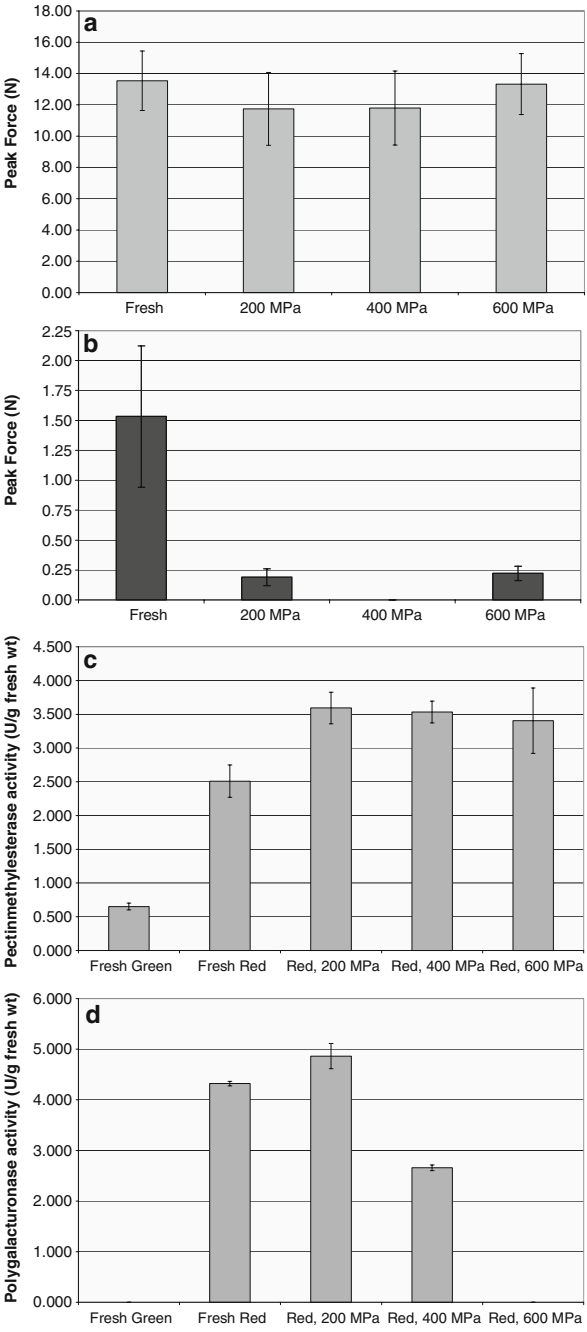

Fig. 5 **a** Graph of peak compression force for fresh and pressure treated unripe tomato pericarp. **b** Graph of peak compression force for fresh and pressure treated ripe tomato pericarp. **c** Graph of pectinmethylesterase activity after different pressure treatments. **d** Graph of polygalacturonase activity after different pressure treatments

region following membrane rupture. A 40% reduction in PG activity is observed following pressure treatment at 400 MPa and total inactivation observed following treatment at 600 MPa.

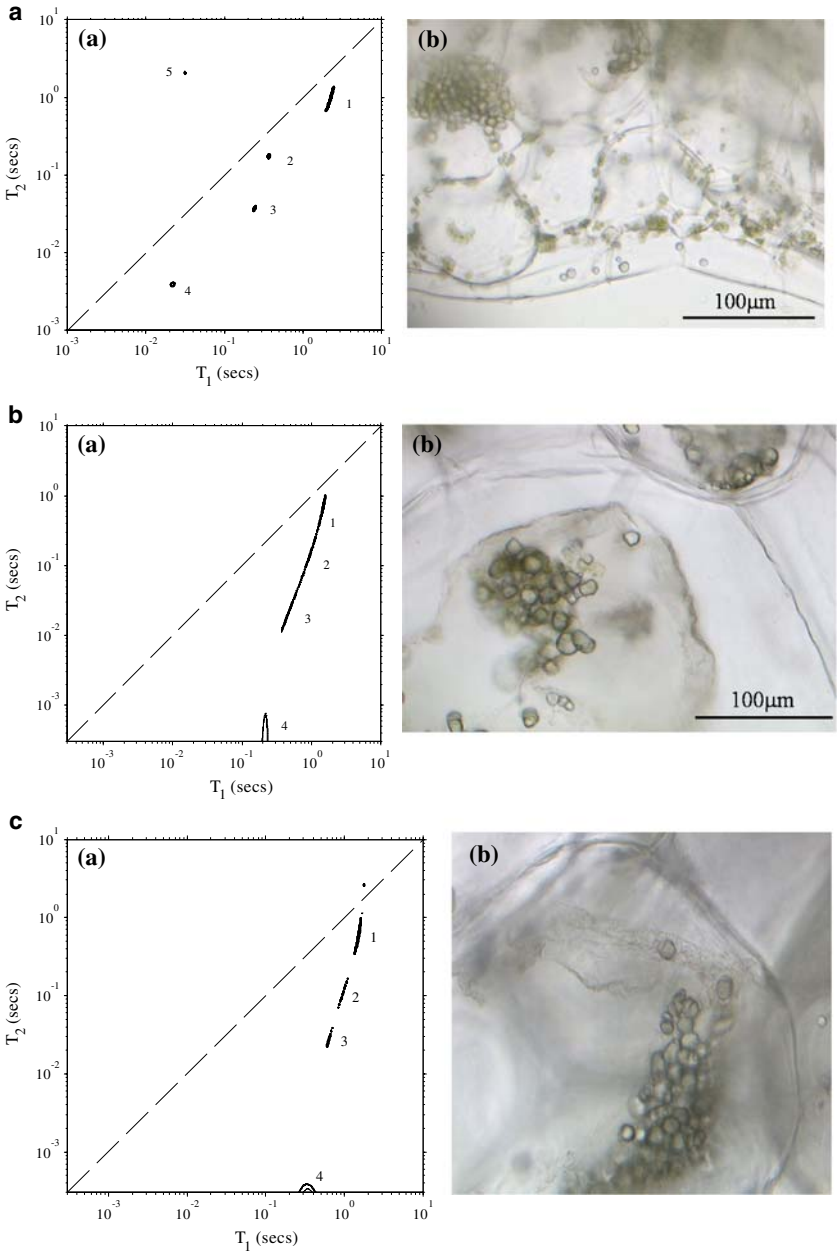

Fig. 6 **A** Unripe tomato pericarp at atmospheric pressure and room temperature *a* experimental T_1–T_2 correlation spectra acquired at spectrometer frequency of 100 MHz with a CPMG pulse spacing of 300 μs, *b* optical microscopy. **B** Unripe tomato pericarp treated at 200 MPa and room temperature *a* experimental T_1–T_2 correlation spectra acquired at spectrometer frequency of 100 MHz with a CPMG pulse spacing of 300 μs, *b* optical microscopy. **C** Unripe tomato pericarp treated at 400 MPa and room temperature *a* experimental T_1–T_2 correlation spectra acquired at spectrometer frequency of 100 MHz with a CPMG pulse spacing of 300 μs, *b* optical microscopy

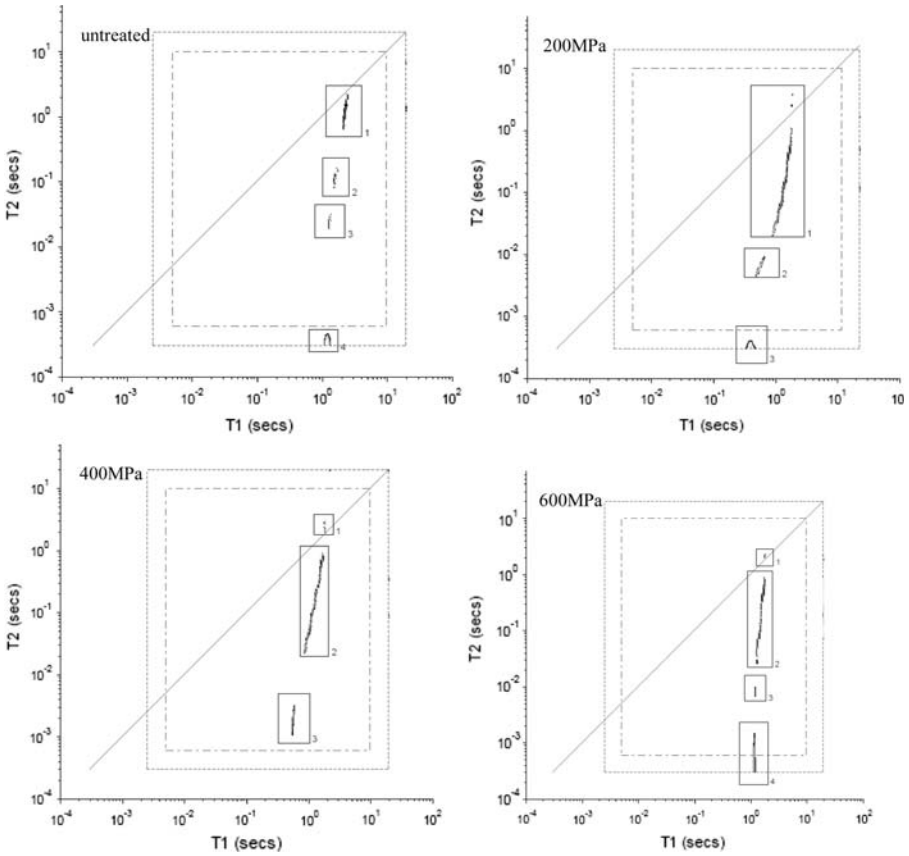

Fig. 7 Experimental T_1–T_2 correlation spectra of ripe tomato pericarp at different pressure treatments acquired at spectrometer frequency of 100 MHz with a CPMG pulse spacing of 300 μs

3.2.3 NMR Relaxometry and Optical Microscopy

Figure 6a shows the 100 MHz T_1–T_2 correlation spectra of unripe tissue at atmospheric pressure and room temperature, which reveals peaks associated with the different cell compartments. As with the 23.4 MHz spectrum in Fig. 3a, peak 1 can be assigned to the water in the vacuole; peak 2 can be associated to the cytoplasm and extra-cellular compartment and peak 3 with the more rigid components of the cell wall respectively. There is also a small peak (peak 4) that may be associated with water inside starch granules, which were also observed in the optical micrographs as shown in Fig. 6a. Treatment at 200 MPa (Fig. 6b) causes loss of compartmentalisation as it is not possible to distinguish the peaks associated with different compartments. This was confirmed by the optical micrograph which showed that the tonoplast along with other membranes have been disrupted causing loss of turgor (Fig. 6b). There is an apparent recovery of compartmentalisation after treatment at higher pressures (400 and 600 MPa) as individual peaks can be

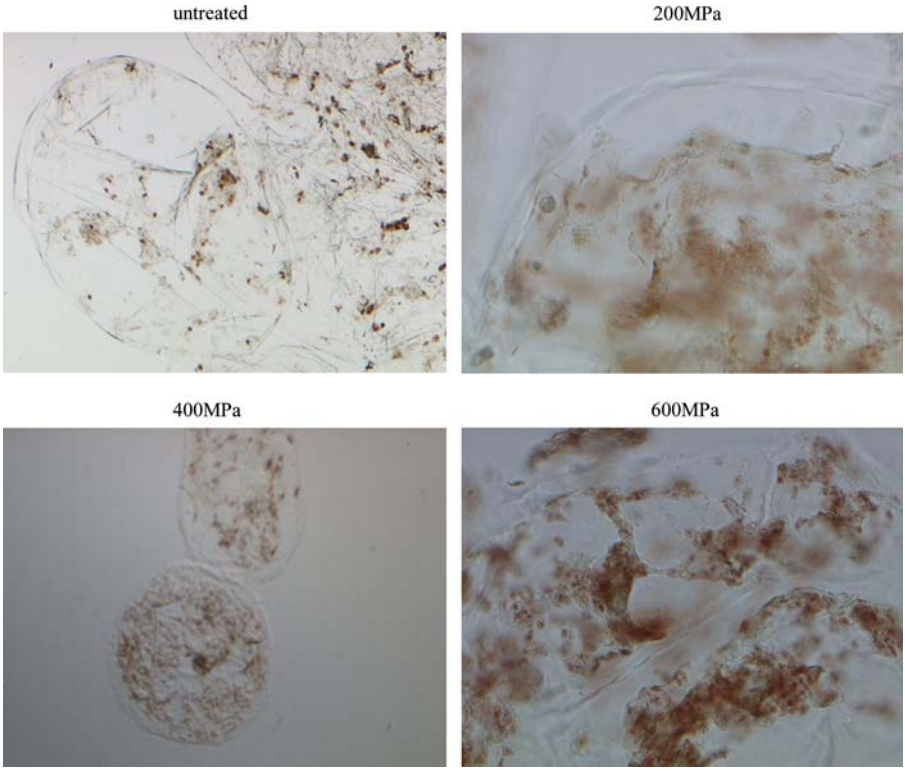

Fig. 8 Optical micrographs of ripe tomato pericarp at different pressure treatments

observed in the T_1–T_2 correlation spectra (e.g. Fig. 6c). The optical micrographs revealed a more 'opaque' cytoplasmatic background when compared to the untreated tissue. At 400 MPa the nucleus is clearly visible showing the opaqueness of the material. Possibly the contents of the cytoplasm and other organelles 'gel' under these higher pressure treatments and even though the membranes maybe ruptured its effect is not so apparent when compared to 200 MPa.

The T_1–T_2 correlation spectra of ripe tissue are shown in Figure 7. As was the case with the untreated ripe tissue, the main peaks (vacuole, cytoplasm and extracellular water and cell wall) can be observed but they are shifted to longer T_1, possibly because of tissue softening. It is notable that the starch peak is not present, which is expected as the fruit ripens starch is broken down into sugars and used for metabolism. Treatment at 200 MPa caused some membrane disruption as shown in the T_1–T_2 correlation spectrum and the optical micrograph where the plasmalemma seems to be intact but away from the cell wall reflecting the loss of turgor. Unlike the unripe tissue, some recovery of compartmentalisation is not observed at higher pressure treatment (400 and 600 MPa) even though the cell contents seem to have gelled as observed by optical microscopy. This may be related to the ease with which cells are separated under pressure as shown by optical microscopy in Fig. 8, thereby destroying gross tissue integrity.

4 Conclusions

2D NMR relaxometry, combined with optical microscopy is a powerful tool for monitoring changes in cell structure induced by HPP at various stages of ripening. The observation that the transverse relaxation time increases with ripening in the early stages M1–M4 when there is no visible change in the green colour of the tomato holds the promise that NMR could be developed as an on-line sensor for sorting tomatoes prior to export. The effects of HPP are also apparent in the NMR relaxation spectra. The rupturing of cell membranes by pressure treatment at 200 MPa is clearly seen as a loss of compartmentalisation of peaks on the spectra. Some of this compartmentalisation can be recovered at higher pressures which is consistent with the gelation of cell contents and the resultant rise in compressive strength. High pressure gelation of the cell contents was also found in our earlier studies on carrot [11, 12] which suggests it is a common mechanism in the high pressure treatment of plant tissue.

Acknowledgments The authors thank the EU (Contract No. 015710-2 Novel Processing Methods for the Production and Distribution of High-Quality and Safe Foods) for financial support for this research. We also gratefully acknowledge the help of Dr. Mary Parker in acquiring the optical micrographs.

References

1. A.A. Kader, L.L. Morris, Correlating subjective and objective measurements of maturation and ripeness of tomatoes, in *Proceedings of 2nd Tomato Quality Workshop* (University of California, USA, 1976), pp. 57–62
2. D.M. Barrett, E. Garcia, J.E. Wayne, Food Sci. Nutr. **38**, 173–258 (1998)
3. I. Verlent, A. Van Loey, C. Smout, T. Duvetter, B. Ly Nguyen, M.E. Hendrickx, J. Sci. Food Agric. **84**, 1839–1847 (2004)
4. L. Alexander, D. Gierson, J. Exp. Bot. **53**(377), 2039–2055 (2002)
5. I. Vovk, B. Simonovska, J. Chromatogr. A **1144**, 90–96 (2007)
6. D. Rodrigo, C. Cortes, E. Clynen, L. Schoofs, A. Van Loey, M. Hendrickx, Food Res. Int. **39**, 440–448 (2005)
7. J.L. Koch, D.J. Nevins, Plant Physiol. **91**, 816–822 (1989)
8. C. Brett, K. Waldron, *Topics in Plant Physiology: 2. Physiology and biochemistry of plant cell walls* (Unwin Hyman, London, 1990)
9. R. Tangwongchai, D.A. Ledward, J.M. Ames, J. Agric. Food Chem. **48**, 1434–1441 (2000)
10. C.M. Shook, T.H. Shellhammer, S.J. Schwartz, J. Agric. Food Chem. **49**, 664–668 (2001)
11. M.E. Furfaro, N. Marigheto, G.K. Moates, K. Cross, M.L. Parker, K.W. Waldron, B.P. Hills, Appl. Magn. Reson. **35**, 521–521 (2009)
12. M.E. Furfaro, N. Marigheto, G.K. Moates, K. Cross, M.L. Parker, K.W. Waldron, B.P. Hills, Appl. Magn. Reson. **35**, 537 (2009)