^{1}H-NMR Study of the Impact of High Pressure and Thermal Processing on Cell Membrane Integrity of Onions

Maria E. Gonzalez, Diane M. Barrett, Michael J. McCarthy, Frank J. Vergeldt, Edo Gerkema, Ariette M. Matser, and Henk Van As

Abstract: Proton nuclear magnetic resonance (^{1}H-NMR) relaxometry was used to study the effects of high pressure and thermal processing on membrane permeability and cell compartmentalization, important components of plant tissue texture. High pressure treated onions were subjected to pressure levels from 20 to 200 MPa at 5 min hold time at initial temperatures of 5 and 20 °C. Thermally treated onions were exposed for 30 min at temperatures from 40 to 90 °C. Loss of membrane integrity was clearly shown by changes in transverse relaxation time (T_2) of water at temperatures of 60 °C and above. Destabilization effects on membranes exposed to high pressure were observed at 200 MPa as indicated by T_2 measurements and cryo-scanning electron microscopy (Cryo-SEM). T_2 relaxation successfully discriminated different degrees of membrane damage based on the T_2 shift of the vacuolar component. Analyses of the average water self-diffusion coefficient indicated less restricted diffusion after membrane rupture occurred in cases of severe thermal treatments. Milder processing treatments yielded lower average diffusion coefficients than the controls. ^{1}H-NMR proved to be an effective method for quantification of cell membrane damage in onions and allowed for the comparison of different food processes based on their impact on tissue integrity.

Keywords: high pressure, NMR, onions, plant cell integrity, thermal processing

Introduction

The quality of fruits and vegetables is greatly related to the degree of tissue integrity. The conversion of plant-based materials into food results in changes to cell structure induced by handling, storage, and processing, and these lead to undesirable biochemical reactions and texture changes (Saltveit 1997; Garcia and Barrett 2005). Cell compartmentalization, that arises from the presence of organelles surrounded by membranes, is in fact the reason that biochemical reactions are physically limited. Texture, one of the most important quality attributes of foods, in plant materials is affected by compartmentalization, where the crisp characteristic of fruits and vegetables is imparted by cellular turgor produced by intact cell membranes supported by intact plant cell walls (Ilker and Szczesniak 1990). Knowledge of cellular and tissue transformations resulting from environmental conditions or processing is a powerful means for improving food engineers understanding of biological systems, to avoid deleterious side effects (Knorr 1994; Angersbach and others 1999; Gomez Galindo and others 2007). Methods that quantify tissue integrity changes resulting from processing will allow for shelf life and quality prediction of plant-based products since the degree of disruption of cellular structure impacts the quality, stability, and safety of food. Therefore, quantitative methods are needed in the food industry (Aguilera 2005).

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Proton nuclear magnetic resonance (^{1}H-NMR) imaging and relaxometry studies have proven to be valuable in the study of plants and plant materials submitted to stress (Van der Weerd and others 2002; Gambhir and others 2005) or processing (Hills and Remigereau 1997; Hills and others 2005; Otero and Prestamo 2009), reflecting anatomical details of the entire tissue and the water status in particular. ^{1}H-NMR relaxometry signals, which are an average over the whole sample, lead to information on the water relations of the plant tissue since the proton signal is dominated by water protons (Van der Weerd and others 2001) and the proton NMR signal intensity is directly proportional to the proton density of the tissue (Westbrook and Kaut 1993). Different compartments can be discriminated in mature plant cells, with the vacuole, a solution filled compartment, occupying up to 95% of the cell volume (Taiz and Zeiger 2006). The temporal behavior of the ^{1}H-NMR signal, obtained from the ^{1}H-NMR transverse relaxation time (T_2), can then be related to the properties of water in different locations or compartments within the tissue and to the interaction of water with macromolecules (Snaar and Van As 1992a). The water exchange rates between these compartments are controlled by the water permeability of the intervening membranes (Van der Weerd and others 2002; Scheenen and others 2002) and the water proton relaxation behavior (T_2) of the compartments will depend on the compartment morphology, permeability of surrounding membranes, exchange with macromolecules, and the intrinsic self diffusion coefficients (Van As 2007, van Duynhoven and others 2010).

Paramagnetic ions and complexes can be used to enhance T_2 relaxation of intracellular water once it is in contact with it. In magnetic resonance imaging, enhanced contrast has been obtained by using gadolinium complexes, which allow internal and external NMR signals from a variety of cells and tissues to be resolved

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(Donker and others 1997). Bertram and others (2004) successfully used gadolinium-diethylene triamine pentaacetic acid (Gd-DTPA) to probe for membrane integrity of cells in post-mortem animal muscles during an NMR relaxation study. Low doses of paramagnetic agents, such as gadolinium, enhance T_2 and longitudinal (T_1) relaxation through electron–nuclear dipolar interactions. These interactions take place only when the water molecules enter within the immediate hydration sphere of the paramagnetic ion and enhance T_2 and T_1 (Donahue and others 1997). Cell membranes of intact cells will act as a barrier and not allow exchange with the infiltrated contrast agent solutions (Shachar-Hill and others 1997; Donker and Van As 1999) impeding T_2 enhancement. Further information of the structure of raw and processed onion tissue can be obtained by studying restricted diffusion. All molecules in a fluid are subjected to Brownian motion. The pulsed field gradient NMR experiment can be used to calculate the bulk diffusion coefficient (D), which will depend on the temperature and viscosity of the fluid and the boundaries encountered that restrict diffusion of water molecules (Van As 2007). In the case where proton relaxation exhibits multi-exponential behavior, as has been shown for plant tissues, the use of multi-spin-echo diffusion sequences can be very helpful (van Dusschotten and others 1995; Duval and others 2005).

High hydrostatic pressure (Butz and others 1994; Prestamo and Arroyo 1998) and traditional thermal processing (Greve and others 1994) alter membrane structure with detrimental effects on cell compartmentalization and tissue integrity. The ability to monitor the exact changes occurring in tissue integrity may allow for process optimization and product quality. The objectives of the present study were to: (1) to verify that ^{1}H-NMR is a useful tool for the quantification of changes in cell membrane integrity after high pressure and thermal processing, and (2) to determine the range of high pressure and thermal treatments that induce changes in membrane permeability of onion tissue.

Materials and Methods

Raw materials

Yellow type onion bulbs, which were approximately 5 cm in diameter and approximately 270 g were obtained from an onion breeding company (De Groot en Slot, Langedijk, The Netherlands) and stored at 4 °C until processing. The papery scales or layers and the first fleshy scale were removed. From each onion bulb a 2-cm thick equatorial slice was obtained and used for further processing and analysis. Two control treatments were included: control 1: raw onion, no vacuum packaging, (0.1 MPa and approximately 20 °C); control 2: raw onion, vacuum packed (easy Pack, Koch, Kansas City, Kans., U.S.A.) (approximately 0 MPa; approximately 20 °C).

Processed sample preparation

High pressure treatment. Equatorial slices were vacuum-packed (approximately 0 MPa) in polyethylene bags and high pressure processed at 50, 100, and 200 MPa at unit initial temperature (T_i) 5 °C (±1 °C) or 20, 50, 100, and 200 MPa at T_i = 20 °C (±1 °C). Samples were held for 5 min at the target pressure. The high pressure unit used had a 2.5 L vessel, 200 MPa maximum-pressure level and water was the pressurizing medium (Resato, Roden, The Netherlands). The unit had water circulation for temperature control with initial temperature of the water 5 or 20 °C according to the experiment. A thermocouple was located at the top of the vessel and the temperature was recorded during build up and hold time. Examples of temperature profiles for the different pressure levels are shown in Figure 1.

Thermal treatment. Equatorial vacuum-packed slices (approximately 0 MPa) were exposed to temperatures of either 20 (raw), 40, 50, 60, 70, or 90 °C for 30 min in a water bath (Lindberg, Model SWB1122A-1, Torrance, Calif., U.S.A.). The 30-min treatment was defined by inserting a thermocouple in the center of the onion slice and measuring the time to reach the target temperature. The water bath temperature was set at target temperature so as to not surpass the desired temperature at any point in the tissue, which resulted in long processing thermal times. After thermal treatment, onion slices were cooled down in iced water and held at 4 °C.

Frozen–thawed treatment. Onion pieces obtained from the equatorial region of the bulb were sized for NMR sample tube dimensions prior to freezing because the tissue was too soft after the freeze–thaw process to permit sizing without additional extensive tissue damage. The pieces were exposed to −20 °C for 4 h and then thawed at room temperature (20 °C). The freeze–thaw cycle was repeated twice. This treatment was included as a negative control indicative of complete cell rupture.

NMR methodology

The NMR sample, a 5 × 5 mm piece obtained from the 3rd scale inwards from the outer most fleshy scale and taken from the middle portion of the onion slice, was put in a covered NMR tube after it reached room temperature.

All NMR experiments were performed using a Maran Ultra console spectrometer (Resonance Instruments, Abingdon, U.K.) operating at 30.9 MHz proton resonance frequency. Decay curves due to transverse relaxation were measured using the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence and the 90 to 180° pulse spacing was 250 μs. The number of echoes was 16384, with a recycle delay of 7.5 s and data were averaged over

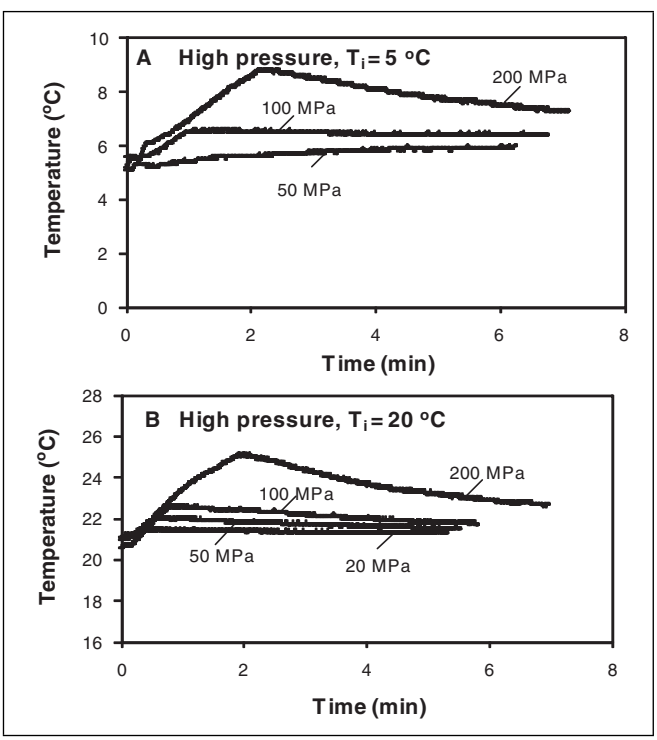

Figure 1–Temperature profile of high pressure experiments at T_i = 5 °C (A) and at T_i = 20 °C (B).

64 scans. The echo decay envelopes were analyzed as a continuous distribution of exponential relaxation times with CONTIN (Provencher 1982) to obtain T_2 distributions or into discrete decay components using SplMod (Wijnaendts and others 1982; Provencher and Vogel 1983) to determine number of components to be analyzed and for comparison of relaxation times of the different components. At least 3 replicates per treatment were evaluated with 3 to 7 replicates depending on the treatment. A general linear model procedure was used to analyze effects of the different treatments on T_2 of the main component using SAS 9.1 statistical package (SAS Insti. Inc., N.C., U.S.A.). Duncan's multiple range test for comparison of means was performed.

T_2 relaxation of raw and thermal treated samples was measured before and after vacuum infiltration in a 15 mL, 25 mM Gd-DTPA solution (Schering AG, Berlin, Germany) with T_2 7 ms. This solution was prepared from a 0.5 M Gd-DTPA stock solution before the experiment (Donker and Van As 1999). Each piece was vacuum infiltrated at 2.7 kPa pressure in a desiccator for 10 min. Three replicates per treatment were obtained. The T_2 distributions of the samples before and after infiltration were compared.

Self-diffusion coefficients of thermal and selected high pressure treatments were determined using a pulsed field gradient stimulated echo pulse sequence followed by a CPMG echo train (PFGSTE-CPMG). The pulse spacing used was 250 μs, the number of echoes was 16384 and the relaxation delay was 7.5 s. The time between the two gradients (Δ) or observation time were 20, 40, 80, 160, 320, 640, and 1280 ms and the duration of the gradients (δ) were 2, 1.5, 1, 1, 0.75, 0.75, and 0.5 ms, respectively. A multiexponential fit was used to obtain the self-diffusion coefficients of the different ^{1}H components within a sample and the weight averaged diffusion coefficient (D_{av}), relative to the amplitude of the NMR signal of the different components was calculated. The averaged diffusion coefficient was expressed relative to the diffusion coefficient of pure water (D_w) at 25 °C, (2.3×10^{-9} m^2/s) and represented as D_{av}/D_w. All measurements were duplicated.

SEM

Cryo-SEM micrographs were obtained at the Wageningen Electron Microscopy Center, Laboratory of Virology, Wageningen Univ., The Netherlands. Raw, freeze–thawed, and 200 MPa (T_i = 5 °C and T_i = 20 °C) samples were analyzed. Samples were mounted on a brass sample holder with TBS (Tissue Freezing Medium EMS, Washington, Pa., U.S.A.). The samples were frozen in liquid nitrogen and subsequently placed in a dedicated cryo-preparation chamber (Oxford Instruments CT 1500 HF, Eynsham, England). In this cryo-preparation chamber, the samples were freeze-dried for 3 min at −90 °C at 8×10^{-4} Pa to remove water vapor contamination. After 3 min, the samples were sputter coated with a layer of 10 nm Pt at the same temperature. The sample was cryo-transferred into the field emission scanning microscope (JEOL 6300F, Japan) on a sample stage at −190 °C. The analysis was performed at a working distance of 16 mm, with secondary electron detection at 3.5 and 5 kV. All images were recorded digitally (Orion, 6 E.L.I. sprl, Charleroi, Belgium) at a scan rate of 100 s (full frame) at the size of 2528 × 2030, 8 bit. The images were optimized and resized for publication by Adobe Photoshop CS4 (Adobe Systems Inc., San Jose, Calif., U.S.A.).

Percent change in weight

Sample weight of onion slices (in high pressure and thermal treatments) and onion pieces (frozen–thawed) were recorded before treatment. After treatment, the onion tissue was blotted dry to remove any excess exudates caused by the processing treatment and final weight was measured. Weight loss was calculated and expressed as the percent change in weight ([initial weight − final weight/initial weight] ∗ 100). A precision balance (Sartorius 1872, Goettingen, Germany) was used for all measurements.

Results and Discussion

Cellular tissue is characterized by the spatial dependence of its NMR parameters, and if diffusive exchange between the cell compartments is slow enough, then the distribution of relaxation times is sensitive to cell morphology and compartmentalization and T_2 peaks may be assigned (Snaar 2002). It can be expected that the cell vacuoles of intact plant tissue have the longest relaxation time and the largest amplitude within the plant cell (Snaar and Van As 1992b; van Dusschoten and others 1995; van der Weerd and others 2002) and the differences in intracellular and extracellular environments, which alter properties of the NMR signal, may lead to information on compartmentalization of the tissue system (van Dusschoten and others 1995; Ratcliffe and Shachar-Hill 2001). This study indicated that T_2 distribution was affected by the severity of the thermal or high pressure process.

T_2 relaxation of raw, thermal-treated, and frozen–thawed onion samples

The T_2 distributions obtained from CPMG experiments of raw untreated onion slices revealed several components present (5; SplMod), each originating from different proton environments within the sample (Figure 2A). The main component of largest relative amplitude (component 1) and assigned to the largest cell

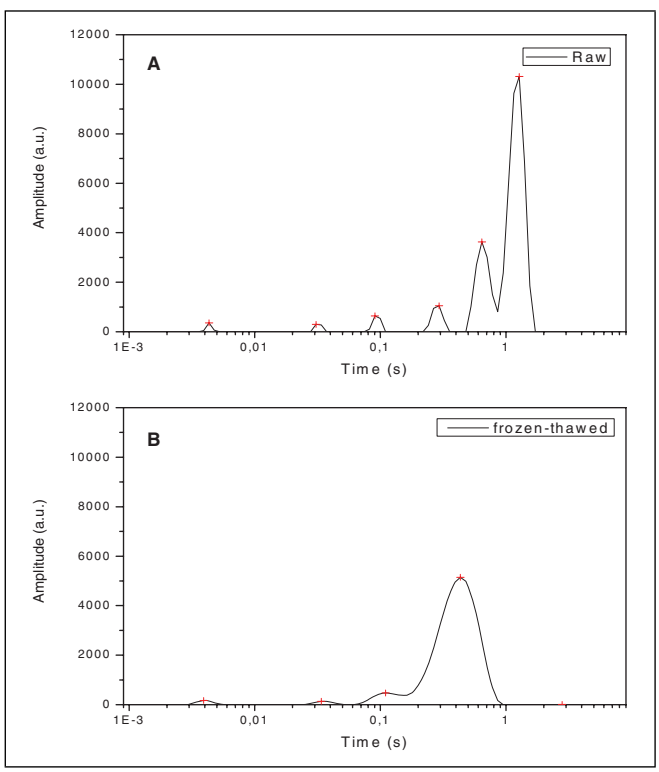

Figure 2–Examples of the distributions of T_2 relaxation obtained by CONTIN analysis of a raw (A) and frozen–thawed (B) piece of onion tissue measured at a spectrometer frequency of 30.9 MHz and a CPMG 90 to 180° pulse spacing of 250 μs.

vacuoles (Snaar and Van As 1992b), had a T_2 mean value of 1.18 s. The 2nd and 3rd components had mean values of 0.71 and 0.26 s, respectively. The identification of several T_2 components (peaks) in the raw treatment indicated that water exchange between components was slow with respect to T_2 and therefore, the cell membranes, primarily the plasma membrane and tonoplast, acted as barriers (Snaar and Van As 1992b; van Dusschoten and others 1995). Snaar and Van As (1992b) assigned peaks to the different cell components of apple parenchyma by following the uptake of Mn^{2+}, a membrane permeable paramagnetic ion, with 3 populations of water of different relaxation characteristics identified as the vacuole, the cytoplasm, and the cell wall/extracellular space. Onion tissue, characterized by epidermal cells, parenchyma cells, and vascular bundles inserted within the parenchyma, is less homogenous than apple parenchyma. The higher number of components resolved in onion tissue most probably relates to the tissue heterogeneity: the different cell types and distribution of cell sizes existing within the onion scale (Quiao and others 2005). The onion outer and inner epidermis are a single cell layer elongated in the longitudinal plane and isodiametric in the transverse plane whereas parenchyma cells are isodiametric in the longitudinal plane and slightly elongated in the transverse plane. Onion parenchyma cells vary from having a uniform size in the middle parenchyma to 1.5 times smaller in the boundary with the outer epidermis (Gonzalez 2009).

The T_2 shift in the main peak observed in the frozen–thawed treatment was indicative of a change in the molecular environment attributed to a loss in cell compartmentalization (Hills and Remigerau 1997). The frozen–thawed treatment was included as a negative control and as evidence of cell injury, where cell membranes are ruptured by the formation of ice crystals (Reid 1994) and the cell contents are released. Temperatures below −11 °C have been shown to cause irreversible damage to onion bulb cells (Palta and others 1977a, 1977b). The characteristic T_2 distribution with fewer components (3 ± 1; SplMod) of the frozen–thawed treatment is shown in Figure 2B. The T_2 of the main component of the frozen–thawed sample was shifted to 0.65 s on average and the 2nd and 3rd components had mean values of 0.36 and 0.11 s, respectively. Cryo-scanning electron micrographs corroborated T_2 results, with intact turgid cells observed in raw tissue (Figure 3A) while frozen–thawed tissue showed a complete loss of cell compartmentalization (Figure 3B). In the thermal treatments studied (30 min exposure), the 40 and 50 °C treatments resembled the raw control, while the 60, 70, and 90 °C showed a T_2 shift of the water in the main component similar to that observed in the frozen–thawed tissue (Figure 4), indicative of loss of cell

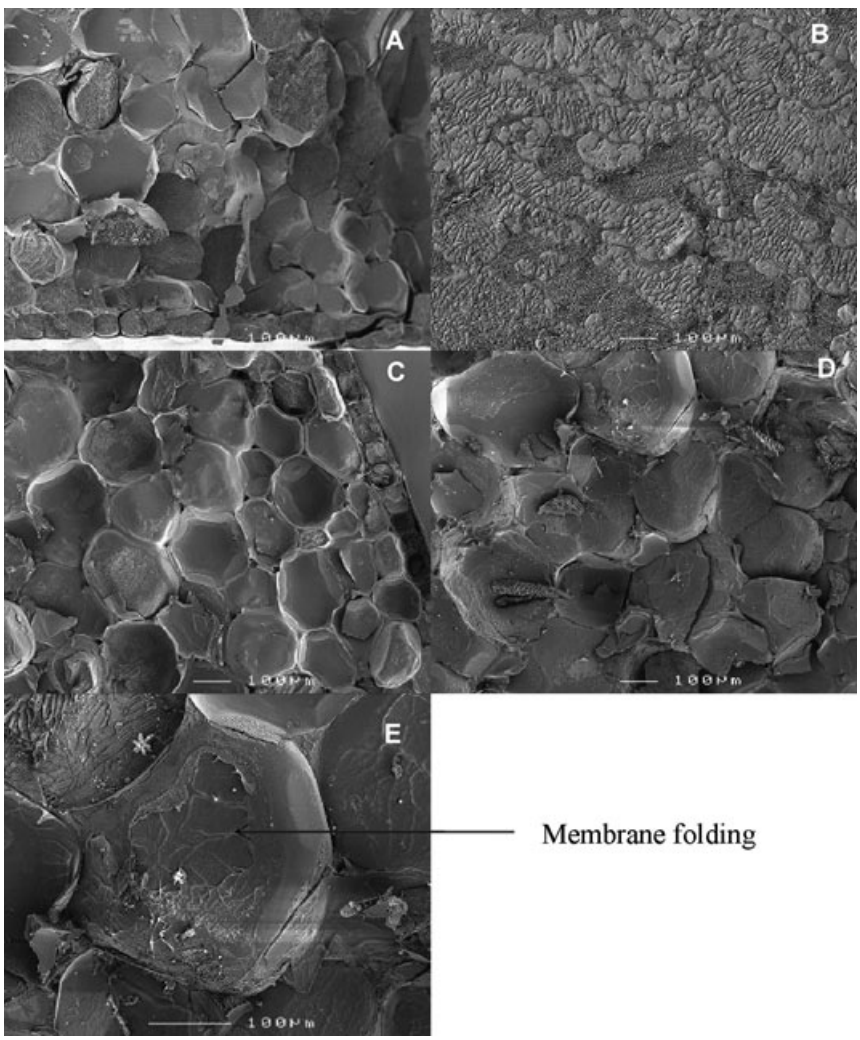

Figure 3–Cryo-SEM of onion cells. Raw tissue (A), frozen–thawed (B), high pressure at T_i = 5 °C (C), high pressure at T_i = 20 °C (D,E).

integrity and increased exchange of water between the different cell compartments.

To provide information on water distribution in onions after processing and to obtain additional knowledge of loss of cell integrity raw, thermal processed and frozen–thawed onion pieces were infiltrated with a solution of 25 mM Gd-DTPA. The Gd-DTPA solution is observed as the T_2 peak around 11 ms in the infiltrated onion piece, which is close to the measured 7 ms of the Gd-DTPA solution. Almost identical T_2 distribution profiles were observed before and after infiltration with Gd-DTPA for the raw and 40 °C (data not shown) and 50 °C thermal treatments (Figure 5). On the contrary, following thermal treatment at 60, 70, and 90 °C and frozen–thawed treatment there was a rapid exchange with the Gd-DTPA solution and a reduction in the T_2 of the intracellular water. The T_2 of the maximum amplitude peak before and after infiltration is shown in Figure 6 for all thermal treatments. The slightly higher T_2 in the raw, vacuum-packed, and 40 °C treatments after infiltration, with respect to same treatments before infiltration, may be explained by the Gd-DTPA solution filling extracellular air spaces and removing susceptibility in homogeneities and leading to increased T_2 values and constant amplitudes in the fresh tissue (Donker and others 1997; Donker and Van As 1999). In intact plant tissue, gadolinium shifted signals represent extracellular material as well as some contribution from intercellular spaces and cell walls, while intracellular signals are not shifted (Shachar-Hill and others 1997). Although Quiquampoix and others (1990) did measure some Gd^{3+} and Gd-$(DTPA)^{2-}$ uptake by maize root vacuoles with Phosphorous ^{31}P NMR, this only happens after several hours of exposure and at sub-millimolar intracellular Gd concentrations. In the present experiment, T_2 of the main component was greatly reduced at 60, 70, and 90 °C and frozen–thawed treatment as a result of the rapid exchange between the high concentration of the Gd-DTPA in extracellular water with the intracellular water, proving that the cell membranes were no longer acting as semipermeable barriers and cell integrity was lost. Gd-DTPA can be used for probing membrane integrity (Donker and Van As 1999) and it verified the results obtained with T_2, indicating that T_2 measurements alone are a valuable tool for the study of cell integrity and changes in membrane permeability in onion tissue. An increase in membrane permeability and corresponding start of the leakage of intracellular water results in a higher probability to exchange with cytoplasm and cell walls of shorter T_2.

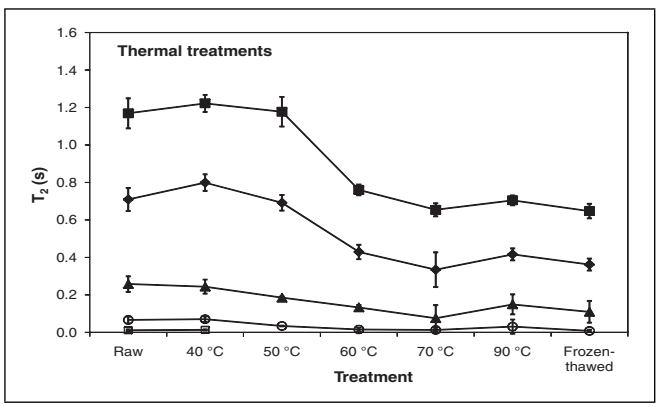

Figure 4–T_2 relaxation of the different components obtained by SplMod analysis (component 1 [■], component 2 [◆], component 3 [▲], component 4 [○], component 5 [□]) of raw, thermally treated (40, 50, 60, 70, and 90 °C), and frozen–thawed onions.

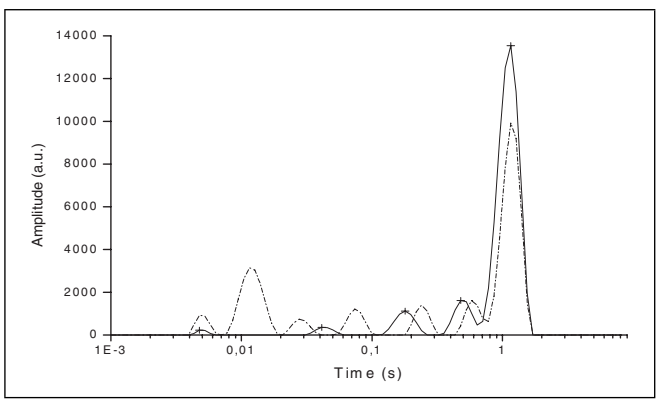

Figure 5–Example of the distributions of T_2 relaxation obtained by CONTIN analysis of a 50 °C before Gd-DTPA infiltration (black line) and after Gd-DTPA infiltration (dotted line).

T_2 relaxation of high pressure treated onion samples

T_2 relaxation was also used to determine the effects of high pressure processing on plant cell integrity and membrane permeability, and to determine the impact of 2 different high pressure unit initial temperature conditions (T_i = 5 and 20 °C). The first temperature was selected to minimize biochemical reactions that may occur as a result of temperature effects. At T_i = 5 °C high pressure experiments, the temperature did not increase above 10 °C (Figure 1A). The higher initial temperature (20 °C) reflects more common conditions in the food processing industry, since many high pressure units do not have temperature control systems. In this case, at the highest pressure level applied (200 MPa), the maximum temperature achieved during pressurization was below 26 °C (Figure 1B). Starting the pressurization with T_i = 5 °C, there was no significant T_2 shift in the pressure range from 50 to 200 MPa with respect to the raw control (Figure 7). On the contrary, when initiating the pressurization at T_i = 20 °C, there was T_2 shift at 200 MPa to 0.95 s (Figure 8). Cryo-scanning electron micrographs of 200 MPa T_i = 5 °C indicated that cells were still intact (Figure 3C) while exposure to 200 MPa at T_i = 20 °C showed initial damage with membrane folding of the plasma membrane also verified by Prestamo and Arroyo (1998) (Figure 3D and 3E).

Previous investigators have found that high pressure processing at 250 MPa caused membrane permeabilization in yeast cells that

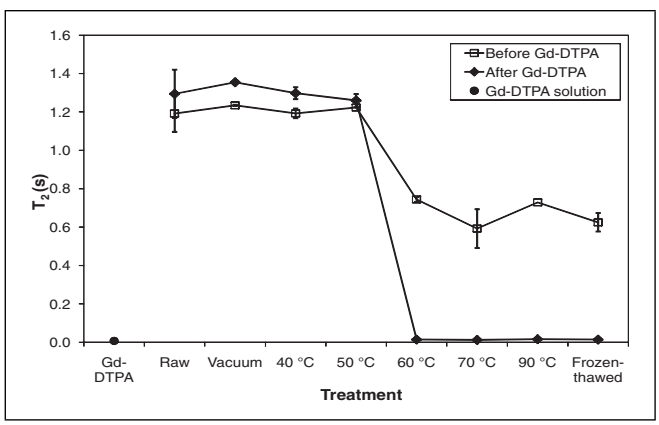

Figure 6–T_2 relaxation of Gd-DTPA solution and that of the average main component of raw, vacuum-packed, thermal (40, 50, 60, 70, and 90 °C) and freeze–thaw treated onions before and after Gd-DTPA infiltration obtained by SplMod analysis.

was not a consequence of cell death since cells were still viable, but rather a consequence of pressure-induced modification of the membrane structure (Perrier-Cornet and others 1999). In another study on *Lactobacillus plantarum* (Wouters and others 1998) at 250 MPa, the activity of ATPase, a membrane bound protein, was reduced and its ability to maintain a difference between the intracellular and extracellular pH was also reduced but no morphological changes could be observed in the membrane. Plant cells seem to be more susceptible to high pressure levels than microorganisms, since at 200 MPa T_i = 20 °C, the T_2 and SEM results in the present study already indicated membrane permeabilization. An effect of the interaction of high pressure and temperature similar to that seen here has also been observed in microorganisms (Ulmer and others 2002).

When T_2 values of the main component of all the treatments were compared, the severity of the treatment and effects on membrane integrity could be discriminated (Table 1). High pressure treated onions at pressures below 200 MPa did not differ statistically from the controls at either T_i = 5 or 20 °C. The 200 MPa, T_i = 20 °C treated onions differed statistically from 200 MPa T_i = 5 °C, with a shorter T_2 indicating less tissue damage at a lower initial high pressure unit temperature. The 200 MPa, T_i = 20 °C treated onions exhibited a less severe effect on cell compartmentalization, compared with the 60, 70, and 90 °C and frozen–thawed treatments. The 90 °C treatment, however, showed longer T_2 than 70 °C, and is clearly in the group of treatments that shows complete loss of cell integrity, as was indicated by the Gd-DTPA experiment.

Averaged diffusion coefficient analysis

Additional information on the structure of raw and processed onion tissue as well as water interactions with the rest of the plant cell material was obtained by studying the weighted diffusion coefficient (Figure 9). The pulsed field gradient NMR experiments used to determine D_{av}/D_w indicated for all treatments that at short observation times (Δ < 20 ms), in the free diffusion regime where time was not long enough for water molecules to reach cell boundaries (Anisimov and others 1998; Van As 2007), there was no difference in D_{av}/D_w among treatments.

At long observation times, thermal treatments 60 and 70 °C and frozen–thawed, showed an increase in D_{av}/D_w with respect to the raw control, which coincided with the loss of cell membrane boundaries indicated by T_2 and Gd-DTPA infiltration experiments. The 40 °C treatment, in which T_2 and Gd-DTPA showed no distortion to the membrane boundaries, had an almost identical D_{av}/D_w with the raw control. On the other hand, the 50 °C treatment, where T_2 and Gd-DTPA showed no rupture or membrane permeabilization, indicated a great decrease in D_{av}/D_w with respect to the raw control at long observation times (Figure 9A). In the high pressure study, the 50 MPa and vacuum-control treatments, which did not indicate destabilization of cell membranes as shown by T_2 results, had very similar diffusion patterns. Following the 150 MPa (T_i = 5 °C) high pressure treatment, there was an increase in D_{av}/D_w with respect to the vacuum-packed sample, though no membrane damage had been indicated by T_2 analysis between 100 and 200 MPa, T_i = 5 °C. Both the 200 MPa (T_i = 5 °C and T_i = 20 °C) treatments clearly indicated restricted diffusion with respect to the vacuum-packed control with a decrease in D_{av}/D_w at long observation times (Figure 9B).

Increased diffusion coefficients as a result of loss in membrane integrity have been observed previously. Ando and others (2009) already determined in onions after frozen–thawed treatments that water diffusion became less restricted due to cell membrane damage. Also, Hernández-Sánchez and others (2007) determined in pears with internal browning, a disorder developed during controlled-atmosphere storage leading to cell

Table 1–T_2 of the main component of the different treatments obtained by SplMod analysis.

Processing type	Pressure (MPa)	Temperature[A] (°C)	T_2 [B] (s)
Frozen–thawed	0.1	−18	0.65[f]
Thermal	0.1	70	0.65[f]
Thermal	0.1	90	0.70[e]
Thermal	0.1	60	0.76[d]
Thermal	0.1	50	1.18[ab]
Thermal	0.1	40	1.22[a]
High pressure	200	20	0.95[c]
High pressure	50	20	1.24[a]
High pressure	100	20	1.30[a]
High pressure	20	20	1.31[a]
High pressure	200	5	1.07[b]
High pressure	50	5	1.1[ab]
High pressure	100	5	1.15[ab]
Raw	0.1	Approximately 20	1.18[ab]
Vacuum packed	0.1	Approximately 20	1.21[a]

[A]Temperature indicated corresponds to the temperature of the water bath or the initial temperature of the high pressure unit accordingly to the processing type.
[B]Values with a common letter do not differ significantly (P < 0.05).

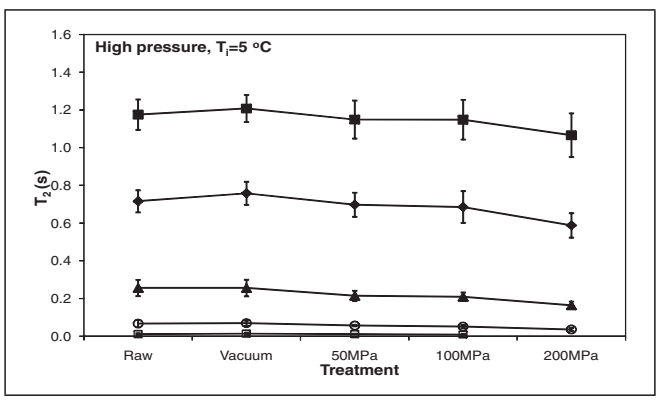

Figure 7–T_2 relaxation of the different components obtained by SplMod analysis (component 1 [■], component 2 [♦], component 3 [▲], component 4 [○], component 5 [□]) of raw, vacuum-packed, and high pressure at T_i = 5 °C treated onions.

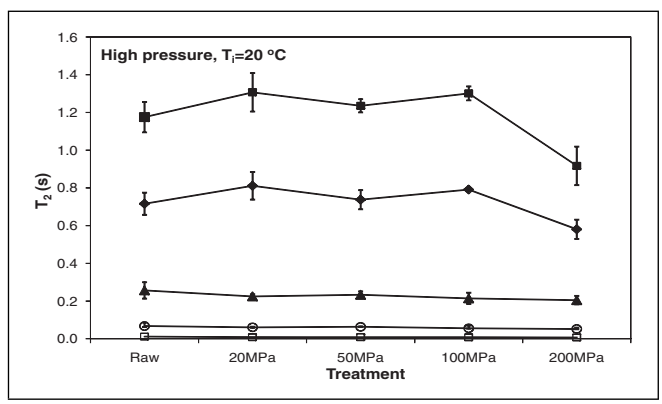

Figure 8–T_2 relaxation of the different components obtained by SplMod analysis (component 1 [■], component 2 [♦], component 3 [▲], component 4 [○], component 5 [□]) of raw and high pressure at T_i = 20 °C treated onions.

de-compartmentalization and browning reactions, using T_2-diffusion correlation spectroscopy, that the 2 compartments determined had higher diffusion coefficients than in the sound tissue. Ionenko and Anisimov (2001) with a spin–echo NMR method demonstrated that the roots of maize seedlings exposed to treatments that destroyed cell membranes, for example, nitrogen vapors, boiling water vapor, diethyl ether, and low temperature (−10 °C), had an increase in the diffusion coefficient with respect to the untreated roots. The slow diffusing component was assigned to the water transport across cell membranes as opposed to the water diffusing in the apoplast and vacuoles (Anisimov and others 1998; Ionenko and others 2006).

The decreases in D_{av}/D_w observed may be a reflection of a stress response from living cells to the adverse conditions, with water movement being restricted within the tissue. A stress response may account for the decrease in the average diffusion coefficient after the 50 °C, 30-min treatment where cells were still alive (Gonzalez and others 2010). Stress response was already observed by Ionenko and others (2006) after exposing maize root seedlings to $HgCl_2$ a water channel blocker. These researchers observed that the diffusion rate of the slow component decreased compared to the control but was re-established after a consecutive treatment with a Hg scavenging agent that unblocked the channels. Still controversial research indicates that vacuoles may be interconnected what could also regulate water permeability (Velikanov and others 2005, Andreev 2008) besides that of the tonoplast.

In the case of the high pressure treatments, a different hypothesis may be put forth for the decrease in D_{av}/D_w at 200 MPa. Changes in cell biopolymers (proteins, polysaccharides, and lipids) that are initiated during high pressure treatments have been reported. Pressure induces changes in polysaccharides, such as in protein structure (unfolding, aggregation, gelation, and so on), which can affect their functionality and the texture/structure of plant foods (Butz and others 2002a, 2002b; Cano and de Ancos 2005). Butz and others (2002a) determined that after high pressure processing, vegetable matrices experienced alterations in physico-chemical properties such as water retention. These researchers found that the water release from tomato pulp measured

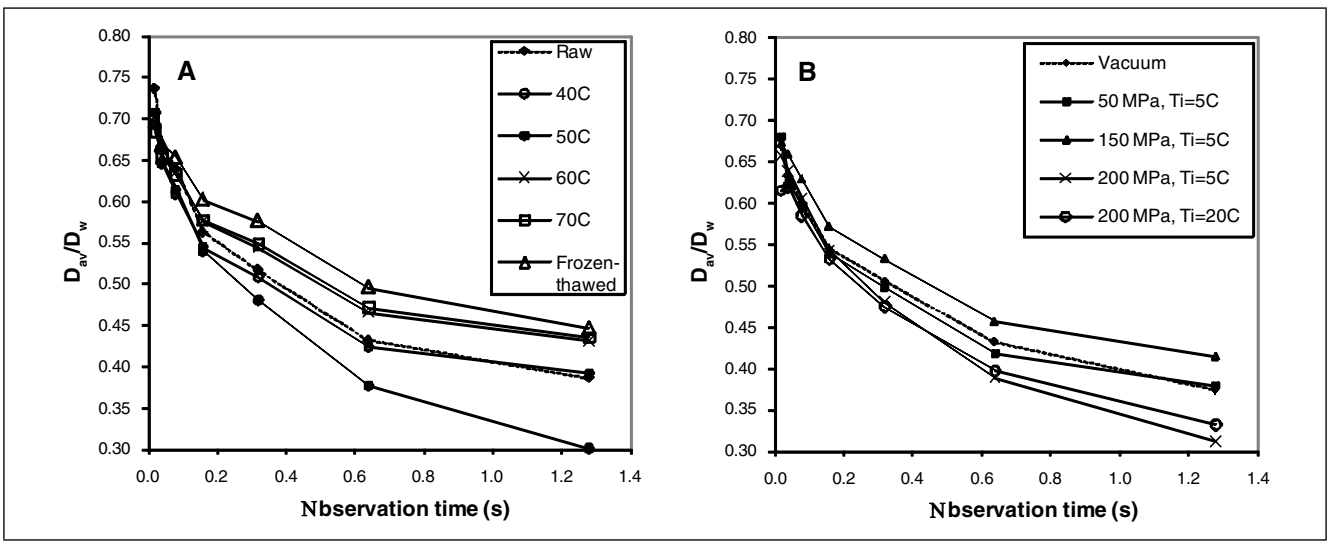

Figure 9–Dependence of weighted diffusion coefficient D_{av}/D_w on observation time for thermally (A) and high pressure (B) treated onions. Data points are the average of 2 measurements.

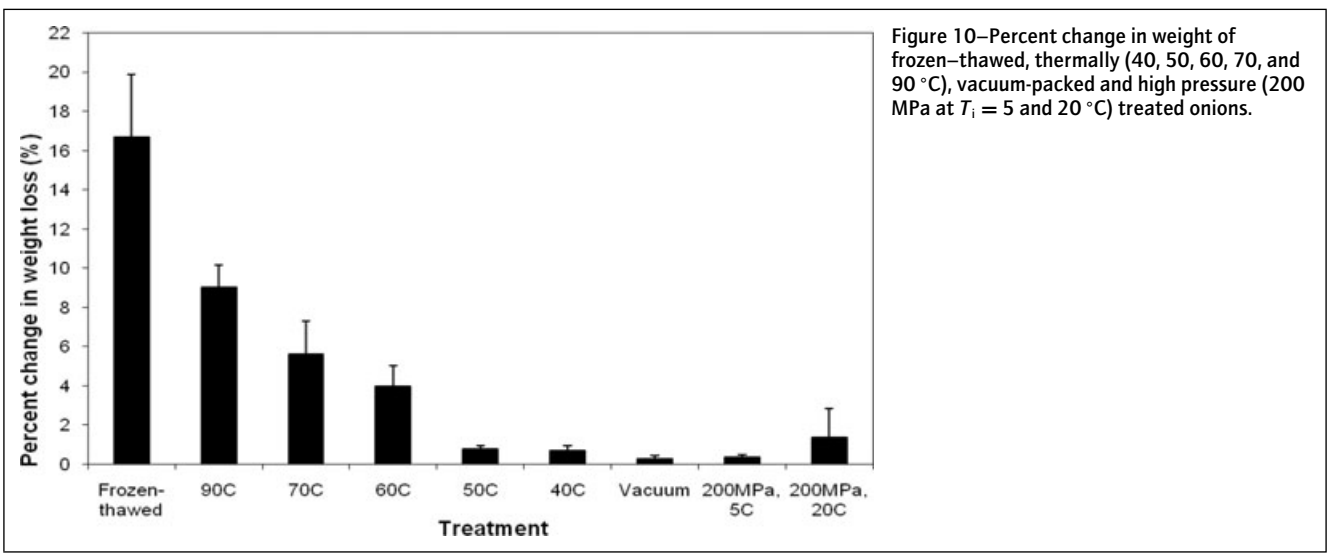

Figure 10–Percent change in weight of frozen–thawed, thermally (40, 50, 60, 70, and 90 °C), vacuum-packed and high pressure (200 MPa at T_i = 5 and 20 °C) treated onions.

after centrifugation indicated that samples treated at 600 MPa for 60 min had much lower water release than the 95 °C, 60-min treated and untreated samples. This change in the water release observed was attributed to the strong effect high pressure has on the structure of macromolecules, affecting binding properties of polar and nonpolar substances. At 200 MPa $T_i = 20$ °C, T_2 and Cryo-SEM images indicated initial membrane damage and Gonzalez and others (2010) indicated reduction in the number of viable cells compared to untreated samples. At 200 MPa $T_i = 5$ °C, there were no changes in membrane permeability or integrity observed by T_2 or Cryo-SEM. If a stress response exists, then it could also account for some reduction in water mobility.

Percent change in weight

Sample weight was recorded before and after each treatment was applied. The greatest percent of weight loss was observed in the frozen sample with an average of 16.7% (Figure 10). The weight losses observed following the thermal treatments were: 9%, 5.6%, 4%, 0.8%, and 0.7% for 90, 70, 60, 50, and 40 °C treatments, respectively, and 0.25% for the raw vacuum-packed control. For all the high pressure treatments below 200 MPa, the percentage of weight loss was insignificant. At 200 MPa, the percent weight loss was 0.3% and 1.3% at $T_i = 5$ °C and $T_i = 20$ °C, respectively. It can be observed that changes in weight loss resembled those found by T_2 measurements.

Conclusions

^{1}H-NMR proved to be an effective method for quantification of cell membrane damage in a plant tissue and may allow for comparison of different food processes based on their impact on tissue integrity. It was possible to distinguish different degrees of membrane damage among the different processing treatments based on changes in T_2 of the vacuolar component. The analysis of averaged diffusion indicated that changes in plant tissue after processing are complex and while severe treatments indicated membrane rupture, mild thermal treatments may induce stress responses while high pressure treatments may induce changes in the macromolecular conformation and hydration of the food matrix, causing less water mobility. Enhanced understanding of tissue cellular changes would be gained with diffusion analysis at pressure levels higher than 200 MPa in onion tissues.

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References

Aguilera JM. 2005. Why food microstructure? J Food Eng 67:3–11.
Ando H, Fukuoka M, Miyawaki O, Watanabe M, Suzuki T. 2009. PFG-NMR study for evaluating freezing damage to onion tissue. Biosci Biotechnol Biochem 73:1257–61.
Andreev IM. 2008. The vacuolar symplast: does it really exist in plants? (Comments on Publications of G.A. Velikanov et al. in Russian Journal of Plant Physiology). Russian J Plant Physiol 55:843–5.
Angersbach A, Heinz V, Knorr D. 1999. Electrophysiological model of intact and processed plant tissues: cell disintegration criteria. Biotechnol Progr 15: 753–62.
Anisimov A, Sorokina NY, Dautova NR. 1998. Water diffusion in biological porous systems: a NMR approach. Magn Reson Imag 16: 565–8.
Bertram HC, Stagsted J, Young JF, Andersen J. 2004. Elucidation of membrane destabilization in post-mortem muscles using an extracellular paramagnetic agent (Gd-DTPA): an NMR study. J Agric Food Chem 52:6320–5.
Butz P, Koller W, Tauscher B, Wolf S. 1994. Ultra-high pressure processing of onions: chemical and sensory changes. Lebensm Wiss Technol 27: 463–7.
Butz P, Edenharder R, Fernandez Garcia A, Fister H, Merkel C, Tauscher B. 2002a. Changes in functional properties of vegetables induced by high pressure treatment. Food Res Int 295–300.
Butz P, Tauscher B. 2002b. Emerging technologies: chemical aspects. Food Res Int 35:279–84.
Cano MP, de Ancos B. 2005. Advances in use of high pressure to processing and preservation. In: Barbosa-Cánovas GV, Tapia MS, Cano MP, editors. Novel food processing technologies. Chapter 13. Boca Raton, Fla.: CRC Press. p 283–309.
Donahue KM, Weisskoff RW, Burstein D. 1997. Water diffusion and exchange as they influence contrast enhancement. J Magn Reson Imag 7:102–10.
Donker HCW, Van As H. 1999. Cell water balance of white button mushrooms (*Agaricus bisporus*) during its post-harvest lifetime studied by quantative magnetic resonance imaging. Biochim Biophys Acta 1427:287–97.
Donker HCW, Van As H, Snijder HJ, Edzes HT. 1997. Quantitative ^{1}H-NMR imaging of water in white button mushrooms (*Agaricus bisporus*). Magn Reson Imag 15:113–21.
Duval FP, Cambert M, Mariette F. 2005. NMR study of tomato pericarp tissue by spin-spin relaxation and water self-diffusion. Appl Magn Reson 28:29–40.
Gambhir PN, Young JC, Slaughter DC, Thompson JF, McCarthy MJ. 2005. Proton spin-spin relaxation time of peel and flesh of navel orange varieties exposed to freezing temperature. J Sci Food Agric 85:2482–6.
Garcia E, Barrett DM. 2005. Fresh-cut fruits. In: Barrett D, Somogyi L, Ramaswamy H, editors. Processing fruits. 2nd ed. Boca Raton, Fla.: CRC Press. p 53–72.
Gomez Galindo F, Rasmusson AG, Widell S, Kaack K, Sjöholm I. 2007. Plant stress physiology: opportunities and challenges for the food industry. Crit Rev Food Sci 46:749–63.
Gonzalez ME. 2009. Quantifiction of cell membrane integrity and its relevance to texture quality of onions: effects of high hydrostatic pressure and thermal processes. [DPhil thesis]. Davis, Calif.: Univ. of California. 139 p.
Gonzalez ME, Jernstedt JA, Slaughter DC, Barrett DM. 2010. Influence of cell integrity on textural properties of raw, high pressure and thermally processed onions. J Food Sci. 75:E409–16.
Greve C, Shackel K, Ahmadi A, McArdle R, Gohlke J, Labavitch J. 1994. Impact of heating on carrot firmness: contribution of cellular turgor. J Agric Food Chem 42:2896–9.
Hernandez-Sanchez N, Hills BP, Barreiro P, Marigheto N. 2007. An NMR study on internal browning in pears. Postharvest Biol Technol 44:260–70.
Hills B, Remigereau B. 1997. NMR studies of changes in subcellular water compartmentation in parenchyma apple tissue during drying and freezing. Int J Food Sci Technol 32:51–61.
Hills B, Costa A, Marigheto N, Wright K. 2005. T1-T2 NMR correlation studies of high pressure processed starch and potato tissue. Appl Magn Reson 28:13–27.
Ilker R, Szczesniak A. 1990. Structural and chemical bases for texture of plant food stuffs. J Text Stud 21:1–36.
Ionenko I, Anisimov AV. 2001. Effect of water deficit and membrane destruction on water diffusion in the tissues of maize seedlings. Biol Plantarum 44:247–52.
Ionenko I, Anisimov AV, Karimova FG. 2006. Water transport in maize roots under the influence of mercuric chloride and water stress: a role of water channels. Biol Plantarum 50:74–80.
Knorr D. 1994. Plant-cell and tissue-cultures as model systems for monitoring the impact of unit operations on plant foods. Trends Food Sci Technol 5:328–31.
Otero L, Prestamo G. 2009. Effects of pressure processing on strawberry studied by nuclear magnetic resonance. Innov Food Sci Emerg 10:434–40.
Palta J, Levitt J, Stadelmann EJ. 1977a. Freezing injury in onion bulb cells. Plant Physiol 60:398–401.
Palta J, Levitt J, Stadelmann EJ. 1977b. Freezing tolerance of onion bulbs and significance of freeze-induced tissue infiltration. Cryobiology 14:614–9.
Perrier-Cornet JM, Hayert M, Gervais P. 1999. Yeast cell mortality related to high-pressure shift: occurrence of cell membrane permeabilization. J Appl Microbiol 87:1–7.
Prestamo G, Arroyo G. 1998. High hydrostatic pressure effects on vegetable structure. J Food Sci 63:878–81.
Provencher S. 1982. CONTIN: a general purpose constrained regularization program for inverting noisy linear algebraic and integral equations. Comput Phys Commun 27:229–42.
Provencher SW, Vogel RH. 1983. Regularization techniques for inverse problems in molecular biology. In: Deuflhard P, Hairer E, editors. Numerical treatment of inverse problems in differential and integral equations. Boston: Birkhäuser. p 304–19.
Quiao Y, Galvosas P, Callaghan PT. 2005. Diffusion correlation NMR spectroscopic study of anisotropic diffusion of water in plant tissues. Biophys J 89:2899–905.
Quiquampoix H, Ratcliffe RG, Ratković S, and Vučinić Ž. 1990. ^{1}H and ^{31}P NMR investigation of gadolinium uptake in maize roots. J Inorg Biochem 38:265–75.
Ratcliffe RG, Shachar-Hill Y. 2001. Probing plant metabolism with NMR. Annu Rev Plant Physiol Plant Mol Biol 52:499–526.
Reid DS. 1994. Basic physical phenomena in the freezing and thawing of plant and animal tissues. In: Mallett L, editor. Frozen food technology. 2nd ed. Glasgow, U.K.: Blackie Academic & Professional. p 1–19.
Saltveit M. 1997. Physical and physiological changes in minimally processed fruits and vegetables. In: Tomas-Barberan FA, Robins R, editors. Phytochemistry of fruits and vegetables. Proceedings of the Phytochemical Society of Europe. 41:205–20.
Scheenen T, Heemskerk A, de Jager A, Vergeldt F, Van As H. 2002. Functional imaging of plants: a nuclear magnetic resonance study of a cucumber plant. Biophys J 82:481–92.
Shachar-Hill Y, Befroy DE, Pfeffer PE, Ratcliffe RG. 1997. Using bulk magnetic susceptibility to resolve internal and external signals in the NMR spectra of plant tissues. J Magn Reson 127:17–25.
Snaar JEM. 2002. NMR of porous bio-systems [DPhil thesis]. Wageningen, The Netherlands: Wageningen Universiteit. 170 p.
Snaar JEM, Van As H. 1992a. A NMR method for the simultaneous measurement of compartmentalized spin–lattice and spin–spin relaxation times in multi compartment systems. J Magn Reson 99:139–48.
Snaar JEM, Van As H. 1992b. Probing water compartments and membrane permeability in plant cells by ^{1}H NMR relaxation measurements. Biophys J 63:1654–8.
Taiz L, Zeiger E. 2006. Plant physiology, 4th ed. Sunderland, Mass.: Sinauer Assoc. Inc.
Ulmer HM, Heberhold H, Fahsel S, Ganzle MG, Winter R, Vogel RF. 2002. Effects of pressure-induced membrane phase transitions on inactivation of HorA, an ATP-dependant multidrug resistance transporter, in *Lactobacillus plantarum*. Appl Environ Microbiol 68:1088–95.
Van As H. 2007. Intact plant MRI for the study of cell water relations, membrane permeability, cell-to-cell and long distance water transport. J Exp Bot 58:743–56.
Van Der Weerd L, Claessens M, Ruttnik T, Vergeldt F, Schaafsma T, Van As H. 2001. Quantitative NMR microscopy of osmotic stress response in maize and pearl millet. J Exp Bot 52:2333–43.

Van Der Weerd L, Claessens MMAE, Efde C, Van As H. 2002. Nuclear magnetic resonance imaging of permeability changes in plants during osmotic stress. Plant Cell Environ 25:1539–49.
van Dusschoten D, de Jager PA, Van As H. 1995. Extracting diffusion constants from echo-time-dependent PFG NMR data using relaxation-time information. J Magn Reson A 116:22–8.
van Duynhoven J, Voda A, Witek M, Van As H. 2010. Time domain NMR applied to food products. Ann R NMR S 69:145–97.
Velikanov GA, Volbueva OV, Belova LP, Gaponenko EM. 2005. Vacuolar symplast as a regulated pathway for water flows in plants. Russian J Plant Physiol 52:326–31.
Westbrook C, Kaut C. 1993. MRI in practice. Oxford, Great Britain: Blackwell Scientific Publications.
Wijnaendts RW, van Resandt RH, Vogel, Provencher SW. 1982. Double beam fluorescence spectrometer with subnanosecond resolution: Application to aqueous tryptophan. Rev Sci Instr 53:1392–7.
Wouters PC, Glaasker E, Smelt PM. 1998. Effects of high pressure on inactivation kinetics and events related to proton efflux in *Lactobacillus plantarum*. Appl Environ Microbiol 64:509–14.