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Effect of pectin methyl esterase (PME) and $CaCl_2$ infusion on the cell integrity of fresh-cut and frozen-thawed mangoes: An NMR relaxometry study

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ABSTRACT

This study describes the use of Nuclear Magnetic Resonance (NMR) relaxometry to understand the effect of pectin methyl esterase (PME) + CaCl2 infusion under different conditions on cell integrity of fresh-cut and frozen–thawed mangoes. Infusion experiments were performed at: atmospheric pressure, vacuum conditions of 50 kPa and 10 kPa. For NMR relaxometry experiments T_2 (spin–spin relaxation time), T_1 (spin–lattice-relaxation times) and 2D T_1–T_2 experiments were performed. Results showed that, as the severity of the vacuum treatment increased, the relaxation times changed significantly ($p < 0.05$). The number of compartments observed in 1-D relaxation spectra of fresh and frozen–thawed mangoes changed with different treatments. The changes in relaxation times were explained due to formation of a gel formed by the interaction of pectin and calcium. 2D T_1–T_2 relaxation maps showed that compartmentalization was retained after vacuum treatment even for frozen–thawed samples. The study showed that NMR relaxometry is a useful tool to analyze the cell integrity of mangoes exposed to different treatments.

1. Introduction

Mango (*Mangifera indica* L.) is a popular tropical fruit that is native to South Asia (Hau, Hau, Hau, & Oppen, 2003). The fruit is mostly grown in tropical climates with India being the world's largest producer (Geethalakshmi, 2011). The high demand for mangoes from other parts of the globe necessitates overseas exportation of the fruit. However, a major issue with mango and its products is texture loss, particularly in fresh cut and frozen mangoes. Fresh cut mangoes suffer from a significant reduction in firmness even with cold storage. Charles, Vidal, Olive, Filgueiras, and Sallanon (2013) reported 1.5 N of firmness loss in the 'Kent' variety of mangoes after 2 days of storage at 6 °C (Charles et al., 2013). Upon freeze thawing, Sriwimon and Boonsupthip (2011) reported approximately 50%–60% loss in texture. The deterioration in texture of frozen–thawed mangoes is related to the formation of ice crystals that damage cell walls and rupture cellular membranes, resulting in release of water and soluble components from intracellular compartments (Sriwimon & Boonsupthip, 2011).

In previous studies, to prevent loss of firmness, a number of methods have been applied such as calcium infusion, ascorbic acid treatment, pectin methyl esterase treatment, ethylene ripening, sugar infusion, exposure to pulsed light and infrared heat as well as cryogenic or air-blast freezing (Charles et al., 2013; Siddiq, Sogi, & Dolan, 2013; Sirijariyawat, Charoenrein, & Barrett, 2012; Sriwimon & Boonsupthip, 2011). Among other treatments, infusion with calcium ions and pectin methyl esterase enzyme showed positive effects on preservation of fruit texture (Draye & Van Cutsern, 2008; Fraeye et al., 2009; Guillemin et al., 2008; Pinheiro & Almeida, 2008). Pectin methyl esterase is an enzyme that cleaves the methyl groups from the galacturonic acid backbone of pectin. Calcium maintains fruit firmness by binding to the negatively charged carboxylic acid groups of the pectin molecules that are de-esterified with PME; forming what is defined as the egg-box gel model (Fig. 1) (Grant, Morris, Rees, Smith, & Thom, 1973; Pinheiro & Almeida, 2008).

Nuclear Magnetic Resonance (NMR) H^1 proton relaxometry has been widely used in the analysis of physiological and biochemical changes in fruits and vegetables (Mariette, Collewet, Davenel, Lucas, & Musse, 2007). Plant cellular tissue contains lipid membranes that compartmentalize water into subcellular structures such as the cytoplasm, vacuoles, cell walls, granules and extracellular space (B. Hills, Benamira, Marigheto, & Wright, 2004). For sound plant tissues, the presence of

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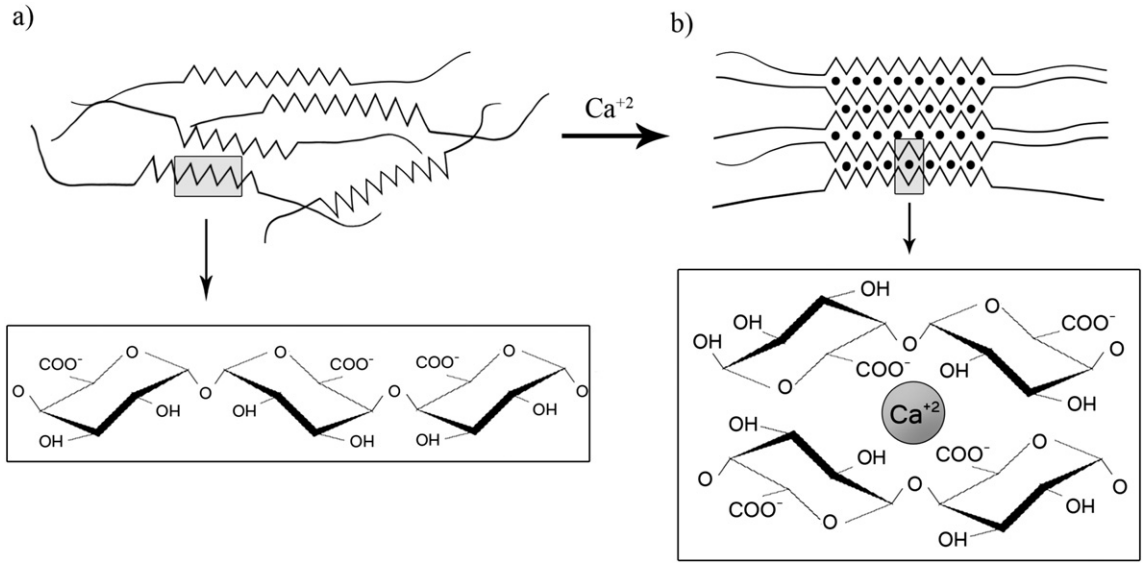

Fig. 1. A schematic representing the Ca^{+2} induced gelation of pectin. (a) Pectin molecules after de-esterification with PME, (b) egg-box model of gelation after subsequent addition of Ca^{+2} ions.

distinct permeability barriers ensure the proton exchange between these structures. This exchange is slow enough in an NMR acquisition time scale that each of these compartments is characterized by a different proton relaxation rate (Hills, 1998, 2006; Hills et al., 2004). The multi-compartment nature of plant tissue is identified by a multi-exponential relaxation in magnetization (Hills & Clark, 2003). By mathematical transformation of the relaxation decay curve, longitudinal (T_1) and transverse (T_2) relaxation times for each compartment can be obtained. Physiological changes in plant tissues are accompanied by changes in water content, distribution and integrity of subcellular compartments which will lead to variations in T_1 and T_2 relaxation times (Zhang & McCarthy, 2013). The changes in relaxation times have been used to investigate ripening, process induced damage, water loss, internal decay and disease related changes in some fruits and vegetables (Altan, Oztop, McCarthy, & McCarthy, 2011; Ersus, Oztop, McCarthy, & Barrett, 2010; Hernandez-Sanchez, Hills, Barreiro, & Marigheto, 2007; Hills & Remigereau, 1997; Marigheto, Moates, Furfaro, Waldron & Hills, 2009; Raffo, Gianferri, Barbieri, & Brosio, 2005; Zhang & McCarthy, 2012).

The use of 2D relaxation spectra assures a more sensitive analysis for compromised samples of different water compartments (Zhang & McCarthy, 2013). 2D T_1–T_2 NMR relaxometry yields information on the correlation between T_1 and T_2 times. Hence, compartments having the same T_2 values could be differentiated based on differences in T_1 (Venturi & Hills, 2010). Compartments with differing water content, composition and proton environment can then be more accurately identified by their intrinsic T_1 and T_2 values. The resolving power of a T_1–T_2 NMR spectrum is especially valuable in investigating complex systems such as plant tissues that display heterogeneity in terms of water distribution both in a macroscopic and microscopic scale (Venturi & Hills, 2010). This method has proven effective in analysis of apple mealiness (Marigheto, Venturi, & Hills, 2008), avocado quality (Marigheto, Duarte, & Hills, 2005), effect of controlled atmospheric storage on pomegranate quality (Zhang & McCarthy, 2013), internal-browning of pears (Hernandez-Sanchez et al., 2007) and starch granule distribution in potatoes (Hills, Costa, Marigheto, & Wright, 2005).

Though NMR has been used for analysis of various fruits and vegetables, there are no studies utilizing NMR for characterization of mangoes. In this study, the purpose was to determine the effects of various PME–$CaCl_2$ infusion treatments on the microstructure of fresh cut and frozen–thawed mangoes using changes in T_1 and T_2 relaxation times and to evaluate the utilization of 1D and 2D NMR relaxometry as tools for analysis of biochemical changes in mangoes.

2. Materials and methods

'Kent' mango variety of ripe mangoes (*M. indica* L.) was purchased from a local supermarket in Davis, CA, USA. The selected fruits were chosen according to uniformity in size and maturity. Maturity was evaluated based on fresh color, texture and soluble solid content. The chosen mangoes' soluble solid content was in the range of 16–19°Brix and firmness was in the range of 26–30 N. During preparation of samples, to minimize variation between samples, the highly diverse parts that correspond to approximately 20% of the total length from both the stem and the blossom end of the fruit were discarded. The mangoes were washed, peeled and cut into cubes of 1.5 cm. For infusion treatments, a stock solution of pectin methyl esterase (commercial PME from *Aspergillus aculeatus*, Novoshape, Novozymes, Bagsvaerd, Denmark) with an activity of 11.53 U/mL was used.

2.1. Infusion experiments

Infusion experiments were carried out as described by Sirijariyawat et al. (2012). As control, untreated mango cubes were used. The infusion solution consisted of 10-g/L $CaCl_2$ and 0.001 U/mL PME (Ca–PME). The PME concentration was selected based on the preliminary studies. To ensure complete immersion of sample into the infusion solution, the sample/infusion solution ratio was chosen to be 1:3 (w/w). Three levels of vacuum pressure, ranging from no vacuum (PATM; atmospheric pressure, 101.325 kPa) to a medium level of vacuum (P50; 50 kPa or 50.6% vacuum) and the highest level of vacuum (P10; 10 kPa or 90.1% vacuum), were used. In all treatments, 35 mango cubes (105 g) were immersed in the infusion solution at room temperature (25 °C) for 10 min. To submerse the samples into the infusion solution, an aluminium mesh was used. For infusion under vacuum conditions, the infusion solution containing the cubes was placed in plastic containers. The containers were then placed in a vacuum oven (Isotemp Vacuum Oven Model 280A, Thermo Fisher Scientific, Pittsburgh, PA, USA) and the vacuum level was adjusted accordingly. The samples were infused for 10 min at both 50 and 10 kPa. After 10 min the vacuum was released to attain atmospheric pressure within 1 min, with subsequent disposal of the solution. Infusion of liquid solution occurred during the vacuum release. Preliminary experiments at different infusion times were performed to determine the infusion time used in the study. Following infusion, samples were laid on paper towels for 5 min, packed into plastic bags and kept at 25 °C for 2 h before analysis or freezing. This ensured

enough time for the reaction between calcium, pectin and the enzyme to take place. Some of the mango cubes were also frozen after infusion in a chest freezer at −80 °C until the central temperature of the samples reached −25 °C (50 min) and subsequently stored at −20 °C for 14 days. Prior to NMR experiments the samples were thawed at 4 °C for 2 h and kept at 25 °C for 30 min. These samples were denoted as "FT", or frozen–thawed samples for the rest of the study. For both before and after freezing experiments an untreated control sample was also used.

2.2. 1D/2D NMR relaxometry experiments

For all treatments and for untreated mangoes (before and after freezing), cubic mangoes were cut into cylindrical pieces with a 4 mm cork borer to fit into a 10 mm NMR tube. T_1, T_2, T_1–T_2 experiments were performed in a 0.367 T (15.635 MHz) system (Spin Core Inc., Gainesville, FL, U.S.A.) with a 10 mm r.f. coil. Spin–lattice relaxation (T_1) measurements were performed using an inversion recovery pulse sequence with a delay time changing in the range of 0.5 ms to 4.61 s with 512 acquisition points, and 4 scans. T_1 data were analyzed using MATLAB. For T_2 measurements, a Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence was used with an echo time (TE) of 4 ms, 4000 echoes, and 16 scans. CYCLOPS phase cycling routine was integrated to the sequences by Spin Core Inc. (Gainesville, FL, U.S.A.). Non-Negative Least Square was (NNLS) applied to the T_2 decay curves to obtain relaxation spectra. PROSPA software (Magritek Inc., Wellington, New Zealand) was used for 1D-NNLS analysis. 2D T_1–T_2 experiments were performed with an Inversion Recovery-CPMG experiment. 2D data sets were analyzed with the NNLS GUI interface developed by the Callaghan Group (Wellington, New Zealand). The number of steps in the T_2 domain was 512 whereas in the T_1 domain 64 inversion times were used with 4 scans. Echo times and inversion times were the same as in the 1D experiments. 6 mango cubes for each treatment were used for the NMR experiments as replicates. Recycle delay in all relaxation experiments was set to 10 s.

2.3. Statistical analysis

To compare the means of the relaxation times, analysis of variance (ANOVA) with Tukey's multiple range test, was used. Differences were considered significant for $p < 0.05$.

3. Results & discussion

3.1. T_1 relaxation time measurements

Fig. 2 shows the longitudinal relaxation times for each treatment that are obtained through a monoexponetial fitting to inversion recovery data. With respect to control, T_1 relaxation times did not exhibit a significant change after Ca–PME infusion under atmospheric conditions (PATM). With increasing severity of vacuum treatment, a general decreasing trend in T_1 times was observed (though not always significant). Freezing and the subsequent thawing (FT) also resulted in an additional decrease in T_1 times. This could be attributed to a reduction in moisture content as T_1 is related to water content more directly (Van As, 2007). However, in a previous study carried out by the authors (Sirijariyawat et al., 2012) in which the same treatments were used to monitor textural changes in 'Kent' mango; after vacuum treatment with either water or Ca–PME solution, moisture content had increased. In fact, moisture content was highest in samples with a 10 kPa vacuum treatment. The increase in water penetration with increasing vacuum severity was explained by the replacement of air-filled pores inside the fruit with water, which is referred to as waterlogging (Sirijariyawat et al., 2012). The increase in moisture should likewise result in an increase in T_1 values. The decline in T_1 despite the rise in moisture content can be explained by the interaction of water with surrounding macromolecules. Ca–PME infusion causes the water to be entrapped within an egg-box shaped cross-link structure produced by the complex between carboxylic acid groups of pectin and calcium ions, which results in a gel (Draye & Van Cutsern, 2008; Grant et al., 1973; Pinheiro & Almeida, 2008). Spin–lattice relaxation time is affected by the rate of energy exchange between spinning H^1 protons and the surrounding lattice. The most efficient energy transfer occurs when the natural motional frequencies of hydrogen protons are at *Larmor frequency* (which is the frequency of the external magnetic field) (Hashemi, Bradley, & Lisanti, 2010). The H^1 protons in water spin at a higher frequency than Larmor frequency of hydrogen. As the mobility of water is restricted, the H^1 protons in water display lower motional frequencies, increasing the efficiency of the spin–lattice energy transfer which results in shorter T_1 times (Hashemi et al., 2010; Hills, 1998). In a number of studies, formation of a gel matrix has been shown to dramatically decrease relaxation times (Dobies, Kusmia, & Jurga, 2005; Fyfe & Blazek, 1997; Knorgen, Arndt, Richter, Kuckling, & Schneider, 2000; Oztop, Rosenberg, Rosenberg, McCarthy, & McCarthy, 2010). Thus, it is safe to conclude that the decrease in T_1 times due to gel formation was high enough to compensate for the effect of increasing moisture content. The fact that, P10 samples exhibited the highest PME activity but also has the shortest T_1 is in agreement with this observation (Sirijariyawat et al., 2012). Mean T_1 time of each sample, thus, could be used to gain an insight on the degree of gelation.

3.2. 1D T_2 relaxation spectra

One dimensional T_2 relaxation measurements are known to yield information on water content, physical properties of water and interaction of water with the surrounding macromolecules (Zhang & McCarthy, 2012). The variations in relaxation spectrum can be used as an indicator for numerous proton related changes within food systems such as an increase in water content, proton exchange between compartments and physiological incidences that causes new protons pools

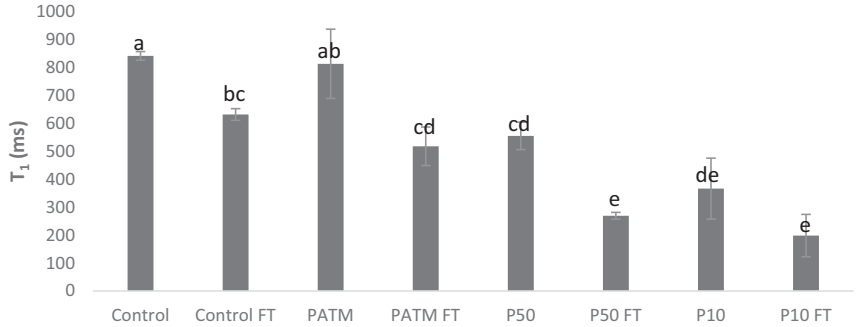

Fig. 2. T_1 relaxation times (mono-exponential fitted) of mango samples after different treatments. Untreated (Control), control after freeze thawing (Control FT); samples infused with 0.001 U/mL of PME + 1% (w/w) $CaCl_2$ solution at atmospheric pressure (PATM), at atmospheric pressure after freeze thawing (PATM FT); at 50 kPa (P50), at 50 kPa after freeze thawing (P50 FT); at 10 kPa (P10), at 10 kPa after freeze thawing (P10 FT).

to emerge (Belton & Capozzi, 2011; Ersus et al., 2010; Hills, 1998; Oztop et al., 2010). For living cells, these proton pools refer to water compartments within the cell (Belton & Capozzi, 2011; Ersus et al., 2010). Fig. 3 shows 1D NMR T2 relaxation spectra of a representative control mango sample. In all of the fresh cut mango samples (control), a total of 4 peaks were observed with each peak referring to a different water compartment. The number of compartments was consistent with the previous studies that used T_2 relaxation spectra for characterizing fruit and vegetable tissues (Hernandez-Sanchez et al., 2007; Marigheto et al., 2008; Musse, Quellec, Cambert, et al., 2009; Musse, Quellec, Devaux, et al., 2009; Zhang & McCarthy, 2012, 2013). According to Fig. 3, and Table 1 the peak with the smallest relative area and short T_2 of 92 ms (Peak 2) is coming from the protons that are associated with the more rigid components of the cell wall. The water in the cell wall resides in small pores and is tightly held by surrounding matrix and water-holding sites (Black & Pritchard, 2002). Close proximity of water and macromolecules accompanied by restriction in water mobility increases the proton exchange between water and the neighboring molecules resulting in shorter T_2 values. The 4th peak with the longest T_2 and relative area was assigned to the vacuole. Vacuoles are the water reservoirs of plant cells embodying 50–80% of cellular water (Kramer, 1983). The water in vacuoles is not highly concentrated with water binding molecules such as sugars and protein, so is in a highly mobile liquid state (Zhang & McCarthy, 2013). Hence, vacuole water should display the longest T_2 relaxation times. Cytoplasm water, on the other hand, is a part of the gel-like cytosol matrix (Raffo et al., 2005) and is expected to exhibit intermediate T_2 relaxation times. Therefore, Peak 3 was assigned to the cytoplasm. The average T_2 of the 1st peak is 28 ms (Table 1). This is too short to be assigned as a water compartment, being around the T_2 relaxation times of solids (Hashemi et al., 2010). Thus, the 1st peak might refer to protons associated with sugar or protein molecules. However, this assignment is made with a higher level of uncertainty. Still a number of studies involve a similar assignment of sugar, starch and protein peaks to T_2 values this short (Hernandez-Sanchez et al., 2007; Hills et al., 2004, 2005).

Precise assignment of peaks might pose some challenges due to cell diversity in terms of size, shape and structure. Moreover, problems such as overlapping peaks and averaging of the signal due to diffusion of water between organelles, can further complicate the assignment (Hills et al., 2004). The resolution of the data might limit the ability to distinguish signals from oil and water in the T_1–T_2 domain and could result in overlapping peaks. The T_2 assignments were made assuming that the diffusive exchange between compartments is slow on the NMR acquisition time scale. However, though slow, the presence of diffusion causes an averaging of the intrinsic relaxation times. This intermediate exchange regime could cause deviations from true relaxation times

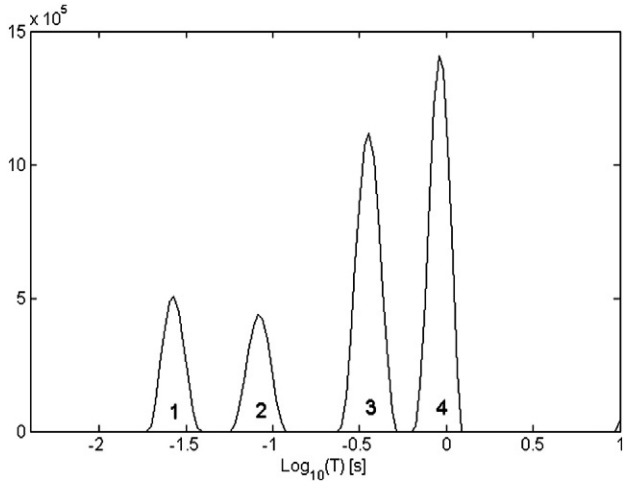

Fig. 3. A representative 1D NMR spectrum of a fresh-cut mango sample (control).

Table 1
Average T_2 relaxation times and percent relative areas (RA) of mango samples after different treatments. Untreated (Control); samples infused with 0.001 U/mL of PME + 1% (w/w) $CaCl_2$ solution at atmospheric pressure (PATM), at 50 kPa (P50), at 10 kPa (P10).

Treatment	Fresh cut			Frozen–thawed	
		T_2 (ms)	RA (%)	T_2 (ms)	RA (%)
Control	Peak 1	28	15%	31	7%
	Peak 2	92	14%		
	Peak 3	379	34%	220	93%
	Peak 4	930	38%		
PATM	Peak 1	22	13%	20	11%
	Peak 2	99	18%	171	79%
	Peak 3	436	22%	367	10%
	Peak 4	958	46%		
P50	Peak 1	30	15%	16	15%
	Peak 2	81	10%	56	43%
	Peak 3	291	24%	136	35%
	Peak 4	736	50%	444	8%
P10	Peak 1	31	17%	18	20%
	Peak 2	84	10%	79	49%
	Peak 3	283	46%	189	25%
	Peak 4	747	27%	513	6%

making it harder to assign the compartments to subcellular units (Hills et al., 2004; Marigheto et al., 2008; Zhang & McCarthy, 2013). Nevertheless, this averaging effect can be used as a tool for analysis. Loss of cell integrity is accompanied by the disruption of subcellular membranes that act as permeability barriers to proton exchange. In the presence of a fast diffusive exchange regime, the previously distinct peaks will merge and the number of peaks in the spectrum will decrease, thus pointing to a change in the cellular structure of the sample tissue (Hills, 1998; Hills et al., 2004).

In Table 1, the spin–spin relaxation times (T_2) and the percent relative areas (RA) that correspond to each peak in the relaxation spectrum (Fig. 3) are shown. The RAs are calculated based on the magnitude of signal intensity coming from each proton pool (Oztop et al., 2010). Relative areas denote the contribution of that component to the overall signal.

3.2.1. Effect of freeze–thawing on untreated fresh-cut mango samples

For untreated mango samples, the number of peaks appearing in the relaxation spectrum decreased from 4 to 2 after freeze thawing (Table 1) with 93% of the signal coming from one, 7% from the other. This decrease in peak number refers to a de-compartmentalization within fruit cells. The T_2 of the Peak 1 that remained after thawing was not different from the Peak 1 of control (that is assigned to sugar) ($p > 0.05$). The second peak of the frozen–thawed samples was observed at a T_2 value that is significantly different from all four peaks of the control and was marginally larger in a relative area. The results clearly indicated a disruption in cell structure. Crystal formation during freezing is known to cause irreversible damage to cellular compartments (Chassagne-Berces et al., 2009). The fact that only peak 1 remained after FT implies the rupture of internal cellular membranes along with the cell walls. The water that is now highly mobile merges and presumably forms the larger proton pool, surrounded by the remains of cell walls and membranes. In a previous study carried out by the authors, using the exact same treatments to monitor textural changes in 'Kent' mango, the control sample exhibited a marginal decrease in firmness after freeze thawing. This was attributed to total disruption of cell integrity, which was confirmed with confocal microscopy images (Sirijariyawat et al., 2012). This result is consistent with the relaxation spectrum obtained in this study for the control samples.

3.2.2. Effect of calcium–PME infusion under atmospheric pressure on fresh cut and frozen–thawed mangoes

Following Ca–PME infusion of mango slices at atmospheric pressure (PATM), in the relaxation spectrum, all 4 peaks were preserved with only minor changes in T_2 times and relative areas (Table 1). While the

relative area of the cell walls did not change significantly ($p > 0.05$), the relative area of the vacuoles displayed a dramatic increase. This can be explained by the water uptake into the cells during Ca–PME infusion. It is normal for swollen cells to store water inside the vacuoles, thereby increasing signal (and RA for that compartment). Nevertheless, looking at these values, it is possible to say that Ca–PME infusion at atmospheric pressure did not alter cell structure significantly. However, after FT treatment of PATM samples, 3 peaks were observed in the relaxation spectrum. Peak 1 which is assigned to protons associated with sugar and starch molecules remained intact as expected. The signal from the cell walls, on the other hand, completely vanished. This suggests that crystal formation during freezing might have caused total disruption of the cell walls, thereby liberating the water that was previously tightly packed inside rigid compartments. As seen in Table 1, the disruption of all subcellular structures gave rise to formation of a new peak that confers significantly different T_2 times (of 171 ms) and a very high signal intensity (79% of total peak area). This new compartment might be related to the change in compartmentalization resulting from to Ca–PME infusion. The fact that the 171 ms relaxation time is quite close to the 200 ms relaxation time of a Ca^{+2} induced low methoxy pectin gel solution, further supports this (Dobies et al., 2005). PME can de-esterify pectin by cleaving the methyl groups. Carboxylic acid groups of the resulting low methyl esterified pectin can then form complexes with Ca^{+2} ions resulting in an egg-box pattern that can entrap water (Draye & Van Cutsern, 2008; Sirijariyawat et al., 2012). The entrapment of water within a continuous solid matrix is crucial to fruit texture, and is highly related to the firmness of fruits and vegetables (Fraeye et al., 2010; Ortiz, Graell, & Lara, 2011). In a previous study by the researchers, samples that had been infused with Ca–PME at atmospheric pressure had higher firmness (though not significant with $p > 0.05$) than the control. Samples infused with water at atmospheric pressure instead of the Ca–PME solution (water control samples) displayed considerably lowered firmness. Nevertheless, Ca–PME infusion was merely enough to compensate for the loss in texture due to water penetration into the fruit. This finding and the NMR results of the FT samples, to a certain extent indicated that Ca–PME infusion was mildly effective at preserving the firmness of mango slices after freezing (Sirijariyawat et al., 2012). However, regardless of firmness, PATM treatment was ineffective in preserving the cellular structure of mangoes after freeze thawing.

3.2.3. Effect of calcium–PME infusion under 50 kPa vacuum on fresh cut and frozen–thawed mangoes

Ca–PME infusion under 50 kPa vacuum (P50) resulted in some minor changes in relative areas and T_2 times of all 3 peaks assigned to subcellular compartments. T_2 times coming from all compartments (except peak 1) displayed significant decreases ($p < 0.05$). Additionally, the peak area of the 4th compartment increased by 30%. An increase in relative area is an indication of growth in size for that particular compartment. The 4th compartment was assigned to vacuoles, and the increase in area of peak 4 can be related with increasing moisture content due to increased rate of water penetration into open pores under vacuum. The reduction in T_2 times were to be expected as a result of entrapment of water inside the Ca^{+2}–pectin continuous matrix. Nevertheless, the general similarity of NMR relaxation spectrum of the 50 kPa vacuum treated samples with the control clearly demonstrates the minimal effect of vacuum treatment on the cellular structure of mangoes.

Unlike Ca–PME treatment under atmospheric pressure, this time after freeze thawing, all four peaks were conserved, yet with significant reduction in T_2 values. Additionally, the relative areas of all water compartments showed significant changes. All water compartments seemed to be intact, even after freeze thawing; this hints at an increase in effectiveness of vacuum treatment in preserving mango cellular tissues. The cell wall peak seems to be preserved despite some minor changes in T_2 relaxation time and relative area. Unlike PATM treatment; no new peak formation, that could be associated with water accumulation in the extracellular space, was observed. This likely indicates that cell walls though damaged, still were intact even after freeze thawing. Some water seems to have diffused from the vacuoles into the cytoplasm. While the cell wall seems to preserve its integrity, the more fragile structure of tonoplast membrane most likely was damaged during freeze–thawing releasing water (Lüttge, 1993). Water loss from vacuoles might be accompanied by a shrinkage in tonoplast membrane, which could result in an increase in solute concentration (Santagapita et al., 2013). This explains the decrease in T2 time of the vacuole peak (peak 4). The decrease in T_2 times for the cytoplasm can be explained by the increased rate of proton exchange between the cellular compartments and macromolecules forming the egg-box crosslinks (Hills, 1998). Still the fact that vacuum infusion at 50 kPa preserved all 3 subcellular compartments is a positive indication for texture conservation. This outcome is in agreement with the previous study conducted by the authors, that reported significantly higher firmness for Ca–PME infusion under 50 kPa vacuum compared to control samples (Sirijariyawat et al., 2012).

3.2.4. Effect of calcium–PME infusion under 10 kPa vacuum on fresh cut and frozen mangoes

The relaxation spectrum results for Ca–PME infusion under the highest vacuum treatment (10 kPa pressure) (P10) followed similar trends to those with 50 kPa vacuum treatment (P50). The T_2 times of all 4 peaks in P50 treatment were not significantly different from the T_2 times of P10 treatment ($p > 0.05$). One crucial difference between the two is in the cell wall relaxation times. For P10, the decrease in T_2 time of the cell wall (peak 2) is not significant ($p > 0.05$), whereas the cell wall of P50 samples after freeze–thawing exhibited 31% decrease in T_2 times. In a previous study by the authors, P10 treatment displayed around 45% higher PME activity compared to P50 which is explained by the higher rate of enzyme and calcium diffusion into the fruit. The high PME activity could result in a higher degree of de-esterification of methyl groups from galacturonic acid chains which, when coupled with a higher Ca^{+2} ion concentration, most likely led to elevated degrees of gelation. This potent gel matrix might have helped preserve cell wall integrity more properly. Compared to P50, in P10 all other compartments similarly displayed significantly shorter reduction in T_2 times after FT ($p < 0.05$). Area distributions also followed similar trends with smaller deviations from control compared to all other treatments. All these indicate a positive correlation between texture preservation and vacuum treatment severity. However, in the previous study by the authors, the firmness of the P10 samples was found to be the same as that of the control, indicating no positive effect of P10 treatment on texture. Yet this was explained by the increased crystal formation due to the higher rates of penetration of water into the fruit pores (Sirijariyawat et al., 2012). Though not accompanied by a higher firmness, 1D T_2 relaxation spectra analysis demonstrated that P10 treatment seems to be the most successful treatment in preserving mangoes' cellular structure.

3.3. 2D relaxation profiles

2D relaxation spectra of a system yields a unique T_1–T_2 profile that acts as a finger-print for water distribution inside the tissue (Venturi & Hills, 2010). Mangoes' T_1–T_2 correlation spectra are given in Fig. 4. The diagonal dashed lined represents the condition where $T_1 = T_2$. The peaks above the $T_1 = T_2$ line is removed from the correlation graphs since it is not possible for the spin–spin relaxation time, T_2, to be larger than the spin–lattice relaxation time, T_1. These peaks, called exchange peaks, arise as a result of mathematical transformation of data and imply spectral densities whose signals do not refer to any physical compartment inside a sample (Hills et al., 2004).

Water in the absence of solutes, tend to have a T_1/T_2 ratio that is very close to unity (Song, 2006). In the presence of chemical and diffusive exchange between water and cell metabolites, T_1/T_2 ratio gets larger. In the T_2 correlation spectrum of fresh-cut mangoes (Fig. 4a), the peak with the largest T_2 and a low T_1/T_2 ratio was assigned to the vacuole

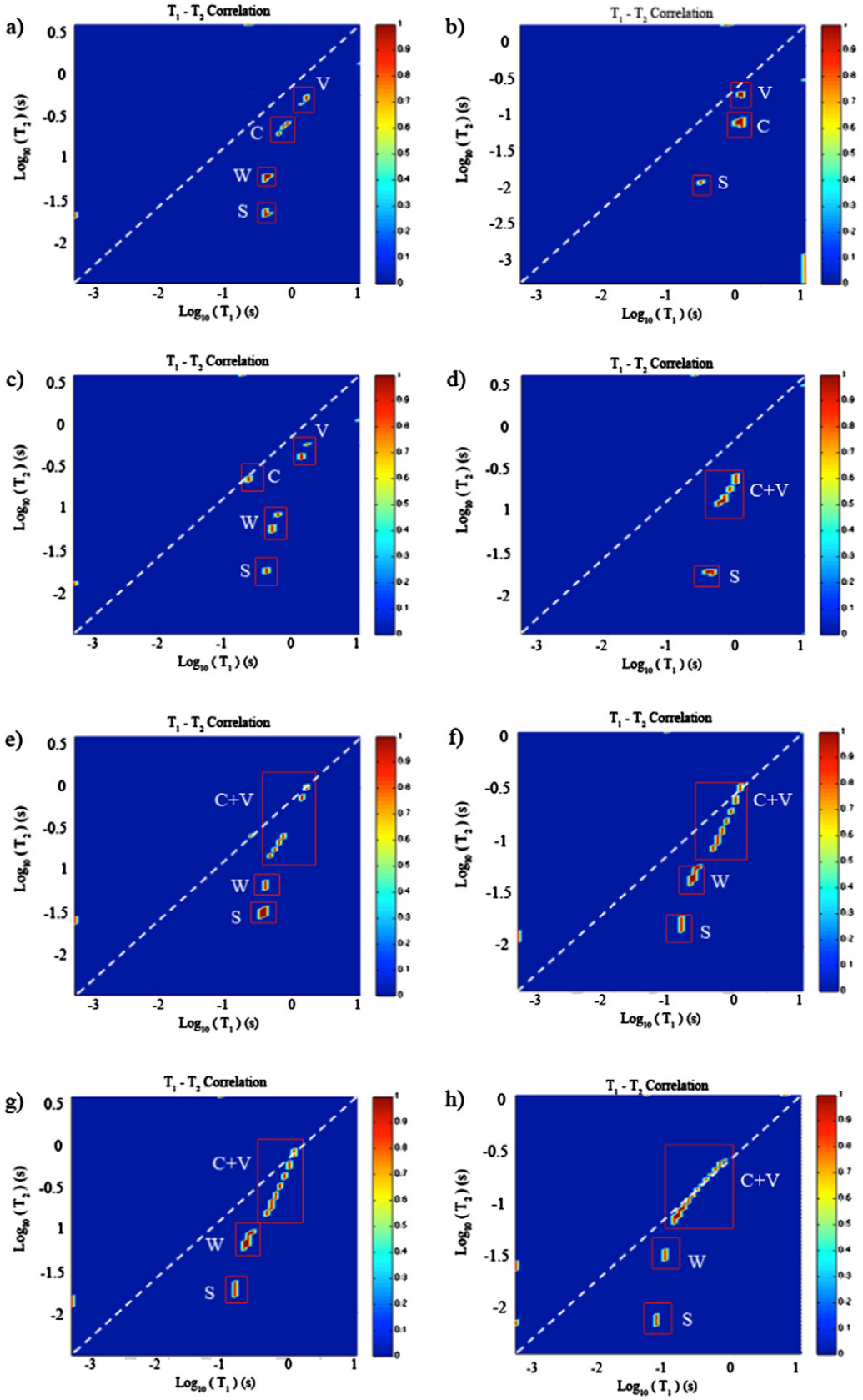

Fig. 4. 2D profiles of relaxation times (x axis: log10 (T_1), y axis: log10 (T_2)) for (a) Untreated (control) (b) Control samples after freeze thawing; samples infused with 0.001 U/mL of PME + 1% (w/w) $CaCl_2$ solution at (c) PATM (d) PATM after freeze thawing; at (e) 50 kPA (f) 50 kPa after freeze thawing; at (g) 10 kPa (h) 10 kPa after freeze thawing.

(shown with "V") since water inside the vacuole is expected to have the highest mobility, hence a slow relaxation rate. The peak below the vacuole peak (shown with "C" in Fig. 4a), had shorter relaxation times and a higher T_1/T_2 ratio. This is the case when interaction of water with macromolecules such as proteins restrains water molecules' rotational frequency and increase the efficiency in magnetic energy transfer (Hashemi et al., 2010). Due to the protein gel-like nature of the cytosol, this peak was assigned to the cytoplasm. The remaining peaks have much higher T_1/T_2 ratios and were assigned to cell wall and sugar (shown with "W" and "S" in Fig. 4a, respectively).

A common observation in all treatments was that after FT, T_2 seemed to decrease. For live tissues, T_2 relaxation time decreased with increasing membrane permeability and size of the cellular compartment (Van As, 2007). Membrane alteration and tissue disruption, owing to crystal formation during freezing, appear to be the main causes of this observation. In control and PATM treatment samples after FT (Fig. 4b & c) the number of distinct peaks decreases. This merging of peaks shows that the exchange of water between cellular compartments is quite fast considering the NMR time scale and suggests that the mass transfer rate limiting barriers have disintegrated (Hills & Remigereau, 1997). For all Ca–PME infusion treatments under vacuum (P50 and P10), the cell wall peak seemed to be conserved with minor changes in T_1/T_2 ratios.

What is worth noting is that, both before and after FT, enzyme infusion seems to cause formation of smaller peaks aligned close to and along the $T_1 = T_2$ diagonal line (shown with "C + V" in Fig. 4). This implies a major change in subcellular compartmentalization. The T_2 and T_1 times of these peaks were highly distributed within the range of 300–120 ms and 250–550 ms., which is close to the relaxation times of calcium ion, induced pectin gels (Dobies et al., 2005; Kerr & Wicker, 2000). These numerous sequentially placed peaks all might refer to pectin gel induced compartments with varying sizes and proton environments which explains the small variations in T_1 and T_2 times. The fact that these are observed in all the Ca–PME induced samples supports this conclusion. Still with the data at hand, it is hard to make 100% confident comments about the source of these peaks. The utilization of a paramagnetic ion could have helped make more concrete assignments in the T_1–T_2 spectrum. Paramagnetic ions, such as Mn^{+2}, enhance the relaxation rate; hence lowering the relaxation time of compartments they can penetrate into (Snaar & Vanas, 1992). Comparison of the degree and order of Mn^{+2} penetration for different proton pools could help assign them to cellular compartments. The method is used by Oztop et al. successfully in characterization of onion tissue by H^1 NMR relaxometry (Ersus et al., 2010).

4. Conclusion

NMR relaxometry was successful in providing insight into the extent and type of disintegration taking place within mango tissues by freezing followed by various pretreatments. The combined information gathered from 1D and 2D relaxation profiles demonstrated the effects of various pretreatments on the texture of mangoes. Infusion of PME and calcium was confirmed to maintain the cellular structure of both fresh cut and frozen mangoes. Additionally the highest vacuum level used for Ca–PME infusion (at 10 kPa) was shown to be the most suitable pretreatment for preservation of cell structure upon freeze thawing. All the information gathered from 1D and 2D NMR experiments was in agreement with a previous study carried out in our laboratory. Hence, this study demonstrated that 1D/2D NMR experiments are useful tools in investigating cellular changes and evaluating the impact of various treatments in the preservation of fresh-cut mangoes.

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