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Water and cell wall contributions to apple mechanical properties

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ABSTRACT

Relations between the apple cortex viscoelastic properties, water dynamics, histological, and chemical characteristics were investigated. Water mobility in four apple genotypes was studied by low-field NMR relaxometry prior and after plasmolysis of the cortex tissue. A discrete and a continuous method for decomposing the multi-exponential T_2 curves were implemented and compared. The results show that both methods of relaxation curve decomposition had close ability to discriminate genotypes before and after plasmolysis. Although the sensitivity of T_2 relaxometry allowed distinguishing microstructures among genotypes even after cellular fluids were mixed and diffused in plasmolyzed tissues, no relaxation component correlated with apple viscoelasticiy. Galactose and arabinose cell wall content were correlated with the storage modulus (E′) prior and after plasmolysis though the correlation signs were opposite and pointed to a potential key role of pectin RGI side chains in regulating apple texture in turgid tissue.

1. Introduction

Texture is one of the major fleshy fruit quality which variation orients consumer choice and affects processing. Improvement of fruit texture relies on instrumental and/or sensory evaluation of genetic collections but the complexity of structures and associated mechanical and physicochemical characteristics underlying this trait makes it difficult to identify genetic markers for selection strategies (Ben Sadok et al., 2015; Longhi, Moretto, Viola, Velasco, & Costa, 2012). Cell walls, cell turgor pressure (Harker, Redgwell, Hallett, Murray, & Carter, 1997) and tissue architecture through size, shape and distribution of cells and intercellular spaces play central roles on fruit mechanical properties and texture perception (Gálvez-López, Laurens, Devaux, & Lahaye, 2012; Ting, Silcock, Bremer, & Biasioli, 2013; Winisdorffer, Musse, Quellec, Barbacci et al., 2015). Cell wall composition and structure received a lot of attention and several enzymes and proteins were identified in the rearrangement and disassembly of the cell wall polysaccharides during fruit softening on ripening (Goulao & Oliveira, 2008). Cell turgor pressure, which has a major effect on fruit firmness (Oey et al., 2007) decreases during fruit ripening possibly due to an increase in solute concentration in the apoplast impacting tissue osmotic equilibrium (Wada, Matthews, & Shackel, 2009) combined with wall relaxation due to its disassembly. The interrelation between cell water compartmentalization, turgor pressure, cell wall mechanical properties and texture has rarely been addressed in texture related works due to the difficulty in assessing some of these variables. In particular, the contribution of cell turgor pressure to tissue mechanical properties is difficult to measure and often passed-by by equilibrating fruit tissues in an osmoticum prior to mechanical measurements (Oey et al., 2007; Videcoq et al., 2017).

NMR relaxometry (Time Domain NMR, TD-NMR) probes water mobility in biological tissue and macromolecular matrices (Foucat & Lahaye, 2014; Hills, 2006; Musse et al., 2009). The microstructure heterogeneity in these systems impacts water diffusion and interactions resulting in complex longitudinal (T_1) and transverse (T_2) exponential relaxations of the NMR signal decay (Furfaro et al., 2009; Snaar & Van As, 1992). In fruit parenchyma, these curves can be decomposed into several components with distinct relaxation times and proportions depending on acquisition parameters and data treatments (Hills & Remigereau, 1997; Marigheto, Venturi, & Hills, 2008). Variations in these components have been used to report on plant tissue microstructural features through a model of water compartmentalization (Snaar & Van As, 1992), on solute content (Marigheto, Wright, & Hills, 2006), and on cell volume in relation to vacuole size (Musse et al., 2013). It has also been studied with regard to fruit texture defects, such as apple mealiness (Marigheto et al., 2008) and to processing (Hills & Remigereau, 1997; Mortensen, Thybo, Bertram, Andersen, & Engelsen, 2005). Recent development in Magnetic Resonance Imaging (MRI) technic allowing extraction of T_1 and T_2 components in localized volumes of tissue represents great value for the non-destructive characterization of fruit microstructural and physicochemical features controlling qualitative traits (Adriaensen et al., 2013; Herremans et al.,

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2014; Winisdorffer, Musse, Quellec, Barbacci et al., 2015; Winisdorffer, Musse, Quellec, Devaux et al., 2015). Applied to texture-contrasted apple, water with long transverse relaxation time was correlated with fruit firmness while the amount of water with short transverse relaxation time was correlated with soft to mealy fruit (Winisdorffer, Musse, Quellec, Devaux et al., 2015). However, the interpretation of the spatial variations of relaxation parameters requires a better understanding of the water environments modulating them. In this work, in line with the MRI study (Winisdorffer, Musse, Quellec, Devaux et al., 2015) the more accessible low-field TD-NMR spectroscopy technique was tested as a phenotyping tool on three apple varieties and one experimental genotype. The water relaxation parameters were evaluated as a mean to probe water mobility in relation with mechanical properties, cell wall chemical composition and histological parameters of the cortex parenchyma tissue. The focus was made on transverse relaxation time T_2, which is more sensitive to small variations in water content and chemical exchange processes than T_1.

2. Materials and methods

2.1. Fruit and sampling

Apple fruits: X1344 (Reinette de Landsberg) referred to as **A**, W43 (progeny from an experimental cross) referred to as **B**, X2838 (Karminje de Sonnarville) referred to as **C** and X9190 (Bonne Hotture) referred to as **D** were selected at random from the INRA orchard production at INRA-IRHS, Angers. X1344, X2838, X9190, W43 were kept for about 4 months at 4 °C prior analysis. Five fruits of close size were analyzed per genotype. Apples were left 24 h at room temperature prior analysis and are referred to in the text as fresh fruit.

Sampling of apple cortex was realized on a 2 cm thick slice at the equator of the fruit. Cylinders of 8 mm diameter and 1 cm height were taken at the apex of a triangle drawn on the slice (Supplementary Fig. 1). One half of one cylinder was subjected to NMR analysis and dynamic mechanical measurement while the other half was used for chemical analysis. For histological analysis, an about 5 mm wide chips was sampled on the diameter of the apple slice (Supplementary Fig. 1). Tissue near the core was removed and samples were stored in ethanol: formaldehyde: acetic acid (85/10/5%) containing 100 mM $CaCl_2$ at 4 °C prior analysis.

2.2. Histological analysis

Sections (200 µm thickness) were cut using a vibrating blade microtome (Microm HM 650 V) and degassed by a short vacuum treatment and overnight stay in water at 4 °C. Images of sections were acquired using a prototype of macrovision system as described (Winisdorffer, Musse, Quellec, Devaux et al., 2015). Images in grey levels were coded between 0 (black) to 255 (white). Field of view was 5.874 × 4.420 mm at a resolution of 3.62 µm per pixel. Four images were taken per fruit or from one representative fruit. Image texture analysis was performed using mathematical morphological procedures as described (Winisdorffer, Musse, Quellec, Devaux et al., 2015) using routines developed on MatLab® software (The MathWorks Inc., Natick, Massachusetts, U.S.A). “Closing” granulometric curves were computed to compare dark objects size. Principal component analysis of the collection of curves was applied for distinguishing the genotypes according to the distributions of dark objects size along the first two components. The scores of each individual along the first two components were then used as measures of the genotypes histological characteristics.

2.3. NMR relaxometry

Excess water was gently wiped out of apple cortex cylinders and weighed prior insertion in the 10 mm NMR tube and covered by a Teflon plug. For fresh samples, relaxometric characteristics were measured immediately. The samples were then taken out of the tube for dynamic mechanical analysis (DMA) and then returned to the tube prior to freezing in liquid nitrogen (10 min). Frozen samples were then kept for 24 h at −20 °C and thawed at room temperature for 24 h prior relaxometric and mechanical analyses.

Transverse relaxation was measured on a Bruker Minispec mq20 (20 MHz, 0.47 T) equipped with a thermostated (± 0.1 °C) ^{1}H probe using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. All experiments were carried out at 20 °C. The 180° pulse separation was 0.2 ms and 32 k (20 k) even echoes were collected for fresh (thawed) samples. Sixty-four scans were acquired with a recycle delay of 15 s (12 s) to avoid saturation, resulting in a total acquisition time of about 30 min (20 min) for fresh (thawed) apple samples.

T_2 relaxation curves were subjected to multi-exponential analysis in two ways: discrete and continuous approaches, respectively by non-linear least-squares fitting (which minimized the sum of quadratic deviations between fitted and experimental values) and inverse Laplace transformation (ILT) of the relaxation signal $S_2(t)$ acquired with the CPMG pulse sequence according on one hand to,

$$S_2(t) = \sum_{j=1}^{n} P_{2j} e^{-t/T_{2j}} + C$$

where P_{2j} is the normalized amplitude/population $\sum_{j=1}^{n} (P_{2j} = 1)$ of the jth component, T_{2j} is the associated to the jth component, C is a constant associated to the relaxation curve offset, and on the other,

$$S_2(t) = \int_0^{\infty} e^{-t/T_2} f(T_2) dT_2 + E(t)$$

where $f(T_2)$ is the probability density and $E(t)$ is the measurements error.

For the discrete analysis, a standard F test was used to discriminate between models with different numbers (n) of exponentials. Conversion of the relaxation signal into a continuous distribution of relaxation components was performed using PDCO, a primal-dual interior method for convex objectives (Saunders, Bunggyoo, Maes, Akle, & Zahr, 2012). PDCO is a convex optimization solver implemented in Matlab® and can be adjusted to solve the LF-NMR relaxometry inverse problem with non-negativity constrains and an L_1 regularization term that stabilizes the solution process without introducing the typical L_2 peak broadening, and makes it possible to resolve close adjacent peaks (Berman, Levi, Parmet, Saunders, & Wiesman, 2013). To extract quantitative information from the continuous analysis, T_2 distribution envelopes were modelled by a sum of peaks with asymmetrical normal shape using an in-house routine developed on Matlab® sofware. Relative peak area, peak width/dispersion and T_2 mean values of each peak were thus determined.

2.4. Dynamic mechanical analysis (DMA)

Dynamic mechanical measures of apple cortex cylinders (1 cm height, 8 mm diameter) were obtained on a Bose ElectroForce 3100 (TA Instrument) equipped with a 22 Newton force sensor. Samples were pre-conditioned by a cycle of 20 sinusoidal strains between 0 and 6% amplitude at a frequency of 10 Hz. After a delay of 2 s at 0.4% strain, measures were taken from 25 cycles at 1 Hz frequency between 0.35 and 0.74% strain. Elastic behaviour of apple cortex was measured by the storage modulus (E′) whereas the amount of energy dissipated as heat was measured by the loss modulus (E″). The ratio between loss and storage modulii (tan ∂) characterized the damping of apple cortex. E′, E″ and tan ∂ were provided by WinTest 7 DMA software (TA Instruments). Storage modulus (E′) is about equal to elastic Young's modulus for a material with low damping, which is the case in the present study.