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Research note

Changes in water status of cherimoya fruit during ripening

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Abstract

Changes in the state of water of cherimoya (*Annona cherimola* Mill.) fruit during ripening were monitored using differential scanning calorimetry (DSC) and magnetic resonance imaging (MRI) techniques. The stage of ripening was determined by analyzing the changes in titratable acidity, firmness and total soluble solids contents. Furthermore, quantitative measurements of solutes such as proline, sucrose, glucose and fructose were also studied. The significant increase in the transverse relaxation time (T_2) values and the loss of flesh firmness during the initial stage of ripening are consistent with the sustained drop in the unfreezable water weight fraction according to the DSC data. The ripe stage of the fruit was marked by minimum longitudinal relaxation time (T_1) values and a rapid upsurge in the unfreezable water weight fraction, greatly influenced by the osmotic adjustments prompted by the significant accumulation of soluble sugars, proline and carboxylates. Changes in structure and solute concentration associated with ripening can be analyzed simultaneously, determining fruit water status by DSC.

Keywords: Transverse and longitudinal relaxation times; Unfreezable water; Solutes; DSC; MRI; Cherimoya

1. Introduction

As water plays an important role in fruit growth, ripening and postharvest storage, accurate measurement of water status is extremely useful, and a considerable amount of work has been done in that direction (Anderson and Richardson, 1982; Burdon and Clark, 2001). Correlations have been shown between nuclear magnetic resonance data and water content and potential in plants (Colire et al., 1988). According to Nagarajan et al. (1993), longitudinal relaxation time (T_1) is the best indicator of the water state, as it furnishes information on both content and availability. The value of transverse relaxation time (T_2) is not generally determined to infer water content but good correlations between T_1 and free water content have been found (McFall et al., 1990). The approach adopted in the present work consisted in ascertaining the content and dynamics of water interactions in cherimoya fruit during ripening using magnetic resonance imaging (MRI) and differential scanning calorimetry (DSC). The term used is the unfreezable water fraction, meaning the amount of water in a system which does not freeze out as ice at subfreezing temperatures (Wang and Kolbe, 1991). In cryobiology this equates to bound water and is osmotically inactive (Wolfe et al., 2002). DSC has been extensively used to determine the amount of unfreezable water in foodstuffs (Biliaderis, 1983) but not as yet in fresh fruit during a developmentally controlled process like ripening.

The progress of ripening is generally defined by the combination of several physicochemical changes. In the case of cherimoya (*Annona cherimola* Mill.) fruit, as for other species of *Annonas*, ethylene production is a late event, and ripening is associated with a sharp early decrease in flesh firmness and a large increase in both soluble solids contents and titratable acidity (Merodio and De La Plaza, 1997). Therefore, the determination of water status in the fruit tissues would be an excellent approach for an integrated analysis of the biochemical and metabolic changes undergone in the course of ripening.

The aim of this work was firstly to determine whether there were variations in the water status of the fruit during ripening, using DSC and MRI, and secondly to determine whether the changes in the content and degree of freedom of water could account for the changes in several specific ripening properties (firmness, titratable acidity and total soluble solids). Also, in the present work the levels of compounds known as osmolites or compatible solutes such as free proline and soluble sugars

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(glucose, fructose and sucrose), which play an important role in the changes occurring in the amount of unfreezable water, were quantified in cherimoya fruit during ripening.

2. Materials and methods

Cherimoya (*Annona cherimola* Mill. cv. Fino de Jete) fruit harvested in January (mid-season) were placed in respiration chambers (20 L) in a continuous flow (100 mL min^{-1}) of humidified air. Three fruit, chosen at random, were used immediately for MRI experiments through the ripening period. Three fruit (each weighing 300 g) were taken at random after 0, 1, 2, 3, and 4 days of storage at 20 °C, and used for flesh firmness analysis, soluble sugars, titrable acidity, proline determinations and DSC measurements. Firstly, fruit were used for flesh firmness determination (three replicates per sample; nine measurements) and then they were peeled, sliced, and frozen in liquid nitrogen for biochemical and DSC analyses.

A DSC822e Mettler-Toledo differential scanning calorimeter (Mettler-Toledo Inc., Columbus, OH, USA) equipped with a liquid nitrogen-cooling accessory was used to study water fusion. The instrument was calibrated using Indium and *n*-octane, and an empty aluminum pan was used as the reference. Frozen pulverized tissues placed in 40 μL aluminum pans hermetically sealed were cooled from 25 to −80 °C at 10 °C/min, left at −80 °C for 5 min and warmed to 25 °C at 10 °C/min. The heating scan was used to determine the latent heat of melting ice (ΔH) by integration of the melting endotherm and the onset freezing temperature (T_0). A method based on the heat of fusion was used to calculate the amount of unfreezable water (Francks, 1991) and was estimated as follows:

$$\mathrm{UFW} = \frac{W_{\mathrm{water}} - (\Delta H_{\mathrm{sample}}/\Delta H_{\mathrm{water}})}{\mathrm{DM}}$$

where $\Delta H_{\mathrm{sample}}$ is the heat of fusion expressed in J, $\Delta H_{\mathrm{water}}$ the normalized heat of fusion of pure water (351.2 ± 1.2 J/g), W_{water} the total water content of the sample expressed in g of water and DM is the dry matter content expressed in g DM.

Samples for DSC analysis were taken from different areas of the mesocarp tissue of each cherimoya fruit in order to obtain a representative population. We selected six areas in each fruit (three fruit per sampling day) and three measurements were made per area.

Magnetic resonance imaging was performed using a Biospec 47/40 spectrometer (Bruker, Karlsruhe, Germany) with a 4.7 T superconducting magnet (Magnex, Oxford, UK). Individual fruit were imaged in the longitudinal plane using a home-made birdcage high-pass ($n = 8$) transmitter and receiver coil (11.3 cm internal diameter). Relaxation measurements were performed on whole fruit using a standard CPMG (Carr–Pucell–Meiboom–Gill) sequence to measure T_2 and an inversion-recovery sequence for T_1, both from the Bruker Paravision library. A multiple spin-echo sequence with a repetition time (TR) of 2 s and echo time (TE) of 13 ms was used for T_2 measurements, accumulating 10 echoes. The inversion times (TI) for T_1 measurements were 15, 50, 100, 200, 300, 500, 700, 1000, 1900 ms, while TE was held constant (10 ms). All images were acquired with a field of view (FOV) of 10 cm, slice thickness of 5 mm and 128 × 256 matrix size. Data were zero-filled before Fourier Transformation to reconstruct a 256 × 256 pixel image. Relaxation time was calculated off-line on a Sun Sparc 10 station, using processing software based on the Interactive Data Language (IDL, Research Systems Inc., Boulder, CO). Signals were read out from rectangular regions of interest (ROI) with different sizes and positions, and each observation was repeated at least five times. Values represent averages of the signals ± S.D. from those calculations. Similar acquisition slices and processing ROIs were chosen daily for the different experiments.

Total soluble solids and titratable acidity, flesh firmness and soluble sugars were determined following the methods of Alique et al. (1994). For proline profile analysis, amino acids extracted by homogenizing frozen tissue in 5% (v/v) ice-cold perchloric acid were analyzed with an amino acid analyzer (Biochrom20 Amino Acid Analyser). Proline was identified by comparison of its retention time with an authentic standard amino acid and quantified as a ratio of the area of the sample peak relative to that of standard samples.

The data from at least three replicates per sample were subjected to analysis of variance (one-way ANOVA) using the LSD test (Statgraphics Program, STSC, Rockville, MD) to determine the level of significance at $P \leq 0.05$.

3. Results and discussion

The numeric values of T_2, measured in the external mesocarp of the fruit (the selection of this area is based on the fact that flesh firmness measurements were performed in this region), increased sharply (80%) in the first three days (Fig. 1). The changes in T_2 appear to have been complete by day 3, as there were no significant changes on subsequent days. Furthermore, the changes in texture data indicated that firmness declined very rapidly between days 1 and 2 of ripening, and by day

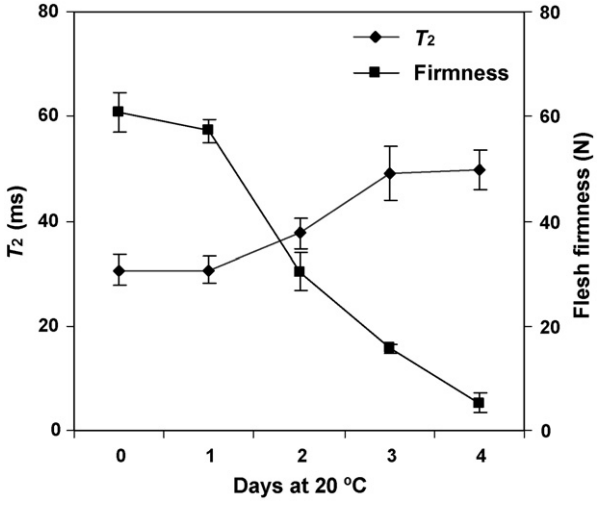

Fig. 1. Variations in the spin–spin relaxation time (T_2) of external mesocarp tissue from serial MR images of the same fruits ($n = 15$) and the flesh firmness of cherimoya fruit ($n = 9$), during ripening at 20 °C. Error bar, ±S.E.