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Characterisation and functionality of fructo-oligosaccharides affecting water status of strawberry fruit (*Fragraria vesca* cv. Mara de Bois) during postharvest storage

María Blanch, Oscar Goñi, María T. Sanchez-Ballesta, María I. Escribano, Carmen Merodio *

Department of Characterization, Quality and Security, Institute of Food Science Technology and Nutrition (ICTAN-CSIC), Jose Antonio Novais 10, Madrid 28040, Spain

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ABSTRACT

Water status and analyses of free sugars, sugar-alcohols and fructo-oligosaccharides (FOS) were carried out in *Fragaria x vesca* treated with different high CO_2 concentrations applied to minimise damage caused by storage at 0 °C. The thermodynamic parameters such as the amount of unfrozen water (U_w), T_g, T'_g, and peak position of the O–H stretching vibration were determined in various saccharides including FOS (1-kestose, nystose and kestopentaose) by infrared spectroscopy studies and differential scanning calorimetry. Beneficial high CO_2 treatment (20%) avoided the reduction of unfreezable water fraction and increased endogenous FOS levels, in contrast to that observed in air-stored and in those exposed to higher CO_2 levels (40%). The direct FOS-water interaction, possibly within the hydrogen-bond network of cellular structures, could explain the maintenance of water status, cell integrity and the low water leakage levels in 20% CO_2-treated fruit at values similar to those found in freshly harvested fruit.

1. Introduction

Strawberries are highly perishable fruit susceptible to water loss and pathogen attack. Consequently, many approaches have been undertaken to improve strawberry postharvest storage procedures in order to maintain fruit quality. Most of the work has focused on the beneficial effect of low temperature and gaseous treatments in controlling storage decay in different varieties of *Fragaria x ananassa*. The effect of high CO_2 treatments on the quality of strawberries has been extensively analysed through changes in colour, titratable acidity, pH, soluble solids and phenolic compounds (Bodelón, Blanch, Sanchez-Ballesta, Escribano, & Merodio, 2010; Gil, Holcroft, & Kader, 1997). However, the molecular mechanism behind the protective role of high CO_2 levels is still not well understood. It has been reported that in other highly perishable fruit such as grapes, high CO_2 levels induced the accumulation of FOS during low temperature storage (Blanch, Sanchez-Ballesta, Escribano, & Merodio, 2011). FOS is a common name for fructose oligomers that are composed mainly of kestose, nystose, and kestopentaose, in which fructosyl units are bound by a β linkage at the position of sucrose and in which the degree of polymerisation is less than 5. There are many positive aspects related to the presence of fructans in plants. Indeed, they have been implicated in abiotic stress-tolerance and they might provide protection from damage to cytosolic proteins and cell membranes (Valluru & Van den Ende, 2008). However, there is little or no thermodynamic information about FOS or comparative studies with other simple saccharides that could explain their protective action against stress conditions.

Fructans may also have functions like carbon storage, and their synthesis results from extended sucrose metabolism. With respect to soluble sugars and starch, variations have been observed in the amount and type of carbohydrates accumulated in fruit both between and within genotypes of a species (Nardozza et al., 2011). Moreover, sugar-alcohols (polyols) are a group of naturally occurring carbohydrate derivates that, in addition to other roles, function in the interconversion of some monosaccharides. It has been reported that *myo*-inositol can be found in most fruit and vegetables, whereas the presence of sorbitol and/or mannitol was related to each species. Specifically, sorbitol is known to be a major constituent of leaves and fruit of the *Rosaceous* species, and can be an important translocating compound in this species. As carbohydrates and sugar-alcohols have similar structures, they are difficult to separate by conventional reversed-phase liquid chromatography, therefore the amperometric detection method was used (Cataldi, Margiotta, & Zambonin, 1998). This method was also used to successfully quantify FOS in accumulating and non-accumulating fructan species during storage (Blanch et al., 2011; Ishiguro, Onodera, Benkeblia, & Shiomi, 2010).

* Corresponding author. Tel.: +34 91 5492300; fax: +34 91 5493627.
E-mail address: merodio@ictan.csic.es (C. Merodio).

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Furthermore, the accumulation of saccharides in plants can contribute to both the water potential (ψ_w) and the protection of macromolecular structures against the destabilising effects of decreasing water activity and/or low temperature storage (Popp & Smirnoff, 1995). We previously reported changes in the water status during ripening and storage in different kinds of fruit, where specifically high CO_2 treatment caused a marked increase in the unfreezable water fraction (Goñi, Fernandez-Caballero, Sanchez-Ballesta, Escribano, & Merodio, 2011). The unfreezable, bound or structured water in living tissue is more likely to play a major role in stress tolerance by maintaining the structural integrity and/or cell wall extensibility, while an increased amount of free water might be able to enhance solute accumulation, leading to better osmotic adjustment and maintenance of the volumes of sub-cellular compartments.

In the present study, our principal aims were to analyse how different high CO_2 treatments during low temperature storage improve: (1) the turnover of nonstructural carbohydrates (glucose, fructose and sucrose), polyols (*myo*-inositol, D-sorbitol and D-mannitol) and FOS, using high-performance anion-exchange chromatography with integrated pulsed amperometric detection (HPAEC-PAD), (2) the thermodynamical properties of FOS using infrared spectroscopy studies and differential scanning calorimetry, and (3) the physiological characterisation of fruit tissue in terms of changes in water potential (ψ_w) and unfreezable water content. Moreover, in order to establish the tolerance limits to high CO_2 concentrations, different treatments (3 days with 20% and 40% CO_2) were applied.

2. Material and methods

2.1. Plant material

Organic remontant strawberries (*Fragaria x vesca* cv. Mara de Bois) harvested from the first flowering from Monjarama orchard, San Sebastian de los Reyes, Madrid (Spain) were stored at 0 °C (±0.5) in three sealed containers with a capacity of 1 m^3. Ten plastic boxes of about 1 kg of strawberries per box were stored in each container for 3 days, with a continuous flow of air or gas mixture containing 20% CO_2 or 40% CO_2. The same O_2 concentration (20%) was maintained in all three lots. Initially, and at the end of the treatments (3 days), 45 strawberries from five boxes were removed at random from each of the treatment groups, frozen in liquid nitrogen and then stored at −80 °C for further analysis.

2.2. Extraction and chromatographic determination of sugars, polyols and fructo-oligosaccharides

To determine sugars (glucose, fructose and sucrose) and polyols (*myo*-inositol, D-sorbitol, and D-mannitol), 1 g of each sample was homogenised in 5 ml of ultra-pure water and centrifuged at 30,000*g* for 20 min, after which the supernatants were filtered through a 0.45 μm pore size membrane. The determination of these compounds was carried out by HPAEC-PAD, equipped with a Metrosep Carb 1–250 IC column (4.6 × 250 mm) as described by Bodelón et al. (2010). The content of each sugar was expressed as mg/g fresh weight (FW) of the sample, each polyol as mg/g FW for *myo*-inositol, and μg/g FW for D-sorbitol and D-mannitol, and the data produced were the means of the three replicates, two different measurements being made.

The quantification of nystose, nystose b and kestopentaose and that of 1-kestose and neokestose, which required pre-treatment with an activated carbon Darco G60, 100 mesh (Sigma, Steinheim, Germany) to remove the mono- and disaccharides, was determined following the method of Blanch et al. (2011). Briefly, the samples were extracted with 85% ethanol, boiled under reflux and centrifuged at 5000*g* for 20 min at 4 °C. The supernatant was evaporated and then redissolved with deionised water, filtered through 0.22 μm filters and analysed by HPAEC-PAD equipped with a CarboPac PA1 carbohydrate column. A three-step PAD protocol was used and the samples (1.5 ml) were injected using an autosampler (model, 838 Advanced Sample Processor, Metrohm, Herisau, Switzerland). FOS identification was performed by comparing the retention time of standard mixtures (1-kestose and nystose from Sigma, Steinheim, Germany, and kestopentaose from Megazyme, Co.Wicklow, Ireland) with those reported in publications, and the FOS content was expressed as μg/g FW of the sample.

2.3. Determination of water status

The total water content (g/100 g FW) was determined after a stable weight had been obtained after drying at 105 °C. The drip loss was determined by a method adapted from Lowithun and Charoenrein (2009). Frozen strawberry samples were stored at −20 °C for 4 and 9 days and then the tissues were thawed at 8 °C for 1 h before measuring drip loss.

The strawberry tissue ψ_w was measured by a Dewpoint PotentialMeter, WP4, (Decagon Devices, Inc., USA). The ψ_w measurements were repeatedly taken from a 2 mm thick strawberry disc cut from the middle portion of four strawberries. The ψ_w of untreated and CO_2-treated strawberries was determined at the onset of low temperature storage. Calibration was at 25 °C with KCl as standard (−2.22 MPa).

The unfreezable water content was determined in frozen pulverised strawberry pulp tissues (10–20 mg) using a differential scanning calorimeter (DSC822e, Mettler-Toledo Inc., USA) equipped with a liquid nitrogen cooling accessory, following the method of Goñi, Escribano, and Merodio (2008). Indium, deionised water and zinc were used for calibration, and an empty aluminium pan was used as the reference. Samples were placed in 100 μl aluminium pans which were hermetically sealed and a method based on fusion heat was used to calculate the amount of unfreezable water content.

2.4. Thermodynamic characterisation of FOS

2.4.1. Determination of glass transition temperatures of the different anhydrous sugars

A DSC822e Mettler-Toledo differential scanning calorimeter was also used to determine the glass transition temperatures (T_g) for the anhydrous sugar glasses. Samples (10–15 mg) were placed in 40 μl hermetically sealed aluminium pans. To remove any residual water from the samples, a pinhole was made in the sealed pan and a preheating gradient was used from 25 to 100 °C at 2 °C/min. During preheating, a dry helium gas was streamed at 100 ml/min to prevent condensation of moisture within the DSC furnace. The dried amorphous sugar was prepared by heating each crystalline compound from −10 °C to a temperature above melting point at 10 °C min^{-1}. Then, samples were cooled to −10 °C at 100 °C/min and left at this temperature for 5 min. In order to erase the thermal history of the sugar, each sample was subjected to a further three heating and cooling cycles. For all samples, the midpoint of the deflection in the heat flow versus temperature curve was taken as the T_g.

2.4.2. Determination of the amount of unfrozen water of the different sugars

The anti-freeze characteristics of the different sugars were evaluated calorimetrically by the amount of unfrozen water (U_w) and ice glass transition temperatures (T'_g) as described by Furuki (2002), but with slight differences using a DSC822e (Mettler-Toledo,