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Calcium and ascorbic acid affect cellular structure and water mobility in apple tissue during osmotic dehydration in sucrose solutions

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ABSTRACT

The effects of the addition of calcium lactate and ascorbic acid to sucrose osmotic solutions on cell viability and microstructure of apple tissue were studied. In addition, water distribution and mobility modification of the different cellular compartments were observed. Fluorescence microscopy, light microscopy and time domain nuclear magnetic resonance (TD-NMR) were respectively used to evaluate cell viability and microstructural changes during osmotic dehydration. Tissues treated in a sucrose–calcium lactate–ascorbic acid solution did not show viability. Calcium lactate had some effects on cell walls and membranes. Sucrose solution visibly preserved the protoplast viability and slightly influenced the water distribution within the apple tissue, as highlighted by TD-NMR, which showed higher proton intensity in the vacuoles and lower intensity in cytoplasm-free spaces compared to other treatments. The presence of ascorbic acid enhanced calcium impregnation, which was associated with permeability changes of the cellular wall and membranes.

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1. Introduction

The concentration of plant foods by immersing solid food pieces in a hypertonic solution consisting of salt, sugar, glycerol, or other humectants is known as osmotic dehydration (OD) (Sereno, Moreira, & Martinez, 2001). This technique reduces the a_w of the product without a phase change because the flow of water from the product into the concentrated solution is compensated by the solutes migration from the solution into the product (Nieto, Vicente, Hodara, Castro, & Alzamora, 2013). This process permits the formulation of products with intermediate moisture content through dewatering and impregnation of desired solutes (Barrera, Betoret, & Fito, 2004). Because of its versatility, OD has a wide range of applications in the development of minimally processed plant foods or as pretreatment for other preservation methods such as freezing or drying (Alzamora, Cerrutti, Guerrero, & López-Malo, 1995; Garcia Loredo, Guerrero, Gomez, & Alzamora, 2013).

The addition of calcium in osmotic solutions has been widely used in plant foods as fortifier and to enhance firmness (Anino, Salvatori, & Alzamora, 2006; Barrera, Betoret, Corell, & Fito, 2009; Mavroudis, Gidley, & Sjöholm, 2012; Silva, Fernandes, & Mauro, 2014a). Fortification using combinations of substances such as calcium and iron (Barrera et al., 2004) or Ca and vitamin C (Silva, Fernandes, & Mauro, 2014b) has also been investigated.

OD causes physical modifications of cell membranes and cell walls, which affects the rheological properties and state of water (Nieto et al., 2013; Vicente, Nieto, Hodara, Castro, & Alzamora, 2012). Knowledge about the microstructure and mass transport in OD of plant tissues is fundamental for controlling production of foods fortified with vitamins and mineral salts. Mass transfer in cellular tissue is influenced by the osmotic pressure and structure properties such as permeability of the plasma membrane and vacuole membrane, cell wall porosity, or even intercellular porosity. The osmotic pressure, in turn, depends on the solute concentration and the salt and acid dissociation because each substance presents specific transport properties through plasma and vacuole membranes or cell wall pores. When the cellular structure is changed, the tissue selectivity is also modified, so that water mobility and distribution are affected.

Osmotic dehydration of plant foods is largely controlled by the cellular membranes, which have different permeabilities to different substances. Biological membranes are composed of phospholipid

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bilayers with intrinsic proteins. Studies have shown that water can cross plant membranes through proteinaceous channels formed by members of the aquaporin superfamily, also called water channels (Weig, Deswarte, & Chrispeels, 1997). Aquaporins are hydrophobic proteins that enhance the biological membrane's permeability to water. They belong to a group of membrane proteins, the major intrinsic proteins (MIP) family of channels, with a molar mass in the range of 26 and 30 kDa (Tyerman, Niemietz, & Bramley, 2002; Weig et al., 1997). These channels increase the permeability of biological membranes to water compared to the lipid bilayers; they are detected by the low activation energy needed to transport water across water channels (Tyerman et al., 2002).

Calcium ions that occupy spaces outside the plasma membrane (apoplast) have a structural role in the cell wall because they interact with pectic acid polymers to form cross-bridges that reinforce the cell adhesion, thereby reducing cell separation, which is one of the major causes of plant tissue softening (Roy et al., 1994). Moreover, calcium can affect water channel activity; however, the significance of the inhibition of plant aquaporins by calcium is complex and has still not been clarified (Maurel, 2007). Conversely, calcium can also cross membranes through cation channels. A vacuolar non-selective Ca^{2+} channel (Peiter et al., 2005) has been identified as a plasma membrane non-selective cation channel (Tapken et al., 2013) in plant cells.

Ascorbic acid (AA) influences the cell physiology; however, little is known about its role in plant tissue. Exposure of *Arabidopsis thaliana* seedlings to ascorbic acid demonstrated that exogenous AA caused grow inhibition and damage in the cellular structure by increasing the ROS (reactive oxygen species) content (Qian et al., 2014). In addition, a very low pH (2–3) can increase the cell wall porosity (Zemke-White, Clements, & Harris, 2000), which increases diffusion of great molecules in the free spaces of the cellular tissue.

The complexity of osmotic dehydration of plant tissues rises when using a multicomponent solution because all the solutes and their respective concentrations affect the membrane permeability and cell wall. Consequently, monitoring the water distribution can be useful to clarify the behavior of the cellular microstructures as osmotic dehydration proceeds. Time domain nuclear magnetic resonance (TD-NMR) is an analytical method that allows the determination of the water content and its mobility in different cell compartments by proton relaxation times of water in foods (Hills & Duce, 1990). It is a non-invasive method suitable for large tissue samples that relates water content and water properties in different proton pools within the tissue with different transverse relaxation times (T_2) of water (Hills & Remigereau, 1997; Panarese et al., 2012; Tylewicz et al., 2011). In fruit samples, the higher the mobility of a proton bearing molecule, the higher the spin–spin (T_2) relaxation time is expected to be. The intensities of proton pools with different transverse relaxation times are a relative measure of the amount of water corresponding to a specific T_2. This technique has been used in OD of plants to evaluate water mobility and distribution within the cellular tissue (Cornillon, 2000; Panarese et al., 2012; Tylewicz et al., 2011). Microscopic techniques can also be important tools to clarify cell viability by using vital dyes. Protoplasts stained with fluorescein diacetate (FDA) allow the estimation of two types of plasma membrane injuries: lysis and the loss of semipermeability (Halperin & Koster, 2006; Koster, Reisdorph, & Ramsay, 2003). Vacuole membrane alterations can be evaluated by the capacity of intact tonoplasts to retain neutral red and provide contrast to vacuoles (Carpita, Sabularse, Montezinos, & Delmer, 1979; Thebud & Santarius, 1982).

A multianalytical approach that combines several techniques such as micro and ultrastructural microscopy, calorimetry and NMR have been successfully employed in investigations of plant foods subjected to mild processing conditions (Panarese et al., 2012; Rocculi et al., 2012; Tylewicz et al., 2011).

The main objective of this work was to investigate the effects of the addition of calcium lactate (CaLac) and ascorbic acid (AA) to sucrose (Suc) osmotic solutions on mass transfer, cell viability and structure of apple tissue, as well as the consequential water distribution and mobility modification among the different cellular compartments.

2. Materials and methods

2.1. Raw materials

Apples (*Malus domestica* Borkh) of the Cripps Pink variety, popularly known by the brand name Pink Lady (Castro, Barrett, Jobling & Mitcham, 2008), were provided by the local market and stored at 5 ± 1 °C for no longer than 2 weeks, during which osmotic dehydration experiments were performed. The average weight of the apples was 233.5 ± 17.7 g, and the soluble solids content was $13.4 \pm 0.3\ g \cdot 100\ g^{-1}$. Apples were cut in cylinders (8-mm diameter) with a manual cork borer and cut to a length of 40 mm using a manual cutter designed for this purpose. Commercial sucrose (refined sugar, Eridania, Italy), L-ascorbic acid (Shandong Luwei Pharmaceutical Co., China) and calcium lactate (calcium-L-lactate 5-hydrate powder, PURACAL® PP Food, Corbion PURAC, Netherlands) were used in the experiments.

2.2. Osmotic dehydration

Apple cylinders were weighed (approximately 0.1 kg) in a mesh basket and immersed in the osmotic solution. Each basket corresponded to a single OD time: 0.5, 1, 2 and 4 h. The OD system consisted of a cylindrical glass vessel containing 4.5 kg of aqueous solution. The cylindrical baskets, coupled with an impeller of a mechanical stirrer, were continuously rotated. Two baskets were prepared for each process time. The syrup-to-fruit ratio was approximately 15:1 (w/w).

The OD was performed with four different aqueous solutions: 40% sucrose (Suc), 40% sucrose + 4% calcium lactate (Suc–CaLac), 40% sucrose + 2% ascorbic acid (Suc–AA) and 40% sucrose + 4% calcium lactate + 2% ascorbic acid (Suc–CaLac–AA). After the pre-established contact period, the samples were removed from the solution, rinsed with distilled water, blotted with absorbing paper, and weighed.

Immediately after the process, analyses of the total solids and soluble solids contents were performed for fresh and osmotically treated samples in triplicate. The proton transverse relaxation time (T_2) was also immediately measured for six replicates. Samples for calcium and ascorbic acid analyses were freeze-dried.

2.3. Analytical methods

The moisture content for 2 g of fresh and treated samples was determined gravimetrically, in triplicate, by drying at 70 °C until a constant weight was achieved. The soluble solids content was determined at 20 °C by measuring the refractive index with a digital refractometer (PR1, Atago, Japan). Water activity was measured in a water activity meter (AquaLab Series mod. CX-2, Decagon, USA).

2.3.1. Ascorbic acid

For ascorbic acid determination, an extraction was performed with 0.5 g of a freeze-dried sample added to 10 ml of meta phosphoric acid (62.5 mM) and sulfuric acid (5 mM) solution. The mixture was vortexed for 2 min and centrifuged at 10,000×g for 10 min at 4 °C. The supernatant was opportunely diluted and filtered through a 0.45 μm nylon filter. Ascorbic acid was determined

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