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Vacuum impregnation modulates the metabolic activity of spinach leaves

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ABSTRACT

In this study calorimetric measurements provided evidence of a drastic increase of spinach leaf gross metabolism as a consequence of vacuum impregnation (VI) at a minimum pressure of 150 mbar with trehalose and sucrose isotonic solutions. When applying VI extracellular air is replaced by the impregnation solution, potentially limiting tissue respiration to any remaining air volume in the tissue. However the observation that impregnated leaves showed photosynthetic activity suggests that not all air was exhausted during VI. Hence impregnation appears to reach a maximum with remaining gas filled compartments. Metabolic inhibitors impregnated together with sugars showed that the short-term metabolic response, causing the drastic increase of gross metabolism upon VI, depends on mitochondrial oxygen consuming pathways. The metabolic effect following mannitol impregnation was comparable with water impregnation, suggesting that the strong metabolic effect reported here is only seen for molecules that can be metabolized and provide energy to the cells.

Industrial relevance: Vacuum impregnation is used to incorporate additives in fruit and vegetable tissues, such as anti-browning agents, microbial preservatives or cryoprotectants. As a promising technology in the food industry, deeper insights on the metabolic consequences of vacuum impregnation are required to define and control the shelf-life of the processed fruits and vegetables.

1. Introduction

Vacuum impregnation (VI) is a unit operation in which porous products are immersed in solutions of various compositions and/or concentrations and subjected to a two-step pressure change. The first step (application of vacuum) consists on the reduction of the pressure in the solid–liquid system. During this step, the gas in the product pores is expanded and flows out until mechanical equilibrium is achieved. When the atmospheric pressure (second step) is restored, the residual gas in the pores is compressed and the external liquid flows into the pores (Tylewicz, Romani, Widell, & Galindo, 2013). VI is widely used in several processes to incorporate additives in fruit and vegetable tissues, such as anti-browning agents, microbial preservatives or cryoprotectants (Barrera, Betoret, Corell, & Fito, 2009; Phoon, Galindo, Vicente, & Dejmek, 2008). There is a large amount of literature focused on the effects of the vacuum level and the structure and mechanical properties of the foodstuff on mass transfer phenomena during VI (Carciofi, Prat, & Laurindo, 2012; Chiralt & Fito, 2003; Fito & Pastor, 1994; Laurindo, Stringari, Paes, & Carciofi, 2007; Mujica-Paz, Valdez-Fragoso, López-Malo, Palou, & Welti-Chanes, 2003; Paes, Stringari, & Laurindo, 2006), as well as the effects of the impregnating liquid on structure and mechanical properties (Guillemin et al., 2008). However, to the best of our knowledge, little is known about the metabolic consequences of VI that are provoked by structural modifications induced by the pressure changes, the impregnated molecules, and/or anaerobic stress. The onset of anaerobic metabolism in sucrose solution-impregnated strawberries was demonstrated after 24 h of storage at 10 °C (Castelló, Fito, & Chiralt, 2010). Recently, we showed that cell membrane vesicles are formed 30 min after the impregnation of apple tissue with different concentrations of sucrose and trehalose, suggesting that the impregnated sugars may not totally remain in the extracellular space, as normally believed, but that at least a fraction of these compounds will be incorporated into the cells (Tylewicz et al., 2013). In this paper, further exploration of short-term metabolic responses is done in spinach using isothermal calorimetry. The calorimetrically measured heat and heat production rate (thermal power) of a biological tissue is related to its metabolic rate and provides a direct indication of integrated metabolic responses, such as respiration and reaction to stress (Criddle, Breidenbach, & Hansen, 1991; Galindo, Wadsö, Vicente & Dejmek, 2008).

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2. Material and methods

2.1. Raw material handling and storage

Baby spinach leaves (*Spinacia oleracea*) grown in southern Sweden were harvested and stored in a packing house storage facility in a normal storage atmosphere (1–2 °C; 90–95% RH). Within 5 days of harvesting, the leaves were washed, air-dried, packed in 65 g bags, as routinely practiced by the producer, and delivered the same day to our laboratory. During the experimental period the packages were stored at 2 ± 1 °C, and experiments were performed within the product expiration time (7 days). Undamaged leaves (30 ± 3 mm in width × 60 ± 3 mm in length) were manually selected.

2.2. Raw material characterization

2.2.1. Porosity

The apparent density (ρ_a) and the true solid–liquid density (ρ_t) of spinach were determined by volume displacement in a pycnometer using appropriate aqueous isotonic sucrose solutions as reference liquid (see below) (Gras, Vidal, Betoret, Chiralt, & Fito, 2003). The true solid–liquid density was also obtained by volume displacement using sample purees obtained by manually grinding the samples using a mortar and pestle. The purees were placed in a Büchner flask and degasified for 10 min by creating vacuum in the flask. The porosity of the sample (ε) is the dimensionless ratio of air volume to total volume, and can have values between 0 and 1 (Eq. 1) (Lozano, Rotstein, & Urbicain, 1980)

$$\varepsilon = 1 - \frac{\rho_a}{\rho_t} \quad (1)$$

2.3. Sample preparation

For calorimetric measurements each leaf, placed in a covered Petri dish, was equilibrated in room light at 25 °C for 15 min and each sample (50 ± 1 mm of height × 30 ± 1 mm of length) was obtained using a sharp microtome blade.

For photosynthesis measurements each leaf, placed in a covered Petri dish, was equilibrated in darkness at 25 °C for 15 min and one disc of 36 mm of diameter was removed from each of twelve spinach leaves with a sharp cork borer. Three discs were placed in the dark inside a Petri dish (untreated samples). The remaining nine discs were divided in three 100 ml beakers and flooded with the impregnating solutions. A 1 mm thick plastic net allowed the samples to remain separated and submerged during the whole VI treatment. The three beakers were placed in the VI vessel (a desiccator) and subjected to VI.

2.4. Impregnating solutions

Isotonic solutions with a_w of 0.999 (AquaLab series 3TE, Decagon Devices Inc., Washington) in equilibrium with spinach leaves were designed with respect to the cell sap. The isotonic solution concentrations were determined by immersion of three spinach leaves (without petioles) in a series of solutions of different sucrose, trehalose or mannitol concentrations, according to Tylewicz et al. (2013). The variation in tissue weight was recorded every 30 min until equilibrium.

When metabolic inhibitors were used, sucrose and trehalose solutions were mixed with inhibitor stock solutions to reach 10 mM salicylhydroxamic acid (SHAM) and 2 mM KCN. KCN and SHAM stocks contained 100 mM in a 20 mM MOPS buffer (pH 7) and 1 M in dimethyl sulfoxide, respectively.

2.5. Automatic vacuum controller system

The automatic vacuum controller system (AVCS, S.I.A., Bologna, Italy) is a programmable device designed to control the pressure acting on the impregnating solution during the impregnation process. The AVCS is connected to the VI chamber by a Teflon tube and includes a number of components: a pressure transmitter, vacuum actuators (valves and vacuum pump), a computer and a programmable logic controller device (PLC, Series 90–30, General Electric, Charlottesville, VA, USA) as illustrated in Fig. 1. The PLC is the core of the AVCS as it manages the vacuum actuators, supervising the value of pressure, time and vacuum release rate by controlling the start and stop of the vacuum pump and the opening and closing of the air inlet and air outlet valves. A software interface (CIMPLICITY Workbench Version 6.10 Service Pack 3, General Electric, Charlottesville, VA, USA) allows the working parameters of the AVCS to be set and controlled (Panarese, Dejmek, Rocculi, & Gómez Galindo, 2013).

2.6. Vacuum impregnation

Based on preliminary experiments to establish maximum weight gain and avoiding visible tissue damage of spinach, a stepwise protocol with a minimum absolute pressure of 150 mbar was chosen. As shown in Fig. 2, pressure was applied in 20 consecutive steps. The chosen pressure profile ensured that cell viability was maintained. Two parameters were set for each pressure step: duration (s) and absolute pressure value (mbar). As outlined in Fig. 2, during the first phase of VI the pressure gradually decreased from the atmospheric value (1000 mbar) to the final reduced pressure value (150 mbar, step 8). During the second phase, vacuum was released and the pressure progressively returned to the atmospheric value. The chosen duration for each step was: 10 s for each step 1 to 4 and step 15 to 20; 20 s for step 5 and steps 12 to 14; 60 s for steps 6, 7 and steps 9 to 11; 120 s for step 8. Thus the VI treatment applied had a total duration of 600 s and was controlled by the AVCS.

2.7. Calorimetry: experimental set-up and heat production measurement

A 20 ml stainless steel ampoule, containing the sample, was closed with a stainless steel lid with bayonet mount and a disposable Teflon seal. Two o-ring sealed lead-throughs were made in the lid. Through each of these a 1 mm stainless steel tube was inserted. These tubes were connected to a 20 ml syringe and to the AVCS, as shown in Fig. 3.

The calorimetric measurements were made with a "microcalorimeter" of the type described in Fig. 2a in Bäckman et al. (1995) and manufactured by Thermometric AB (now TA Instruments, New Castle, DE, USA). This was placed in a precision water thermostat. The 24 h baseline noise of the instrument without the VI had a standard deviation of about 0.4 μW.

The sealed stainless steel ampoule containing the sample was placed in the calorimeter ampoule holder (Fig. 3) at 25 °C. When the sample

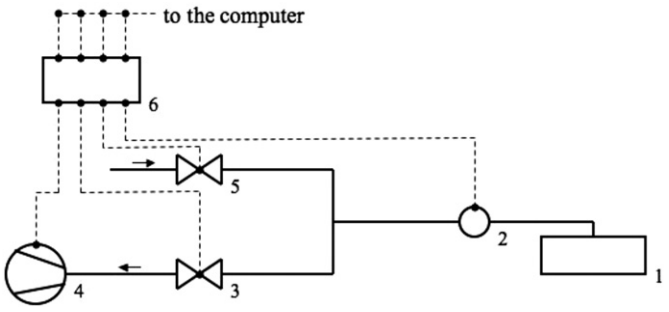

Fig. 1. Schematic illustration of the AVCS: 1. Treatment chamber, 2. Pressure transmitter, 3. Valve regulating air outlet, 4. Vacuum pump, 5. Valve regulating air inlet, 6. PLC. Arrows indicate the direction of air flow. Solid lines represent pneumatic connections, dashed lines represent electrical connections.