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X-ray microtomography provides new insights into vacuum impregnation of spinach leaves

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ABSTRACT

Vacuum impregnation is used in the food industry to facilitate the impregnation of porous products with, e.g. firming, antioxidant, antimicrobial or cryoprotective agents. X-ray micro-tomography (μCT) was used to study the process of vacuum impregnation in spinach leaves. Low (300 mbar absolute pressure) and mild vacuum (150 mbar absolute pressure) impregnation protocols were used to impregnate an isotonic solution of trehalose in the leaves and μCT was used to make observations of the cross section of the impregnated samples and quantify their porosity. Results revealed that the free volume in the spongy mesophyll is easier to impregnate than the spaces around the palisade mesophyll. The low vacuum impregnation protocol provoked less impregnation close to the edge of the leaf than in its centre, probably accounting for an influence of the tissue structure on impregnation. The vacuum impregnation protocols tested in this investigation drastically decreased the proportion of large pores (>100 μm) and increased the proportion of small pores (<50 μm). The mild vacuum impregnation protocol, which was designed on the basis of measured apparent porosity, did not achieve full impregnation of the tissue.

1. Introduction

Vacuum impregnation (VI) of a porous tissue involves the removal of the gas normally contained in open pores and its replacement with a liquid. VI is used in the food industry to facilitate the impregnation of various products with, e.g. firming, antioxidant, antimicrobial or cryoprotective agents (Hironaka et al., 2011; Phoon et al., 2008; Barrera et al., 2009; Gras et al., 2003). In a VI process, porous products are immersed in a solution of different compositions and/or concentrations and subjected to a two-step pressure change. The first step (vacuum increase) consists on the reduction of the pressure in a solid-liquid system. During this step, the gas in the product pores is expanded and partially flows out until mechanical equilibrium is achieved. When the atmospheric pressure (second step) is restored, the residual air in the pores compresses and the external liquid flows into the pores due to the action of a hydrodynamic mechanism (HDM) (Fito et al., 1996; Fito, 1994; Fito and Pastor, 1994). The filling of the pores is affected by capillary pressure which depends on pore size, surface tension of the liquid and wetting angle between the liquid and the pore walls (Tylewicz et al., 2012). The pressure changes can also promote deformation (that could provoke an increase of volume) of the product due to viscoelastic properties of its solid matrix (deformation-relaxation phenomena, DRP) (Carciofi et al., 2012; Salvatori et al., 1997; Fito et al., 1996).

The complicated network of highly interconnected intercellular air spaces consisting of tortuous paths and clusters contributes to both anisotropy and heterogeneity of the tissue. Tylewicz et al. (2012) suggested that the flow of the impregnated liquid in the tissue is strongly influenced by the topology and geometry of this network. Using gas in scattering media absorption spectroscopy (GASMAS), these authors found that apples in which air was not totally exhausted during impregnation keep an internal reduced pressure which rises slowly towards ambient temperature over a

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time scale of hours after the operation is terminated. This finding suggests that, in the case of apple parenchyma, the interconnected air spaces expose at least in part an essentially hydrophobic surface and the Laplace pressure term in Fito's model can be locally negative. The liquid flow will be arrested if the liquid interface arrives to a pore so narrow that the driving pressure would not overcome the capillary pressure. Therefore, pressure equilibration can only be achieved either by gas diffusion in gas phase or by gradual wetting of the pores.

During vacuum impregnation, the liquid will convectively penetrate into the pores at a time scale given by pore sizes, the driving pressure difference and liquid viscosity. Fito and Chiralt (2000) calculated that the time scale for liquid flooding of apple air space is of the order of 1 s for a low viscosity liquid. This time scale was confirmed by the *in situ* time lapse microscopy results published by Panarese et al. (2013) where the impregnation of apple tissue was seen as soon as the restoration of the atmospheric pressure was started. These microscopic observations were also performed in spinach leaves, where impregnation occurred later. The difference between the impregnation of both materials was attributed to their different microstructure, pore size and wetting angle.

Impregnation might not be homogeneous in the heterogeneous matrix of the plant tissue due to differences in pore size distribution, morphology and porosity. These differences in impregnation have been confirmed by high resolution X-ray microtomography (μCT). Schulze et al. (2012) reported that VI resulted in a higher isotonic solution uptake in samples of the inner apple cortex compared to the outer part. Smaller cells and lower connectivity of intercellular spaces were attributed to be the cause of the lower impregnation results.

μCT is a non-destructive imaging technology to scrutinize the internal structures of plant tissues and characterize their 3D microstructure at the level of single cells and pores, determining size and shape distributions (Verboven et al., 2015; Herremans et al., 2013). The objective of this paper was to measure the 3D microstructural changes due to impregnation of spinach leaves and on the resulting tissue porosity.

2. Materials and methods

2.1. Raw material handling and storage

Locally grown baby spinach (*Spinacia oleracea* cv. Gazzelle) was harvested, washed, packed in 5 kg bags and transported to our laboratory within two hours after harvesting. Three hundred grams of spinach leaves (55 ± 5 mm of length) were packed in 30 × 30 cm OPPE, heat-sealed, laser perforated bags and stored at 4 °C until used in experiments which were performed within 3 days.

2.2. Impregnating solution

An isotonic solution in equilibrium with spinach leaves was designed with respect to the cell sap. The isotonic solution concentration was determined by immersing three spinach leaves (without petioles) in a series of solutions of different trehalose concentrations, according to Tylewicz et al. (2013). The variation of tissue weight was recorded every 30 min until equilibrium.

2.3. Apparent porosity

The pycnometer method described by Gras et al. (2002) was used to determine the spinach apparent density (ρ_a, in Kg m^{-3}) and the real solid-liquid density (ρ_r, in Kg m^{-3}). The apparent density was measured in leaf pieces by volume displacement in a pycnometer using the respective aqueous isotonic solution as reference liquid. The real solid-liquid density was also obtained by volume displacement, but in this case in samples purees, obtained by manually mashing the leaf samples using a mortar and pestle. The purees were degasified for 10 min by applying vacuum using a manual water pump. The total apparent porosity of the sample (ε) is the ratio of the apparent density and the real solid-liquid density (Eq. (1)) (Lozano et al., 1980):

$$\varepsilon = \left(1 - \frac{\rho_a}{\rho_r}\right) \times 100 \quad (1)$$

2.4. Vacuum impregnation

Vacuum impregnation was carried out at room temperature (20 ± 2 °C) in a chamber connected to a vacuum pump (PIAB Lab Vac, Sigma-Aldrich). The spinach leaves (at least 6), which petiole was removed using a sharp scalpel, were immersed in the impregnating solution during the entire procedure. Based on preliminary experiments to establish maximum weight gain and avoiding tissue damage of spinach, a two cycles, stepwise protocol with a minimum absolute pressure of 150 mbar was chosen (also referred as mild vacuum protocol in this paper). The value of the apparent porosity was used as a reference value for understanding the maximum levels of weight gain that could be achieved (Tylewicz et al., 2012). During vacuum impregnation, the pressure gradually decreased from the atmospheric value (1000 mbar) to the final reduced pressure value (150 mbar) in 10 min. The pressure was kept at 150 mbar for 2 min before releasing the vacuum and letting the pressure to progressively return to the atmospheric value in 10 min. This impregnation cycle was repeated once more to ensure maximum weight gain.

In a separate experiment, aiming at achieving partial impregnation of the leaf samples (also referred as low vacuum protocol in this paper), the pressure gradually decreased from the atmospheric value to a reduced pressure value of 300 mbar in 7.5 min. When vacuum was released, the pressure progressively returned to the atmospheric value in 8.5 min.

After impregnation, the leaf samples were gently blotted with tissue and weighed to determine their weight gain. For μCT, samples from leaves totally or partially impregnated in the trehalose solution and two controls (non-impregnated leaves and leaves kept in the impregnating solution overnight (no vacuum)) were prepared.

2.5. X-ray computed tomography (CT)

2.5.1. Sample preparation

After weighing, leaves (3 per each treatment) were placed in a closed Petri dish in which a tissue wet in distilled water was previously placed for saturation. Each leaf was placed on a polystyrene foam mounting stage with the upper surface in contact with the stage. A layer of Parafilm® was gently wrapped around the lower surface of the sample and the stage to avoid sample dehydration and to keep the sample in a vertical position during the scan (Fig. 1).

2.5.2. Scanning

The samples were scanned within 15 min after completing the vacuum impregnation procedure. Each leaf was scanned using an X-ray CT system (Tomohawk, AEA Technology, UK) using an X-ray source Philips HOMX 161 (Philips Medical Systems Gmbh, Hamburg, Germany) at a voltage of 70 kV and current of 0.5 mA. A 2-mm aluminum filter was placed before the detector to increase