VISUALIZATION OF VACUUM IMPREGNATION TREATMENT AND ITS CONSEQUENCES IN BABY SPINACH LEAVES BY X-RAY TOMOGRAPHY

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Summary: Vacuum impregnation is used in the food industry to facilitate the impregnation of porous products e.g. sugar, salt, antioxidant, antimicrobial or cryoprotective agents. In this study, X-ray tomography was used to study the process of vacuum impregnation in spinach leaves. The impregnation protocols was used to impregnate an isotonic solution into the extracellular space of the plant tissue. The *in-situ* mechanism of VI treatment and the changes in leaf thickness before and after VI using the same leaves are the aims of this study.

1. INTRODUCTION

Vacuum impregnation (VI) has been widely used as a pre-treatment method prior to minimal processing, freezing, or drying of fruits and vegetables (Chiralt et al., 1999; Fito et al., 2001). During VI, porous materials are immersed in solutions of different compositions and/or concentrations, and subjected to two-step pressure changes. The first step occurs when vacuum is applied to the solid-liquid system, the gas inside the pores expands and the native liquid flows out until mechanical equilibrium is achieved. The second step occurs when the atmospheric pressure is restored, the residual gas in the pores is compressed and the external liquid flows into the pores, replacing the air (Tylewicz, Romani, Widell, & Gómez Galindo, 2013). VI is, therefore, a controlled way to access the intercellular space and introduce different compounds that modify the structural, functional, or nutritional properties of plant tissues, depending on the type of molecules impregnated (Chiralt et al., 1999). However, to the best of our knowledge, lack of research has been done on the *in-situ* mechanism of VI and how it affect the leaves thickness after the treatment. The homogeneity of the solution introduced in the leaves also remain unsolved. Therefore, the aim of this study was to visualize the *in-situ* process of VI and measure the change of thickness in the spinach leaves after VI treatment.

2. EXPERIMENTAL METHOD

The experiments were performed at the Tomography Laboratory in Division of Solids Mechanic, LTH, Lund

University, Sweden. The baby spinach leaves were grown in a greenhouse at the Biology Department of Lund University. On the day of experiment, leaves from five weeks old spinach were harvested. The length of the leaf blade was about 5.0 ± 0.1 cm with 2.0 ± 0.1 cm petiole. The leaves were placed in a saturated humidity container and transported to the tomography laboratory within 10 min. The leaf was placed in a rectangular plastic chamber filled with isotonic sucrose solution with 33 % Lymphoprep™ in order to create the contrast. The image was scanned using Zeiss X radia XRM 520 X-ray tomogram and the exposure time used was about 2.5 sec per hour and it took about 2 h for imaging. The voltage used was 80/90 KV, objective 4X, pixel size 3.500, current 90 mAmp, and intensity of > 5000. For the *in-situ* impregnation with VI machine, the set-up of the chamber for the vacuum pressure to be applied during the treatment is still under construction.

3. RESULTS

The result shows the cross section of the spinach leaves when immersed in the sucrose solution and Lymphoprep™ (Figure 1). The resolution was quite promising in which the microstructure of the leaves can be recognized e.g. mesophyll palisade, spongy mesophyll, vein, epidermis, calcium oxalate crystals and air spaces. As can be seen, the image of cells was against the pore space is visible. However, the contrast issue comes into contact when the cells was filled with surrounding liquid. The dye that has been used was helpful to some extent to create contrast. Several trials have been launched before getting the image shown here, as some challenges have been encountered during the experiments. Issues with movement of leaves, which was resulted from the movement of the solution into the extracellular space of the tissue (osmosis effect), has resulted in some noise during imaging. The limited contrast obtained when the pores of the plant tissue was filled with liquid adds up to the challenges during imaging with biological sample.

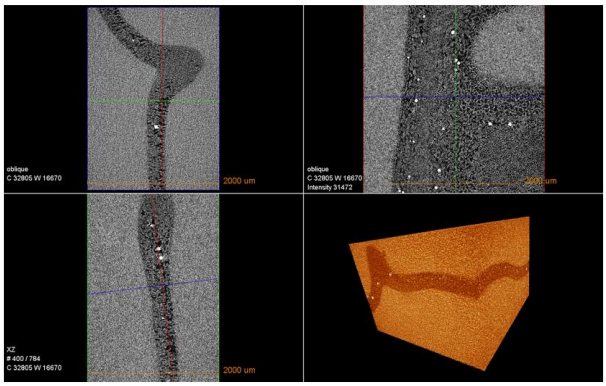

Figure 1. Images of spinach leaf when immersed in rectangular plastic chamber filled with 33 % Lymphoprep™ and isotonic sucrose solution.

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