Influence of Vacuum Impregnation with Different Substances on the Metabolic Heat Production and Sugar Metabolism of Spinach Leaves

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Abstract Vacuum impregnation (VI) has been widely used as pre-treatment prior to, e.g., minimal processing, freezing, or drying of fruit and vegetables. Most of the investigations have focused on the applicability of VI to modify physicochemical, sensory, and nutritive characteristics. However, little attention has been paid to the metabolic consequences of impregnating different substances into the plant tissue. This study explores short- and long-term metabolic responses of baby spinach leaves at 5 °C after impregnation with several substances that are commonly used in food industry, e.g., sucrose, calcium lactate, citric acid, and ascorbic acid. Short-term metabolic response of impregnated spinach leaves was measured with isothermal calorimetry for 7 h. Results demonstrated that leaves impregnated with calcium lactate and sucrose showed a drastic increase in metabolic heat production, but no change was recorded in leaves impregnated with solutions of ascorbic acid or citric acid. Long-term metabolic responses were evaluated by measuring sugars and starch. The different impregnation solutes provoked specific changes in the carbohydrate composition during cold storage and the concentrations at each time point are likely to be the result of mobilization of starch, which

sharply decreased during storage, and solute-specific differences in metabolization and interconversion of sugars.

Keywords Isothermal calorimetry · Vacuum impregnation · Metabolic heat production · Spinach · Sugar metabolism

Introduction

Vacuum impregnation (VI) is a unit operation that allows the introduction of solutions into the porous structures of fruit and vegetables. It has been widely studied as a pre-treatment method prior to, e.g., minimal processing, freezing, or drying of fruit and vegetables (Chiralt et al. 1999). 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 et al. 2013). Therefore, VI is 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).

Application of VI has been extensively studied, particularly with regard to the modification of the physicochemical properties and sensory attributes of food products (Betoret et al. 2003; Codoñer-Franch et al. 2013; Derossi et al. 2010; Fito et al. 2001; Gras et al. 2003; Moreno et al. 2004). A significant interest for VI has been received from the food industry since it has the potential to improve a number of issues related to food production and quality. Impregnation of fruit and vegetables with ascorbic acid or citric acid solutions with concentrations ranging

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from 10 mg/L to 20 g/L has been extensively used in order to inhibit enzymatic browning during storage and reduce microbial growth (Bieganska-Marecik and Czapski 2007; Blanda et al. 2008; Radziejewska-Kubzdela et al. 2007; Shah and Nath 2008; Yurttas et al. 2014). Similarly, VI of calcium lactate at concentrations ranging from 0.5 to 2.5 g/L has been shown to improve the texture of minimally processed apples, pears, carrots, and lettuce (Alandes et al. 2009; Anino et al. 2006; Martín-Diana et al. 2006; Rico et al. 2007) and to enhance the rigidity and brittleness in carrot and eggplant when impregnated together with sucrose (Gras et al. 2003). Luna-Guzmán and Barrett (2000) showed that dipping fresh cut cantaloupe cylinders in calcium lactate solution significantly increased the firmness throughout cold storage. VI with sucrose is normally used to extend shelf life by reducing the water activity (Moreno et al. 2004, 2000; Perez-Cabrera et al. 2011), as well as to shorten dehydration time (Pallas et al. 2013).

To the best of our knowledge, little attention has been paid to the metabolic response of the plant tissue once different substances are impregnated into the structure. However, recent findings pointing to the direction of the metabolization of the impregnated molecules open a wide range of questions and possibilities. Panarese et al. (2014) presented evidence that substances that are commonly used for VI, such as sugars, affect the metabolic activity of the cells within short time scales after the treatment. Detailed metabolic consequences, however, still remain to be understood. Metabolic effects might also be true for the impregnation of other substances such as ascorbic acid, as suggested by the results reported by Rocculi et al. (2007) where simple immersion of potato slices in this compound provoked an increase in their gross metabolic activity and depletion of reducing sugars. As pointed out by Gómez Galindo and Yusof (2014), VI of plant tissues is not only about mass transfer, quality attributes, and structure but also how the metabolically active cells in the impregnated tissue may be affected.

The main purpose of this study was to explore the metabolic consequences of impregnating different substances, commonly used in the food industry such as sucrose, calcium lactate, citric acid, and ascorbic acid, into baby spinach leaves. Short-term metabolic response was evaluated by measuring the metabolic heat production using isothermal calorimetry. Calorimetrically measured heat production rates are proportional to metabolic activity (Wadsö and Gómez Galindo 2009). Long-term metabolic response was evaluated by starch and sugar analysis during 4 days of cold storage at 5 °C.

Material and Methods

Plant Material

Baby spinach leaves (*Spinacia oleracea* cv. Misano F1) were grown in a greenhouse with 16 h of light at 20 °C during day

and night times. The greenhouse lamps were 400-W metal halide lamps with a photosynthetic photon flux of 100 $\mu\text{mol}/\text{m}^2/\text{s}$. Two seeds were sowed 1.5 cm below the soil surface and 4.0 cm apart from each other. The dimensions of each plant growing tray were 54 cm $\times$ 32 cm and there were 42 spinach plants grown in each tray. The spinach trays were watered every second day. Leaves from 5-week-old spinach were harvested. At the time of harvesting, the length of each leaf blade was 7.0 ± 0.1 cm with 2.0 ± 0.1 -cm petiole and the width at the center of the leaf was 3.0 ± 0.3 cm. Leaves were harvested at 10 am, which was 4 h after the start of the light period and only the non-shaded leaves were used. Leaves were harvested randomly from plants located in five trays, placed into sealed plastic bags, and transported to the laboratory within 10 min. For each experimental replication, the harvesting of the leaves was done from five new trays where the plants were 5 weeks old.

Solutions

An isotonic sucrose solution 0.6 mol/L (pH 5.8) in equilibrium with the spinach leaves was designed with respect to the cell sap. The isotonic solution concentration was determined by immersing three spinach leaves in a series of solutions with different concentrations. The variation of tissue weight was recorded every hour until equilibrium. Ascorbic acid and citric acid solutions were prepared at 5.7 (pH 3.2) and 1.0 mmol/L (pH 3.2), respectively. Calcium lactate solution was prepared at 50 mmol/L (pH 7.1). These concentrations were chosen based on the most commonly used concentrations for VI of fruits and vegetables (Bieganska-Marecik and Czapski 2007; Radziejewska-Kubzdela et al. 2014; Yurttas et al. 2014). The chosen concentrations did not noticeably change the taste of the leaves immediately after VI. The impregnating solutions were kept at 5 °C before the VI treatment took place.

Vacuum Impregnation

Four leaves (3.8 ± 0.1 g) were submerged in the solutions and immediately subjected to VI, which was carried out in a chamber connected to a vacuum controller (SIA, Bologna, Italy) and a vacuum pump, as described by Panarese et al. (2013). The chamber was covered with aluminum foil to keep dark condition during VI. The setup was placed in a temperature-controlled room so that the impregnation was carried out at 5.0 ± 0.1 °C.

Based on preliminary experiments to establish maximum weight gain and avoid tissue damage, a protocol with a minimum absolute pressure of 150 mbar was chosen. The chosen pressure profile ensured that the cell viability was maintained; cell viability after VI was verified by vital staining with fluorescein diacetate (FDA) as described by Phoon et al. (2008). During the first phase of VI, the pressure was gradually

decreased from 1000 to 150 mbar in 11 min and was kept at 150 mbar for 1 min. During the second phase, the vacuum was released and the pressure progressively increased to atmospheric pressure during 7 min and was kept at atmospheric pressure for 13 min. The total treatment time was 32 min and this cycle was repeated twice. After VI, the excess solution on the surface of the spinach leaves was removed with tissue paper and the leaves were immediately transferred to calorimetry ampoules.

Short-term Metabolic Response: Isothermal Calorimetry Measurements of Heat Production

The calorimetric measurements were performed with a two-channel isothermal calorimeter (Biocal 2000, Calmetrix Inc., USA), where each calorimeter is equipped with its own reference cell. The reference cell was made up of aluminum (91.0 g). The primary output from the heat flow sensors in the calorimeter (voltage) was recorded every minute by a computer. The corresponding thermal powers (heat production rates) were calculated according to Eq. 1.

$$P = \varepsilon \frac{(V_s - V_{bl})}{m} \quad (1)$$

where P is the specific thermal power of the spinach sample ($\mu\text{W g}^{-1}$), ε is the calibration coefficient of the calorimeter ($\mu\text{W } \mu\text{V}^{-1}$), V_s the voltage signal from the calorimeter (μV), V_{bl} the voltage recorded for the baseline (μW), and m is the mass of the sample (g). Baselines were recorded before every new measurement.

Isothermal calorimetry measurements were performed at 5.0 °C. No condensation or temperature fluctuations were registered in the equipment as it was placed in the cold room at 5.0 ± 0.1 °C. Eight leaves (7.8 ± 0.1 g) were placed in a 1.1-L closed plastic container with wet tissue on the bottom for 3 h at 5.0 ± 0.1 °C in the darkness. The lid of the plastic containers was perforated with small holes (1 mm) to ensure gas exchange during storage. After this incubation period, the leaves were subjected to two treatments, described below. Four replications were made for each of the impregnated solutions and the control.

- i) The leaves were placed into sealed 125-mL plastic ampoules which were placed in the calorimeter. After the initial disturbance, the signal was recorded for 2 h. They were then removed from the ampoule and VI was applied. The leaves were subsequently placed back in the ampoule and the calorimeter. After the initial disturbance, the signal was recorded for 2 more hours.
- ii) Untreated leaves (control) were placed in the ampoule and after the initial disturbance, the signal was recorded for 2 h. They were removed from the ampoule for 64 min but VI was not applied; instead, the leaves were

placed in a container with water saturated atmosphere. The leaves were subsequently placed back in the ampoule and the calorimeter. After the initial disturbance, the signal was recorded for 2 more hours.

In a separate experiment, the signal from the untreated leaves was recorded for 6 consecutive hours.

Long-term Metabolic Effects: Sugar, Starch, and Color Analysis

The untreated and impregnated spinach leaves were placed in 1.1-L closed plastic containers with wet tissue on the bottom for 4 days at 5.0 ± 0.1 °C in darkness. During these 4 days of cold storage, there were no visual signs of senescence or microbial degradation in the leaves. Samples were taken 15 min, 2, and 4 days after impregnation for sugar and color analysis. Starch was analyzed 2 and 4 days after impregnation. For all analysis, each of the three replications was done by repeating the procedures from the harvesting of the leaves. Three measurements were performed for each time point in each replication.

For sugar analysis, the extraction was done according to the method described by Toledo et al. (2003). Of freeze dried spinach tissue, 0.30 g was placed into a reflux tube containing a boiling solution of 20 mL of 99% ethanol for 10 min and later cooled under running water. After this, 50 mL 80% ethanol solution was added, and the extract was evaporated in vacuum at 50 °C. The extracts of spinach tissue and ethanol solution were filtered and centrifuged at 13000 g for 25 min. The supernatant was analyzed enzymatically for sucrose, D-glucose, and D-fructose by using the Megazyme K-SUFRG 06/14 Assay Procedure (Megazyme, Megazyme International, Ireland). The absorbance of the blank and samples was measured at 340 nm using a Varian Cary® 50 UV-Vis spectrophotometer (Varian Inc., Santa Clara, CA, USA).

The total starch analysis was carried out according to the method described by Buysse and Merckx (1993) with certain modifications. Of freeze dried spinach tissue, 0.10 g was hydrolyzed with 100 mL of 1-M sulfuric acid at 100 °C for 3 h, with occasional stirring. Of the hydrolyzed sample solution, 1.0 mL was pipetted into a test tube and 1.0 mL of Milli-Q water, 0.05 mL of 80% phenol, and 5 mL of concentrated sulfuric acid were added. Tubes were vortexed and incubated at 25 °C in a water bath for 10 min. The absorbance of the blank and samples was measured at 490 nm using a Varian Cary® 50 UV-Vis spectrophotometer. The standard curve was prepared by diluting a glucose stock solution (20 g/L) in de-ionized water to obtain solutions with concentrations ranging from 0 to 500 mg/L. The concentrations were determined against the glucose standard curve and the starch content of the samples was estimated by the glucose equivalent, multiplying the concentration with a 0.9 factor (Nielsen 2010). Knowing that before hydrolysis the spinach samples have a

mixture of starch and sugars, a separate experiment was designed to evaluate the influence of those sugars on the phenol reaction after acid hydrolysis. Essay tubes were prepared by adding the concentrations of sucrose, glucose, fructose measured enzymatically on each sample, and the amount of starch measured after acid hydrolysis of each of the spinach samples (potato starch was used). Other tubes were prepared by adding the same concentrations of sucrose, glucose, and fructose as in the first tubes but without starch. All the tubes were subjected to acid hydrolysis and phenol reaction as described above. The difference in absorbance was used to calculate the amount of starch in the samples against the glucose standard curve. For every time point after VI, the concentration of the starch was expressed as relative to the levels at the time of treatment. At least three measurements were performed for each time point.

Color measurements were performed using a spectrophotometer (model CM-700d, Minolta Corporation, Japan) in a room at 5.0 ± 0.1 °C. The L^* , a^* , and b^* values of the spinach leaves were recorded before VI, 15 min, 2, and 4 days after VI with each of the studied solutions. Measurements were done on three different points of a leaf. Ten leaves per time point and three replicates were used for the measurements. The color of the untreated leaves was also measured at the same time points (control).

Statistical Analysis

The statistical significance ($p < 0.05$) of the treatments was tested by means of one-way analysis of variance (ANOVA) using Excel (Microsoft Office, Redmond, WA USA). The Tukey-Kramer multiple comparison test was used to evaluate true differences in treatment means.

Results

Short-term Metabolic Responses

Effects of Different Impregnating Solutions on Metabolic Heat Production at 5 °C

Typical raw calorimetric data are shown in Fig. 1. The calorimetric signal was disturbed each time the ampoule was placed in the calorimeter. The signal returned to the recording range after about 1 h.

The results from the calorimetric measurements on spinach leaves when impregnated with sucrose, calcium lactate, citric acid, or ascorbic acid solutions are reported in Fig. 2. In this figure, to facilitate the comparison between the treatments, the signals during the 1-h stabilization periods are not shown, and the continuous measurement of the control sample was added (dashed line). Although a transient change on thermal power shorter than 2 h cannot be excluded, a doubling of the thermal power was observed when the leaves were impregnated with calcium lactate solution; an increase in thermal power was also seen for the sucrose solution. No change in thermal power was detected when leaves were impregnated with ascorbic acid or citric acid solutions.

Long-term Metabolic Responses

Effects of Different Impregnating Solutions on the Concentration of Sugars During Storage at 5 °C

The effect of impregnating different solutions into spinach leaves during 4 days of cold storage is shown in Figs. 3 to 6.

Fig. 1 An example of the raw calorimetric data obtained using the following measurement sequence. The leaves were placed in the ampoule for 3 h. The first 1 h was needed to stabilize the signal and the signal from the metabolic activity of the sample was recorded for the next 2 h. The leaves were then removed from the ampoule for 1 h for the VI.

The leaves were subsequently placed back in the ampoule and the signal was recorded. Again, the first 1 h is the stabilization of the signal and the metabolic activity of the impregnated sample was recorded for the next 2 h. All measurements were performed in the dark

Fig. 2 Calorimetric measurements of metabolic thermal power of untreated (•) and VI-treated spinach leaves with 50-mmol/L calcium lactate (pH 7.1) (x), 0.6-mol/L sucrose (pH 5.8) (▲), 5.7-mmol/L ascorbic acid (pH 3.2) (■), and 1.0-mmol/L citric acid (pH 3.2) (◆) at 5 °C in the darkness. In a separate experiment, the signal from the untreated leaves (*dashed line*) was recorded for 6 consecutive hours. Statistically significant difference of the different curves ($p < 0.05$) is represented by *different letters* above the recorded thermal power curves. Bars represent the standard deviation of the mean of four measurements

Fig. 3 Concentration of sucrose, glucose, and fructose in leaves **a** impregnated with 0.6-mol/L sucrose solution (pH 5.8) (*filled symbols*) and **b** untreated leaves (*empty symbols*) during 4 days at 5 °C in the darkness. The first point of the VI-treated leaves represents the concentration of the sugars 15 min after VI. Statistically significant difference ($p < 0.05$) within each curve is represented by *different letters*. Bars represent the standard deviation of the mean of three measurements

Figure 3A shows that the impregnated sucrose is being metabolized at a high rate during the storage period, reaching a final concentration of 260 mg/g (DM) at the end of the storage time. This shows that about 50% of the exogenously fed sucrose has been metabolized during 4 days of storage. For glucose, the concentration increased significantly from 36 (DM) to 70 mg/g (DM) during cold storage, whereas for fructose, no change was observed.

Figure 3B shows the concentration of sucrose, glucose, and fructose in the untreated leaves. Over the storage period, sucrose and glucose were significantly decreased, reaching a final concentration of 24 (DM) and 9 mg/g (DM), respectively. A significant increase of fructose was observed at the end of the storage period, with final concentration of 6 mg/g (DM).

Figure 4 shows the changes of sucrose, glucose, and fructose in leaves impregnated with calcium lactate during the studied storage period in comparison with the control. For

the impregnated samples, a significant increase in glucose was detected 15 min after treatment and throughout storage period, whereas for fructose, the concentration increased after 2 days of storage. In contrast, in the control samples, sucrose and glucose concentrations showed a steady decrease over the storage period. The concentration of fructose in the calcium lactate-impregnated sample increased to a lower extent in comparison with the control leaves.

When leaves were impregnated with citric acid (Fig. 5), the concentration of sucrose over the storage period was declining in a way that is comparable to the control. For the VI samples, the concentration of glucose remained unchanged over the storage period, whereas a steady decrease was observed in the control. The concentration of fructose increased over 4 days of storage.

When leaves were impregnated with ascorbic acid (Fig. 6), the concentration of sucrose was unchanged during 4 days of storage. In contrast, there was a steady decrease in sucrose

Fig. 4 Concentration of sucrose, glucose, and fructose in baby spinach leaves impregnated with 50-mmol/L calcium lactate (*filled symbols*) and untreated leaves (*empty symbols*) during 4 days at 5 °C in the darkness. The first point for the VI-treated leaves represents the concentration of the sugars 15 min after VI. Statistically significant difference ($p < 0.05$) within each curve is represented by *different letters*. Bars represent the standard deviation of the mean of three measurements

Fig. 5 Concentration of sucrose, glucose, and fructose in baby spinach leaves impregnated with 1.0-mmol/L citric acid (pH 3.2) (filled symbols) and untreated leaves (empty symbols) during 4 days at 5 °C in the darkness. The first point of the VI-treated leaves represents the concentration of the sugars 15 min after VI. Statistically significant difference ($p < 0.05$) within each curve is represented by different letters. Bars represent the standard deviation of the mean of three measurements

content in the control. For the impregnated leaves, the concentration of glucose was significantly increased after 4 days, in contrast to the steady decrease observed in the control. The changes in the concentration of fructose in the impregnated leaves were comparable to those of the control samples. However, the concentration increased to a higher extent in the impregnated samples.

Effects of Different Impregnating Solutions on Starch Concentration During Storage at 5 °C

To account for an observed high variability in the initial concentration of starch in the leaves used, the concentration of starch during storage after impregnation with solutions of sucrose, calcium lactate, citric acid, and ascorbic acid is reported relative to the start concentration in Fig. 7. There was a steady decrease of the starch in the control as well as all impregnated

leaves. Among all substances, sucrose-impregnated leaves showed the most drastic decrease after 2 days of storage, with a 63% starch reduction. In citric acid-impregnated leaves, the concentration of starch decreased about 40% during the first 2 days but did not decrease much more between days 2 and 4.

Long-term Quality Changes: Effects of Different Impregnating Solutions on Leaf Color During Storage at 5 °C

Figure 8A–C shows the changes of color in spinach leaves impregnated with sucrose, calcium lactate, citric acid, or ascorbic acid solution during the studied period in comparison with the non-impregnated leaves. Shortly after VI, the impregnated leaves show darker color (lower L^* value) as compared to the control and the greenness of the leaves (a^*) which significantly increased in all impregnated leaves. The values for

Fig. 6 Concentration of sucrose, glucose, and fructose in baby spinach leaves impregnated with 5.7-mmol/L ascorbic acid (pH 3.2) (filled symbols) and untreated leaves (empty symbols) during 4 days at 5 °C in the darkness. The first point of the VI-treated leaves represents the concentration of the sugars 15 min after VI. Statistically significant difference ($p < 0.05$) within each curve is represented by different letters. Bars represent the standard deviation of the mean of three measurements

Fig. 7 Relative concentration of starch in baby spinach leaves with an initial content of 0.393 ± 0.006 g/kg (DM). The leaves were not impregnated (control), impregnated with sucrose solution 0.6 mol/L (pH 5.8), 50-mmol/L calcium lactate, 1.0-mmol/L citric acid (pH 3.2), and 5.7-mmol/L ascorbic acid (pH 3.2) during 4 days at 5 °C in the darkness. Statistically significant difference ($p < 0.05$) within each curve is represented by different letters. Bars represent the standard deviation of the mean of three measurements

yellowness (b^*) was generally lower than in the untreated sample, but changes were not significant. After 2 days of storage, the color parameters of the impregnated leaves had reverted back to their original color, and impregnated samples and control were similar regarding the measured parameters.

Discussion

A deeper understanding of the metabolic consequences of impregnating different molecules into the structure of a plant tissue is of key importance for product development, since the product composition and shelf life are strongly dependent on the tissue metabolic activity. In this study, we provide evidence that the different substances impregnated into baby spinach leaves have different effects on the short-term gross metabolic activity of the leaves and provoke different changes to their carbohydrate pools. During storage in the dark, the starch decreases drastically (clearly shown in Fig. 7), which

Fig. 8 Effect of different impregnating solutions on color changes during storage at 5 °C in the darkness. Spinach leaves were either untreated or impregnated with sucrose, calcium lactate, citric acid, or ascorbic acid solutions. The leaves were stored for 4 days. Color parameters: **a** L^* (from 0 black to 100 white), **b** a^* (from $-a^*$ green to $+a^*$ red), and **c** b^* (from $-b^*$ blue to $+b^*$ yellow). Statistically significant difference ($p < 0.05$) within each curve is represented by different letters. Values represent means $\pm$ standard error mean of three measurements

was expected as, in nature, starch is mobilized during the night (Zeeman et al. 2007).

In this study, calorimetric measurements provide evidence of a significant increase in spinach leaf gross metabolism as a consequence of impregnation with sucrose (Fig. 2), which is consistent with previous observations by our group (Panarese et al. 2014). In contrast, the gross metabolic activity of the control remains practically unchanged after the first 3 h of measurement. According to Panarese et al. (2014), the sucrose-induced increase might be caused by the metabolism of the impregnated sucrose. Sucrose might be taken up and metabolized by the cells. Sucrose loaded into the apoplast can be co-transported with protons into the symplast, driven by the plasma membrane ATPase, which pumps protons into the apoplast (Voitsekhovskaya et al. 2002). The sucrose can then be stored in the vacuole or directly metabolized into glucose and fructose derivatives, before entering respiratory or biosynthetic pathways. The increased metabolic activity observed by isothermal calorimetry may be the cause of the

dramatic and sustained decrease in the concentration of the impregnated sucrose and the drastic loss of starch after the impregnation (Figs. 3 and 7). The total concentration of the sugars may thus be the final result of the mobilization of starch and the metabolism of sugars occurring during the storage period, whereas the relative changes observed between glucose and fructose concentrations should reflect also interconversions within sugar metabolism.

The gross metabolic activity of the baby spinach leaves increased drastically after impregnation with calcium lactate, accounting to the largest increase in metabolic activity among the studied substances. Externally fed lactate has shown to be rapidly metabolized by spinach leaves and leaves of other species of higher plants such as lettuce and soybean (Betsche 1983). This lactate metabolism was shown to occur in the light and the dark. A fast, mitochondrial lactate metabolism has also been reported in potato tubers (Paventi et al. 2007). However, the carbon supplied with the lactate represents only a small percentage of the carbon supplied with

the sucrose in the sucrose impregnated leaves, but the resulting energy burst is much higher (Fig. 2). Therefore, the calorimetry results (Fig. 2) suggest that the energy burst caused by the impregnation of calcium lactate is the result of a metabolic stimulation of the respiratory pathway by lactate as an effector rather than as a substrate. The energy burst caused by lactate impregnation may be caused by the mobilization of other carbon sources such as starch. During storage in the dark, mobilization of starch would explain the significant increase in fructose 2 days after impregnation and in glucose 4 days after impregnation. As, in the darkness, glucose is easier metabolized by the leaves than fructose (Sagishima et al. 1989), the overall effect was an earlier detection of the increase of fructose. However, the steady state concentrations of fructose and glucose will be influenced by glucose to fructose interconversion and glucose and fructose metabolism.

The impregnation of citric and ascorbic acid into the leaves did not provoke an increase of their gross metabolic activity, which is the measurement of short-term metabolic responses (Fig. 2). Interestingly, the effect of the impregnation with these acids is only noticeable through the sugar and starch composition analysis, days after the impregnation (Figs. 5 and 6). Upon VI with these solutions (at pH 3.2), the leaf's apoplastic pH might have been considerably affected, which may have provoked changes in respiration (Lambers et al. 1998) and/or the alteration of gene expression patterns as reported by Lager et al. 2010, although this study was performed at higher pH values (4.5 to 6.0). As with the other impregnated substances, the measured concentrations of sugars will be the final result of the balance between sugar metabolism, starch mobilization, and sugar interconversions, differentially affected by the impregnated solutes.

Conclusions

This study explores metabolic responses of baby spinach tissue that follows the application of VI with commonly used substances for impregnation of fruit and vegetables. The following are the main results:

- i. Introducing different substances into baby spinach leaves by vacuum impregnation provoked different effects on the short-term gross metabolic activity measured with isothermal calorimetry. Sucrose and calcium lactate provoked rapid increases in the gross metabolic activity of the leaves while no change was detected after impregnation of citric or ascorbic acid.
- ii. Different changes in sugar composition (sucrose, glucose, and fructose) were detected, depending on the impregnated substance. The total sugar concentrations at each time point is likely the result of the balance between

metabolization of the sugars and mobilization of starch; the concentration of which decreases in the impregnated leaves during 4 days of cold storage in darkness.

- iii. The detected effects of the impregnation with different substances are likely to be a consequence of their metabolism by the cells in the tissue.

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References

- Alandes, L., Pérez-Munuera, I., Llorca, E., Quiles, A., & Hernando, I. (2009). Use of calcium lactate to improve structure of “Flor de Invierno” fresh-cut pears. *Postharvest Biology and Technology*, 53(3), 145–151.
- Anino, S. V., Salvatori, D. M., & Alzamora, S. M. (2006). Changes in calcium level and mechanical properties of apple tissue due to impregnation with calcium salts. *Food Research International*, 39(2), 154–164.
- Betoret, N., Puente, L., Díaz, M. J., Pagán, M. J., García, M. J., Gras, M. L., et al. (2003). Development of probiotic-enriched dried fruits by vacuum impregnation. *Journal of Food Engineering*, 56(2–3), 273–277.
- Betsche, T. (1983). L-Lactate dehydrogenase from leaves of higher plants. Occurrence and metabolism of lactate under aerobic conditions. *Phytochemistry*, 22(6), 1341–1344.
- Bieganska-Marecik, R., & Czapski, J. (2007). Effect of minimal processing on changes in the texture of vacuum-packaged apple slices. *Polish Journal of Food and Nutrition Sciences*, 57(2), 161–166.
- Blanda, G., Cerretani, L., Bendini, A., Cardinali, A., Scarpellini, A., & Lercker, G. (2008). Effect of vacuum impregnation on the phenolic content of Granny Smith and Stark Delicious frozen apple cvv. *European Food Research and Technology*, 226(5), 1229–1237.
- Buyse, J. A. N., & Merckx, R. (1993). An improved colorimetric method to quantify sugar content of plant tissue. *Journal of Experimental Botany*, 44(10), 1627–1629.
- Chiralt, A., Fito, P., Andrés, A., Barat, J., Martínez-Monzó, J., Martínez-Navarrete, N., ... Oliveira, J. (1999). Vacuum impregnation: a tool in minimally processing of foods. In *Processing foods: quality optimization and process assessment* (pp. 341–356). Boca Raton: CRC Press.
- Codoñer-Franch, P., Betoret, E., Betoret, N., López-Jaén, A. B., Valls-Belles, V., & Fito, P. (2013). Dried apples enriched with mandarin juice by vacuum impregnation improve antioxidant capacity and decrease inflammation in obese children. *Nutrición Hospitalaria*, 28(3), 1177–1183.
- Derossi, A., De Pilli, T., & Severini, C. (2010). Reduction in the pH of vegetables by vacuum impregnation: a study on pepper. *Journal of Food Engineering*, 99(1), 9–15.
- Fito, P., Chiralt, A., Betoret, N., Gras, M., Cháfer, M., Martínez-Monzó, J., et al. (2001). Vacuum impregnation and osmotic dehydration in matrix engineering: application in functional fresh food development. *Journal of Food Engineering*, 49(2), 175–183.

- Gómez Galindo, F., & Yusof, N. L. (2014). New insights into the dynamics of vacuum impregnation of plant tissues and its metabolic consequences. *Journal of the Science of Food and Agriculture*, 95(6), 1127–1130.
- Gras, M., Vidal, D., Betoret, N., Chiralt, A., & Fito, P. (2003). Calcium fortification of vegetables by vacuum impregnation: interactions with cellular matrix. *Journal of Food Engineering*, 56(2), 279–284.
- Lambers, H., Stuart Chapin III, F., Pons, T. L. (1998). Photosynthesis, respiration and long-distance transport. In: *Plant Physiological Ecology* (1st ed., pp. 10–152). New York: Springer.
- Lager, I., Andréasson, O., Dunbar, T. L., Andréasson, E., Escobar, M. A., Rasmusson, A. G. (2010). Changes in external pH rapidly alter plant gene expression and modulate auxin and elicitor responses. *Plant, Cell & Environment*, 33, 1513–1528.
- Luna-Guzmán, I., & Barrett, D. M. (2000). Comparison of calcium chloride and calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupes. *Postharvest Biology and Technology*, 19(1), 61–72.
- Martín-Diana, A. B., Rico, D., Frías, J., Henehan, G. T. M., Mulcahy, J., Barat, J. M., & Barry-Ryan, C. (2006). Effect of calcium lactate and heat-shock on texture in fresh-cut lettuce during storage. *Journal of Food Engineering*, 77(4), 1069–1077.
- Moreno, J., Bugueño, G., Velasco, V., Petzold, G., & Tabilo-Munizaga, G. (2004). Osmotic dehydration and vacuum impregnation on physicochemical properties of Chilean papaya (*Carica candamarcensis*). *Journal of Food Science*, 69(3), 102–106.
- Moreno, J., Chiralt, A., Escriche, I., & Serra, J. (2000). Effect of blanching/osmotic dehydration combined methods on quality and stability of minimally processed strawberries. *Food Research International*, 33(7), 609–616.
- Nielsen, S. S. (2010). *Food analysis*. New York: Springer.
- Pallas, L. A., Pegg, R. B., & Kerr, W. L. (2013). Quality factors, antioxidant activity, and sensory properties of jet-tube dried rabbiteye blueberries. *Journal of the Science of Food and Agriculture*, 93(8), 1887–1897.
- Panarese, V., Dejmek, P., Rocculi, P., & Gómez Galindo, F. (2013). Microscopic studies providing insight into the mechanisms of mass transfer in vacuum impregnation. *Innovative Food Science & Emerging Technologies*, 18, 169–176.
- Panarese, V., Rocculi, P., Baldi, E., Wadsö, L., Rasmusson, A. G., & Gómez Galindo, F. (2014). Vacuum impregnation modulates the metabolic activity of spinach leaves. *Innovative Food Science & Emerging Technologies*, 26, 286–293.
- Paventi, G., Pizzuto, R., Chieppa, G., & Passarella, S. (2007). L-Lactate metabolism in potato tuber mitochondria. *FEBS Journal*, 274(6), 1459–1469.
- Perez-Cabrera, L., Chafer, M., Chiralt, A., & Gonzalez-Martinez, C. (2011). Effectiveness of antibrowning agents applied by vacuum impregnation on minimally processed pear. *LWT-Food Science and Technology*, 44(10), 2273–2280.
- Phoon, P. Y., Gómez Galindo, F., Vicente, A., & Dejmek, P. (2008). Pulsed electric field in combination with vacuum impregnation with trehalose improves the freezing tolerance of spinach leaves. *Journal of Food Engineering*, 88(1), 144–148.
- Radziejewska-Kubzdela, E., Biegańska-Marecik, R., & Kidoń, M. (2014). Applicability of vacuum impregnation to modify physicochemical, sensory and nutritive characteristics of plant origin products—a review. *International Journal of Molecular Sciences*, 15(9), 16577–16610.
- Radziejewska-Kubzdela, E., Czapski, J., & Czaczyk, K. (2007). The effect of pretreatment of shredded celeriac using solutions of enzymatic browning inhibitors on the quality of minimally processed product. *Journal of Food Quality*, 30(5), 594–608.
- Rico, D., Martín-Diana, A. B., Frias, J. M., Barat, J., Henehan, G., & Barry-Ryan, C. (2007). Improvement in texture using calcium lactate and heat-shock treatments for stored ready-to-eat carrots. *Journal of Food Engineering*, 79(4), 1196–1206.
- Rocculi, P., Gómez Galindo, F., Mendoza, F., Wadsö, L., Romani, S., Dalla Rosa, M., & Sjöholm, I. (2007). Effects of the application of anti-browning substances on the metabolic activity and sugar composition of fresh-cut potatoes. *Postharvest Biology and Technology*, 43(1), 151–157.
- Sagishima, K., Kubota, K., & Ashihara, H. (1989). Uptake and metabolism of sugars by suspension cultured *Catharanthus roseus* cells. *Annals of Botany*, 64(2), 185–193.
- Shah, N. S., & Nath, N. (2008). Changes in qualities of minimally processed litchis: effect of antibrowning agents, osmo-vacuum drying and moderate vacuum packaging. *LWT-Food Science and Technology*, 41(4), 660–668.
- Toledo, M. E. A., Ueda, Y., Imahori, Y., & Ayaki, M. (2003). L-Ascorbic acid metabolism in spinach (*Spinacia oleracea* L.) during postharvest storage in light and dark. *Postharvest Biology and Technology*, 28(1), 47–57.
- Tylewicz, U., Romani, S., Widell, S., & Gómez Galindo, F. (2013). Induction of vesicle formation by exposing apple tissue to vacuum impregnation. *Food and Bioprocess Technology*, 6(4), 1099–1104.
- Voitsekhovskaya, O., Heber, U., Wiese, C., Lohaus, G., Heldt, H.-W., & Gamalei, Y. V. (2002). Energized uptake of sugars from the apoplast of leaves: a study of some plants possessing different minor vein anatomy. *Russian Journal of Plant Physiology*, 49(1), 44–53.
- Wadsö, L., & Gómez Galindo, F. (2009). Isothermal calorimetry for biological applications in food science and technology. *Food Control*, 20(10), 956–961.
- Yurttas, Z. S., Moreira, R. G., & Castell-Perez, E. (2014). Combined vacuum impregnation and electron-beam irradiation treatment to extend the storage life of sliced white button mushrooms (*Agaricus bisporus*). *Journal of Food Science*, 79(1), 39–46.
- Zeeman, S. C., Smith, S. M., & Smith, A. M. (2007). The diurnal metabolism of leaf starch. *Biochemical Journal*, 401(1), 13–28.