Study on Metabolic Consequences of Vacuum Impregnation of Apple Tissue

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ABSTRACT

Vacuum Impregnation in food industry is an interesting process particularly in sem-dry fruit processing. At the beginning of the process the pressure is reduced and the internal gas in the product pores is expanded and partially flows out, after that, when atmospheric pressure is restored and residual gas is compressed, the external liquid flows into the pores. Although vacuum impregnation process has been widely studied, the metabolic consequences of the vacuum impregnation with different solutions on fruit tissue are still not well understood. The aim of this work was to study the metabolic consequences of vacuum impregnation on apple tissue. In particular, the respiration rate and the possibility of vesicular transport within the cells were explored.

The apple pieces were vacuum impregnated with isotonic sucrose solution. The respiration rate was measured on fresh and vacuum impregnated samples. The endocytosis marker FM 4-64 was impregnated together with the sucrose solution in presence or absence of Chloroquine. The samples were examined under fluorescent light in a Nikon upright microscope (Eclipse Ti-U, Nikon Co, Japan) equipped with a Nikon digital video camera (digital sight DS-Qi1Mc, Nikon Co, Japan) at a magnification of 4, 10 and 20 x.

The respiration rate of apple pieces was lower after the vacuum impregnation process respect to untreated samples. The microscopic observation showed small vesicle formation around the plasma membrane 0.5 h after the vacuum impregnation treatment. The density of vesicles became higher during storage at 10°C, and 24h after vacuum impregnation treatment very high density of vesicles was observed giving impression that they are accumulating within the cells.

Keywords: apple; vacuum impregnation; endocytosis; respiration rate

INTRODUCTION

Vacuum Impregnation in food industry is an interesting process particularly in sem-dry fruit processing. At the beginning of the process the pressure is reduced and the internal gas in the product pores is expanded and partially flows out, after that, when atmospheric pressure is restored and residual gas is compressed, the external liquid flows into the pores.[1] Vacuum Impregnation is, therefore, widely used in several processes to incorporate different additives in the tissue of fruit and vegetables such as anti-browning agents, microbial preservatives or cryoprotectants, improving their quality upon processing.[2,3,4]

Little is known on the metabolic consequences of vacuum impregnation. Igual et al.[5] and Castelló et al. [6] reported that the respiration rate of vacuum impregnated fruits significantly dropped after the treatment with an increase of the respiration quotient (RQ) that suggested the onset of anaerobic metabolism. However, to the best of our knowledge, metabolic responses upon vacuum impregnation that might be provoked by the changes in pressure experienced during the operation, structural modifications and/or anaerobic stress have not been explored.

The objective of this work was to study the metabolic consequences of vacuum impregnation on apple tissue. The possibility of vesicular transport within the cells was also explored.

MATERIALS & METHODS

Apples cv Aroma (12 ± 0.53 °Brix) grown in the south of Sweden were used. Vacuum impregnation was carried out at room temperature (20 ± 2 °C) in a chamber connected to a vacuum pump (Piab Lab Vac, Sigma-Aldrich). Medium-size apples (70±5mm of height and 80±5mm of width) were manually washed,
peeled and cut into rectangular samples (10 x 40 x 5 mm), selected from the inner part of the parenchyma tissue. Immediately after cutting, the samples were weighted and placed into the isotonic sucrose solution and vacuum impregnated for 13 min. This duration comprised a gradual increase of the vacuum for 4 min, a holding time of 5 min at 15 kPa (absolute pressure) and a gradual release of the vacuum for 4 min. The vacuum impregnation was repeated until maximum weight gain (18.1 ± 1.8 %), which was achieved after three cycles of vacuum impregnation at 15 kPa.

The respiration rate was measured on fresh and vacuum impregnated samples 3, 6, 9 h after storage at 10°C. A closed system was chosen to measure the respiration rate. Apple samples were placed in hermetic glass jars and closed for 3 h. The measurements of percentage of consumed O₂ and produced CO₂ were performed using an O₂/CO₂ gas analyzer (MFA II S/L, Witt-Gasetechnik, Witten, Germany) and expressed as ml O₂ or CO₂ kg⁻¹h⁻¹.

In a separated experiment the apple pieces (5 x 10 x 5 mm) were impregnated with isotonic sucrose solution plus endocytosis marker 16μM FM 4-64 in presence or absence of 1mM Chloroquine, known as endosome formation inhibitor. Solutions were prepared in 0.1 M sodium phosphate buffer (pH 5.8). Vacuum impregnated samples were cut into 2.5 x 5.0 mm slices using a sharp scalpel and examined under fluorescent light in a Nikon upright microscope (Eclipse Ti-U, Nikon Co, Japan) equipped with a Nikon digital video camera (digital sight DS-Qi1Mc, Nikon Co, Japan) at a magnification of 4, 10 and 20 x: 0.5, 3 and 24 h after vacuum impregnation. During the time frame of the experiments, the samples were stored in closed vessels under saturated atmosphere at 10 °C in the darkness.

**RESULTS & DISCUSSION**

**Respiration rate**

Table 1 shows the respiration rate in terms of O₂ consumption (RRO₂) and CO₂ production (RRCO₂) of fresh and vacuum impregnated apple pieces. The obtained results show that vacuum impregnation process implied changes in the respiration behaviour of apple tissue. In both, fresh and treated samples the time of storage caused the decrease of respiration rate. The O₂ consumption and CO₂ production of vacuum impregnated samples were lower than those of fresh sample. The results are in disagreement with these found by Castelló et al. [6] where they observed the increase of CO₂ production suggesting the anaerobic pathways in strawberries impregnated in isotonic glucose solution. Igual et al. [5] observed that immediately after treatment (time 0), the respiration rate (RRO₂) of VI persimmon was significantly lower than that of the fresh one, although this pattern does not appear in the CO₂ production.

<table>
<thead>
<tr>
<th>Time of storage</th>
<th>Fresh</th>
<th>Vacuum Impregnated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RRO₂ (mlO₂kg⁻¹h⁻¹)</td>
<td>RRCO₂ (mlCO₂kg⁻¹h⁻¹)</td>
</tr>
<tr>
<td>3h</td>
<td>10.61 ± 0.68</td>
<td>10.02 ± 0.60</td>
</tr>
<tr>
<td>6h</td>
<td>10.03 ± 0.59</td>
<td>7.85 ± 0.59</td>
</tr>
<tr>
<td>9h</td>
<td>9.28 ± 0.59</td>
<td>7.84 ± 1.18</td>
</tr>
<tr>
<td>3h</td>
<td>13.57 ± 0.59</td>
<td>8.26 ± 0.69</td>
</tr>
<tr>
<td>6h</td>
<td>12.39 ± 0.60</td>
<td>7.07 ± 0.69</td>
</tr>
<tr>
<td>9h</td>
<td>8.73 ± 0.98</td>
<td>5.30 ± 1.14</td>
</tr>
</tbody>
</table>

Table 1. Respiration rate of fresh and vacuum impregnated apple pieces.

**Vesiculation**

Intracellular endocytic vesicles localized with FM4-64, a general fluorescent marker for vesiculation [7,8], were found in the vacuum impregnated apples with the isotonic sucrose solution (Figure 1a,b,c). Vesicles were already found when samples were analysed 30 min after vacuum impregnation (indicated by arrows in Figure 1a and b). When a time course analysis was performed, both an increase of vesicles density and a higher internalization of the vesicles in the cells were observed (indicated with circles in Figures 1b and c).
Figure 1. Microscopic observations of apple tissue impregnated with isotonic sucrose solutions and FM4-64 (a) 0.5h, (b) 3h and (c) 24h after vacuum impregnation.

Figure 2. Microscopic observations of apple tissue impregnated with isotonic sucrose solution in presence of both FM4-64 and chloroquine. Lack of inhibition by chloroquine is shown in (a) 30 min after vacuum impregnation, and only a sporadic endosomes can be seen in (b) 3h after treatment. 24h after vacuum impregnation complete inhibition of endocytosis in the cells is shown in (c).
The effect of chloroquine impregnation on apple tissue is shown in Figure 2a,b,c. Chloroquine seems to have an effect in the long term accumulation of the vesicles rather than in the early endosomal formation, as shown in Figure 2a, where endosomes around the cell membranes are still present 30 min after vacuum impregnation. Three hours after vacuum impregnation, a sporadic endosomes can still be seen in the cells (Figure 2b). However, the chloroquine shows a dramatic inhibition of vesicle accumulation in the cells 24 h after vacuum impregnation (Figure 2c).

The results presented here provide evidence that vacuum impregnation, under the conditions used in the present study, induces vesiculation in cells of mature apple fruits. The vesicles were formed already after 30 min, and remained for 24 h (Figure 1). The membranes were visualized using the endocytic marker FM 4-64, which is inserted into the outer leaflet of the plasma membrane to pass into the cell by endocytosis.[9,10] In experiments where FM 4-64 was injected into the cytoplasm of e.g., tobacco cells, no internal staining was found, in contrast, this phenomena was observed when the dye was injected into the vacuole. [11] Therefore, we believe that in our experiment the stain probably did not enter because of permeabilised membranes that might have arisen during impregnation. This interpretation is strengthened by that the amine chloroquine, which is known to interfere with the formation of acid-inside vesicles such as endosomes, resulted in less vesicles formed (Figure 2).

Once inside, the vesicles and their contents may either end up in the vacuole to be degraded or may be recycled back to the plasma membranes. [12,13] The fact that the staining remains for at least 24 h suggest that the membranes and their content are somehow stopped on their way to the vacuole or other organelles that take part in degradation.

CONCLUSIONS

The conclusions pointed out that vacuum impregnation has a great effect on metabolism of apple tissue. The respiration rate of apple pieces was lower after the vacuum impregnation process respect to untreated samples. The microscopic observations showed that vacuum impregnation of apple tissue results in the formation of vesicles inside the cell that probably are formed at the plasma membrane and end up inside the cell. This may allow also substances such as sucrose, present in the impregnation solution to be taken up, but to what extent remains unknown.

Results obtained in the research showed, for the first time at our knowledge, that vacuum impregnation provokes metabolic reactions at the cell membrane level and it is not simply a process implying transformations in the extracellular space. The origin and technological consequences of the reported vesiculation is the subject of current investigations in our group.

REFERENCES

