Calcium and Iron Distribution in Fortified Vacuum-impregnated Fruits Determined by Electron Dispersion X-ray Microanalysis

NOELIA BETORET, JAVIER MARTINEZ-MONZO, PEDRO J. FITO, AND PEDRO FITO

ABSTRACT: Fresh apple cylinders were vacuum-impregnated with aqueous solutions containing calcium and iron gluconates (calcium and iron concentration up to 114.7 g/L and 2.98 g/L respectively). The study demonstrated an important effect of Ca concentration on the response of apple tissue to vacuum impregnation (VI), increasing matrix elasticity, and diminishing external liquid net fluxes. None of the effect was attributed to Fe. The location and distribution of the cations were analyzed by Electron Dispersive X-ray Microanalysis (EDXMA). The results showed that they were located mainly in the intercellular spaces, and their distribution was homogeneous. A semiempirical relationship has been used to quantify the amount of incorporated calcium by EDXMA.

Keywords: functional food, fruits, minerals, EDXMA, vacuum impregnation

Introduction

Traditional methods and new technologies can be used to obtain new fortified products. Some of them such as vacuum impregnation (VI) are suitable for introducing components included in a liquid phase (solution, suspension, or emulsion) into a porous structure of a solid matrix. This technique is being investigated to incorporate physiological active compounds (minerals, probiotics, and vitamins) into the structure of fruits and vegetables (Fito and others 1998; Gras and others 2002). This response, which is affected by the viscoelastic behavior of the fruit tissue, some physicochemical properties of the impregnation media, and working conditions, can be assessed using characteristic impregnation parameters such as the volumetric fraction of the incorporated liquid (X), volumetric deformation (γ), and effective porosity (ε) (Fito and others 1996). The location and distribution of the active compounds incorporated affect some physical and chemical properties, as well as some nutritive and sensorial attributes of the impregnated fruit. X-ray microanalysis can be used to analyze very specific regions, giving a reasonable description of the distribution of elements along a plant tissue (Konishi and others 1998). When this technique is applied to FFE, the information may be useful to describe mass transfer phenomena during and after the process.

In this work, the response of apple tissue to vacuum impregnation with an aqueous solution of sucrose, calcium, and iron is analyzed using the characteristic impregnation parameters. The location and distribution of the incorporated ions are analyzed by Electron Dispersive X-ray Microanalysis (EDXMA). Finally, both the characteristic impregnation parameters and EDXMA are used to quantify the amount of ions incorporated in the impregnated fruit.

Materials and Methods

Raw material

Apples (Granny Smith) from a local market, with a starch index of 3, were used as raw material. The apples were vertically cut into cylindrically shaped samples (40 mm in length and 18 mm in dia). Three cylinders from each fresh apple were obtained from the parenchymatic tissue avoiding the core and removing the peel. Each one was submitted to a VI treatment.

Impregnation media

Aqueous solutions of sucrose, calcium gluconate, and iron gluconate in different concentrations were used as the liquid phase in VI experiments. The composition of the solutions is shown in Table 1. Sucrose was used in solutions 1, 2, and 3 to adjust its water activity to the water activity of apple samples and in solution 4 to get an hypertonic solution. Calcium and iron salts were used jointly because no interference between them was hoped.

VI experiments

VI experiments were carried out in a pilot plant at the Food Technology Dept. of the Polytechnic Univ. of Valencia, Spain. A 50 mbar vacuum pressure was applied to the samples immersed in impregnation solution for 10 min, and then atmospheric pressure was re-stored, leaving the samples in the liquid solution for an additional 10-min period. Sample weight was monitored during the process to calculate the VI parameters (X, γ, and ε) according to the following equation (Salvatori and others 1998).

\[ X - \gamma = \varepsilon \left( \frac{1}{r} - \frac{\gamma}{r} \right) \]  

(1)

Electron dispersive X-ray microanalysis (EDXMA)

After the VI treatment, a small section from the middle of each vacuum-impregnated cylinder was excised, mounted on stainless steel stub, and rapidly frozen in liquid nitrogen slush. The sample
was transferred to a preparation chamber, cooled at −180 °C, and fractured with a liquid nitrogen-cooled scalpel blade just above the level of the stub. Ice was removed from the cell surface by exposing the sample to a high vacuum at −85 °C for 15 min. After this etching process, the sample was recooled to −180 °C and evaporatively coated with gold to produce an electrically conducting surface. The specimen was then taken to the scanning electron microscope (SEM) (JEOL JSM-5410 microscope). The EDXMA was performed in the SEM using an acceleration voltage of 30 kV, a takeoff angle of 45°, and a working distance (sample to final lens of the SEM) of 10 mm. Spectra from 0 to 20 keV were collected with the beam focused in a rectangular area on the central portion of the zone being analyzed. The surface of the sample was divided into 6 regions of the same width as the sample and 1/6 of the total length, as shown in Figure 1. Different areas belonging to intracellular space, cellular surface, and intercellular space, and randomly distributed along each region were selected. In each region, 12 to 16 raised areas with a flat surface were analyzed.

In all cases the counts per second (cps) were kept at 800. The spectra were analyzed using the Link ZAF-P/B program and the results of the individual elements were presented as (P-B)/B ratios (peak minus background divided by background). These values were referred to those of gold, which were thought to be constant (peak minus background divided by background). These values of R are a function of the surface concentration of the cation referred to surface gold concentration. The deformations are then more energy-consuming, but the effect of calcium and iron on the structural behavior of the samples is evident, showing that as the tissue of the impregnated samples is more concentrated in Ca, it becomes more elastic.

Results

VI treatment

Table 2 shows the characteristic impregnation parameters (X, γ, and ε) for aqueous solutions of sucrose, calcium gluconate, and iron gluconate as a impregnation media. Impregnation with solution 1, an isotonic solution of sucrose, gives an X value equal to 24.48%, a volumetric deformation close to 2%, and an effective porosity of 22%. These values are similar to those reported by Chiralt and others in 1999. Nevertheless, lower values in the volumetric fraction of the incorporated liquid and in the sample residual deformation, were observed when more calcium and iron were included in the impregnation media (solutions 2, 3, and 4 of Table 1).

Because the low concentration of minerals included in the solutions does not affect their viscosity very much the differences observed in the VI parameters should be mainly attributed to changes in the viscoelastic behavior of the fruit solid matrix. These differences related to a higher elasticity of the sample may be caused by changes in the structural properties of the tissue due to the interactions between calcium and other components of the fruit matrix. In a previous paper (Gras and others 2003), interactions between Ca and pectin of middle lamellae have been reported, producing changes in the sample textural behavior that becomes more elastic. The deformations are then more energy-consuming, but the tissue also shows more reversibility in the relaxations. As a consequence, the final values of X and γ in the impregnated samples are lower when elasticity increases. In Figure 2 the experimental values of X and γ have been plotted against the Ca concentration values. The effect of Ca ions reinforcing the tissue structure is evident, showing that as the tissue of the impregnated samples is more concentrated in Ca, it becomes more elastic.

Impregnation solution 4, with a lower water activity and higher sucrose, calcium, and iron concentration, leads to the lowest X and γ values, probably due both to the higher solution viscosity value and to the action of mass transfer phenomena promoted by the aw gradients. In isotonic conditions, effective porosity values do not have significant differences, leading to the conclusion that the effect of calcium and iron on the structural behavior of the samples

![Figure 1—Cryo-scanning electron microscopy observation of an impregnated apple sample, showing the 6 regions defined from the interphene to the center of the sample. Each one of the regions was submitted to EDXMA.](image1)

![Figure 2—Effect of calcium gluconate concentration in the impregnation liquid on the impregnation (X) and deformation (γ) volumetric fractions of samples](image2)
will not affect the coupling of the hydrodynamic flow of the liquid phase into the pores and the deformation-relaxation phenomena of the solid matrix in the porous food, as explained by Eq. 1.

The values of the volumetric fraction of the sample occupied by the impregnation solution have been used in Eq. 2 to estimate theoretical calcium and iron concentrations in the impregnated apple samples, as proposed by Fito and others (2001). The results have been expressed as the percentage of the recommended daily intake (RDI) in 100 g of final product (Table 2). It can be observed that any of the solutions analyzed are useful to prepare fruit products with more than 10% of RDI in 100 g of the product.

\[
x_c = \frac{x_{Ca} \rho_{Ca} + x_{Fe}}{1 + x_{Fe} \rho_{Fe} / \rho_{Ca}}
\] (2)
Calcium and iron distribution by EDXMA . . .

where \(x_{\text{imp}}\), \(x_{\text{lm}}\), and \(x_{\text{ff}}\) refer to mineral concentration in impregnated fruit, liquid media, and fresh fruit respectively; \(p_{\text{lm}}\) and \(p_{\text{ff}}\) refer to density of liquid media and fresh fruit.

**Electro disperse X-ray microanalysis.**

Examples of spectra obtained from the analysis in 3 areas located at intracellular space, cellular surface, and extracellular space are represented by Figure 3. Peaks belonging to the response from Au, K, Ca, and Fe were labeled. The Au layer provided the sharpest peak; the peaks coming from the Ca and Fe incorporated in impregnated samples are also important. It can be observed that there is a sharper calcium and iron peak in spectra from the intracellular spaces, and a potassium peak in those from the intracellular spaces. The results were used to determine (1) the predominant location of each ion incorporated in the cellular tissue, (2) the concentration profile of each element along the analyzed tissue, (3) approximated and empirical relationships to quantify the calcium concentrations in impregnated apple samples.

**Determining predominant location of each ion incorporated in cellular tissue.** The mean values and standard deviation resulting from the calcium and iron analysis in the 3 locations (intracellular, cellular surface, and extracellular) distributed along the 6 regions in the samples impregnated with solution 3 are represented in Figure 4. The calcium and iron concentrations detected in the 3 locations of the impregnated samples are higher than those detected in nonimpregnated samples. The comparison between calcium and iron concentrations in the different locations on the impregnated samples reveals a decrease from extracellular to intracellular spaces. In the analysis, the peak of potassium also shows differences in each area. The mean values and standard deviations are shown in Figure 4c. The results show that the high potassium concentration detected in the intracellular space of fresh apple tissue decreases in impregnated tissue. However, the potassium levels detected in the intracellular space and cellular surface increase slightly as a result of the VI treatment.

**Determining the concentration profile of each element along the analyzed tissue.** Figure 5 shows the mean concentration values obtained from the analysis of the extracellular spaces in apple samples impregnated with the solution 3 compared with the distance to the interphone. The results show practically horizontal ion concentration profiles. The low value of the profiles slope leads one to conclude that pressure and time conditions used in the 1st stage of the VI treatment were correct and guarantee the total and homogeneous impregnation of the sample by the external liquid solution.

**Determining approximated and empirical relationships to quantify calcium concentrations in impregnated apple samples.**

The agreement between the calcium content theoretically estimated by Eq. 2 and the EDXMA reading of calcium in the vacuum-impregnated samples is shown in Figure 6. There is a linear relationship with good correlation between both values. In the case of iron, the lower concentration and the high variability in the results of the EDXMA make it difficult to obtain a similar relationship.

**Conclusions**

**Table 2**—Response of apple tissue to vacuum impregnation (X, \(\gamma\), \(\epsilon\)) with the solutions shown in Table 1, and mineral content in the vacuum-impregnated samples calculated with the Eq. 2*.

<table>
<thead>
<tr>
<th>Solution</th>
<th>(x_{\text{imp}})</th>
<th>(x_{\text{lm}})</th>
<th>(x_{\text{ff}})</th>
<th>(\text{mg Ca/g sample})</th>
<th>(\text{mg Fe/g sample})</th>
<th>(%\text{RDI (100 g of VI apple)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution 1</td>
<td>0.2448 ± 0.0002</td>
<td>0.019 ± 0.0008</td>
<td>0.225 ± 0.018</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0%</td>
</tr>
<tr>
<td>Solution 2</td>
<td>0.208 ± 0.015</td>
<td>0.017 ± 0.004</td>
<td>0.202 ± 0.015</td>
<td>0.86 ± 0.05</td>
<td>0.0194 ± 0.0011</td>
<td>13.27%</td>
</tr>
<tr>
<td>Solution 3</td>
<td>0.142 ± 0.008</td>
<td>−0.036 ± 0.006</td>
<td>0.187 ± 0.011</td>
<td>1.28 ± 0.03</td>
<td>0.0287 ± 0.0008</td>
<td>18.06%</td>
</tr>
<tr>
<td>Solution 4</td>
<td>0.06 ± 0.02</td>
<td>−0.121 ± 0.005</td>
<td>0.18 ± 0.02</td>
<td>0.64 ± 0.19</td>
<td>0.010 ± 0.004</td>
<td>11.15%</td>
</tr>
</tbody>
</table>


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\* where \(x_{\text{imp}}, x_{\text{lm}}, \text{and } x_{\text{ff}}\) refer to mineral concentration in impregnated fruit, liquid media, and fresh fruit respectively; \(p_{\text{lm}}\) and \(p_{\text{ff}}\) refer to density of liquid media and fresh fruit.

**Figure 5**—Distribution of calcium (a) and iron (b) along vacuum-impregnated samples. Bars represent R values for each region (Figure 2).

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**Conclusions**

\(V\) of apple tissue with sucrose aqueous solutions containing calcium and iron gluconates is a useful procedure to produce functional fresh fruits. The amount of incorporated cations supplies up to 30% of RDI in 100 g of fruit. The cations remain mainly homoge-
neously located in the extracellular spaces of tissue. Ca^{2+} promotes important changes in fruit matrix viscoelastic behavior, increasing elasticity and diminishing the final values of VI parameters, proportional to Ca concentration. EDXMA has been an adequate tool to assess how the cations remain distributed in the tissue. A semiempirical relationship has been deduced to calculate Ca concentrations in the fruit tissue from the EDXMA measurements.

Acknowledgments

We would like to thank the UE INCO Programm and the Spanish Ministry of Science and Technology for economical support. We would like also to thank the Foreign Language Co-ordination Office at the Polytechnic Univ. of Valencia and Gretchen Reid at Univ. of Guelph (Canada) for their help in revising this paper.

Nomenclature

RDI: Recommended Daily Intake in United States (1100 mg Ca/d; 16 mg Fe/d)
VI: Vacuum Impregnation
EDXMA: Electron Dispersive X-Ray Microanalysis
FFF: Functional Fresh Foods

References