Changes in Apple Liquid Phase Concentration Throughout Equilibrium in Osmotic Dehydration

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ABSTRACT: Previous results on apple tissue equilibration during osmotic dehydration showed that, at very long processing times, the solute concentration of the fruit liquid phase and the osmotic solution were the same. In the present study, changes in apple liquid phase composition throughout equilibrium in osmotic dehydration were analyzed and modeled. Results showed that, by the time osmosed samples reached the maximum weight and volume loss, solute concentration of the fruit liquid phase was higher than that of the osmotic solution. The reported overconcentration could be explained in terms of the apple structure shrinkage that occurred during the osmotic dehydration with highly concentrated osmotic solutions due to the elastic response of the food structure to the loss of water and intake of solutes. The fruit liquid phase overconcentration rate was observed to depend on the concentration of the osmotic solution, the processing temperature, the sample size, and shape of the cellular tissue.

Keywords: equilibrium, liquid phase concentration, osmotic dehydration, over-concentration

Introduction

Apple tissue has been widely used as a model food for a better understanding of the osmotic dehydration process in fruits and vegetables due to its homogeneous tissue structure and sample preparation (Barat and others 1998, 1999; Fito and others 1998; Salvatori and others 1998, 1999a, 1999b). Among different aspects, osmotic dehydration research has focused on the influence of the process conditions on osmotic dehydration kinetics of apple tissue and its evolution to an equilibration stage (Barat and others 1998, 2001a, 2001b). Previous work on apple tissue changes in weight and volume throughout its equilibration in osmotic dehydration at different process conditions showed the existence of a pseudo-equilibrium stage when the concentration of the apple liquid phase and the osmotic solution were very close (Barat and others 1998). At this stage, structure relaxation was reported to promote a bulk flux of osmotic solution into the fruit tissue. According to this, true equilibrium would be achieved when no changes either in sample composition or weight would occur. At the equilibrium stage both activity and pressure gradients disappeared, and the solid cellular matrix showed a full relaxation. After sufficiently long processing times, the solute concentration in the liquid phase of the osmotically dehydrated samples and that corresponding to the surrounding osmotic solution were also observed to be the same. Nevertheless, little information was reported on compositional changes in apple tissue subjected to long-term osmotic treatments.

The aim of this work was to study and model the main changes in apple liquid phase composition throughout its evolution to an equilibrium stage in order to corroborate the important role of cell wall viscoelastic properties on cellular structure equilibration.

Materials and Methods

Preliminary studies

A preliminary study was done to check the equilibrium point in osmotic dehydration of apple (var. Granny smith) cuboids (30 × 16 × 3.5 mm). Apples were peeled and tissue samples were cut along the stem-base direction. Equilibrium studies were done at 40 and 50 °C with sucrose osmotic solutions of 5, 15, 25, 35, 45, 55, and 65 Brix. Three samples from the same fruit piece were used at each experimental condition. Samples were placed in a flask with a cover Twist-off containing 300 g of different nonstirred sucrose solutions and were immersed for a maximum of 360 h (in the case of lower concentrated solutions). The flask was placed in a thermostated bath. Sodium azide (Sigma, U.K., 1000 ppm) was added to the osmotic solution to prevent microbial growth. Water activity, moisture, and soluble solids content were measured in triplicate for both the fruit samples and the osmotic solution at the end of the experiment.

Kinetics of apple tissue osmotic dehydration toward equilibrium

Additional experiments were set up in order to further study the preliminary results from the previous experiment (see Results and Discussion).

Apples (var. Granny smith) were peeled and cut into cylindrical shape (2 cm in length × 2 cm in diameter) with the longitudinal axes in the stem-base direction. Four samples from the same fruit piece were placed in a flask containing nonstirred sucrose solution (1:25 w/w sample to osmotic solution ratio) (35 and 55 Brix) and subjected to long-term osmotic dehydration. Samples were immersed by means of a metallic grid and kept at constant temperature (30, 40, and 50 °C) by placing the flask in an oven. Potassium sorbate (2000 ppm) was added to the osmotic solution to stabilize samples microbiologically. In order to corroborate the effect of the structure in sample behavior during the osmotic equilibration, not only fresh, but also thawed samples were subjected to the osmotic dehydration at 50 °C with a 55 Brix sucrose solution. Freezing of apple cylinders was carried out by storing the enveloped samples at −18 °C for 24 h. Subsequent thawing was carried out at room temperature.
Changes in sample weight, volume, moisture, and soluble solids content throughout the process were analyzed in triplicate, as well as changes in the osmotic solution composition.

**Analytical determinations**

Volume was determined with a pycnometer for liquids using the osmotic solution as reference liquid. Moisture content of samples was determined by holding them in a vacuum oven until constant weight was reached (AOAC 1980). Soluble solids were analyzed by measuring the refractive index with a thermostated refractometer (Atago, NAR T3, Japan) at 20 °C. Water activity was measured with a 3-cell Novasina (Novasina AG, Zurich, Switzerland) water activity meter at 25 °C.

**Mathematical modeling**

In order to study in detail the osmotic dehydration operation and to be able to describe the different processes and flux kinetics involved in the process, a mathematical model describing the transfer of solutes and water through the tissue together with an elastic response of the cellular material against the solute and water transfer was proposed. This model was fitted to the experimental results obtained in the kinetic studies toward the equilibrium.

**Hypothesis.** The hypotheses of the model applied to our study were:

1. The osmotic dehydration can be characterized by the transfer of water and solutes between 3 compartments: (a) the osmotic solution, (b) the extracellular space of the apple sample, and (c) the intracellular space of the apple tissue. As a result of the mass transfer, changes in volume occur.
2. The main fluxes involved in the osmotic dehydration process correspond to water, sucrose added to the osmotic solution, and apple-native-soluble solids transfer.
3. Gradients inside the compartments lump in the transfer coefficients, and the driving forces are measured as the differences of average properties between compartments.
4. The physical properties of the tissue remain constant during the experiment.
5. The osmotic pressure (\(\pi\)) across a semipermeable membrane as a function of the temperature was approximated using Van’t Hoff law (Eq. 1):

\[
\pi = \sigma^*CRT
\]

where \(\sigma^*\) accumulates the activity coefficient, the reflection coefficient, and the proportionality coefficient between the molar concentration and Brix degrees for each solute.

6. The net volumetric flux of water across a non-ideal membrane (\(J_v(t)\)) can be defined as (Eq. 2):

\[
J_v(t) = A_m \frac{dV}{dt} = k_f(\Delta p(t) - \Delta \pi(t))
\]

where \(k_f\) is the volumetric rate change constant, \(\Delta p\) is the pressure gradient, and \(\Delta \pi\) is the osmotic pressure gradient, considering a purely elastic response from a compartment in the tissue. The hydraulic permeability \(L_p\) is defined as the ratio between \(k_f\) and \(A_m\).

7. For each compartment the pressure change (\(p(t)\)) depends on the volume change (\(V(t)\)) (Eq. 3):

\[
p(t) = p_0 + \text{Elast}(V(t) - V_0)
\]

where \(p_0\) is the initial pressure of the system (1 atm), \((V(t) - V_0)\) is the decrease in volume and Elast stands for the elastic response of the compartment for a change in volume.

8. The flux of a given solute \(S\) between 2 compartments, neglecting coupling of transport is (Eq. 4)

\[
\frac{dS}{dt} = -k_p^*S
\]

where \(k_p^*\) is the effective permeability of the membrane and \(S\) is the concentration of the solute. Two main groups of solutes were considered (i) the osmotic solution solids (A, mainly sucrose) and (ii) native apple solutes (B, mainly glucose and fructose).

**System of ordinary differential equations (ODE).** From these hypothesis the following system of ODEs was built (Eqs. 5 to 13):

\[
\frac{dV_1}{dt} = -L_p^*(p_1 - p_2 - (\pi_{A_1} - \pi_{A_2} + \pi_{B_1} - \pi_{B_2}))
\]

(5)

\[
\frac{dV_2}{dt} = L_p^*(p_1 - p_2 - (\pi_{A_2} - \pi_{A_3} + \pi_{B_2} - \pi_{B_3}))
\]

(6)

\[
\frac{dV_3}{dt} = L_p^*(p_2 - p_3 - (\pi_{A_3} - \pi_{A_1} + \pi_{B_3} - \pi_{B_1}))
\]

(7)

\[
V_1 \frac{dA_1}{dt} = -k_{A_1}^*(A_1 - A_2)
\]

(8)

\[
V_2 \frac{dA_2}{dt} = k_{A_1}^*(A_1 - A_2) - k_{A_2}^*(A_2 - A_3)
\]

(9)

\[
V_3 \frac{dA_3}{dt} = k_{A_2}^*(A_2 - A_3)
\]

(10)

\[
V_1 \frac{dB_1}{dt} = -k_{B_1}^*(B_1 - B_2)
\]

(11)

\[
V_2 \frac{dB_2}{dt} = k_{B_1}^*(B_1 - B_2) - k_{B_2}^*(B_2 - B_3)
\]

(12)

\[
V_3 \frac{dB_3}{dt} = k_{B_2}^*(B_2 - B_3)
\]

(13)

The subscripts 1, 2, and 3 denote the osmotic solution, extracellular space, and intracellular space compartments, respectively, and \(A\) and \(B\) species stand for osmotic solution solutes and native apple-soluble solids, respectively.

**Auxiliary equations.** To calculate osmotic pressure and pressure at each compartment Eq. 14 was used:

\[
\forall i = 1, 2, 3 \pi_i = \sigma^* A_i(t) RT; \pi_B = \sigma^* B_i(t) RT
\]

\[
p_i(t) = p_0 + \text{Elast} \cdot (V_i(t) - V_0)
\]

(14)

**Initial conditions.** The initial volume of the osmotic solution was 100 mL, which was employed as initial condition for Eq. 5, and the volume of each cylinder (6.28 cm³) was divided between extracellular space and intracellular space considering the porosity of the apple tissue (0.21), so as to provide initial conditions for Eqs. 6 and 7. The initial weight of the apple samples in the model was set to 5 g.

The Brix degree of the osmotic solution provided the initial condition for Eq. 8.

A significant amount of sucrose was present in the extracellular and the intracellular space of the apple (0.82 Brix), which was used as initial conditions for Eqs. 9 and 10.

The extracellular space and the osmotic solution were considered to have a negligible content of apple-soluble solids and a zero initial condition was used for Eqs. 11 and 12.
The soluble solids Brix content of the sample was measured to an average of 13.66 and was considered as the initial point for the intracellular space (Eq. 13).

**Observable quantities.** The predicted values obtained by means of the proposed equations were (Eqs. 15 to 19)

\[
\text{Brix}_{\text{cal}} = A_1 + B_1
\]
\[
\text{Brix}_{\text{apple}} = ((A_2 + B_2) \cdot V_2 + (A_3 + B_3) \cdot V_3)/(V_2 + V_3)
\]
\[
\Delta V^P_t = (V_2 + V_3 - (V_{2,0} + V_{3,0}))/(V_{2,0} + V_{3,0})
\]
\[
\Delta M^P_t = ((A_2 + B_2)/100 \cdot \rho_2 \cdot V_2 + (A_3 + B_3)/100 \cdot \rho_3 \cdot V_3)
\]
\[
\Delta \sigma_t = ((A_{2,0} + B_{2,0})/100 \cdot \rho_{2,0} \cdot V_{2,0} + (A_{3,0} + B_{3,0})/100 \cdot \rho_{3,0} \cdot V_{3,0})/M_0
\]

The subscripts 1, 2, and 3 denote the osmotic solution, extracellular space, and intracellular space, respectively, and \( \rho \) is the density of the osmotic solution, estimated as (Barat, J, private communication)

\[
\rho = 2 \times 10^{-5} \times \text{Brix}^2 + 0.0038 \times \text{Brix} + 0.9982
\]

where \( \rho \) is the density of the osmotic solution, extracellular space, or intracellular space and Brix is the accumulated Brix degree of the compartment, including sucrose and apple-native-soluble solids.

**Numerical methods.** Model building and individual fitting of each of the experiments were performed using JSim v1.602 (Bassingthwaighte 2005). Sensitivity analysis was performed by forward approximation with a \( \Delta \) of 0.01 in each parameter. The fitting of the model to the whole experimental data was performed using simulations employing the subroutine DLSODA from the ODEPACK library (ODEPACK 2001) and the multiresponse nonlinear regression subroutine DODRC from the ODRPACK library (ODRPACK 1992; Boggs and others 1992). In order to simplify the regression of the whole experimental data set and given the complexity of the model, the following simplifications were assumed:

9. The extracellular space of the cell was assumed to be 0.01, therefore assuming an elastic coefficient of 0 for compartment 2.

10. To avoid the high collinearity between the activity coefficients, the \( \sigma^* \) coefficient was fixed to 1 and \( \sigma^*A \) was estimated and forced to be bigger than 1.

**Results and Discussion**

**Preliminary studies**

Solutes concentration (Brix) of the osmosed apples and the osmotic solutions at each sampling time are shown in Figure 1. In all experiments, the soluble solids content of the liquid phase of the apple tissue was significantly higher \( (P < 0.05) \) than that of the corresponding osmotic solution for all the osmotic solutions employed. The above mentioned differences were found to be bigger as the processing temperature increased. In addition, water activity values of the osmotic solutions employed were found to be higher than those reached by fruit samples that were in contact with them (Figure 2). These differences in water activity values were only statistically significant for the most concentrated solutions (55 and 65 Brix) and decreased as the osmotic solution became more diluted. In previous studies on long-term osmotic dehydration of apple cylinders (Barat and others 1998), the concentration of the fruit liquid phase and the osmotic solution at equilibrium were found to be the same, thus suggesting that contact time between apple samples and each osmotic solution in the present study (up to 15 d for the lower Brix solutions) was not long enough to reach the equilibrium stage. According to these results, the fruit liquid phase might experience certain overconcentration on its way to the equilibrium. Therefore, studying changes in weight, volume, and composition throughout long-term osmotic treatments were considered of special importance.
interest in following experiments carried out at 30, 40, and 50 °C, when differences in concentration between the fruit liquid phase and the correspondent osmotic solution were found to be greater. In such experiments, the influence of sample size and shape on its evolution to the equilibrium stage was evaluated by processing apple cylinders instead of cuboids. As it was reported that the fruit liquid phase overconcentration rate increased with the concentration of the osmotic solution, 35 and 55 Brix sucrose solutions were employed as osmotic agents.

**Study of samples evolution to the equilibrium stage**

Changes in weight, volume, and composition throughout long-term osmotic treatments of apple cylinders in different sucrose solutions (35 and 55 Brix) at 50 °C, calculated according to Eqs. 1 to 3 (Fito and Chiralt 1996), are shown in Figure 3.

\[
\Delta M_i^t = \frac{M_i^t - M_i^0}{M_i^0} \\
\Delta M_i = \frac{M_i^t - M_i^0}{M_i^0} \times x_i^0 \\
\Delta V_i^t = \frac{V_i^t - V_i^0}{V_i^0}
\]

In the above equations \(M_i^0\) (mass of sample at time 0 or \(t\) in kg; \(x_i\) (mass fraction of water (\(w\)) or soluble solids (\(ss\)) at time 0 or \(t\); and \(V_i^0\) (volume of sample at time 0 or \(t\)).

![Figure 3 — Changes throughout osmotic treatment in 35 and 55 Brix sucrose solutions at 50 °C.](image)
Results corroborated the existence of 2 periods in porous food equilibration (Barat and others 1998; Figure 3). During the 1st period (<24 h), water loss and soluble solids uptake would be mainly promoted by activity gradients resulting in a moisture content decrease and a soluble solids content increase. As expected, mass fluxes in the system during the 1st period involved considerable weight and volume losses, which noticeably increased with the concentration of the osmotic solution. During the 2nd period, both water and soluble solids gain were observed to promote an important recovery of weight and volume with small changes in samples composition, thus suggesting that mass fluxes in the system were mainly promoted by pressure gradients. As reported by Barat and others (1998), initial cell wall shrinkage and deformation caused by water loss might be associated with stress accumulation in the solid matrix. Subsequent relaxation of the structure might result in an uptake of the external solution in which the product remained immersed. This could explain the mass and volume gain observed during the 2nd period. For both osmotic solution concentrations tested, weight recovery was shown to be greater than volume recovery and mass values became higher than the initial ones. Weight and volume recovery were faster as the concentration of the osmotic solution decreased.

In terms of water and soluble solids content, equality between the samples liquid phase composition and that of the osmotic solution would be achieved during the 1st period. By the time samples reached maximum weight and volume losses, the concentration of the fruit liquid phase was observed to be higher than that of the sucrose solution in contact with it. Compositional differences between the fruit liquid phase and the osmotic solution were observed to be more noticeable when working with highly concentrated osmotic solution (55 Brix). Changes occurring during the 2nd period (>24 h) slowly reduced differences in concentration between the fruit liquid phase and the osmotic solution in contact with it. When comparing the liquid phase composition of apple samples of different shape on its way to the equilibrium with a 55 Brix sucrose solution at 50 °C, significantly higher soluble solids content (P < 0.05) was observed in the case of working with apple cuboids, thus suggesting the important role of sample size and shape on their evolution to an equilibrium stage.

Overconcentration observed in the fruit liquid phase on its way to an equilibrium stage could be related to shrinkage observed in apple samples at the end of the 1st period. The pressure gradients between the samples and the external solution and(or) the higher density of cell membranes (which represents a barrier for the native soluble solids outflow) could be the reason for the overconcentration observed in the liquid phase.

At the beginning of the osmotic treatment, both pressure and activity gradients between the samples and the osmotic solution in contact with them would promote water to flow toward the external solution. Even when compositional equality between the fruit liquid phase and the osmotic solution was reached, weight and volume were still reported to decrease for a short period of time. At this stage, a gradient of osmotic pressures should appear to balance the stress accumulated in the solid matrix during fruit contraction, thus justifying the observed overconcentration phenomena. Mass transport at this period might be proportional to pressure fall inside the product, which was expected to be higher when working at higher temperatures or with higher concentrated solutions. That would explain higher overconcentration rates observed in apple cuboids processed at 50 °C or in apple cylinders processed with a 55 Brix sucrose solution. Although neither weight loss nor volume reduction at the 1st step of apple cuboids equilibration with a 55 Brix sucrose solution at 50 °C was evaluated, greater overconcentration rate reported when working with apple cuboids suggested that these would be higher than those achieved by apple cylinders.

Once samples reached their maximum weight loss and volume reduction, the structure might relax and lead inner pressure to equilibrium with the external one by means of a bulk flux of osmotic solution into the fruit tissue (Figure 3). At this step, concentration

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Table 1 – Estimated parameters from the regression of the osmotic dehydration data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Estimate</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{a1}$</td>
<td>h$^{-1}$</td>
<td>0.32</td>
<td>0.04</td>
</tr>
<tr>
<td>$k_{b1}$</td>
<td>h$^{-1}$</td>
<td>0.19</td>
<td>0.08</td>
</tr>
<tr>
<td>$k_{a2}$</td>
<td>h$^{-1}$</td>
<td>0.023</td>
<td>0.01</td>
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<tr>
<td>$k_{b2}$</td>
<td>h$^{-1}$</td>
<td>0.016</td>
<td>0.009</td>
</tr>
<tr>
<td>$L_{a1}$</td>
<td>Pa$^{-1}$ cm$^2$ s$^{-1}$</td>
<td>0.0050</td>
<td>0.0007</td>
</tr>
<tr>
<td>$L_{b1}$</td>
<td>Pa$^{-1}$ cm$^2$ s$^{-1}$</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Elast$_3$</td>
<td>Pa cm$^{-3}$</td>
<td>0.992</td>
<td>0.02</td>
</tr>
<tr>
<td>$\sigma_{a}$</td>
<td>Mol l$^{-1}$ Brix$^{-1}$</td>
<td>1.19</td>
<td>0.15</td>
</tr>
<tr>
<td>Deviance</td>
<td></td>
<td>575.38</td>
<td>2.86</td>
</tr>
</tbody>
</table>

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Figure 4 – Normalized model parameters (estimated parameter divided by the average of all the experiments) estimated from individually fitted experiment (a: $k_{a1}$, b: $k_{b1}$, c: $k_{a2}$, d: $k_{b2}$, e: $L_{a1}$; f: $L_{b1}$; g: Elast$_3$, h: Elast$_3$; i: $\sigma_{a}$, j: $\sigma_{b}$)
gradients might promote water to flow toward the fruit liquid phase till the equilibrium between the osmotic and volumetric pressures is reached. As reported by Barat and others (1998), true equilibrium would be achieved when no changes either in sample composition or weight would occur. In such a state, differences in both activity and pressure would disappear, and the solid cellular matrix would become fully relaxed (Figure 3).

**Model fitting and sensitivity analysis**

The coefficients estimated by individual fitting of each of the performed experiments are shown in Figure 4. Although the standard errors of the coefficients were high, the fact that estimated coefficients did not differ greatly between experiments lead us to assume that a single group of parameters could be used to explain all the experiments together.

The estimated parameters, together with their associated standard errors are presented in Table 1. It can be seen that in general the transfer coefficients through the cell membrane \((k^*_A, k^*_B, L^*_p)\) are generally smaller than the transfer coefficients through the vegetable cell wall \((k^*_A, k^*_B, L^*_p)\), except for the hydraulic transport coefficient \(L^*_p\). It also can be seen that sucrose molecules seem to have a slightly higher activity coefficient \(\sigma^*_A (1.19 \pm 0.15)\) than fructose (1.0).

Examples of typical fits of the model to the different responses can be seen in Figure 5 and 6. In general, the model systematically over-predicted the soluble solids gain of the experiment, but predicted reasonably well the patterns of the evolution of Brix degrees of both the solution and the apple-soluble solids, the volume change of the sample, and the water losses. The model, in general, was able to predict the overconcentration phenomena in the sample, showing how the shrinkage of the sample and the elastic stress allowed for a temporary overconcentration of the solutes inside the sample. After sufficient time to relax the structure of the sample was achieved, this overconcentration phenomenon disappeared and the solutes final equilibrium was reached. It is important to note that this phenomenon can only be explained by taking into consideration the

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**Figure 5** — Experimental data and global model prediction of an osmotic drying experiment in 35 Brix sucrose solution at 50 °C.
existence of the 3 compartments mentioned before: the extracellular space acts as a buffer so that elastic tensions can be developed and volume changes can be delayed with concentration fluxes and overconcentration can occur. Further studies considering concentration gradients and effective diffusivity would be needed to assess the effect of dimensions and volume of the sample into this phenomenon.

A sensitivity analysis of the model and the estimated parameters were performed by approximating the derivatives of the model prediction over time for an osmotic dehydration with a 35 Brix sucrose at 40 °C. The parameters contributing most significantly to the responses of the model were the hydraulic permeabilities \( L_{p_1} \) and \( L_{p_2} \), which are shown in Figure 7. It can be seen how the overconcentration phenomena that occurs early in the osmotic drying is associated with a change in the derivatives associated with the hydraulic transport coefficient in the extracellular space \( (L_{p_1}) \). In this way any phenomenon that increases the water permeability through the cell wall (for example, pulse vacuum treatments) or the vegetable cell membrane (for example, freeze thawing) is foreseen to affect most the kinetics of the osmotic dehydration.

The present results confirmed the important role of tissue elastic properties on sample behavior during the osmotic dehydration. Therefore, different behavior would be expected in osmotic equilibration of samples submitted to different structural changes. Mass changes throughout osmotic treatment of frozen-thawed samples in 55 Brix sucrose solutions at 50 °C (Figure 8) corroborated this assumption. Results showed that cell membrane differential permeability, which was reported to limit solutes transfer and to promote large-scale transfer of water during osmotic dehydration (Mauro and others 2002), might be lost when the cellular structure was destroyed by prior freezing and thawing. As a result, soluble solids could penetrate more easily. On the other hand, no significant differences in composition between sample liquid phase and the external solution at the equilibrium stage were found when working with frozen-thawed samples. According to what was previously reported, structural damage taking part during the freezing-thawing
process might lead to a lower volume contraction and therefore, to a lower overconcentration rate.

Conclusions

An overconcentration of the apple liquid phase was observed when compared to the osmotic solution close to the point of maximum weight and volume during sample equilibration. The observed overconcentration seems to be closely related with tissue shrinkage, since this phenomena was higher for the samples processed with the higher osmotic solution concentration. The proposed model fitted to the experimental data reproduces the mentioned overconcentration related to the accumulated stress in apple tissue. When apple samples were frozen and thawed before osmotic dehydration, neither shrinkage nor overconcentration was observed, reinforcing the above mentioned hypothesis.

References

Apple changes throughout equilibrium...


Queries

Q1  Author: Please provide year and origin of ‘var. Granny smith’.
Q2  Author: Please provide manufacturer’s details of ‘thermostated bath’.
Q3  Author: Please provide city where ‘Sigma’ is located.
Q4  Author: Please provide city where ‘Atago’ is located.
Q5  Author: Please check the suggested citing of reference ‘Boggs and others 1992’.
Q6  Author: Reference ‘Hindmarsh 1983’ is not cited. Please check.