Seguí Gil, L.; Fito Suñer, PJ.; Fito Maupoey, P. (2012). Understanding osmotic dehydration of tissue structured foods by means of a cellular approach. Journal of Food Engineering. 110(2):240-247. doi:10.1016/j.jfoodeng.2011.05.012.

Understanding osmotic dehydration of tissue structured foods by means of a cellular approach

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ABSTRACT

This contribution presents a study on osmotic dehydration of tissue structured foods based on a microstructural approach in which simplified systems such as isolated apple cells and protoplasts have been used. An appropriate description of the microstructure of the raw material and its evolution during processing has been evidenced as critical in order to better understand and describe osmotic dehydration processes; as a direct consequence, it is stated that predictive models should incorporate this microstructural information so as to be more reliable. Microstructural changes observed by examining the isolated cells under the microscope along the treatments have been used to identify critical points that separate the stages that a cell undergoes, and which depend also on its particular response to the osmotic treatment (lysis, shrinkage or complete plasmolysis). Irreversible thermodynamics has been used to mathematically describe the process by distinguishing two main stages: one at which significant deformation-relaxation phenomena are coupled with mass transfer, and another one at which the former may be neglected.

Key words: *microstructure, osmotic dehydration, mass transfer, deformation-relaxation phenomena, irreversible thermodynamics.*

NOMENCLATURE

a_j activity of component j, (—).

J_j molar flux of component j, ($mol \cdot m^{-2} s^{-1}$).

L_j phenomenological coefficient of component j, ($mol^2 \cdot J^{-1} \cdot m^{-2} \cdot s^{-1}$).

Mr_j molecular weight of component j, ($kg \cdot mol^{-1}$).

P pressure, (Pa).

R universal gas constant, ($J \cdot mol^{-1} \cdot K^{-1}$).

S surface area, (m^2).

T temperature, (K).

t time, (s).

V volume, (m^3).

$\overline{V_j}$ partial molar volume of species j ($m^3 \cdot mol^{-1}$).

w_j mass fraction of component j ($kg \cdot kg^{-1}$).

x_j molar fraction of component j ($mol \cdot mol^{-1}$).

Greeks

ρ density, ($kg \cdot m^{-3}$).

μ_j chemical potential of component j, ($J \cdot mol^{-1}$).

Subscripts, superscripts and abbreviations.

ss soluble solids.

t time.

w water.

CW cell wall.

DEF mechanical deformations

EP external phase.

EXP experimental

IRD irreversible deformations.

InP incipient plasmolysis

IP internal phase.

MB membrane breakage

OS osmotic solution.

PM plasma membrane.

RD reversible deformations.

SEP protoplast separation.

1. INTRODUCTION

The complexity and heterogeneity of biological materials make osmotic dehydration (OD) modelling a difficult subject to face, and constitute the main reason why OD models have generally made use of a macroscopic approach in which the tissue is assumed to be homogeneous and isotropic. It is frequent to find applications of Fick's Second law to obtain an approximate solution, in which an apparent or effective diffusivity is used to account for all variables. Aguilera et al. (2003) reported that the use of an effective diffusivity obtained from the Second Fick's law is not only questionable from the theoretical standpoint, but reduces all structural effects and related mechanisms to a single parameter. Accordingly, several authors emphasize the relevance of food structure and how it is modified during processing in order to clearly understand mass transfer phenomena in cellular tissues (Aguilera et al. 2003, 2005;

Barat et al. 2001, Fito et al., 2007; Lewicki and Porzecka-Pawlak, 2005, Mebatsion et al., 2006). In contrast, it has also been reported that food engineers still make extensive use of the approximations of Fick's Second law to calculate an apparent diffusivity, thus underestimating the importance of microstructure (Aguilera, 2005; Mebatsion et al., 2006).

Food materials are multicomponent and multiphasic systems organized in microstructural elements that respond differently to the imposed process conditions. Structural modifications during processing originate changes in the food properties, not only properties that are related to quality attributes such as texture or colour, but this structural modifications also originate changes in mass transfer properties and, consequently, mass transfer mechanisms change during the process. Knowing the response of each structural element and how this affects the product properties, including mass transfer properties, is essential for understanding osmotic dehydration and mathematically describe the process. It seems evident that models need to incorporate structural information in order to be reliable.

Osmotic dehydration has been extensively studied under a macroscopic perspective, and microscopic approaches in which structural changes are analyzed are also present in the literature (Barat et al., 1998; Ferrando and Spiess, 2001; Lewicki and Porzecka-Pawlak, 2005; Mavroudis et al., 1998, 2004; Nieto et al., 2004); however, studies in which the whole tissue is analyzed do not offer the possibility of characterizing the individual response of each structural element, which in turn leads to a partial misunderstanding of the properties and the mechanisms involved in the mass transfer process. Nonetheless, in the context of *food product engineering*, it has been noticed an increasing interest in microstructural approaches that emphasize the role of structure with the aim of facing the challenge of real food modelling (Aguilera, 2006; Ferrando and Spiess, 2002; Fito et

al., 2007; Mebatsion et al., 2008). In line with that, the possibility of controlling the microstructure for designing products of specific desired properties has also been discussed (Aguilera, 2006; Fito et al., 2007).

The study of individual structural elements can be better achieved by analysing simplified systems at the microscopic level. In this sense, isolation techniques appear as an attractive tool for food engineering purposes, since it allows us to obtain simplified living systems that can be directly observed under the microscope in the course of the process. Previous steps to this work comprise the study of mass transfer phenomena during OD of apple isolated protoplasts (Seguí et al., 2006) and the analysis of the microstructural response of isolated cells during OD treatments (Seguí et al., 2010). In the latter, the importance of the role of the microstructure and its evolution during processing in order to understand the mechanisms involved in dehydration and, consequently, to develop models that describe micro and macroscopic changes was evidenced. In the present work, this microstructural information is used to make a comprehensive interpretation of mass transfer and deformation-relaxation phenomena during the osmotic dehydration of isolated apple cells, and to model their response by means of irreversible thermodynamics.

2. MATERIALS AND METHODS

2.1. Miniaturised experiments

Apple cells (*Malus domestica* var. Fuji) were enzymatically isolated and equilibrated in a manitol solution (a_w = 0.986), and subsequently monitored and measured during OD experiments with sucrose solutions (25, 35, 45% (w/w)). The isolation procedure and the osmotic experiments, including monitoring and measurements, have been previously

126 described (Seguí et al, 2010). Briefly, cells were isolated from apple parenchyma in a
127 digestion medium containing pectinase. Subsequent osmotic experiments were carried
128 out in a heating/cooling stage at constant temperature (30 ºC), placed under a light
129 microscope, and monitored by a CCD camera incorporated to the microscope and
130 connected to a computer. Images of the cells being dehydrated were saved during the
131 treatment and next analyzed and measured (Adobe Photoshop, v. 7.0; ImageJ, 1.36b
132 free version). The projected cross area and major axis of each cell protoplast were
133 measured; volumes (V^{PM}) were calculated considering cells as spheroids obtained by
134 rotating the ellipses about their major axis.
135 For comparison purposes, apple protoplasts were also isolated from the same plant
136 material using the isolation procedure described in Seguí et al. (2006), equilibrated in
137 manitol (a_w = 0.984), dehydrated in 45% (w/w) sucrose and measured. In this case,
138 volumes were directly obtained from the projected cross areas (A^{PM}), due to the
139 spherical shape of isolated protoplasts.
140

141 **2.2. Quantification of mass transfer across the plasmalemma**

142 Progress of cell protoplast volume was used to calculate water fluxes across the plasma
143 membrane (J_w^{PM}) by means of equation 1.

144 $$J_w^{PM} = \frac{-\Delta\left(V^{PM} \cdot \rho_{ss}^{IP} \cdot w_w^{IP}\right)}{\overline{S^{PM}} \cdot \Delta t \cdot Mr_w} \qquad (1)$$

145 Where, V^{PM} is the cell protoplast volume, ρ^{IP}_{ss} the density of the solution inside the
146 protoplast as a function of soluble solids content, w^{IP}_w the water mass fraction inside the
147 protoplast, $\overline{S^{PM}}$ the mean protoplast surface area, Δt the time interval between two
148 consecutive images, and Mr_w the water molecular weight. As in Seguí et al. (2006), the

149 solution inside the protoplast was identified as internal phase (IP), and the solution
150 outside the protoplast as the external phase (EP).
151 The water mass fraction inside the protoplast (w^{IP}_w) at initial time was calculated from
152 the value of the molar water fraction inside the protoplast (x^{IP}_w) obtained by applying
153 the Norrish equation (Norrish, 1966) to the water activity of the manitol solution in
154 which the cells had been equilibrated ($a_w{}^{IP}_0$ = 0.986). The Norrish equation coefficient
155 (K) was obtained for a combination of different components using the proportional
156 values obtained from bibliographic data (Yamaki and Ino, 1992). Subsequent values of
157 w^{IP}_w were obtained applying the mathematical approach developed in Seguí et al.
158 (2006) for apple isolated protoplasts, which considers the plasma membrane
159 impermeable to solutes and assumes a homogeneous water concentration in both the
160 internal and external phases at each measured time. Since the plasma membrane
161 constitutes the interface, the EP comprises all the solution outside the protoplast
162 independently on the presence of a cell wall.
163

164 **2.3. Using Irreversible Thermodynamics to describe cell dehydration**

165 Regarding the driving force that promotes mass transfer, this can be analysed by means
166 of equations based on the diffusional mechanism or irreversible thermodynamics,
167 depending on whether the driving force is defined as the difference in concentration
168 between phases or the difference in the chemical potential, respectively (Gekas, 1992).
169 The thermodynamic equilibrium of a system is usually analysed in terms of the Gibbs
170 free energy (Fito et al., 2007) as stated by equation 2:

171 $$dG = VdP - SdT + \sum \left(\frac{\partial G}{\partial n_i} \right)_{T,P,n_{j \neq i}} dn_i \quad (2)$$

172 The partial derivative in equation 2 is the chemical potential of component i (μ_i), which
173 refers exclusively to molecular mobility and represents a variation in the Gibbs free
174 energy of the system when there is an infinitesimal change in the number of moles of
175 component i (P, T and j ≠ i being constant). On the other hand, equation 3, obtained by
176 dividing equation 2 by dn_i, suggests that a variation in the number of moles of
177 component i may be coupled with pressure and temperature changes.

178 $$\frac{dG}{dn_i} = V\frac{dP}{dn_i} - S\frac{dT}{dn_i} + \mu_i \qquad (3)$$

179 If applying equation 3 to a cellular system where the semipermeable membrane that
180 separates the internal and external phases allows only the water to flow through it, mass
181 transfer is necessarily coupled with mechanical deformations of the cellular structure
182 (Fito et al., 2007). Consequently, the extended definition of the chemical potential must
183 be used when analysing mass transfer phenomena in a cellular tissue, which in the case
184 of water is defined by equation 4 (Gekas, 2001).

185 $$\mu_w^{ext} = \overline{G_w} = RT\,ln\,a_w + \overline{V_w}P \qquad (4)$$

186 Therefore, and focusing on the experiments analysed in this work, the driving force that
187 promotes mass transfer during the OD of an apple isolated cell can be defined as the
188 gradient of the extended water chemical potential (equation 5).

189 $$\Delta\mu_w^{ext} = RT\Delta\,ln\,a_w + \overline{V_w}\Delta P \qquad (5)$$

190 The pressure term in the stated equation stands not only for the difference in pressure
191 between the inner and the outer side of the plasma membrane (P^{EP}-P^{IP}), but also for the
192 mechanical deformations that the cell undergoes, these being either reversible (RD:
193 elastic) or irreversible (IRD: ruptures or viscous) (equation 6). A pressure term opposite
194 in sign (positive) to the compositional one (negative) implies a reduction in the driving
195 force.

196 $$\Delta\mu_w^{ext} = RT\Delta ln\, a_w + \overline{V_w}\left[\left(P^{EP} - P^{IP}\right) + \Delta P_{RD} + \Delta P_{IRD}\right] \quad (6)$$

197

198 ## 3. RESULTS AND DISCUSSION

199

200 ### 3.1. Water flux across the plasmalemma

201 Molar water flux across the plasma membrane of the isolated cells was calculated as
202 stated by equation 1. Figure 1 shows the mean water flux variation in the course of
203 treatments with 45, 35 and 25% (w/w) sucrose solutions. As expected, higher OS
204 concentrations produced higher mean water fluxes. Initially, mean water fluxes increase
205 until reaching a maximum, which appears earlier at more concentrated OS, in
206 correlation with the phenomenon of incipient plasmolysis (Seguí et al., 2010). Although
207 initial values may be transiently affected by the adaptation of the system to the new
208 imposed conditions, the plot illustrates how the cell wall prevents the protoplast from
209 shrinking at the beginning of the treatment, thus reducing the driving force and the
210 water flux. During this period, it is expected that the available free energy is used not
211 only for transferring water molecules through the plasma membrane, but also for
212 deforming the cellular structure. If the cell wall shrinks together with the protoplast
213 during most of the treatment, as occurs when an OS of 25% sucrose (w/w) is used
214 (Seguí et al., 2010), the maximum does not appear due to a low $\Delta\mu_w$ value when
215 incipient plasmolysis takes place; however, if at incipient plasmolysis $\Delta\mu_w$ is still
216 considerable, the water flux increases to a maximum. Eventually, water fluxes decrease
217 towards values that were considered negligible after five minutes of processing.

218

219 ### 3.2. Modelling mass transfer and deformation-relaxation phenomena based on
220 microscopic studies

As mentioned before, (micro)structural information is essential for understanding mass transfer phenomena in complex systems, as well as for developing mathematical models. In this section, the microstructural information obtained in a previous work (Seguí et al., 2010) is used to interpret and describe the mass transfer and deformation-relaxation phenomena that occur during OD of isolated cells, as well as to deduce the equations that need to be used in each case.

3.2.1. Identification of critical points and stages of the dehydration process as a function of cell response

In a previous work (Seguí et al., 2010) cells were classified depending on the phenomenon undergone in response to the osmotic treatment: cell shrinkage, cell lysis and cell plasmolysis (type I, II). Distinction among these responses is essential for mass transfer analysis since the mechanisms that are involved in the mass transfer process are different in each case. The path that a cell may follow after soaking it in the OS is schematized in figure 2. As deduced from the analysis of the structure-property relationships (Seguí et al., 2010), a cell will follow one or another path (A-C) as a function of both the gradient imposed and cell morphology.

During processing, *critical points* make differences in the mechanisms involved in the dehydration process. These critical points separate the *stages* that each cell experiments, and depend on the cell response.

- (A) Cell lysis: In the case of cells which membrane breaks during the dehydration process, *membrane breakage* (MB) is a critical point as it represents the loss of cell compartmentation.
- (B) Shrinkage: The moment at which the plasma membrane starts to detach from the cell wall, i.e. *incipient plasmolysis* (InP), is identified as a critical point. It is

important to point out that, as deduced in Seguí et al. (2010), incipient plasmolysis occurs at a similar degree of cell deformation for each osmotic gradient applied.

- (C) Complete plasmolysis: If the cell undergoes full plasmolysis two critical points are distinguished: *incipient plasmolysis*, as in the previous case; and *complete separation* (SEP) of the protoplast, which has also been proved to occur at a specific degree of deformation, for a given osmotic gradient (Seguí et al. 2010).

During OD of isolated cells deformations occur as a consequence of water loss, this being responsible for pressure gradients that appear coupled with mass transfer phenomena, at least during some part of the process. According to Eq. 6, the pressure gradient included in the extended definition of the water chemical potential explains not only the difference in the hydrostatic pressure, but also the free energy consumed or released in deformation-relaxation. Depending on the cell response (A-C) and on the stage of the process the pressure or compositional terms may prevail over the other, or act as the only driving force for mass transfer. Focusing on the identified responses: equation 6 does not apply to response A (cell lysis), since cell compartmentation is lost and internal and external phases mix; In case of shrinkage (response B), mass transfer will be coupled with mechanical deformation during the whole process, although the energy used in deformation will be different before and after the critical point *incipient plasmolysis*; For a cell that undergoes response C (complete plasmolysis), *complete separation* of the plasma membrane clearly separates two stages: a first stage, during which significant deformation phenomena exist and the pressure term should different from zero ($\overline{V_w}\cdot\Delta P \neq 0$); and a second one, which starts immediately after protoplast

separation, during which the protoplast is not slowed down by the cell wall and the pressure term might be neglected ($\overline{V_w}\cdot\Delta P \approx 0$).

These two main stages ($\overline{V_w}\cdot\Delta P \neq 0$ and $\overline{V_w}\cdot\Delta P \approx 0$) separated by the critical point *complete separation* were experimentally verified in completely plasmolysed cells when plotting individual transmembrane water fluxes against protoplast relative deformation (V^{PM}_t/V^{PM}_0) (figure 3). It is important to remember that although the separation time varied from 75 to 210 seconds among cells, protoplast relative deformation at which separation took place was similar for all cases [$(V^{PM}_t/V^{PM}_0)^{SEP} = 0.0617 \pm 0.008$] (Seguí et al., 2010). These results suggested that processing time does not provide adequate information to determine which mechanisms are governing the mass transfer process; in contrast, protoplast relative deformation indicates the stage of the process that the cell is undergoing. Again, the importance of the microstructure and how it is modified during processing is revealed as critical for understanding and modelling the process.

In figure 3, the discontinuous line that represents complete separation of the membrane separates the two stages. During the first period ($\overline{V_w}\cdot\Delta P \neq 0$), uncertain deformation-relaxation phenomena driven by cell individual shape and microstructure are coupled with mass transfer, originating different patterns of the water flux curves. This stage ends at $V^{PM}_t/V^{PM}_0 = 0.617 \pm 0.008$, indicating the beginning of the period at which $\overline{V_w}\cdot\Delta P \approx 0$. The discontinuous ellipse drawn in figure 3 comprises residual water fluxes that appeared at the end of the osmotic treatment. These values were deduced to be the consequence of the homogenisation of the external phase at long times of the process. Yao and LeMaguer (1986) suggested that an intensive water flow near the interface during OD may wash back the solute penetration in the tissue, in our case the space between the plasma membrane and the cell wall; according to this, the fluid near the

plasma membrane (the boundary layer) may experiment a dilution effect due to the wash back of solutes which becomes less significant as the water flux decreases. When approaching the equilibrium, the convective water flux going out the protoplast minimises and the boundary layer homogenises with the rest of the OS, which originates a rise in the solute concentration near the interface and a subsequent flux of water. This dilution effect would originate an overestimation of the solute concentration in the EP following high water flux values, as well as an underestimation when the EP homogenises and promotes water fluxes at long times.

3.2.2. Modelling the dehydration process by means of irreversible thermodynamics

Up to now, critical points have been defined and the existence of at least two differentiated stages has been experimentally proved in cells that plasmolysed completely: a stage during which significant deformation phenomena exist and another one in which these are not perceptible. In terms of the extended definition of the water chemical potential, this means that the pressure term must be taken into account ($\overline{V_w}\cdot\Delta P \neq 0$), or may be neglected ($\overline{V_w}\cdot\Delta P \approx 0$).

After complete membrane separation (SEP), the simplification in the extended water chemical potential leads to the simplification of equation 6 to the compositional term. Thus, kinetics of water transfer through the plasmalemma may be analysed by determining the phenomenological coefficient (L_w) obtained from the following equation based on irreversible thermodynamics:

$$J_w^{PM} = -L_w \cdot RT \cdot ln\left(\frac{a_w^{EP}}{a_w^{IP}}\right) \qquad (7)$$

Where L_w is the water phenomenological coefficient, T the temperature of the system, R the universal gas constant, and $a^{EP}{}_w$ and $a^{IP}{}_w$ the water activities in the external and internal phases.

In Seguí et al. (2006) it was corroborated for apple isolated protoplasts that equation 7 can be used to model water transfer across the plasma membrane in the absence of a cell wall; however, in the case of apple isolated cells the application of equation 7 is restricted to the period at which the plasmalemma is not longer stretched by the cell wall. The application of this equation is also subjected to the hypothesis that the cell wall is not significantly influencing the transfer of water or solutes during this stage, which needs to be corroborated by comparing the results with mass transfer coefficients obtained for apple isolated protoplasts.

Experimental values for cells and protoplasts were fitted to equation 7 by simple linear regression. In the case of the isolated cells, points before full separation were not fitted to the straight line since they belong to stage $\overline{V_w} \cdot \Delta P \neq 0$. Table 1 summarizes the fit results obtained for the experimental data. The P-value obtained from the ANOVA analysis (Statgraphics plus 5.1) was > 0.05 for the phenomenological coefficients calculated, indicating that no significant differences exist between the means of the phenomenological coefficients obtained for isolated protoplasts and isolated cells (the latter during stage $\overline{V_w} \cdot \Delta P \approx 0$).

On the one hand, these results corroborate that, in the experimental conditions assayed and after complete protoplast separation, the cell wall does not represent a significant barrier to mass transfer; however, analysing the experimental data in more detail reveal that some differences exist. First, it can be appreciated that the correlation coefficients (R^2) obtained in the fittings for isolated protoplasts are appreciably better than the obtained for isolated cells, which becomes more significant in the case of type-II cells, some of which showed a very poor R^2. In figure 4, the differences among cells and protoplasts are illustrated. This representation suggests that, kinetics of the dehydration process is faster in the case of the isolated cells just after complete separation, and it

reduces as the process continues. These differences in the behaviour of cells and protoplasts might be explained by the differences in the way they accommodate an excess of plasma membrane material. While a shrinking isolated cell may be able to accommodate the plasma membrane surface in the Hechtian structures through membrane strands formation (Ferrando & Spiess, 2001; Oparka et al., 1994; Seguí et al., 2010) an isolated protoplast must activate the mechanisms for membrane material exchange (Wolfe et al., 1985). According to this, the accommodation of plasma membrane surface through strands formation could origin a faster dehydration rate in the case of apple isolated cells. Nevertheless, isolated protoplasts did not lose their spherical shape during the OD process, suggesting that the membrane material exchange is sufficiently fast to restore the membrane tension during protoplast contraction. Other mechanisms that may be enhancing the mass transfer rate in cells are the related to a negative pressure gradient term which might affect the water flux at t^{SEP} as a consequence of the mechanical deformations associated to the full detachment of the plasma membrane; this will be explained later in more detail. In any case, whichever is the dominant mechanism, the increase in the water flux out of the protoplast around SEP accentuates the dilution effect in the boundary layer causing the water flux to decrease faster, as it can be seen in figure 4. Likewise, the cell wall could act as a physical barrier for convection fluxes, widening the boundary layer and also enhancing the dilution effect.

Coupling of mass transfer and deformation-relaxation phenomena

Modelling water transfer after complete membrane separation has been simplified by reducing the gradient of the extended water chemical potential (Eq. 6) to the gradient of water activities (Eq. 7); however, modelling water transfer before that requires the use

of the extended water chemical potential since during this stage the driving force may be greatly modified by the term $\overline{V_w}\cdot\Delta P$. Taking into account that phenomenological coefficients may be calculated at any time provided that water fluxes and water activities are known; an experimental phenomenological coefficient (L_w^{EXP}) which accounts for all the resistances acting in the system at a particular time may be calculated (Equation 8). Accordingly, L_w^{EXP} coefficients obtained in the absence of cell wall deformations account for water transfer across the plasma membrane when $\overline{V_w}\cdot\Delta P \approx 0$. [Note that equation 8 considers two driving forces but one flux and one phenomenological coefficient; this is applicable on condition that the membrane is perfectly semipermeable (reflection coefficient = 1) and only water transport takes place; otherwise, the Onsager principle of reciprocity should have been applied].

$$J_w^{PM} = L_w\cdot\left(RT\Delta \ln a_w + \overline{V_w}\Delta P\right) \qquad (8)$$

Calculating an experimental phenomenological coefficient at different points of the treatment allows us to subtract resistances (Equation 9) and attribute the difference to the pressure term in equation 6, thus quantifying the energy consumed in irreversible deformations or stored/released via elastic ones.

$$\overline{V_w}\Delta P = \left(\frac{1}{L_w^{\Delta P\approx 0}} - \frac{1}{L_w^{EXP}}\right)\times J_w^{PM} \qquad (9)$$

The results of applying equation 9 to experimental data are shown in figure 5 for cells soaked in 45% (w/w) sucrose. As occurred with water fluxes, results did not show a clear time dependency and only some general features could be extracted; in contrast, the pressure gradient showed a particular pattern when representing it against protoplast relative deformation. In general terms it can be deduced that stages at which $\overline{V_w}\cdot\Delta P \approx 0$ and $\overline{V_w}\cdot\Delta P \neq 0$ are again identifiable and separated by protoplast deformation at the separation time $V^{PM}_t/V^{PM}_0 = 0.617 \pm 0.008$. There were also evident differences

393 between the cells classified as type-II (thin lines) and the classified as type-I (thick
394 lines), which exhibited a more similar pattern during all the process.
395 In type-I cells the pressure term was positive before complete separation, showing
396 higher values at the beginning of the treatment, suggesting that an important part of the
397 available energy is used in deforming the structure. Before incipient plasmolysis the cell
398 wall is deformed together with the plasma membrane so that both structures consume
399 energy (Seguí et al., 2010), either irreversibly (viscous) or elastically (the membrane
400 pulls the cell wall and stretches). Irreversible deformations consume energy that cannot
401 be recovered ($\overline{V_w}\cdot\Delta P>0$), whereas in elastic deformations the energy is stored
402 ($\overline{V_w}\cdot\Delta P>0$) and may be later released ($\overline{V_w}\cdot\Delta P<0$).
403 Once the cell wall does not resist more deformations, the membrane continuous
404 deforming until the stretching forces are higher than the needed for breaking the
405 membrane attachment sites, which occurs at incipient plasmolysis. Since this moment,
406 the three phenomena take place simultaneously and cannot be separated; nevertheless,
407 the predominant mechanisms may be identified since a positive pressure gradient
408 ($\overline{V_w}\cdot\Delta P>0$) indicates that energy consumption or storage prevails; whereas a negative
409 pressure gradient ($\overline{V_w}\cdot\Delta P<0$) indicates that the release of the previously stored energy
410 is prevailing. The oscillating shape of a curve also provides information about the
411 degree of participation of dissipative structures in the process; this suggest cycles of
412 energy storage/release which origin relies on elastic deformations.
413 As suggested in Seguí et al. (2010) the degree of cell wall/membrane interaction
414 depended on the concentration of the OS used, but also on the morphology of the cell.
415 In general terms, small gradients promote slow dehydration rates and, consequently, the
416 elastic deformation of the PM and the CW; whereas fast dehydration rates promote

membrane breakage and rupture of membrane attachment sites, thus reducing PM and CW elastic deformations. Not elongated cells with certain degree of poligonality also facilitate the separation of the plasma membrane, resulting in shorter times for incipient and complete plasmolysis. In conclusion, scarce membrane/wall interaction implies that less energy is stored in the system by deforming elastic structures and, therefore, that the pressure gradient is predominantly positive before IP, which is the case of completely plasmolyzed cells type-I (figure 5). In contrast, cells that exhibit higher degree of membrane/wall interaction during more time (elongated-rounded cells and/or cells dehydrated in less concentrated OS) store/release more energy during the treatment. Upholding this, the pressure gradient for the type-II plasmolysed cells plotted in figure 5 exhibit an oscillating curve in which negative pressure gradients prevail at some points of the dehydration process.

3.3. A comprehensive interpretation of osmotic dehydration based on the cellular approach.

Based on microstructural observations and experimental data, a more comprehensive interpretation of the dehydration process is presented in this section. For illustration, figure 6 shows the evolution of a cell during osmotic dehydration, since the beginning of the treatment until complete plasmolysis. If plasmolysing completely (response C), this cell will undergo three different stages: from time zero to InP, from InP to SEP and from SEP to the end of the treatment. If the cell's response is shrinkage (B), it will undergo the first two stages.

1st stage: time zero – incipient plasmolysis

When a cell is soaked in a hypertonic solution, the gradient of chemical potential imposed to the system originates a water flux out of the protoplast (J_w), which shrinks. The protoplast, which is linked to the cell wall at some points (attachment sites), stretches the wall (F_S) and deforms it. The cell wall is a viscoelastic structure, thus its deformation will be partially reversible (elastic) and partially irreversible (viscous). Protoplast and cell wall will deform together as long as the stretching forces appearing as a consequence of protoplast shrinkage are not enough to break the membrane-to-wall attachment sites. This first stage ends with incipient plasmolysis and in most of the cases it is not perceptible.

The equations used to describe this stage must take into account the coupling of water transfer with deformation-relaxation phenomena. In terms of irreversible thermodynamics some of the energy is consumed in mechanical deformations ($\overline{V_w}\cdot\Delta P > 0$), so the extended definition of the water chemical potential must be used.

2nd stage: incipient plasmolysis – membrane separation

As the water transfers through the plasma membrane, the stretching forces between membrane and cell wall (F_S and its opposite F_S') raise until: (i) the local membrane-to-wall attachments break (if $F_S > F_B$), (ii) the plasma membrane ruptures (cell lysis), or (iii) the pressure and compositional terms balance and the flux ceases. Usually, case (iii) is combined with case (i), since the pressure and compositional terms balance after a period during which intermittent membrane retention/detachment takes place. In biological structures the existence of elastic or viscoelastic structures such as the plasma membrane and the cell wall implies the possibility of storing part of the available free energy on the structure; both represent dissipative structures able to store energy that can be later released in the system. Unless membrane lysis occurs (response A:

466 breakage), and as long as the plasmalemma is retained at some point(s), some free
467 energy is stored via the stretching of the plasma membrane and the elastic deformation
468 of the cell wall. In terms of equations 6, the pressure gradient accounts for the stored
469 energy (+) that, if released (-), promotes mass transfer.

470 Focusing on case (i) (which follows for responses B and C) when $F_S > F_B$, the
471 membrane detaches and contracts elastically. As a direct consequence, an intracellular
472 volume between the plasma membrane and the cell wall is created, thus acting as a
473 driving force for a volumetric flow of osmotic solution (J^{OS}) that occupies the
474 intracellular space. On the one hand, this volumetric flux may enhance mass transfer by
475 narrowing the boundary layer and increasing the solute concentration near the interface;
476 on the other hand, the elastic contraction of the membrane may cause a slight increase
477 in the internal pressure over the external one and originate a hydrodynamic water flux
478 out of the protoplast. This means that part of the free energy that accumulates in the
479 structure during its deformation due to membrane stretching is regularly released as the
480 membrane detaches from the cell wall, thus promoting water transfer. This mechanism
481 might be maximized at full protoplast separation, when the plasma membrane separates
482 completely (figure 3); in that case, this could be responsible for the acceleration of mass
483 transfer kinetics in isolated cells close to SEP as compared with apple isolated
484 protoplasts (figure 4).

485 If the cell does not plasmolyse completely (response classified as B: shrinkage), the flux
486 ceases when the compositional term compensates the pressure term (case iii), the
487 membrane remaining stretched (not breaking or detaching). In this case, the system
488 tends to relax at long times of the dehydration process. Depending on the possibilities of
489 the structure, the mechanism used to release this energy would be different: In some
490 cases, the stretched membrane relaxed and re-accommodated in its original position

491 thanks to a loss of volume that took place at other sites (figure 2 in Seguí et al., 2010);
492 In other cases, it could also occur that the membrane tension, if maintained for a
493 sufficient time, activated the mechanisms for membrane material incorporation (Wolfe
494 et al., 1985) reducing the tension of the membrane and also relaxing the structure.
495 Whichever is the case, the excess of free energy associated with a pressure gradient that
496 accumulated in the viscoelastic matrix during its deformation, is later released to the
497 system causing the relaxation of the structure; this relaxation would then promote the
498 entrance of a hydrodynamic flux of OS into the intracellular space (Barat et al., 1998).
499 This is also supported by the results discussed in Seguí et al. (2010), where it was
500 noticed an increase in the projected area of cells (cell wall delimited area) at long times,
501 mainly in treatments in which the cell wall deformed to a major degree during the
502 process.

503 If using irreversible thermodynamics for modelling the process, the equation used at
504 this stage must take into account the coupling of water transfer with deformation-
505 relaxation phenomena: the energy is consumed in irreversible deformations and stored-
506 released via elastic deformations ($\overline{V_w}\cdot\Delta P \neq 0$).

507

508 ***3rd stage: membrane separation – end of the process***

509 After the complete separation of the plasma membrane, the protoplast is not longer held
510 back by the cell wall and may shrink freely. During this stage, mass transfer
511 mechanisms are quite similar to those acting during dehydration of an isolated
512 protoplast. Nevertheless, the presence of the cell wall as a physical barrier, as well as
513 the existence of elastic structures that may link the protoplast to the cell wall (Hechtian
514 structures) must be taken into account. In this case, the pressure term may be neglected
515 ($\overline{V_w}\cdot\Delta P \approx 0$) and the water chemical potential simplified to the compositional term.

4. CONCLUSIONS

This study has evidenced the importance of a proper knowledge of the microstructure and how it is modified during processing in the study of osmotic dehydration of tissue structured foods, in which mass transfer takes place coupled with deformation-relaxation phenomena. In combination with a previous work (Seguí et al., 2010) it has been proved that the appropriate description of the microstructure helps predict the response of the cells; furthermore, identifying some critical microstructural modifications undergone by cells during processing is essential for better understanding mass transfer and deformation-relaxation phenomena, and thus being able to describe the process properly. In particular, different microstructural responses have been described and they have been divided in stages thanks to the identification of critical points, which also correspond to specific changes observed at the microscopic level.

On the other hand, the equations used for modelling each stage of the process must be different and must consider the phenomena that take place at each specific period. The use of one or other equation depends on microstructural changes and not of processing time: e.g. degree of protoplast shrinkage might be used to determine the stage of the dehydration process and thus the equation that describes it more adequately. It is therefore deduced that predictive models should incorporate microstructural information in order to be more feasible.

In conclusion, these results demonstrate that including microstructural information in the mathematical models, instead of generalising a kinetic coefficient for an idealised isotropic tissue, is essential for understanding and describing osmotic dehydration of tissue structured foods more accurately, and hence offer better results.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministerio de Educación y Ciencia (Spain) for financial support, and the organizing committee of the International Conference on Food Innovation 2010 (FoodInnova2010) for granting this work with the best oral communication for young scientists award.

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