

OSMOTIC DEHYDRATION OF CUT APPLE: MASS TRANSFER KINETICS AND MICROSTRUCTURAL CHANGES

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Apple cubes were osmotically dehydrated with 40 °Bx sucrose and sorbitol solutions. Light microscopy was used to observe the microstructure of fresh and osmotically dehydrated samples. Peleg's model could fit the experimental data and describe the mass transfer kinetics of water loss (*WL*) and solid gain (*SG*). The use of sorbitol as osmotic agent, the increase of temperature and concentration of the solution increased the *WL* during the osmotic dehydration. The average cellular parameters, area and perimeter (size), and circularity, elongation, roundness, and compactness (shape) of fresh samples were $14.28 \pm 6.65 \times 10^3 \mu\text{m}^2$ and 0.486 mm, and 0.73, 1.56, 0.70, 0.83, respectively. The osmotically dehydrated samples presented a decrease in area, circularity, roundness and compactness and an increase in the elongation of the cells, and these changes were higher in samples treated with sorbitol.

Keywords: apple, osmotic dehydration, sorbitol, sucrose, mathematical modelling, microstructure

The osmotic dehydration (OD) allows to preserve fruit reducing their initial water content down to 50%. The osmotic agent most used in the OD of fruit is sucrose because of its effectiveness, convenience, and desirable flavour (LENART, 1996). Alternative solutes have been used, such as sorbitol (CHAUHAN et al., 2011; RODRÍGUEZ et al., 2013; BROCHIER et al., 2014), which is a prebiotic with proven health properties (CHAUHAN et al., 2011; PATEL & GOYAL, 2012). Besides, sorbitol is less calorific and has a relative sweetness of around 60% compared with sucrose (SILVEIRA & JONAS, 2002).

Most food processes that involve heat and mass transfer cause many macroscopic and microscopic modifications in the plant tissue (NIETO et al., 2004; LEWICKI & PORZECKA-PAWLAK, 2005). These structural modifications, consequently, alter the mass transfer mechanisms during these processes (SEGÚI et al., 2012). The light microscopy has been used to observe structural changes in osmotically dehydrated products of apple and pumpkin (QUILES et al., 2003; NIETO et al., 2004; MAYOR et al., 2008). In the light microscopy images, these authors observed the folding of cell wall, rupture of cellular membranes, and cellular shrinkage after the osmotic process. Changes in geometrical cellular parameters were also observed.

The higher the difference between the concentration of the solute in the osmotic solution and the food, the greater the mass transfer is (KHAN, 2012). The mass transfer involves water loss (*WL*) and solid gain (*SG*), but in some cases a high increase in the *SG* is not desired. MAYOR and co-workers (2006) osmotically dehydrated pumpkin and found that the concentration of the osmotic solution had more influence on the equilibrium values than the

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temperature. During OD of red cabbage with sugar beet molasses, MISLJENOVIC and co-workers (2009) noted that the increase of the concentration of the molasses and the immersion time tended to increase the dry matter, consequently, the *SG*.

The objectives of this study were: i) to carry out the OD of apple cubes, using two different solutions with sucrose and sorbitol at 40 °Bx, and to study the effect of the temperature on the *WL* and *SG*; ii) to test the adequacy of the fitness of Peleg's model to describe the *WL* and the *SG* of the product during the OD process; iii) to evaluate the microstructural changes of the apple tissue after the OD with the two different solutes.

1. Materials and methods

The osmotic dehydration processes were performed at CBQF of UCP while the light microscopy analyses were carried out at Faculdade de Ciências of UP.

1.1. Samples

Apples (*Malus* spp., variety Royal Gala) were supplied by Campotec, Portugal, and stored at 4 °C. The fruit were washed and sanitized with aqueous solution with 7500 ppm active chlorine for 5 min. Then, they were cut in cubes (12 mm) with a vegetable cutter (Secret de Gourmet, France) and immersed in a solution with 0.9% sodium chloride for 3 min to prevent enzymatic browning. The samples were blotted gently with tissue paper in order to remove the excess of sodium chloride solution from the surface. The soluble solids content of the apple was 17.0±0.7 °Bx (Hand refractometer, Atago, China).

1.2. Osmotic dehydration process

The osmotic solutions were prepared with ultra-pure water and commercial sucrose and sorbitol (Fagron Iberica, Spain). The OD was carried out in beakers placed in a shaking incubator (Wiggenhauser, Germany) at constant temperature and agitation (50 r.p.m.). Apple samples were immersed in the osmotic solution at 40 °Brix and submitted to temperatures of 25, 40, and 60 °C. The mass ratio of sample to solution used was 1:4. The apple cubes were removed from the solution at different times. Then, the samples were rinsed with ultra-pure water to remove the solution adhered to the surface and blotted with tissue paper to remove the excess of water from the surface. The experiments were performed in duplicate.

1.3. Moisture content determination

The moisture content was determined by placing the fresh and osmotically dehydrated samples in an oven (FP115, Binder, Tuttlingen, Germany) at 105 °C until constant weight (AOAC, 2002). The determinations were performed in triplicate.

1.4. Osmotic dehydration parameters and mathematical model

The parameters of water loss (*WL*) and solid gain (*SG*) were determined using the following equations:

$$WL = \frac{w_{w0} - w_w}{w_0} \quad (1)$$

$$SG = \frac{w_s - w_{s0}}{w_0} \quad (2)$$

where w_{w_0} is the initial moisture content, w_w is the moisture content at time t , w_0 is the initial weight of the sample, w_s is the dry matter at time t of OD, and w_{s0} is the initial dry matter of sample, all in kg.

PELEG's model (1988) was used to fit the WL and SG data. The WL and SG are given by:

$$WL \text{ or } SG = \frac{t}{k_1 + k_2 \cdot t} \quad (3)$$

where k_1 and k_2 are the Peleg constants for WL or SG .

The constant k_1 relates to the initial rate of the mass transfer and the constant k_2 relates to equilibrium values, WL_∞ or SG_∞ , as may be seen below:

$$\left(\frac{d(WL \text{ or } SG)}{dt} \right)_{t \rightarrow 0} = \lim_{t \rightarrow 0} \left[\frac{d\left(\frac{t}{k_1 + k_2 \cdot t}\right)}{dt} \right] = \frac{1}{k_1} \quad (4)$$

$$\lim_{t \rightarrow \infty} (WL \text{ or } SG) = (WL \text{ or } SG)_\infty = \lim_{t \rightarrow \infty} \frac{t}{k_1 + k_2 \cdot t} = \frac{1}{k_2} \quad (5)$$

1.5. Light microscopy analysis

For the analysis by light microscopy, the samples were cut in parallelepipeds (12×12×15 mm) with the vegetable cutter and immersed in a solution with 0.9% sodium chloride for 3 minutes. With the aim to obtain a good structural and compositional homogeneity of the samples, the parallelepipeds were taken from the same parts of the fruit (Fig. 1). The samples were blotted gently with tissue paper in order to remove the excess of sodium chloride solution from the surface. These samples were then immersed in 60 °Bx osmotic solutions of sucrose and sorbitol for 14 hours at 60 °C, using a mass ratio of sample to solution of 1:4. Then, the samples were fixed in 2.5% glutaraldehyde in 1.25% PIPES buffer at pH 7–7.2 for 24 hours at room temperature (ca. 20 °C) (MAYOR et al., 2008). For the osmotically dehydrated samples, the fixing solution was added to the osmotic solution at the same concentration of the solution at the end of OD, which was 53.6 and 52.4 °Bx for sucrose and sorbitol, respectively. After that, they were dehydrated in a water/ethanol series and embedded in LR White resin (London Resin Co., Basingstoke, UK). Sections (6 µm) of the resin blocks were obtained with a microtome (Jung RM 2035, Leica, Germany). The sections were stained with an aqueous solution Azure II 0.5%, Methylene Blue 0.5%, Borax 0.5% for 30 seconds. After that, they were washed with distilled water and mounted on a glass slide (MAYOR et al., 2008).

The microimages were obtained under a light microscope equipped with a digital camera (BA310, Motic, China) and connected to a computer. The image acquisition was performed with an interface (Motic Images Plus 2.0 ML). The image analysis of the cells was performed using the free software "ImageJ" (version 1.51g), which allows to obtain geometric cellular parameters, such as area, perimeter, length of the major axis, length of the minor axis, circularity, elongation, roundness, aspect ratio, and compactness.

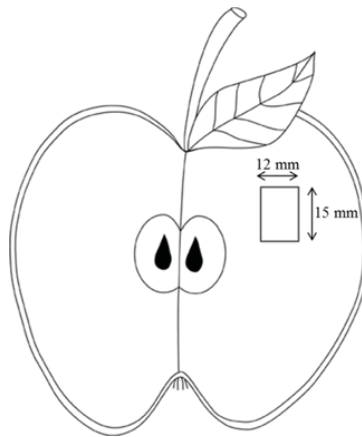


Fig. 1. Location of sampling of apple tissue

1.6. Statistical analysis

The statistical analysis was performed using IBM SPSS® Statistics 20.0 for Windows® (2012, SPSS Inc., Chicago, USA). The normality of the data was tested using the Kolmogorov–Smirnov test. When the normality of data was not verified, an alternative non-parametric test, Kruskal–Wallis, was used. In this case, the Mann–Whitney test was subsequently performed to detect which values were significantly different.

The adequacy of the model fit was evaluated by the determination coefficient (R^2) and by the residual analysis. This was performed in order to check the assumptions of independence, randomness, and normality (mean equal to zero and constant variance). Randomness and homoscedasticity was assessed by visual inspection of the dispersion of residuals vs. the values predicted by the model. The normality of the residuals was evaluated by Kolmogorov–Smirnov test.

In all tests and analysis performed, the significance level assumed was 5%.

2. Results and discussion

2.1. Mass transfer kinetics

Higher WL and SG rates were noted in the first two hours of the process (Figs 2 and 3), which were probably due to the large differences of osmotic pressure between the solutions and the samples at the beginning of the process. Also during this period, these rates were higher in the OD with sorbitol solutions. This might be explained by the lower viscosity of sorbitol solutions attributed to the higher molar concentration of these solutions, 2.198 M in comparison with 1.170 M for sucrose solutions (40 °Bx), as discussed by Assis and co-workers (2017).

Peleg's model satisfied all assumptions described above in section 1.6, and it was able to describe the mass transfer kinetics of WL and SG of apple cubes at the OD conditions used. The R^2 values of the fits of WL were higher than 0.95 (Table 1). The initial rate of WL ,

reflected in $1/k_1$, was higher using sorbitol for all tested conditions. $1/k_1$ also presented the highest value in experiments with the sorbitol solution at 60 °C. The increase in temperature increased the rate of the WL . The equilibrium values of WL ($1/k_2$) varied from 0.312 to 0.552 h kg DM/kg water. At 25 and 40 °C, the WL at equilibrium was higher in experiments with sorbitol in relation to sucrose.

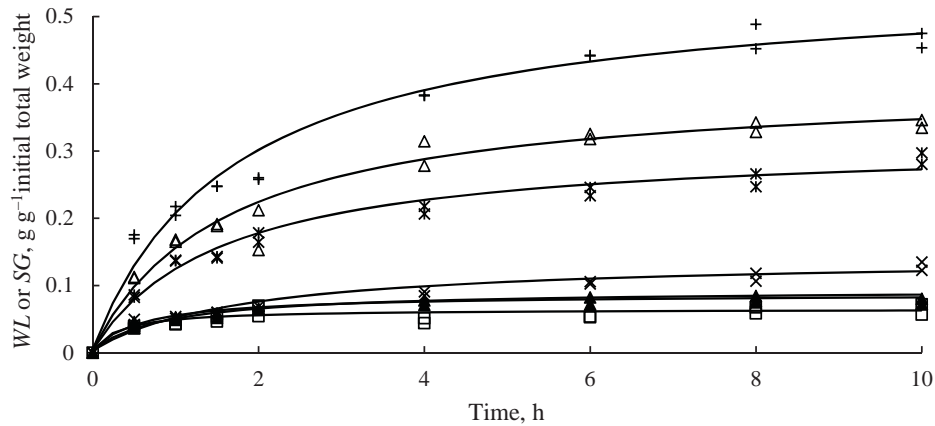


Fig. 2. Experimental data and the fit of Peleg's model of WL and SG of apple cubes osmotically dehydrated in a 40 °Bx sucrose solution and at 25, 40, and 60 °C, using a mass ratio of sample to solution of 1:4. *: WL 25 °C; Δ : WL 40 °C; +: WL 60 °C; \blacktriangle : SG 25 °C; \square : SG 40 °C; \times : SG 60 °C; — Peleg's model fit.

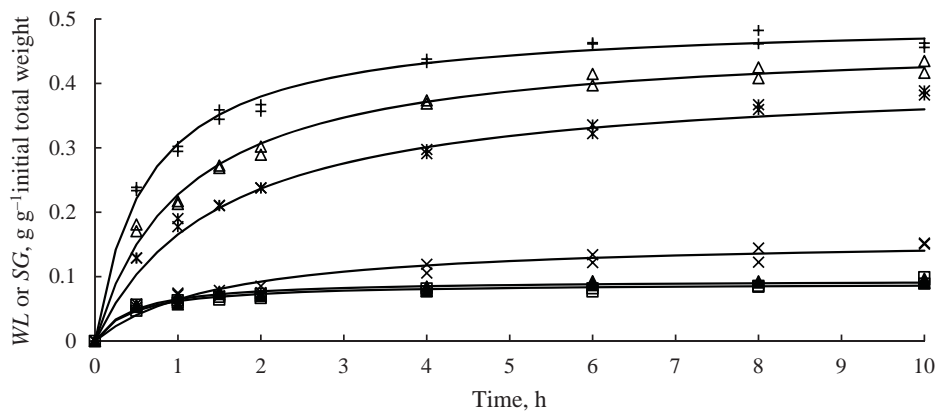


Fig. 3. Experimental data and the fit of Peleg's model of WL and SG of apple cubes osmotically dehydrated in a 40 °Bx sorbitol solution and at 25, 40, and 60 °C, using a mass ratio of sample to solution of 1:4. *: WL 25 °C; Δ : WL 40 °C; +: WL 60 °C; \blacktriangle : SG 25 °C; \square : SG 40 °C; \times : SG 60 °C; — Peleg's model fit.

Table 1. Parameters of the fit of Peleg's model of *WL* and *SG* during OD of apple cubes at 40 °Bx and mass ratio of sample to solution 1:4

Temperature (°C)	Solute	Peleg					
		<i>WL</i>			<i>SG</i>		
		$k_1 \pm$ margin of error (h kg kg ⁻¹)	$k_2 \pm$ margin of error (kg kg ⁻¹)	R ²	$k_1 \pm$ margin of error (h kg kg ⁻¹)	$k_2 \pm$ margin of error (kg kg ⁻¹)	R ²
25	sucrose	5.03±0.86	3.21±0.22	0.974	7.28±1.76	11.90±0.71	0.957
25	sorbitol	3.34±0.44	2.39±0.12	0.985	4.84±1.18	10.55±0.54	0.961
40	sucrose	4.06±0.89	2.50±0.23	0.959	5.49±3.64	16.18±1.88	0.804
40	sorbitol	2.27±0.26	2.13±0.09	0.989	5.02±1.41	11.14±0.65	0.949
60	sucrose	3.09±0.58	1.81±0.15	0.969	13.13±3.50	7.15±0.85	0.937
60	sorbitol	1.26±0.14	2.00±0.06	0.991	9.26±2.52	6.21±0.67	0.934

Margin of error is the half width of the confidence interval at 95%.

The R² values of the fits of *SG* were higher than 0.8. The solute did not show an influence on the initial rate of *SG* (k_1 -values). The experiments carried out at 60 °C resulted in a lower *SG*, while no significant differences were observed between 25 and 40 °C. In relation to k_2 parameter, the use of sorbitol as osmotic agent promoted a higher *SG* at the end of the process at 25 and 40 °C.

A similar behaviour for experiments at 60 °Bx (Assis et al., 2017) and 40 °Bx was noted: the same trend in k_1 parameter was noted, and the *WL* increased with temperature. With respect to the *WL*, experiments with sucrose and sorbitol did not follow the same trend in what concerns k_1 parameter, but *WL* tended to be higher in experiments at 60 °Bx than at 40 °Bx. At the equilibrium ($1/k_2$ -values), the samples treated with 60 °Bx solutions presented higher *WL* than the ones treated with 40 °Bx. The results of LAZARIDES and co-workers (1995) obtained in OD of apple slices were in agreement with these findings. With respect to the *SG*, there were no significant differences in the initial rate of *SG* between OD at 40 °Bx and OD at 60 °Bx, neither at 25 nor at 40 °C, but, at the end of the process, the *SG* was higher in samples treated with 60 °Bx solutions.

ROOPA and co-workers (2012) observed that the increase of the solute concentration, solution temperature, and dehydration time increased the *WL* and the *SG* of carambola slices. PALOU and co-workers (1994) also used Peleg's equation (PELEG, 1988) to describe the mass transfer kinetics during the OD of papaya. They found the same k_1 for *SG* during OD in 60 and 70 °Bx sucrose syrups and a lower k_2 for 70 °Bx. WALISZEWSKI and co-workers (2002) dehydrated pineapple slabs with sucrose solutions, and found that the *WL* and *SG* at the equilibrium were significantly higher in the OD with more concentrated solutions.

2.2. Microscopy results

After 14 hours of OD at 60 °Bx, it was considered that the equilibrium was achieved, i.e., the maxima *WL* and *SG* occurred. The initial moisture content of the fresh apples was 5.431±0.205 kg water/kg DM. At the end of the OD process, the moisture content was reduced to 0.966±0.028 and 1.034±0.040 kg water/kg DM, using sucrose and sorbitol solutions, respectively. Thus, both solutes resulted in a moisture loss of around 80%. In a previous work, ASSIS and co-workers (2017) also observed that, at the end of the OD process, these

two solutes resulted in the same *WL*. However, the solute used in the osmotic solution had an influence on the initial *WL* rate.

The microscopy analysis of the apple parenchyma was performed before (control) and after the OD with sucrose and sorbitol (Fig. 4). For this part of the study, the OD was performed with 60 °Bx solutions, because *WL* at the equilibrium was higher at this concentration than at 40 °Bx, as discussed above.

The cells submitted to the osmotic treatment presented different forms: the cells of the fresh sample were round and the ones of the dehydrated samples were wrinkled. The presence of intercellular spaces could also be noted (Fig. 4B and C). In Figure 5, it is possible to observe a region with vascular site. The cells near this site present different orientation and irregular shape. Therefore, these were not considered in the measurements.

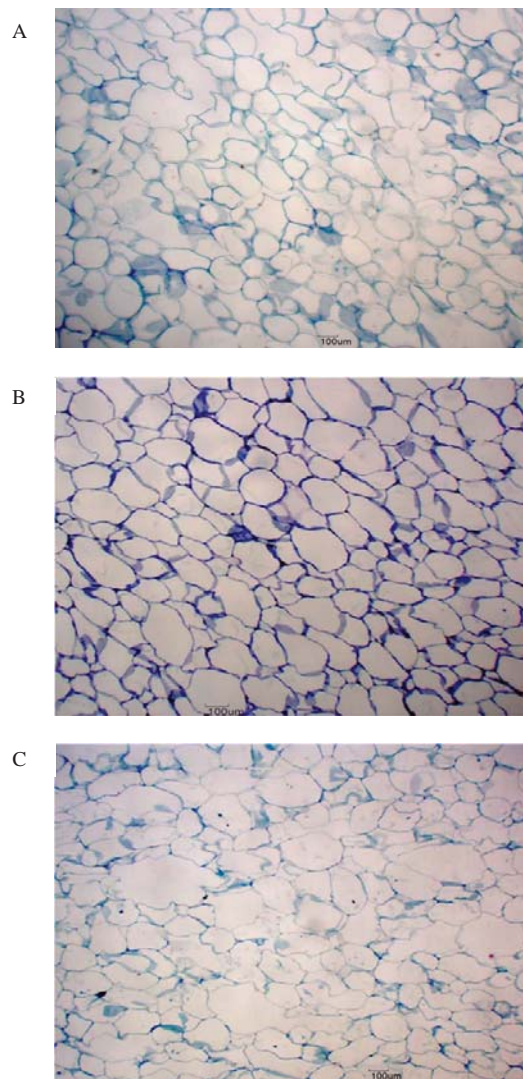


Fig. 4. Microstructure of apple parenchyma analysed before (A) and after the osmotic dehydration with sucrose (B) and sorbitol (C)

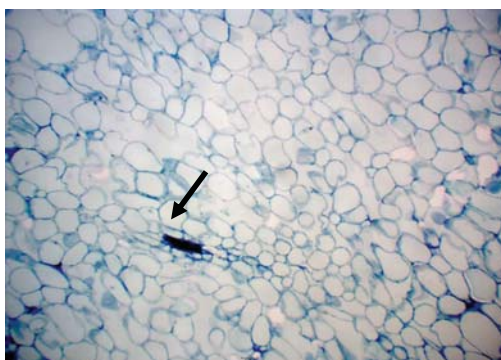


Fig. 5. Microstructure of apple parenchyma with vascular site (arrow)

The area of the cells of fresh samples, $14.28 \pm 6.65 \times 10^3 \mu\text{m}^2$ (Table 2), is in the range of those obtained by LEWICKI and PAWLAK (2003), LEWICKI and PORZECKA-PAWLAK (2005), and MAYOR and co-workers (2005) for different apple varieties. The area decreased significantly with the osmotic treatment, but there were no significant differences between the solutes used, sucrose and sorbitol. However, the perimeter of the cells was not affected by the OD (Table 2). MAYOR and co-workers (2008) also observed that the area of the tissue cells of pumpkin decreased and their perimeter was maintained during the OD process.

Table 2. Average cellular size and shape parameters of fresh and osmotically dehydrated apple samples

Pre-treatment	Area $\times 10^{-3}$ (μm^2)	Perimeter (μm)	Circularity	Elongation	Roundness	Compactness
Fresh	14.28 ± 6.65^a	485.6 ± 128.4^a	0.73 ± 0.11^a	1.56 ± 0.33^c	0.70 ± 0.12^a	0.83 ± 0.07^a
OD with sucrose solution	13.60 ± 8.07^b	487.8 ± 137.9^a	0.71 ± 0.09^b	1.60 ± 0.34^b	0.65 ± 0.14^b	0.80 ± 0.09^b
OD with sorbitol solution	12.91 ± 7.30^b	497.5 ± 159.2^a	0.64 ± 0.11^c	1.90 ± 0.51^a	0.56 ± 0.16^c	0.74 ± 0.11^c

Margin of error is the half width of the confidence interval at 95%.

Different letters in each column mean significantly different values at $P < 0.05$.

At the end of the osmotic process, folding of the cell wall, plasmolysis, and cellular shrinkage were observed (Figs 4B and C). No differences were observed in the intercellular spaces, but there was a reduction of the volume induced by the shrinkage of the samples. The OD affected all shape parameters. Roundness, compactness, and elongation of the fresh sample were 0.70, 0.83, and 1.56, respectively (Table 2). These values are near the values found by KARUNASENA and co-workers (2014) for the same apple variety. The roundness and compactness decreased after the OD process and the cells became more elongated (Table 2). The maximum decrease in roundness and the highest elongation were obtained in samples osmotically dehydrated with sorbitol solutions. The circularity was also affected by the OD

process. The highest reduction (from 0.73 to 0.64) was observed for sorbitol solutions. The decrease of this shape parameter could be explained by the fact that the initial *WL* was higher when the sorbitol was used as osmotic agent, thus, causing more changes in the structure of the tissue in comparison with the tissue of the samples osmotically dehydrated with sucrose solution.

3. Conclusions

Peleg's model was able to describe the mass transfer kinetics of water loss and solid gain in the osmotic dehydration of apple cubes in 40 °Bx sucrose and sorbitol solutions. The use of sorbitol, the increase of the temperature and solute concentration increased the water loss during the process. This osmotic dehydration process resulted in shrinkage of the cells of the apple tissues and, consequently, in volume reduction, plasmolysis and folding of the cell walls. It caused significant changes in size and shape parameters of the cells, and the use of sorbitol as osmotic agent enhanced these changes.

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