CrossMark

Unfreezable Water in Apple Treated by Pulsed Electric Fields: Impact of Osmotic Impregnation in Glycerol Solutions

Oleksii Parniakov[1] · Olivier Bals[1] · Viacheslav Mykhailyk[2] · Nikolai Lebovka[1,3] · Eugene Vorobiev[1]

Abstract The effects of pulsed electric fields (PEF) and osmotic impregnation in glycerol solution on the amount of unfreezable water in apple were determined by means of low-temperature differential scanning calorimetry (DSC). The obtained data were compared with behaviour of pure water–glycerol solutions (sample WG). PEF treatment was applied using a near-rectangular monopolar generator with pulse duration of 100 µs at electric field strength of 800 V/cm. The osmotic impregnation of PEF-treated apple discs was done using water–glycerol (sample AWG) and apple juice–glycerol (sample AJG) osmotic solutions at different concentration of water or juice in glycerol, $W = 30$–100 wt%. The data evidenced that for the PEF-treated samples the glycerol was able to penetrate successfully inside apple tissue. The state diagrams for WG, AWG and AJG samples were rather similar. It was observed that free water existed only for moisture content above some minimum value. The unfreezable water content was the largest in AJG, followed by WG and AWG. The juice concentration in glycerol $W \approx 80$ wt% was found to be optimal for preservation of the texture of PEF-treated samples.

Nomenclature

°Brix	Dry matter content of intrinsic juice in the sample
d	Diameter of apple disk (mm)
E	Electric field strength (V/cm)
F	Force (N)
F^*	Texture index
h	Thickness of apple disk (mm)
ΔH	Enthalpy (J/g)
m	Total mass of water in sample (g)
m^u	Mass of unfreezable water (g)
m^f	Mass of free or freezable water (g)
m_d	MASS of dry matter in the sample (g)
m_s	Mass of the sample (g)
n	Number of pulses in one train
N	Number of trains of pulses
R	Fraction of unfreezable water, m_u/m
t	Time of osmotic treatment (min)
t_{PEF}	Time of PEF treatment (s)
t_i	Pulse duration (µs)
Δt	Pulse repetition time (µs)
Δt_t	Pause between trains (s)
T	Temperature (K)
ΔT	Temperature increase (K)
W	Wet basis content of water or juice in glycerol (% wt)
W_s	Wet basis moisture content in the sample
W_d	Dry basis moisture content in the sample
W^u	Wet basis unfreezable moisture content in the sample
W_d^u	Dry basis unfreezable moisture content in the sample
Z	Electrical conductivity disintegration index

✉ Nikolai Lebovka
nikolai.lebovka@utc.fr; lebovka@gmail.com

1 Université de Technologie de Compiègne, Laboratoire de Transformations Intégrées de la Matière Renouvelable, Sorbonne Universités, EA 4297, Centre de Recherches de Royallieu, BP 20529, 60205 Compiègne Cedex, France

2 Institute of Engineering Thermal Physics, National Academy of Sciences of Ukraine, 2a, str. Zheljabova, Kyiv 03057, Ukraine

3 Institute of BioColloidal Chemistry named after F. D. Ovcharenko, National Academy of Sciences of Ukraine, 42, Vernadsky av, Kyiv 03142, Ukraine

🙋 Springer

Greeks

σ Electrical conductivity (S/cm)

Subscripts

d Completely damaged

i Initial, intact

f Final

Abbreviations

PEF Pulsed electric field

WG Water-glycerol sample

AWG PEF-treated and osmotically treated apple in water + glycerol mixture

AJG PEF-treated and osmotically treated apple in juice + glycerol mixture

Introduction

A growing interest has been shown for partially dehydrated or intermediate-moisture foods mainly due to their acceptable eating quality and reasonable storage stability (several months) under ambient conditions (Barbosa-Cánovas et al. 2008).

Usually, the moisture content of these products is in the range of 20–50 wt% and some amount of their water is bound by osmotic agents such as sugars (sucrose, glucose and fructose), alcohols (sorbitol, glycerol), salts (sodium chloride) or other substances preventing the growth of microorganisms. The convective drying (Calín-Sánchez et al. 2015), freeze-drying (Pei et al. 2014) and osmotic treatment (Garcia Loredo et al. 2013) are the most popular techniques for removal of water from fruit and vegetable tissues. The process of freezing of the partially dehydrated foods, known as dehydrofreezing, is also rather popular (James et al. 2014). In addition, it is noteworthy that dehydrofrozen fruits and vegetables have better quality than conventionally frozen products (Li and Sun 2002).

Different examples of successful applications of pulsed electric field (PEF) for the enhancement of the dehydration of fruit and vegetable tissues were demonstrated (Donsi et al. 2010; Vorobiev and Lebovka 2011). The efficiency of PEF was explained by electroporation phenomenon (Lebovka and Vorobiev 2010) and this technique allows avoidance of undesirable changes in materials that are typical for other techniques, such as thermal, chemical and enzymatic ones (Donsi et al. 2011; Jaeger et al. 2012; Odriozola-Serrano et al. 2013; Raso and Heinz 2006). Moreover, the positive effects of PEF on drying, freezing and freeze-drying processes in different biomaterials were demonstrated (Ben Ammar et al. 2010; Jalte et al. 2009; Shynkaryk et al. 2008; Wiktor et al. 2015). The substantial improvement of the freezing tolerance of spinach leaves was obtained using the PEF-assisted vacuum impregnation of a cryoprotectant (trehalose) inside the cells (Phoon et al. 2008). The combined PEF treatment associated with cryoprotectant and texturizing agents (different salts, glycerol, trehalose and sucrose) allowed prevention of tissue softening after defrosting (Shayanfar et al. 2013, 2014).

Recently, the effects of PEF and osmotic impregnation in aqueous glycerol solution (20 wt%) on the structure of apple tissue and its freezing/thawing behaviour were studied (Parniakov et al. 2015). It was shown that glycerol acted as a cryoprotectant and texturing agent, and the pronounced reducing of both freezing and thawing duration, as well as strengthening of the apple texture were observed after the PEF treatment. In foods and beverages, glycerol serves as humectant, sweetener and hygroscopic ingredient, allowing more water at a specific water activity (Farahnaky et al. 2009). It should be noted that glycerol is easily digested, non-toxic and is recognized as safe by food and drug administration. At low temperatures, glycerol may serve as penetrating cryoprotectant that inhibits the growth of ice crystals (Fuller 2004). Due to its strong interactions with water in the plant system, some parts of water become unfreezable or bound. The concept of bound water (Wolfe et al. 2002) is of great significance with regard to texture, chemical deterioration and microbial stability of foods (Blanch et al. 2015). The amount of unfreezable water in aqueous glycerol solutions was experimentally deduced from the differential scanning calorimetry measurements (DSC) (Weng et al. 2011b) and was estimated using molecular dynamics simulations (Dashnau et al. 2006; Weng et al. 2011a; Zhang et al. 2013a, 2013b). Data have shown that glycerol molecules serve as "water blocker" and bound water fraction increases with rising glycerol concentration. The temperature effect on the bound water fraction was unessential (Weng et al. 2011a). However, impact of PEF-assisted osmotic glycerol impregnation on the changes in unfreezable water content in biological tissues was not previously discussed. Impregnation of glycerol or other osmotic agents such as sugars, alcohols, salts can prevent the growth of microorganisms and can be useful for improving the quality of the dehydrofrozen fruits and vegetables (Li and Sun 2002).

Therefore, this manuscript studies unfreezable water content in apple tissue treated by PEF and subjected to osmotic impregnation in water–glycerol and juice–glycerol solutions (30–100 wt%). The content of unfreezable water was determined using the low-temperature DSC. The obtained data were compared with thermal behaviour of pure water–glycerol solutions.

Materials and Methods

Materials

Commercial apples (*Jonagold*) were purchased in a local supermarket (Compiegne, France). They were stored (not more than 1 week) in a plastic bag in a laboratory refrigerator at 278 K until required. The moisture content, measured by

drying 25 g of the fresh apple tissue at 378 K to constant weight, was about 82 wt%. The apple disc-shaped samples (29 mm in diameter and 10 mm in thickness) were manually prepared immediately before experiments using a special cylindrical knife.

Electric Field Treatment

Pulsed electric field (PEF) treatment was applied using a monopolar PEF generator (5 kV–1 kA, Hazemeyer, Saint-Quentin, France). The PEF generator provided pulses of a near-rectangular shape, and N trains of pulses were applied. Each separate series consisted of n pulses with pulse duration t_i, time interval between pulses Δt and pause Δt_t after each train. The total time of PEF treatment was regulated by the variation of the number of series N and was calculated as $t_{PEF} = N \cdot n \cdot t_i$. The current and voltage values were measured during the period between two consecutive series of pulses. The following protocol was used in PEF experiments: $E = 800$ V/cm, $n = 10$, $t_i = 100$ μs, $\Delta t = 100$ ms, $\Delta t_t = 10$ s, and the value of N was varied in order to obtain the desirable t_{PEF}, e.g. $N = 100$ corresponded to $t_{PEF} = 0.1$ s. The chosen protocol of successive trains with long pause after each train allowed controlling the plant tissue permeabilization without any significant temperature elevation ($\Delta T \leq 3$ K) during PEF treatment. Electrical treatment cell consisted of a Teflon cylindrical tube with $\approx$110-mm inner diameter and an electrode at the bottom. The seven apple disc-shaped samples (with diameter of $d = 29$ mm) were placed inside the cell on the bottom electrode and covered with fresh apple juice. After that, the second electrode was put on the top of the samples. The distance between the electrodes was determined by the thickness of the sample ($h = 10$ mm). Fresh juice was chosen as a natural medium in order to reduce the sample degradation and to improve electrical contact between the electrodes and the sample. The temperature inside the geometrical centre of the central sample was recorded in the online mode by a teflon-coated thermocouple Thermocoax type 2 (AB 25 NN). All data (electrical conductivity, voltage, current, temperature) were collected using a data logger and software adapted by Service Electronique UTC, Compiègne, France.

The degree of apple tissue damage was estimated by electrical conductivity disintegration index, Z (Lebovka et al. 2002):

$$Z = (\sigma - \sigma_i)/(\sigma_d - \sigma_i), \tag{1}$$

where σ is the measured electrical conductivity and subscripts i and d refer to the conductivities of the intact and completely damaged tissue, respectively. All values of σ were measured at the same temperature, $T = 293$ K. The value of σ_d was estimated as the maximum attainable level of σ for the given mode of treatment. It can be attained using long-lasting treatment (e.g. treatment during the time of 1 s at 800 V/cm for PEF treatment).

The $Z(t_{PEF})$ dependence for the same apple variety (*Jonagold*) was previously investigated in detail (Parniakov et al. 2015). In this work, the two protocols of PEF treatment were applied for obtaining the apple samples with high ($Z \approx 0.95$, $t_{PEF} = 0.1$ s) and intermediate ($Z \approx 0.6$, $t_{PEF} = 0.004$ s) damage degree.

Osmotic Impregnation

The osmotic impregnation of water–glycerol (sample AWG) and apple juice–glycerol (sample AJG) osmotic solutions was studied at different water or AJG content, $W = 30$–100 wt%. The apple juice was freshly prepared from the same apples (*Jonagold*) used in the experiments. The anhydrous glycerol (BioUltra, Sigma-Aldrich) and double distilled water with electrical conductivity of $\approx$0.18 μS/cm were used throughout this study.

After the PEF treatment, the samples were placed into a 600-ml beaker, the solid–liquid ratio in the beaker was maintained at 1:20 (w/w), the temperature was held at $T = 293$ K. Samples were carefully kept under agitation using a magnetic stirrer (0–1, 250 min^{-1}, Bioblock Scientific, Heidolph, Germany) and the beaker was closed in order to avoid any evaporation. The level of osmotic impregnation was controlled by periodical measurements of the changes in °Brix of intrinsic juice in the sample. In order to measure °Brix, the sample was taken from the osmotic solution periodically and the juice was expressed from this sample. °Brix was measured using a refractometer PR-201 (Atago, Japan) which reflected the concentration of total soluble matter in intrinsic juice inside the sample. Figure 1 presents examples of excess values of Δ°Brix = °Brix $-$ °Brix$_i$ for PEF-treated apples ($Z \approx 0.95$) in juice–glycerol solutions (sample AJG) at different values of W. The value of Δ°Brix increased slowly with increase of t in untreated tissue. The observed changes in °Brix of intrinsic juice during osmotic treatment can reflect the saturation of tissue by the osmotic component (glycerol) and, also, the migration of water outside the tissue into osmotic solution (sample dehydration). Such behaviour evidently reflects the impact of electroporation on the enhancement of osmotic impregnation of glycerol by apple tissue and the effects of apple tissue dehydration. Similar osmotic effects were reported for different fruit and vegetable tissues subjected to electrical treatment (Vorobiev and Lebovka 2011).

It is interesting that the values of Δ°Brix in PEF-treated samples practically got saturated after 210 min ($\approx$3.5 h) of osmotic impregnation, reaching the levels that are close to the values of Δ°Brix ≈ 13.5 ($W = 80$ wt%) and Δ°Brix ≈ 42.0 ($W = 30$ wt%) in apple juice–glycerol mixtures. In this work, the time of osmotic impregnation was always fixed at 3.5 h.
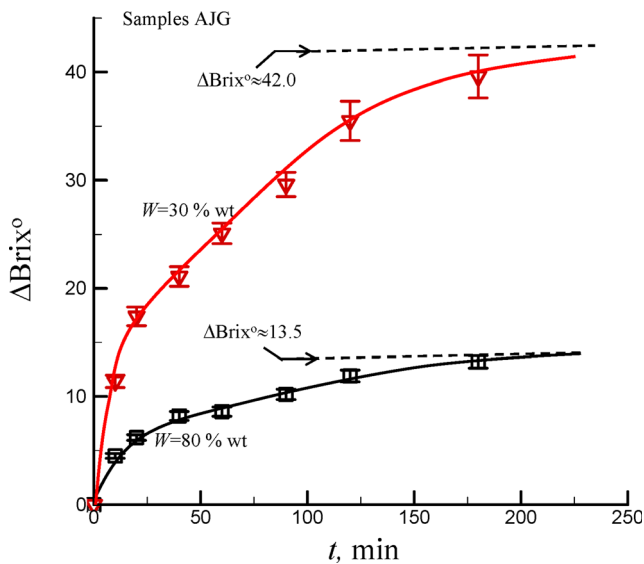
Fig. 1 Examples of $\Delta°\text{Brix} = °\text{Brix} − °\text{Brix}_i$ of intrinsic juice versus the time of osmotic impregnation t for PEF-treated apples ($Z \approx 0.95$) in juice–glycerol solutions (samples AJG) at different values of W. Here, subscript i refers to the initial value of $°\text{Brix}_i$ at $t = 0$

Differential Scanning Calorimetry Experiments

The low-temperature DSC was used to determine the amount of bound water in untreated, PEF-treated and PEF-treated and subjected to osmotic impregnation apples. Note that tightly bound water remains fluidic below freezing temperature (273 K) and does not display a distinct phase transition. The DSC method allows subdividing the "free" and "bound" water by analysing low-temperature thermograms. The samples were sealed using the standard 120-μl aluminium pans by pressing. Then, pans were placed into the calorimeter cell of DSC131 evo (Setaram, France). During the measurements, the calorimeter cell was blown by dried gaseous helium at the rate of 30 ml/min.

In order to avoid the effects of thermal history upon the phase behaviour of the studied aqueous glycerol systems (Bohon and Conway 1972), all DSC measurements were done using the same protocol: (1) cooling (293 K → 123 K) with the high rate of 32 K/min (in order to prevent the growth of big ice crystals) and holding for 5 min at 123 K; (2) rapid heating (123 K → 173 K) with the rate of 32 K/min followed by slow heating (173 K → 293 K) with the small rate of 4 K/min (in order to measure the thermal effects more accurately).

The amount of unfreezable water, m^u, was calculated as

$$m^u = m − m^f \tag{2}$$

where m is the total mass of water in the sample [$m = m_s W_s$; m_s and W_s are the mass and the moisture content (wet basis moisture content) in a sample, respectively] and m^f is the mass of a free (freezable water).

The mass of freezable water m^f was experimentally determined by the comparison of the melting enthalpies of a given

sample (ΔH_s) and the reference sample consisting of distilled water (ΔH_r):

$$m^f = m_s \Delta H_s / \Delta H_r \tag{3}$$

where m_s is the total mass of the sample (sample WG for water–glycerol mixture or samples AJG and AWG for PEF-treated apple subjected to osmotic impregnation).

The mass of dry matter in the sample was determined as $m_d = m_s - m$. Dry basis moisture content W_d in the sample was defined as the ratio of the mass of water to the mass of dry matter, $W_d = m/m_d$ and $W_d^u = m^u/m_d$ for total water and unfreezable water, respectively. Fraction of unfreezable water R was defined as the ratio of unfreezable and total water masses m^u/m.

Textural Properties

The textural properties of tissues were measured using a Texture Analyser TA-XT plus (Rhéo, Champlan, France). The apple samples were placed on the flat platform of the texture analyser and stress relaxation tests were carried out. Pre-loading with 15 % deformation compensated non-uniformity of the structure. In the force relaxation tests, the applied force was set at the level of 5 N, the speed of piston displacement was 1.0 mm/s and stress relaxation curves were recorded with 0.1 s resolution during 60 s.

In order to quantify the difference in the sample texture at different modes of apple tissue treatment, the texture index defined as

$$F^* = (F − F_i)/(F_d − F_i) \tag{4}$$

was used.

Here, F is the force measured at $t = 50$ s, and subscripts i and d refer to the values of intact and completely damaged tissue, respectively. The value of F_d was estimated as the minimum level of F attainable after long-lasting ($\approx$90 h) freezing and thawing treatment of apple issue. Texture index is convenient for understanding the viscoelastic properties of tissues. The value of F^* is equal to 0 for completely damaged tissue, while for intact material F^* is 1.0.

Statistical Analysis

All experiments and measurements of characteristics were repeated using, at least, five replicates. One-way analysis of variance was used for statistical analysis of the data using the Statgraphics plus (version 5.1, Statpoint Technologies Inc., Warrenton, VA). For each analysis, significance level of 5 % was assumed. The error bars presented on the figures correspond to the standard deviations.

Results and Discussions

Figure 2a presents the examples of DSC melting curves for water + glycerol mixtures (sample WG) at different water content W (on wet basis). At concentration of water above $W \approx 40$ wt%, the endothermic peaks of water melting (a first-order solid–liquid transition) in the interval 223–273 K were observed and the increase of concentration of water resulted in an increase of melting temperature T_m (the formation of a liquid phase from a solid). Note that for $W < 80$ wt%, the DSC patterns also shown the typical second-order transition glass-transition peaks at the low-temperature interval ≈158–193 K (Fig. 2b). Moreover, at rather low water content the
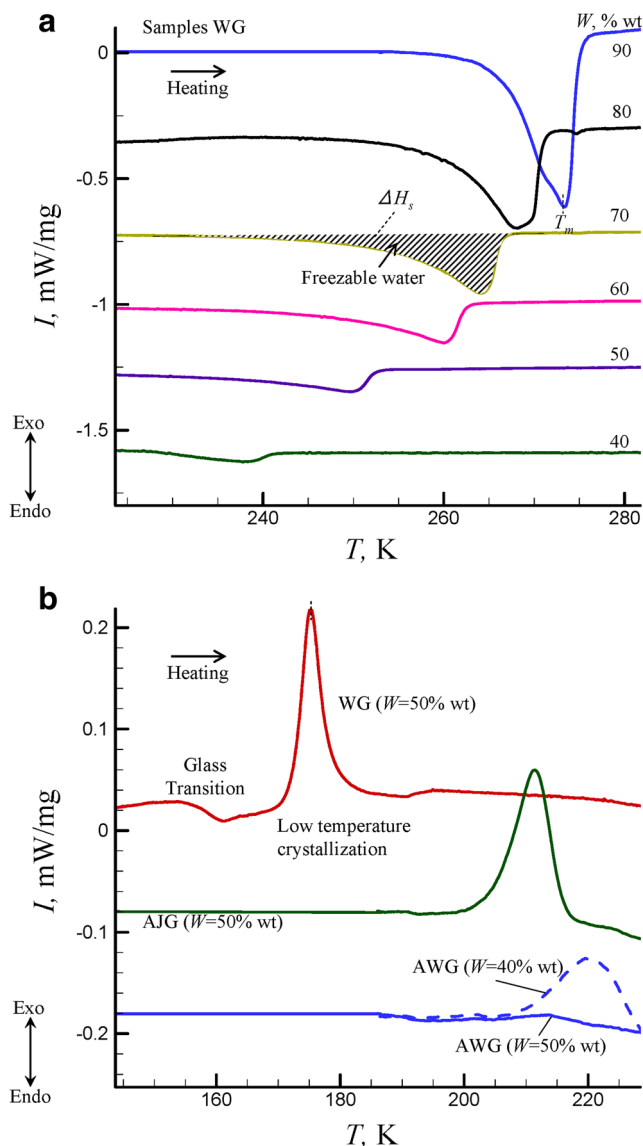
Fig. 2 DSC melting curves for water + glycerol mixtures (samples WG) at different concentration of water W (**a**) and DSC low-temperature heating curves for WG ($W = 50$ wt%) mixture, PEF-treated ($Z = 0.95$) and subjected to osmotic impregnation apples (samples AJG and AWG) at different concentration of water or juice W (**b**)
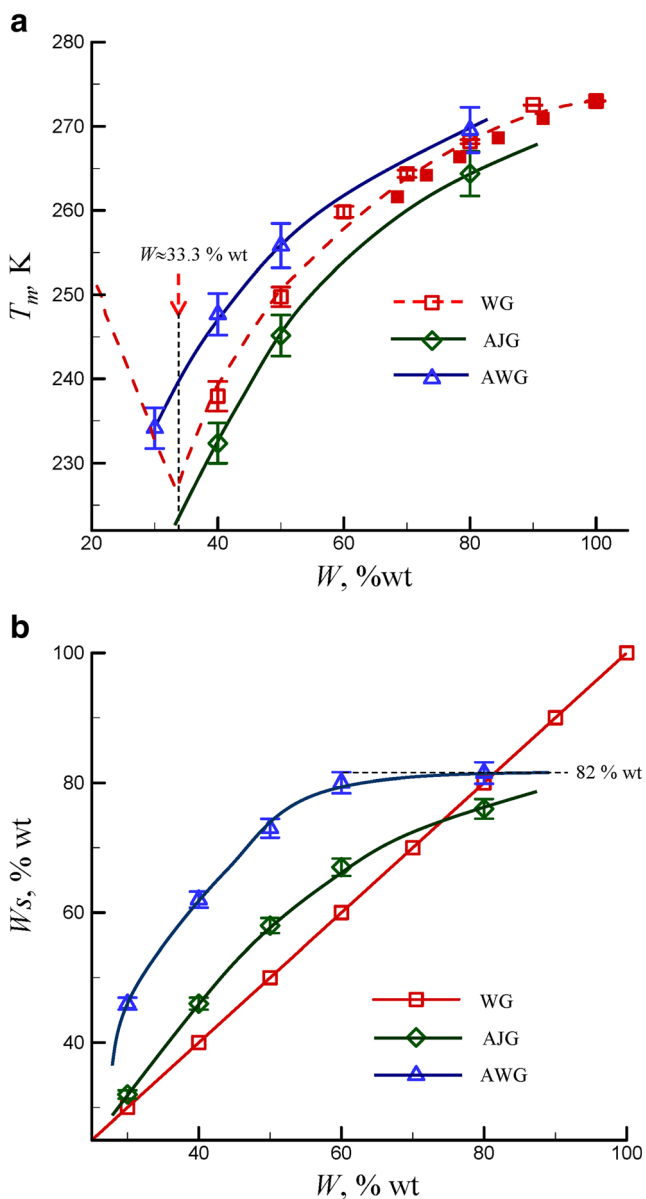
low-temperature crystallization was observed, e.g. at ≈175 K for $W \approx 50$ wt%. This exothermic transition corresponds to the formation of a crystalline (or more stable amorphous) phase from a glassy state. However, the melting peaks become insignificant for $W < 40$ wt%. and glycerol tends to supercool, rather than crystallize. The observed data for WG mixtures were in good correspondence with that obtained earlier for the same systems (Bohon and Conway 1972; Weng et al. 2011b).

Note that similar DSC melting curves were also observed for PEF-treated and subjected to osmotic impregnation apples in juice + glycerol (samples AJG) and in water + glycerol (sample AWG) mixtures at different concentration of water W. These data were used for determination of melting temperature T_m and amount of the unfreezable water (using Eqs. (2)–(3)). It is interesting that the phenomenon of low-temperature crystallization during heating was also observed in PEF-treated and subjected to osmotic impregnation samples AJG and AWG at appropriated values of W and T (Fig. 2b). The observed thermal behaviour reflects that glycerol was able to penetrate successfully inside PEF-treated tissue. This conclusion is in correspondence with our data reported in a previous study (Parniakov et al. 2015).

Figure 3a presents melting point T_m versus concentration of water or apple juice W for WG sample or PEF-treated ($Z = 0.95$) and subjected to osmotic impregnation samples AJG and AWG. The obtained $T_m(W)$ for WG sample were in good correspondence with previously reported data (dashed line (Association 1963; Bohon and Conway 1972) and filled squares (Weng et al. 2011b)). At $W \approx 33.3$ wt% WG solution forms eutectic mixture with a melting point of 226.7 K.
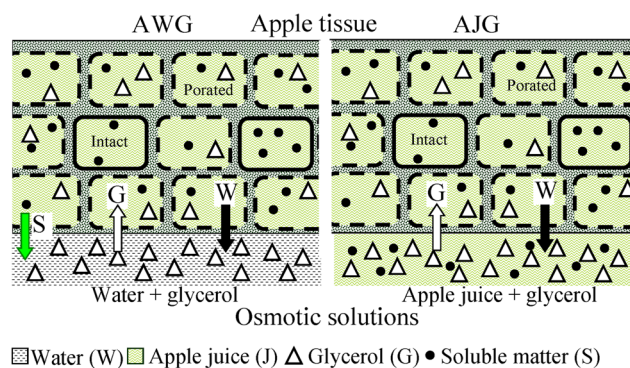
The state diagrams for WG, AWG and AJG samples were rather similar (Fig. 3a). It is interesting that melting curve $T_m(W)$ for WG solutions was located between the melting curves for the samples AWG (upper curve) and AJG (lower curve). The difference between WG and AJG samples may reflect the supplementary effect of the apple tissue on the depression of melting temperature T_m of WG mixture trapped inside tissue. On the other hand, the elevation of the melting temperature T_m for the sample AWG may reflect the osmotic effects related with the difference of the aqueous medium inside the tissue (juice) and outside (water + glycerol). Figure 3b compares the concentrations of water inside sample W_s and in osmotic solution W. Note that for the WG sample $W_s = W$. However, the concentration of water inside the samples AWG and AJG, W_s, was higher than that in osmotic solutions, W, at least for $W < 80$ wt%.

Figure 4 presents a schematic illustration of the possible differences of mass transfer processes for PEF-treated and subjected to osmotic impregnation apple tissues in water + glycerol (sample AWG) and in apple juice + glycerol (sample AJG) solutions. It is well-known that water in food tissues has heterogeneous structure and can be divided into intercellular

Fig. 4 Schematic illustration of mass transfer processes for the PEF treated and subjected to osmotic impregnation apple tissues in water + glycerol (sample AWG) and apple juice + glycerol (sample AJG) solutions

Fig. 3 Melting point T_m (**a**) and concentration of water in the sample W_s (**b**) versus concentration of water (samples WG, AWG) or juice (sample AJG) in glycerol, W. The samples of apples (AJG and AWG) were PEF-treated ($Z = 0.95$) and then subjected to osmotic impregnation. *Dashed line* in **a** shows concentration dependence of freezing point for WG solutions (Association 1963; Bohon and Conway 1972), *filled squares* correspond to the reported DSC data (Weng, Li, et al. 2011), *arrow* at $W \approx 33.3$ wt% shows eutectic concentration with a melting point of 226.7 K

or capillary water (free water), multilayer water (weakly bound water) and monolayer water (tightly bound to the polar sites of apple tissues) (Okos et al. 1992). Osmotic impregnation of the sample AWG results in the glycerol gain by the tissue, but also leads to the water and solutes loss. Note that active mass transfer can occur only between electroporated cells and osmotic solution. However, the intact cells are mostly isolated from the mass transfer processes. The similar mass

transfer processes take place during osmotic impregnation of the sample AJG with the exception of exchange of the soluble matters. In this case, the soluble matter is present inside both apple tissue and juice–glycerol solution. Therefore, the exchange of the soluble matter may occur only for the small concentration of juice.

The studies have shown that the application of PEF treatment following glycerol osmotic impregnation may greatly affect the amount of unfreezable water in samples. PEF treatment destroys membrane integrity and causes redistribution of moisture between the cytoplasm and cell wall regions. Unfreezable moisture content for PEF-treated apple ($W_d^u = 0.53 \pm 0.02$, $W^u = 0.095 \pm 0.003$) was higher than that for intact apples ($W_d^u = 0.42 \pm 0.02$, $W^u = 0.075 \pm 0.003$), and it can reflect the enhancement of interactions between the cytoplasm fluids and cell wall compartments. Note that obtained value of W^u for intact apples is in good correspondence with recently estimated value of amount of bound water in apples (Mykhailyk and Lebovka 2014) and is comparable with that corresponding to the monolayer moisture content of fresh apple, $W = 0.039$–0.084 (Mrad et al. 2012). It evidenced that unfreezable moisture in fresh apple presumably corresponds to monolayer water (tightly bound to the polar sites of apple tissues) (Okos et al. 1992).

Figure 5 presents the unfreezable moisture contents W_d^u versus the total moisture contents W_d (on dry basis) in the samples WG, AJG and AWG. The unfreezable water content W_d^u increased with increasing of the total water content W_d (Fig. 5). The obtained data evidenced that the free water can be present in the samples only for the moisture content above some minimum value, i.e. for $W_d > (W_d^u)_{min}$ (Inset to Fig. 5). The most pronounced unfreezable water content was observed for the sample AJG and the values of W_d^u may be arranged in the order AWG < WG < AJG.
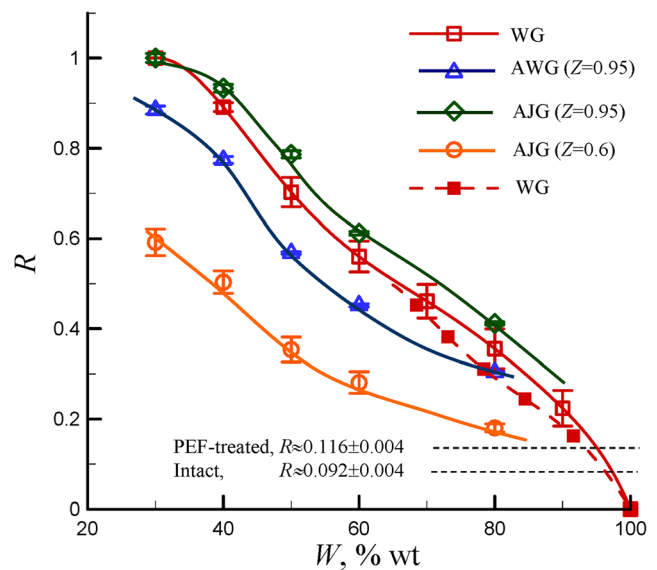
It is interesting that for the water glycerol mixture, the value of $(W_d^u)_{min} = 0.60 \pm 0.05$ corresponds to the following number of bound water molecules by one glycerol molecule (hydration number) $N_w^u/N_g = (W_d^u)_{min} M_g/M_w = 3.07 \pm 0.25$.
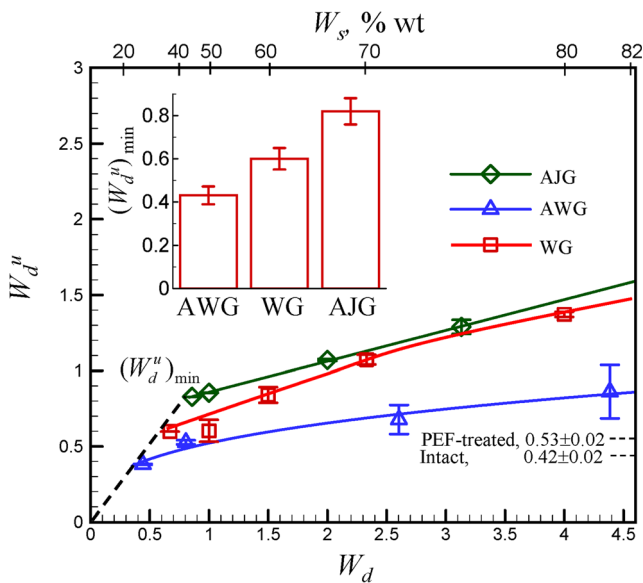
Fig. 5 Unfreezable moisture contents W_d^u versus the total moisture contents W_d (both are on dry basis) for the samples WG, AJG and AWG. The W_d^u attains the minimum value of $(W_d^u)_{min}$ when the free water is absent in the sample. Inset shows $(W_d^u)_{min}$ values for the samples AWG, WG and AWG

Fig. 6 Fraction of unfreezable water R versus concentration of water or juice W in the osmotic solutions for the samples WG, AJG ($Z = 0.95$ and $Z = 0.6$) and AWG ($Z = 0.95$). The samples of apples (AJG and AWG) were PEF-treated and subjected to osmotic impregnation. The *filled squares connected by dashed line* are the data of previously reported DSC measurements for water glycerol solutions (Weng, Li, et al. 2011)

Where $M_g = 92.09$ and $M_w = 18.02$ are the molecular weight of glycerol and water, respectively. It was suggested that bound water molecules in the first hydration shell become more likely to be associated with the glycerol OH groups than the alkyl backbone (Dashnau et al. 2006). The hydration number increased with the rising water content in full correspondence with previously reported data (Weng et al. 2011a).

Figure 6 presents the fraction of unfreezable water R versus the concentration W of water or juice in osmotic solutions for different samples WG, AJG and AWG. Note that our data for water glycerol solutions (sample WG) were in satisfactory correspondence with previously reported DSC measurements for water glycerol solutions (the filled squares connected by dashed line) (Weng et al. 2011b). Before osmotic treatment the values of R were 0.092 ± 0.004 and 0.116 ± 0.004 for intact and PEF-treated ($Z = 0.95$) apple tissues. The PEF treatment for the samples AJG was done to a high ($Z = 0.95$) and intermediate ($Z = 0.6$) levels of Z. For the samples WG, AJG ($Z = 0.95$) the water inside them became totally unfreezable (i.e. $R \approx 1$), below the concentrations that approximately correspond to the eutectic concentration, $W \approx 33.3$ wt%. However, for the samples AWG and AJG ($Z = 0.6$), the fraction of unfreezable water was noticeably smaller than 1, even for osmotic treatment in eutectic mixtures. It evidently reflected the poor penetration of glycerol inside partially electroporated apple tissue for the sample AJG ($Z = 0.6$) and presence of mass transfer of soluble matter from intensively electroporated apple tissue into the WG osmotic solution for the sample AWG ($Z = 0.95$). At the given value of W the values of R were arranged in the raw: AJG ($Z = 0.95$) > WG > AWG > AJG ($Z = 0.6$).

Note that subjection to osmotic impregnation of PEF-treated apples can result in noticeable changes of the textural properties of samples. Figure 7 presents the example of the texture index F^* versus concentration of juice in osmotic solution W for the sample AJG ($Z = 0.95$). The value of F^* gone through the maximum at $W \approx 80$ wt% ($F^* = 0.93 \pm 0.05$) with increasing of W. Therefore, the osmotic treatment in juice–glycerol mixture with juice content of concentration $W \approx 80$ wt% was optimal for
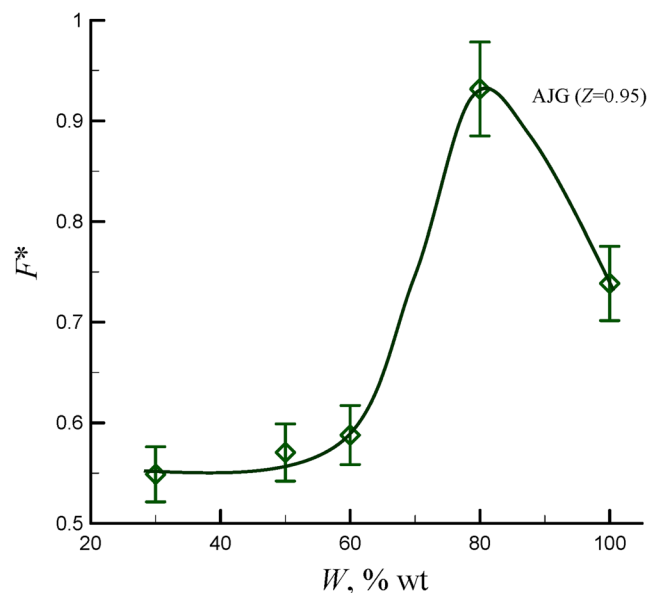
Fig. 7 Texture index F^* versus concentration of juice in osmotic solution W for the sample AJG ($Z = 0.95$). The relaxation measurements were done at $T = 298$ K

preservation of the texture of PEF-treated samples. The high value of texture index $F^* = 1.29 \pm 0.35$ was also observed previously for AWG ($Z = 0.95$) sample (Parniakov et al. 2015).

Conclusion

The content of unfreezable water in PEF-treated and subjected to osmotic impregnation in glycerol apple was determined by means of low-temperature DSC in dependence of water or apple juice content in glycerol. The data evidenced that, for PEF-treated samples, the glycerol was able to penetrate successfully inside apple tissue. The state diagrams for WG, AWG and AJG samples were rather similar. The melting curve $T_m(W)$ for WG solutions was located between that for the samples AWG and AJG. The mass transfer processes between electroporated cells and osmotic solution may explain the observed behaviour. For the sample AWG, the glycerol and water are transferred inside the tissue, while soluble matter is transferred into the osmotic solution. From other hand for the sample AJG the exchange of the soluble matter between apple tissue and osmotic solution is not so important. The mass transfer processes between apple and osmotic solution were noticeably constrained for partially electroporated or intact tissues. The free water was observed in the samples only for the moisture content above some minimum value, i.e. for $W_d > (W_d^u)_{min}$. The most pronounced unfreezable water content was observed for the sample AJG and the values of W_d^u may be arranged in the row AWG < WG < AJG. The impact of osmotic impregnation on the texture of PEF-treated apples was also demonstrated. The juice concentration in glycerol $W \approx 80$ wt% was found to be optimal for preservation of the texture of PEF-treated samples.

The obtained experimental data may be useful for optimisation of the scaled up industrial applications of PEF-assisted dehydrofreezing of fruits and vegetables, e.g. sliced apples, potatoes, carrots and onions. The proposed method allows facilitation the non-thermal dehydration of products as well as the impregnation of special additives that prevent oxidation and greying. More detailed investigations on impact of PEF-assisted dehydrofreezing on the colour, flavour and texture of the rehydrated products is desirable in future.

References

Association, G. P. (1963). Physical properties of glycerine and its solutions. Glycerine Producers' Association.

Barbosa-Cánovas, G. V, Fontana Jr., A. J., Schmidt, S. J., & Labuza, T. P. (2008). Water activity in foods: fundamentals and applications. In P. S. Taoukis & M. Richardson (Eds.), Water Activity in Foods (pp. 273–312). Blackwell Publishing Ltd.

Ben Ammar, J., Lanoiselle, J.-L., Lebovka, N. I., Van Hecke, E., & Vorobiev, E. (2010). Effect of a pulsed electric field and osmotic treatment on freezing of potato tissue. Food Biophysics, 5(3), 247–254.

Blanch, M., Sanchez-Ballesta, M. T., Escribano, M., & Merodio, C. (2015). The relationship between bound water and carbohydrate reserves in association with cellular integrity in fragaria vesca stored under different conditions. Food and Bioprocess Technology, 8(4), 875–884.

Bohon, R. L., & Conway, W. T. (1972). DTA studies on the glycerol-water system. Thermochimica Acta, 4(3–5), 321–341.

Calín-Sánchez, Á., Kharaghani, A., Lech, K., Figiel, A., Carbonell-Barrachina, Á., & Tsotsas, E. (2015). Drying kinetics and microstructural and SensoryProperties of black chokeberry (aronia melanocarpa) as affected by drying method. Food and Bioprocess Technology, 8(1), 63–74.

Dashnau, J. L., Nucci, N. V., Sharp, K. A., & Vanderkooi, J. M. (2006). Hydrogen bonding and the cryoprotective properties of glycerol/water mixtures. The Journal of Physical Chemistry B, 110(27), 13670–13677.

Donsi, F., Ferrari, G., & Pataro, G. (2010). Applications of pulsed electric field treatments for the enhancement of mass transfer from vegetable tissue. Food Engineering Reviews, 2(2), 109–130.

Donsi, F., Ferrari, G., Maresca, P., & Pataro, G. (2011). Food quality: control, analysis and consumer concerns. In D. A. Medina & A. M. Laine (Eds.), Food Quality: Control, Analysis and Consumer Concerns (pp. 505–554). Nova Science Publishers, Inc.

Farahnaky, A., Ansari, S., & Majzoobi, M. (2009). Effect of glycerol on the moisture sorption isotherms of figs. Journal of Food Engineering, 93(4), 468–473. doi:10.1016/j.jfoodeng.2009.02.014.

Fuller, B. J. (2004). Cryoprotectants: the essential antifreezes to protect life in the frozen state. CryoLetters, 25, 375–388.

Garcia Loredo, A., Guerrero, S., Gomez, P., & Alzamora, S. (2013). Relationships between rheological properties, texture and structure of apple (Granny Smith var.) affected by blanching and/or osmotic dehydration. Food and Bioprocess Technology, 6(2), 475–488. doi:10.1007/s11947-011-0701-9.

Jaeger, H., Reineke, K., Schoessler, K., & Knorr, D. (2012). Effects of emerging processing technologies on food material properties. In B. Bhandari & Y. H. Roos (Eds.), Food Materials Science and Engineering (pp. 222–262). Wiley.

Jalte, M., Lanoiselle, J.-L., Lebovka, N., & Vorobiev, E. (2009). Freezing of potato tissue pre-treated by pulsed electric fields. Food Science and Technology, 42(2), 576–580.

James, C., Purnell, G., & James, S. (2014). A critical review of dehydrofreezing of fruits and vegetables. Food and Bioprocess Technology, xx, 1–16.

Lebovka, N., & Vorobiev, E. (2010). Advanced electroporation techniques in biology and medicine. (A. G. Pakhomov, D. Miklavcic, & M. S. Markov, Eds.). Boca Raton: CRC Press.

Lebovka, N. I., Bazhal, M. I., & Vorobiev, E. (2002). Estimation of characteristic damage time of food materials in pulsed-electric fields. Journal of Food Engineering, 54(4), 337–346. doi:10.1016/S0260-8774(01)00220-5.

Li, B., & Sun, D.-W. (2002). Novel methods for rapid freezing and thawing of foods вЂ"a review. Journal of Food Engineering, 54(3), 175–182.

Mrad, N. D., Bonazzi, C., Boudhrioua, N., Kechaou, N., & Courtois, F. (2012). Moisture sorption isotherms, thermodynamic properties, and glass transition of pears and apples. Drying Technology, 30(13), 1397–1406. doi:10.1080/07373937.2012.683843.

Mykhailyk, V., & Lebovka, N. (2014). Specific heat of apple at different moisture contents and temperatures. Journal of Food Engineering, 123(0), 32–35. doi:10.1016/j.jfoodeng.2013.09.015.

Odriozola-Serrano, I., Aguiló-Aguayo, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2013). Pulsed electric fields processing effects on quality and health-related constituents of plant-based foods. *Trends in Food Science & Technology, 29*(2), 98–107.

Okos, M. R., Narsimhan, G., Singh, R. K., & Weitnauer, A. C. (1992). Food dehydration. In D. R. Heldman, & D. B. Lund (Eds.), *Handbook of food engineering* (pp. 437–562). New York: Marcel Dekker, Inc.

Parniakov, O., Lebovka, N. I., Bals, O., & Vorobiev, E. (2015). Effect of electric field and osmotic pre-treatments on quality of apples after freezing-thawing. *Innovative Food Science & Emerging Technologies, 29*, 23–30.

Pei, F., Shi, Y., Mariga, A., Yang, W., Tang, X., Zhao, L., et al. (2014). Comparison of freeze-drying and freeze-drying combined with microwave vacuum drying methods on drying kinetics and rehydration characteristics of button mushroom (Agaricus bisporus) slices. *Food and Bioprocess Technology, 7*(6), 1629–1639. doi:10.1007/s11947-013-1199-0.

Phoon, P. Y., Galindo, F. G., Vicente, A., & Dejmek, P. (2008). Pulsed electric field in combination with vacuum impregnation with trehalose improves the freezing tolerance of spinach leaves. *Journal of Food Engineering, 88*(1), 144–148.

Raso, J., & Heinz, V. (Eds.) (2006). *Pulsed electric field technology for the food industry. Fundamentals and applications*. New York: Springer.

Shayanfar, S., Chauhan, O. P., Toepfl, S., & Heinz, V. (2013). The interaction of pulsed electric fields and texturizing–antifreezing agents in quality retention of defrosted potato strips. *International Journal of Food Science and Technology, 48*(6), 1289–1295.

Shayanfar, S., Chauhan, O. P., Toepfl, S., & Heinz, V. (2014). Pulsed electric field treatment prior to freezing carrot discs significantly maintains their initial quality parameters after thawing, (4). doi:10.1111/ijfs.12421

Shynkaryk, M. V., Lebovka, N. I., & Vorobiev, E. (2008). Pulsed electric fields and temperature effects on drying and rehydration of red beetroots. *Drying Technology, 26*(6), 695–704. doi:10.1080/07373930802046260.

Vorobiev, E., & Lebovka, N. (2011). Pulse electric field-assisted extraction. In N. Lebovka, E. Vorobiev, & F. Chemat (Eds.), *Enhancing Extraction Processes in the Food Industry* (p. 592). Boca Raton: CRC Press, Taylor & Francis LLC.

Weng, L., Chen, C., Zuo, J., & Li, W. (2011a). Molecular dynamics study of effects of temperature and concentration on hydrogen-bond abilities of ethylene glycol and glycerol: implications for cryopreservation. *The Journal of Physical Chemistry A, 115*(18), 4729–4737. doi:10.1021/jp111162w.

Weng, L., Li, W., & Zuo, J. (2011b). Two applications of the thermogram of the alcohol/water binary system with compositions of cryobiological interests. *Cryobiology, 62*(3), 210–217. doi:10.1016/j.cryobiol.2011.03.005.

Wiktor, A., Schulz, M., Voigt, E., Witrowa-Rajchert, D., & Knorr, D. (2015). The effect of pulsed electric field treatment on immersion freezing, thawing and selected properties of apple tissue. *Journal of Food Engineering, 146*, 8–16. doi:10.1016/j.jfoodeng.2014.08.013.

Wolfe, J., Bryant, G., & Koster, K. L. (2002). What is "unfreezable water", how unfreezable is it and how much is there? *CryoLetters, 23*, 157–166.

Zhang, N., Li, W., Chen, C., Zuo, J., & Weng, L. (2013a). Molecular dynamics investigation of the effects of concentration on hydrogen bonding in aqueous solutions of methanol, ethylene glycol and glycerol. *Bulletin of the Korean Chemical Society, 34*(9), 2711–2719.

Zhang, N., Li, W., Chen, C., Zuo, J., & Weng, L. (2013b). Molecular dynamics study on water self-diffusion in aqueous mixtures of methanol, ethylene glycol and glycerol: investigations from the point of view of hydrogen bonding. *Molecular Physics, 111*(7), 939–949. doi:10.1080/00268976.2012.760050.