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Pulsed electric field enhanced freeze-drying of apple tissue

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Abstract: The influence of pulsed electric field (PEF) on freeze-drying of apple tissue was investigated. The freeze-drying was performed with different parameters of PEF treatment, and PEF treatment on the drying characters, microstructure, rehydration ratio, effective diffusion coefficient and hardness of apple tissue were discussed separately. The results indicated that PEF utilization as a pretreatment of apples enhances the drying process. The drying time was shortened by 17.73% at most, specific energy consumption decreased by 24.74% at most, and the rehydration ratio was improved by 65.22% at most for PEF treatment samples respectively, compared with the untreated samples. The effective diffusion coefficients varied from 2.60×10^{-8} m^2/s to 4.20×10^{-8} m^2/s for PEF pretreated samples, and was 2.40×10^{-8} m^2/s for untreated samples drying at 75°C, the hardness of the untreated apple tissue was about 144.4 N which was decreased to 39.5–115.0 N after PEF treatment.

Keywords: freeze-drying; pulsed electric field; apple; drying properties

A growing demand for drying foods with a high nutritional quality caused the non-thermal technologies to be more and more popular in processing of foodstuffs (Ahmed *et al.* 2016; Moreno *et al.* 2017). Pulsed electric field (PEF) as an innovative non-thermal food-processing methods, has been widely used for food preservation (Botero-Uribe *et al.* 2017; Liu *et al.* 2017). It offers advantages such as increasing rehydration rate and mass transfer properties without undesirable changes in food tissues, reducing the energy consumption, improving the sensory (color, texture and flavor) and maintaining the nutrient elements (Yildiz *et al.* 2016).

PEF treatment depends on short, high voltage pulses to food material, placed between two electrodes, generating electric fields, which usually span from 0.1 to 10 kV/cm for drying (Dalvi-Isfahan *et al.* 2016). Moreover, numerous scientific data indicate that PEF leads to the electroporation phenomenon manifested as local structural changes and cell membrane breakdowns, hence it application improves drying rate and enhances the mass transfer (Yu *et al.* 2017). Different parameters of PEF have different influence on drying properties (*e.g.*, microstructure, drying time and drying rate) of fruits and vegetables (Wu & Zhang 2014; Parniakov et *al.* 2016).

The aim of this study was to evaluate the impact of PEF pretreatment on the responses of apple tissue in the processes of freeze-drying. Moreover, the effect of PEF pretreatment on drying properties, such as structure, drying characteristics, rehydration ratio, effective diffusion coefficient D_{eff} and hardness were discussed separately.

MATERIAL AND METHODS

Raw material. Fuji apple were bought on a local market located in Taiyuan (China) and stored at 4 ± 1°C and humidity of 80–90% until required. Before experiment (similar in shape and maturity, without any physical damage, with similar colour

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and texture), the apples were washed with tap water, and were sliced parallel to the main axis of the apple, then cut into 20 mm long, 20 mm width and 10 mm thick from the middle parenchyma region of the fruit. After cutting, slices were subjected to PEF treatment. To ensure the stability of the material quality all drying procedures were performed using the same batch of the material.

Methods. The apple samples were initially treated by PEF, and then freeze-drying experiments were done. Finally, the drying properties of the samples were analysed.

PEF treatment. Pulsed electric field treatment was conducted using generator (BTX ECM 830; Harvard Apparatus, USA) with monopolar, rectangular- shaped pulses. The sample was placed directly between two parallel stainless steel rectangular electrodes of 20 mm × 20 mm dimension, and the space between two electrodes was adjustable. The interval between pulses was set at 900 ms in order to minimize the temperature increase during electric field application. The parameters of PEF treatment were selected on the basis of preliminary study results. The apple samples were treated at 1–2 kV/cm, 10–60 pulses, and the average value of specific energy input was 0.026 kJ/g. Selected PEF parameters that were used in the experiment are listed in Table 1. Moreover, the pulse width was set to 90 µs. 30 samples were pretreated by the same PEF process. The untreated samples served as the control.

Freeze-drying experiments. The freeze-drying experiment was performed with online moisture monitoring system (Wu *et al.* 2014), which was designed based on the working condition of the JDG-0.2 freeze-drying machine (Kejin Vacuum Freeze-drying Technics Co., China), and a watt-hour meter was installed to measure energy electric power consumption. The chamber pressure range for the equipment is 10–130 Pa, and the temperature of the heating board is 20–120°C. After PEF treatment, 30 samples (average weight of 91.5 g) were pre-freezing in refrigerator (BCD-130E; Haier, China) for 10 h at the temperature of –40°C. In the drying process, the chamber pressure was set to 35 Pa, the temperature of the heating board and condenser was set at 75°C and –45°C, respectively. Before the drying experiments, the moisture content was determined by drying 10 g of fresh apples to constant weight at 100°C for at least 12 h according to the direct drying method using the air blowing thermostatic oven (GB 5009.3-2010; China).

Table 1. The parameters of pulsed elecric field (PEF) used in the investigation

Trial number	Electric field intensity (kV/cm)	Pulse number (pulse)
1	1	10
2	1	35
3	1	60
4	1.5	10
5	1.5	35
6	1.5	60
7	2	10
8	2	35
9	2	60
10	0	0

Microstructure. After freeze-drying experiments, the dried samples that untreated and PEF treated were randomly chosen for microscopic examination. The internal structure of apples tissue was coated with a fine gold layer in an anion-sputtering apparatus (LDM150D; Taiwan Jingda, China) and then examined under a scanning electron microscope at 100 × magnification (FESEM, JSM-7100F; JEOL, Japan). The fresh sample was freeze-dried (ZL-10TD; Zollo, China) during 24 h, in order to remove any residual moisture.

Mechanical properties. The texture of dried apple that untreated and PEF treated were measured as the stress at maximum force using a puncture test. The measurements were performed in a texture analyser (TA-XT Plus; Texture Technologies Corp., UK) at a constant speed of 0.5 mm/s using a cylindrical puncture probe 2.5 mm in diameter (P/2.5R, Texture Technologies Corp., UK). The penetration depth was 5 mm, the experiments were repeated three times for different groups. The hardness (maximum force required to compress the sample) values were recorded in N.

Analytical and calculation methods

Energy consumption. 10 groups of apple samples were investigated for energy consumption measurement. All the samples were spread in a layer and were dehydrated until the freeze-drying curve did not change during 0.5 hour. The total energy consumption during drying was calculated from an ampere meter reading (kWh). The specific energy consumption needed for drying a gram (g) of apple samples was defined as:

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$$\eta = \frac{P}{m_0} \quad (1)$$

where: m_0 – initial mass (g); P – electricity consumption (kJ)

Rehydration ratio. Rehydration experiments were performed by immersing a weighed amount of dried samples into water at 20°C for 30 minutes. And the surface water of samples was removed by absorbent paper for 4–5 times and then reweighed. Each experiment was repeated for three times and the results were given as averages. The rehydration ratio was calculated by the following equation:

$$\text{Rehydration ratio} = \frac{g_f}{g_d} \quad (2)$$

where: g_f *and* g_d are mass of the samples before and after rehydration, respectively.

Effective Diffusion Coefficient. In this study, apple slices were assumed to have an infinite plate (negligible shrink or expanded) with uniform initial humidity distribution, negligible external resistance, and constant diffusivity. Therefore, effective diffusion coefficient was estimated using one of the simplifications of Fick's second law of diffusion by Crank in 1975 (Therdthai & Northongkom 2011).

$$MR = \frac{8}{\pi^2} \times \exp\left(-\frac{\pi^2 D_{eff} t}{4L^2}\right) \quad (3)$$

where: L – the half thickness (m)

D_{eff} can be measured from the plot of ln(MR) against time. The diffusion coefficient was calculated by substituting the experimental data in the previous equation. In practice, effective moisture diffusion coefficient can be obtained by plotting the ln(MR) curve against time and then calculating its slope from equation:

$$\ln(MR) = \ln\left(\frac{8}{\pi^2}\right) - \left(\frac{\pi^2 D_{eff}}{4L^2}\right)t \quad (4)$$

The moisture ratio during drying was calculated using equation (Ding *et al.* 2015)

$$MR = \frac{M_t - M_e}{M_i - M_e} \quad (5)$$

where : M_t – moisture content at any time (kg water/kg dry solid), M_i – initial moisture content (kg water/kg dry solid), and M_e – equilibrium moisture content of the apple samples (kg water/kg dry solid), which is equal to the final moisture content.

Statistical analysis. A statistical analysis of the data was performed using an analysis of variance (ANOVA) with 95% confidence levels (with significance determined by $P < 0.05$) using SPSS 19.0 (IBM; USA).

RESULTS AND DISCUSSION

Influence on tissue structure. There is no significant difference of moisture content was obtained with different PEF treatments. The moisture content of fresh apples and PEF-treated samples were (84.63 ± 0.50%) wet basis and (84.85 ± 0.50%) wet basis, respectively. Figure 1 showed the microscopic pictures of apple tissue with different treatments. The results demonstrated that there was a considerable difference in the microstructure of apple tissues after PEF treatment at various parameters. The higher the electric field parameters applied, the greater the potential to irreversibly alter the permeability of biological cells and thereby the appearance and properties of apple cells.

As shown in Figure 1, the tissue structure of untreated fresh sample revealed that the most regular in shape and arrangement spaces, which was smooth without any damages. After freeze-drying, the microstructure of the untreated sample was characterized by a high density, a compact structure and the absence of visible micro-pores. Moreover, the cell walls collapsed in samples. This is due to the untreated samples had low levels of cellular permeability, and the internal diffusion was relatively slow, the heat and moisture were not easily evaporated, which led to the collapse phenomenon (Wu *et al.* 2011; Lammerskitten *et al.* 2019).

Compared with untreated samples, regardless of the PEF level applied, dried material was characterized by strongly porous and irregular structure (Figure 1). When pulse number was 10 pulses, electric field intensity was 1 kV/cm, the structure had a relative integrity, and regular-shaped. This was because PEF treatment could increase the cell membrane permeability, accelerate intracellular water diffusion, resulting in good preservation of tissue structure (Traffano-Schiffo *et al.* 2016). The tissue structure integrity could be affected by PEF parameters during drying. When the cells are exposed to an external electric field, the accumulation of oppositely charged ions on both sides of membrane causes membrane thickness reduction. Further increase of the electric field up to the critical values (0.5–5 kV/cm for plant cells) cause pores formation and loss of cell membrane semi-permeability (Mouazen & Nemenyi 1999). Hence, after PEF application, the permeability of membranes was increased and even form electroporation on cell

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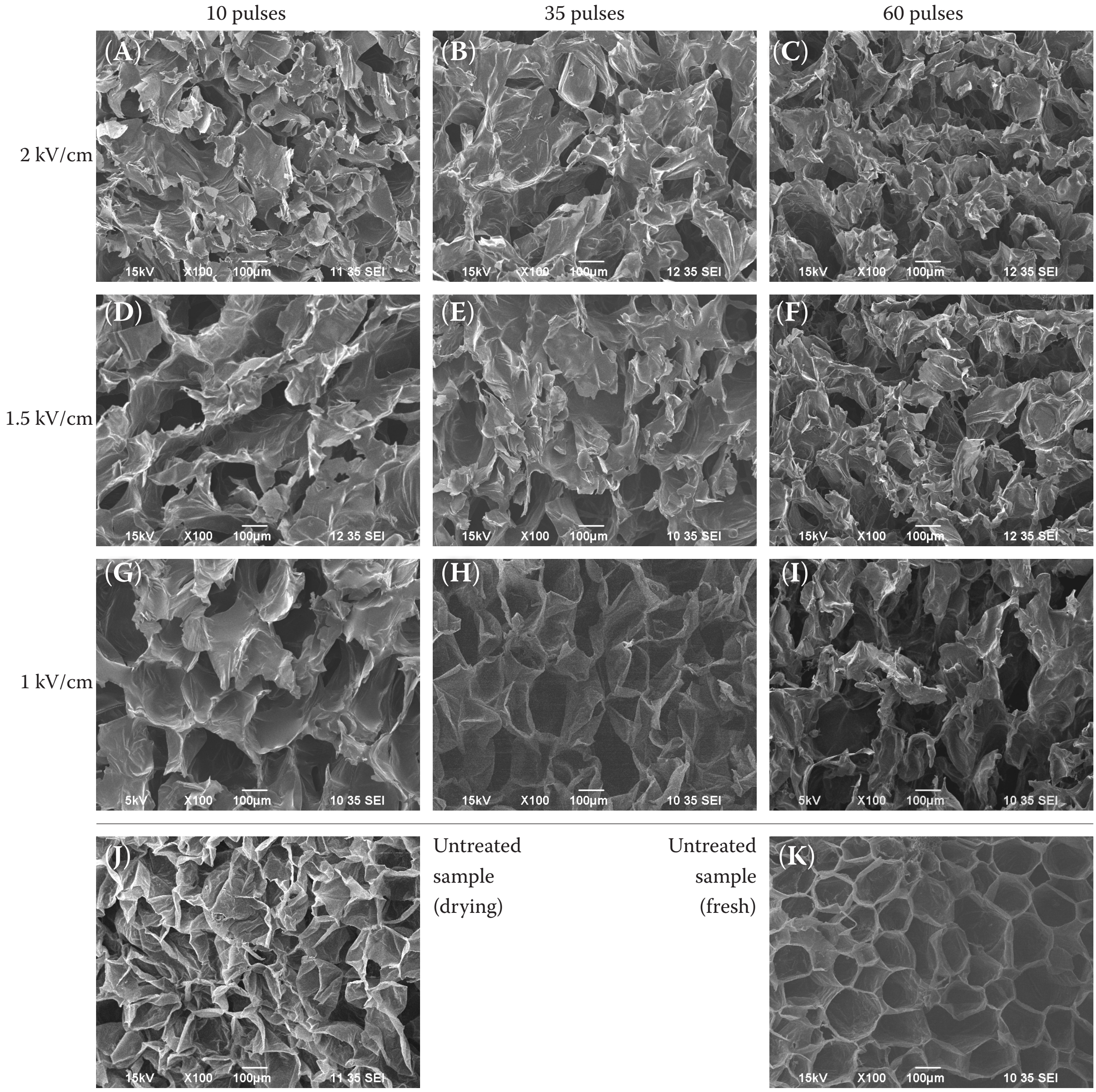

Figure 1. SEM images of apple tissue at a magnification of 100×

(**A**)–(**I**) freeze dried at 75°C under different electric field intensity and pulse numbers; (**J**) freeze dried at 75°C, untreated; (**K**) fresh sample, untreated

membranes, as the drying progresses, thermal stress and electroporation results in the weakening of the membrane until it burst, which led to complete destruction of the cell structure.

Influence on drying characteristics. The experimental data are presented in Table 2. The moisture ratio curve of apple samples treated with different PEF parameters was shown in Figure 2. The *MR* decreased exponentially with time in the samples. It is clear that the moisture ratio of apple samples was significantly reduced under PEF compared with that of control. The *MR* was reached 0.2 in only 190 minutes for PEF treatment, on average, but the *MR* of untreated sample, was reached 0.2 in 230 minutes. The drying time was found to be as 7.50 h for control group, whereas, after the PEF application it took 6.64 h average, this results can be seen in Table 2. At beginning of drying, the rate of moisture removal was rapid due to the amount of moisture

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Table 2. Drying experiment results of apple tissue with different treatment

	No	Energy consumption (kJ/g)	Drying time (hours)	D_{eff} (m^2/s; 10^{-8})	Rehydration ratio
PEF treatment	1	377.77	6.17	2.64 (R^2 = 0.99)	6.84 ± 0.05[a]
	2	424.20	6.50	3.00 (R^2 = 0.98)	6.67 ± 0.03[a]
	3	387.95	6.88	2.70 (R^2=0.97)	7.11 ± 0.06[a]
	4	380.47	6.33	2.90 (R^2 = 0.97)	6.43 ± 0.04[a]
	5	372.17	6.17	2.60 (R^2 = 0.95)	5.99 ± 0.02[a]
	6	430.82	6.33	3.60 (R^2 = 0.96)	5.71 ± 0.03[b]
	7	486.23	7.21	3.40 (R^2 = 0.98)	5.50 ± 0.06[b]
	8	450.42	7.18	3.30 (R^2 = 0.93)	5.34 ± 0.04[b]
	9	466.82	7.01	4.20 (R^2 = 0.95)	5.68 ± 0.02[b]
Untreated sample		494.51	7.50	2.40 (R^2 = 0.98)	4.14 ± 0.02[c]

Results are shown as the mean ± standard deviation (s.d.); different letters within columns indicate statistically significant differences (P < 0.05)

on the surface of the apple slices. With the effect of the applied electric field, the cell membrane permeability was increased, intracellular water was more easily diffused out of the cells, and part of the drying time could be saved (Wu *et al.* 2011).

The energy consumption of apple treated with different electric fields and control group were shown in Table 2. From Table 2, it can be seen that the specific energy consumption of apple that treated by PEF were less than that of control group. The experimental results indicate that the group of 1.5 kV/cm, 35 pulses is the most efficient drying method in terms of saving energy, followed by the group of 1 kV/cm, 10 pulses. The specific energy consumption was 372.17 kJ/g (1.5 kV/cm, 35 pulses) while that of the control group was 494.51 kJ/g. Therefore, PEF treatment could save the energy approximately 24.74% compared with the control group. PEF as a non-thermal techniques, it offer the advantages of low processing temperatures, and low energy utilization, hence the evaporation rate of water was significantly enhanced while a low amount of heat was produced (Taiwo *et al.* 2003).

Influence on rehydration ratio. The rehydration characteristics of the dried product were used as a quality index because they could indicate the physical and chemical changes of samples during drying (Bai *et al.* 2012). When dehydrated products are immersed in water, complex phenomena take place, which influence the properties of the rehydrated product. The effect of the different treatment on rehydration ratio was

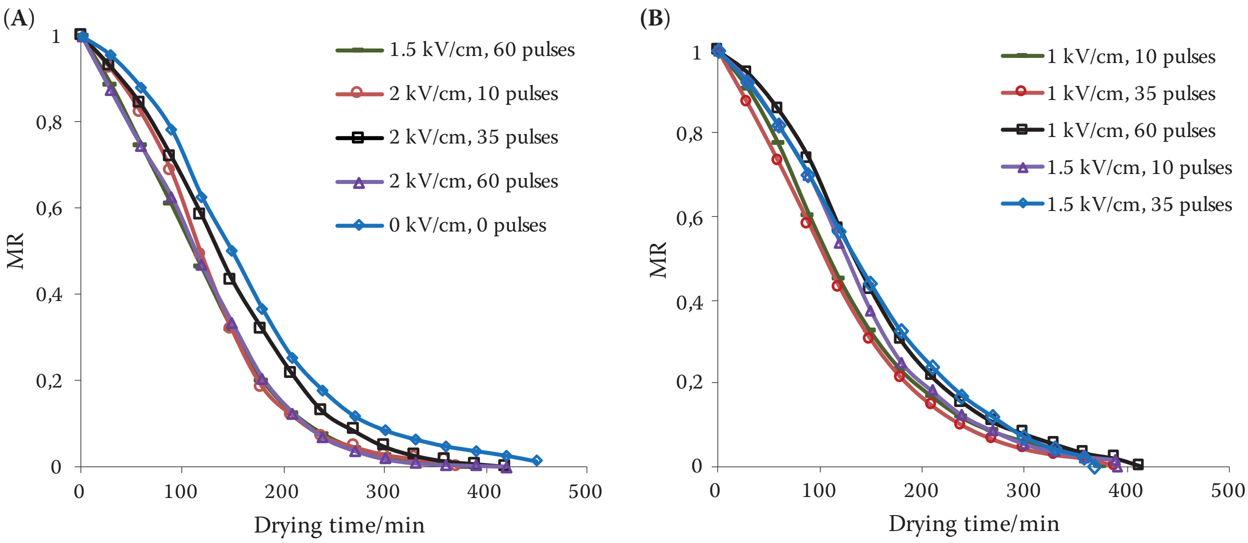

Figure 2. Moisture ratio of apples with different treatment

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shown in Table 2. In the present study the non-thermal heating could result in more rehydration and water absorption capacity (Bajgai & Hashinaga 2007). As can be seen from Table 2, the apple sample with 1 kV/cm, 60 pulses had a good rehydration capability, and the rehydration ratio was 7.11 ± 0.06. The rehydration ratio of untreated sample showed poor rehydration capability, it was 4.14 ± 0.02. Therefore, PEF treatment could greatly improve the rehydration ratio of apple slices, compared with the control group. PEF dried apple exhibited less shrinkage, high absorption of water and better rehydration than untreated samples. The membrane permeability of the untreated samples was low in the drying stage, which led to the collapse phenomena and greater resulted in the loss of cellular integrity in the ground tissues. Moreover, collapse can reduce the porosity of dried materials, influencing moisture distribution, rehydration capacity, resulting in undesirable hardening of the product (Link *et al.* 2017). These factors led to a decrease in rehydration ratio.

Influence on effective diffusion coefficient D_{eff}. The values of effective diffusion coefficients D_{eff} for PEF pretreated and untreated apple tissues were presented in Table 2. All data sets were fitted by the model Crank and presented good fit. In particular, R^2 values were higher than 0.93 in all cases. The D_{eff} value varied from 2.60×10^{-8} m^2/s to 4.20×10^{-8} m^2/s for PEF pretreated samples. An average D_{eff} value of 3.10×10^{-8} m^2/s was obtained for PEF-treated apple slices dried with freeze-drying at 75°C, and the D_{eff} value of untreated samples was 2.40×10^{-8} m^2/s. The D_{eff} is a parameter sensitive to the pre-treatment procedure, and the highest values of D_{eff} was observed for 2 kV/cm, 60 pulses (Table 2). According to the results, the D_{eff} value was showed the clear tendency to accelerate with the increase of electric field intensity and pulse number. Similar to other study, the D_{eff} was reported as to be more accelerated by PEF treatment before drying than untreated samples (Yildiz *et al.* 2016). PEF as an innovative technology resulting in a negligible ohmic heating of processed apples. Ohmic heating during PEF treatment leads to enhanced permeabilization of cell membranes and, in turn, improves the increased moisture diffusivity (Singh *et al.* 2013).

Influence on hardness. Figure 3 showed that the different PEF treatments had a significant effect on the hardness response of the dried apple slices ($P < 0.001$). It can be seen that the hardness of the untreated apple sample was about 144.4 N which was decreased from 39.5 N (1 kV/cm, 10 pulses) to 115.0 N (2 kV/cm, 60 pulses) after PEF treatment. Indeed, the magnitude of electric field intensity affects the degree of membrane disruption. According to Alam et al. (2018), the disruption of the cell membrane and the loss of turgor, induced by PEF, can affect the mechanical properties of the carrot at higher temperature. In this study, when the drying temperature was 75°C with different electric field parameters, the hardness of apple tissue was decreased with PEF treatment, moreover, the hardness of apple tissue increased with the increase of electric field intensity and pulse number.

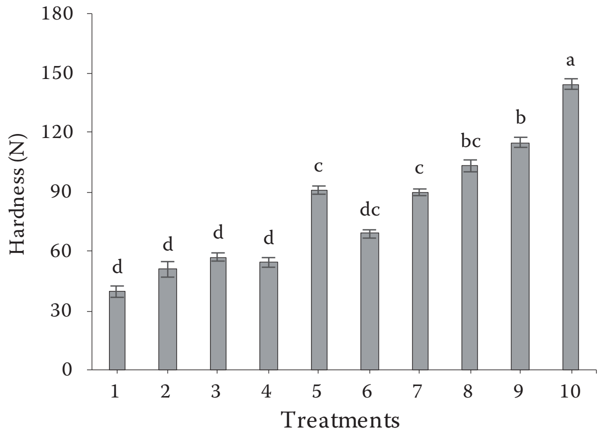

Figure 3. Effect of different treatment on dried apple hardness (mean ± s.d.; $P < 0.05$)

The mechanical properties was related to the change of microstructure of apple slices. Apple as a porous material, dried material had strongly porous and irregular structure after freeze-drying. The porosity and porous structure had a great influence on the mechanical properties of fruits. PEF treatment could increase the drying rate, the moisture inside the apple slices was quickly vaporized, leaving behind the large pores, this led to a decreasing the hardness of apple slices. However, the drying rate of untreated samples was relative low, the collapse was found to be proportional to the moisture content which was being lost during the process (Karathanos *et al.* 1996). The collapse phenomena was more evident with increase in drying time, cell undergo aggregation, polymerization due to dehydration and the loss of structural integrity, this microstructure was the essential, cause which led to the increase of the hardness.

CONCLUSION

This paper presented experimental results for the effects of PEF treatment on microstructure, effective diffusion coefficient, drying characteristics, rehydra-

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tion ratio and hardness of apple slices. PEF was found to be effective on the textural and drying properties of apple slices. The result showed that the tissue structure integrity could be affected by PEF parameters during drying and brought about reduction in an average time of 0.86 h after PEF treatment when compared to the control group. Moreover, PEF treatment could save the energy approximately 24.74% and greatly improve the rehydration ratio of apple tissue compared with the control group. However, the hardness of apple slices decreased with PEF treatment, moreover, the hardness of apple tissue increased with the increase of electric field intensity and pulse number. These data will provide support for application widely of PEF treatment for assistance of freeze-drying of fruit and vegetable tissues.

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