Defence Life Science Journal, Vol. 04, No. 01, January 2019, pp. 38-44, DOI : 10.14429/dlsj.4.12153

Cell Permeabilisation, Microstructure and Quality of Dehydrated Apple Slices Treated with Pulsed Electric Field During Blanching

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ABSTRACT

Effect of pulsed electric field as a blanching pretreatment on cell permeabilisation, microstructure and quality of dehydrated apple slices was studied. Apple slices were pulsed electric field pretreated (1.0 kV/cm, 1.5 kV/cm, and 2.0 kV/cm using 25 and 75 pulses) at a temperature of 60 °C and 80 °C in water followed by dehydration at 60 °C in a cabinet dryer. Cell disintegration index was found to increase significantly ($p<0.05$) with increase in the electric field strength, number of pulses and blanching temperature and thereby reducing the drying time. The dehydrated slices showed lower hardness and higher lightness (L^*) values for the samples treated at higher electric field strength for longer durations. Scanning electron microscopic studies of samples revealed better retention of cellular integrity when pre-treated at a low level of PEF (1.0 kV/cm using 25 pulses) and blanched at low temperature (60 °C). However, the samples treated to a level of 1.5 kV/cm and 75 pulses of PEF with subsequent blanching temperature 80 °C was found to yield optimum cell permeabilisation. The study suggests that PEF can be used as an effective blanching pretreatment for achieve good quality dehydrated apple slices in less drying time.

Keywords: Pulse electric field; Apple; Blanching; Dehydration; Microstructure; Scanning electron microscope

1. INTRODUCTION

The frontiers of pulsed electric field (PEF) technology have been explored in several areas of food processing in recent years. It has find application in solid foods also besides liquid and semi-liquid foods. Pulsed electric field has been in use for several liquid foods such as fruit or vegetable juices, milk etc. to achieve pasteurisation or sterilisation effects where pulsed electric field has been found effective against microbial inactivation and thereby increasing the shelf-life of the products[1-3]. Pulsed electric field processing causes increase in membrane permeability, typically known as electroporation. When external electric field is applied to biological cells, it induces trans-membrane potential. When this trans-membrane potential goes beyond a specified threshold value (0.2 V - 1.0 V), applied electric field causes a momentary loss of semi-permeability of the cell membranes, which is called as electro-permeabilisation[4]. Several workers have studied effect of pulsed electric field treatment on cell membrane permeabilisation of plant and animal tissues. The increase in cell membrane permeability may be of reversible or irreversible in nature depending on the applied electric field strength. Formation of irreversible permeabilicell membranes in plant tissues can be used for a variety of processing applications where cell membrane permeation is desirable, such as extraction of juice from plant materials, extraction of plant metabolites, increase in mass and heat transfer during food processing operations[5-6]. Several researchers have studied cell membrane permeabilisation in different plant tissues. In the case of onion tissues above electric field strength of 0.35 kV/cm, it was possible to distinguish the individual permeabilised cells[7]. López[8], *et al.* studied the effect of pulsed electric field on extraction of phenolic compounds in grapes during fermentation and reported increased extractability of anthocyanin and phenolic compounds due to PEF treatment. Belghiti and Vorobiew[9] reported increased sugar extraction from sugar beets treated with pulsed electric field. Ade-Omowaye[10], *et al.* reviewed use of pulsed electric field for dehydration of plant tissues and reported increased cell permeabilisation due to PEF treatment resulting in increased dehydration rates. The effect of moderately applied electric field pulses on the diffusion coefficient of soluble substances from apple slices were studied by Jemai and Vorobiev[11]. The technology has been found promising in the extraction of oils from maize, olive and soybeans with improved phytosterols and oil recovery[12]. Pulsed electric field processing has found its application in improving the freeze tolerance by higher impregnation of trehalose in to the spinach leaf tissues due to PEF treatment[13]. Pulsed electric field application has also been found to improve the freezing quality of potatoes[14]. Several reports exist with regards to increased mass transfer during dehydration of apples[15-16], radish[17], rice[18], carrot[19] etc., as well as osmotic dehydration of apples[20], bell pepper[21], and carrots[22] due to pulsed electric field treatments. Angersbach[5], *et*

Received : 21 October 2017, Revised : 23 May 2018
Accepted : 05 June 2018, Online published : 31 December 2018

al. suggested that cell membrane permeabilisation depends on electrical energy input, pulse duration and number of pulses. However, very limited data is available with regards to electric field strength, pulse numbers and treatment temperature on cell membrane permeability and other physicochemical parameters of PEF treated fruits. Further, it is hypothesised that electric field strength, treatment time as well as treatment temperature can affect the physical characteristics and sensory acceptability of treated plant materials. Therefore, present work was undertaken to study the effect of different electric field strength, number of pulses and treatment temperature on cell membrane permeability, structural changes, color, hardness and sensory properties of the apple slices.

2. MATERIALS AND METHODS

2.1 Raw Material and Precutting

Apples (*Jonagold var.*) were procured from local market of Quakenbruck, Germany. The apples with any blemishes or injury were discarded and only sound apples of good quality were selected for the study. The average weight of apples varied from 147-166 g. The apples were stored at 4 °C in a cold room prior to experimental study. The apples were taken out from the cold room, washed in tap water and sliced using a mechanical slicer (Graef, DVE GS, Quakenbrück, Germany). The thickness of unpeeled slices was maintained uniform at 3 mm. The apple slices were given pulsed electric field blanching pretreatment immediately after cutting.

2.2 Pulsed Electric Field Pretreatment and Dehydration

The apple slices (100 g - 110 g) were put in the pulsed electric field treatment chamber (18 cm x 13 cm x 20 cm) containing water (3 l) at specified temperatures. The treatment chamber is connected with a batch type pulsed electric field equipment (Elcrack, DIL, Quakenbruck, Germany). The PEF system provided mono-polar pulses of near-rectangular shape. The treatment was done at 4 Hz frequency having 25 µs pulse duration. Two stainless steel electrodes were fitted in the treatment chamber at a distance of 13 cm. The experiment was carried out at electric field strengths of 1.0 kV/cm, 1.5 kV/cm, and 2.0 kV/cm using 25 pulses and 75 pulses at a temperature of 60 °C and 80 °C. The PEF treated slices were immediately taken out from the water, drained on a wire mesh and spread on tissue paper to remove the surface moisture. The slices were dehydrated up to a moisture content of 6 % - 7% (fresh weight basis) in hot air dehydration unit (Heraeus D6450, Hanau, Germany) maintained at 60 °C where the velocity of hot air of 12 m/s flowing horizontally. Untreated samples as well as those blanched in water at 90 °C for 60 s were also dehydrated in similar conditions for comparative evaluations.

2.3 Energy Input During PEF Pretreatment

The energy input during pulsed electric field treatment was calculated based on electric field strength, number of pulses employed and capacitance of the electric field chamber using modified equation of Knorr and Angersbach[24]:

$$w_{total} = 0.5 \times C \times U^2 \times n$$

where
W_{Total} = Total energy input (kJ/kg)
C = Capacitance = 0.05 µF
U = Electric field strength (kV/cm)
n = Number of pulses

2.4 Cell Permeabilisation

Cell disintegration index was calculated as a measure to visualize the cell permeabilisation of the tissues. Electrical conductivities of the PEF treated apple slices were measured using impedance measurement equipment (DIL, Quakenbruck, Germany) in the frequency range of 0.5 kHz to 5 MHz for calculation cell disintegration index. Impedance was measured in intact apple tissue and PEF pretreated samples (cylindrical) (diameter 2 cm, length 3 cm) and the data were recorded in computer using software (DIL, Quakenbruck, Germany). Cell disintegration index was calculated from the impedance data using following equation as described by Knorr and Angersbach[24]:

$$Z_p = 1 - b * \frac{(K_h^{'} - K_l^{'})}{(K_h - K_l)}$$

where

$$b = \frac{K_h}{K_h^{'}}$$

K_l' and K_l are electrical conductivity of treated and untreated materials, respectively, in low frequency field (i.e. 1 kHz - 5 kHz); whereas, K_h' and K_h are electrical conductivity of treated and untreated materials, respectively, in high frequency field (i.e. 1 MHz - 5 MHz). The value of Z_p varies from 0 - 1; Z_p = 0 for intact cells, and Z_p = 1 for total disintegration.

2.5 CIE Colour Values

Surface color of dehydrated apple slices was recorded using a colour meter (CM 600d, Konica Minolta, Tokyo, Japan) as reflected in CIE (L^*, a^*, b^*) colour space. Standard illuminant D65 and 10º observer were used for the measurements. The equipment was calibrated using black and white standard ceramic tiles before taking readings of experimental samples.

2.6 Textural Evaluation

Hardness of dehydrated apple slices were measured using a texture analyzer (TAXT$_2$, Stable Microsystems, UK) using a conical probe and 25 kg load cell. The data were recorded using an analyser via a software programme (Texture Expert, version 1.22, Stable Microsystems, UK). All measurements were taken in triplicate.

2.7 Microstructure

Surface and internal features of dehydrated apple slices were observed using a scanning electron microscope (JSM-6460 LV, Jeol LTD, Tokyo, Japan). Apple slices with a size of 1.0 cm × 0.5 cm were cut using a sharp blade, mounted on a conductive putty (Leit-C-Plast, Plano GmbH, Germany) and coated with about 20 nm gold particles in a sputter coater (SCD 040, Balzer Union Ltd., Lichtenstein). Specimens were observed with the electron microscope fitted with a scanning attachment at 15 kV acceleration voltage.

2.8 Sensory Acceptability

The sensory evaluation of the dehydrated apple slices was carried out by a panel of 30 persons including staff members of the laboratory. The samples were presented to the panelists under normal lighting conditions coded with random numbers in a sensory room maintained at 20 ºC. Colour, texture and overall acceptability of samples were evaluated using a 9 point hedonic scale[25]. The average values of the sensory parameters were used for the analysis.

2.9 Drying Time

The PEF treated, water blanched and untreated samples were put in a drying chamber (Heraeus D6450, Hanau, Germany) maintained at 60°C temperature with hot air velocity of 12 m/s flowing horizontally and the samples were dried up to 6-7 % moisture content (fresh weight basis). The time taken to reach this moisture content was recorded and reported in min.

2.10 Statistical Analysis

All measurements were carried out in triplicates for physicochemical parameters and data obtained were analysed statistically by one-way ANOVA (Analysis of Variance) using Statistica 7 software (Stat Soft, Tulsa, OK, USA).

3. RESULTS AND DISCUSSION

3.1 Cell Membrane Permeability and Drying Time

The cell disintegration index varied from 0.34 to 0.91 among different experimental samples in the electric field range of 1-2 kV/cm at 60 °C and 80 °C (Table 1). The maximum cell disintegration index was observed in the case of samples treated at 1.5 kV/cm at 80 °C and 75 pulses. Cell disintegration index was found to vary with increase in temperature and number of pulses at the same electric field strength. The cell disintegration increased significantly ($p<0.05$) with increase in temperature. Increase in number of pulses also increased the cell disintegration index significantly ($p<0.05$) indicating dependency of treatment duration on cell disintegration. Increase in electric field strength from 1.0 kV/cm to 1.5 kV/cm also increased the cell disintegration significantly ($p<0.05$), whereas, further increase in electric field strength showed lesser effect on it. The cell disintegration in the sample treated at 1.5 kV/cm, 80 °C and 75 pulses was found to be even significantly ($p<0.05$) higher than blanched samples (90 °C, 60 s) which can be attributed to combined effect of PEF and temperature. The study indicated that PEF treatment at lower temperature (80 °C) for much shorter time (18.75 s) can result in better cell disintegration compared to blanching of slices at higher temperature (90 °C) for much longer time (60 s). There was increase in cell disintegration with increase in energy input during PEF treatment from 0.35 kJ/kg to 1.06 kJ/kg as shown in Fig. 1. At an energy input of 1.41 kJ/kg, the cell disintegration decreased compared to that at 1.06 kJ/kg, however, it further increased to highest at an energy input of 2.38 kJ/kg and decreased again at 4.23 kJ/kg energy input. Therefore, it can be concluded that maximum cell disintegration can be achieved at an energy input of 2.38 kJ/kg and there is no need to go further in order to make cost effective treatment with low energy input during PEF treatment. It is also clear from the Fig. 1 that there is significant effect of temperature used during treatment on cell disintegration which was found to be of much higher magnitude at 80 °C as compared to 60 °C. The time taken during drying was found to have direct correlation with cell disintegration index. The samples where cell disintegration was higher showed shorter drying time (Table 1). It is obvious from the results that pulsed electric field treatment caused openings in the cell membranes (electroporation) causing faster movements of moisture during hot air dehydration. Increase in cell disintegration means more openings in the cell wall of plant cells causing increased mobility of solute or water molecules through the cell wall and thereby causing faster drying of the material[19,24]. Liu[26], *et al.* performed PEF treatment using electric field strength of $E = 600$ V/cm and total treatment time of $t_{PEF} = 0.1$ s to reach a high level of potato tissue disintegration, whereas, Janositz[27] *et al.* observed similar impact of pulsed electric fields on the diffusion characteristics of potato slices. Traffano-Schiffo[28] *et al.* also found that PEF

Table 1. Effect of electric field strength, number of pulses and treatment temperature on cell disintegration index, hardness and drying time (n=6)

Electric field strength (kV/cm)	Number of pulses	Temperature (°C)	Cell disintegration index	Hardness (N)	Drying time (min)
Untreated	-	-	0.00	4.76^{l}	210^{h}
1.0	25	60	0.34^{a}	4.62^{k}	180^{f}
		80	0.62^{e}	3.92^{e}	170^{e}
	75	60	0.71^{g}	4.35^{i}	175^{ef}
		80	0.80^{i}	3.38^{a}	145^{c}
1.5	25	60	0.41^{c}	4.35^{i}	200^{g}
		80	0.63^{f}	4.17^{f}	135^{b}
	75	60	0.61^{d}	4.19^{g}	160^{d}
		80	0.91^{l}	3.86^{d}	125^{a}
2.0	25	60	0.36^{b}	4.41^{g}	175^{ef}
		80	0.63^{f}	3.83^{c}	150^{c}
	75	60	0.74^{h}	4.26^{h}	170^{e}
		80	0.81^{j}	3.37^{a}	140^{bc}
Blanched	-	90	0.82^{k}	3.46^{b}	160^{d}
CD ($p < 0.05$)			0.01	0.02	6.31
±SEM			0.004	0.005	2.18

Values with different superscript in the same column differ significantly ($p<0.05$). CD: Critical difference. SEM: Standard error of mean.

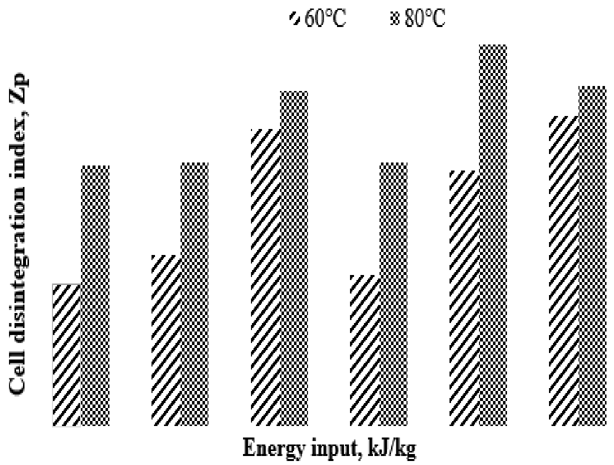


Figure 1. Effect of energy input during PEF treatment on cell disintegration index of apple.

pre-treatment increased the mass transfer during the osmotic dehydration of organic kiwifruit. PEF treatment maintains quality as compared to thermal processing and has advantages of less energy consumption, shorter processing time[6,10]. Similar results have been observed during PEF treated osmotic dehydration of apples[20], carrots[22], etc. where PEF pretreatment was found to improve the dehydration process. PEF treatment had been found to noticeable decrease the drying time (by 22 % – 27 % at *Td* = 40 °C – 70 °C) in potatoes[26]. Pulsed electric field pretreatment can significantly reduce the total energy input during dehydration process by means of opening the cells due to PEF and thereby increasing the rate of water removal during dehydration, and thus reducing the drying time[29].

3.2 Hardness

Hardness of the dehydrated apple slices varied from 3.37 N to 4.62 N among different experimental samples (Table 1). The untreated samples showed a significantly ($p<0.05$) higher hardness compared to PEF treated or blanched samples. The electric field strength, number of pulses used and temperature during PEF treatment affected significantly ($p<0.05$) the hardness of the dehydrated slices. The hardness was found to decrease with increase in electric field strength, number of pulses as well as temperature during treatment. The samples treated at 1 kV/cm electric field strength using 75 pulses and at 80 °C showed minimum hardness for the apple slices indicating crispy nature of the product. This sample had also showed lesser hardness even than blanched samples. Crispiness of slices depends on the internal structure of the tissues. If there is case hardening on the surface of the material the product will show more hardness. Pulsed electric field treatment caused pores in the cells which resulted in faster drying rates leading to porous tissue structure in the dehydrated material and this is the reason for lesser hardness of the PEF treated materials. The textural properties of a tissue are dependent on structure as well as morphology of plant tissue, cellular dimensions, intercellular spaces, presence of cell constituents, starch, and other components[30-31]. Lebovka[15], *et al.* reported that after PEF treatment, the tissues lost their initial strength, whereas, elastic modulus and fracture stress were found to decrease with increase of time of PEF treatment in potatoes, carrots and apples. The PEF treatment in combination with mild heat pretreatment caused additional softening of the tissues resulting in textural changes. The combined mode of treatment depends on the type of plant tissue.

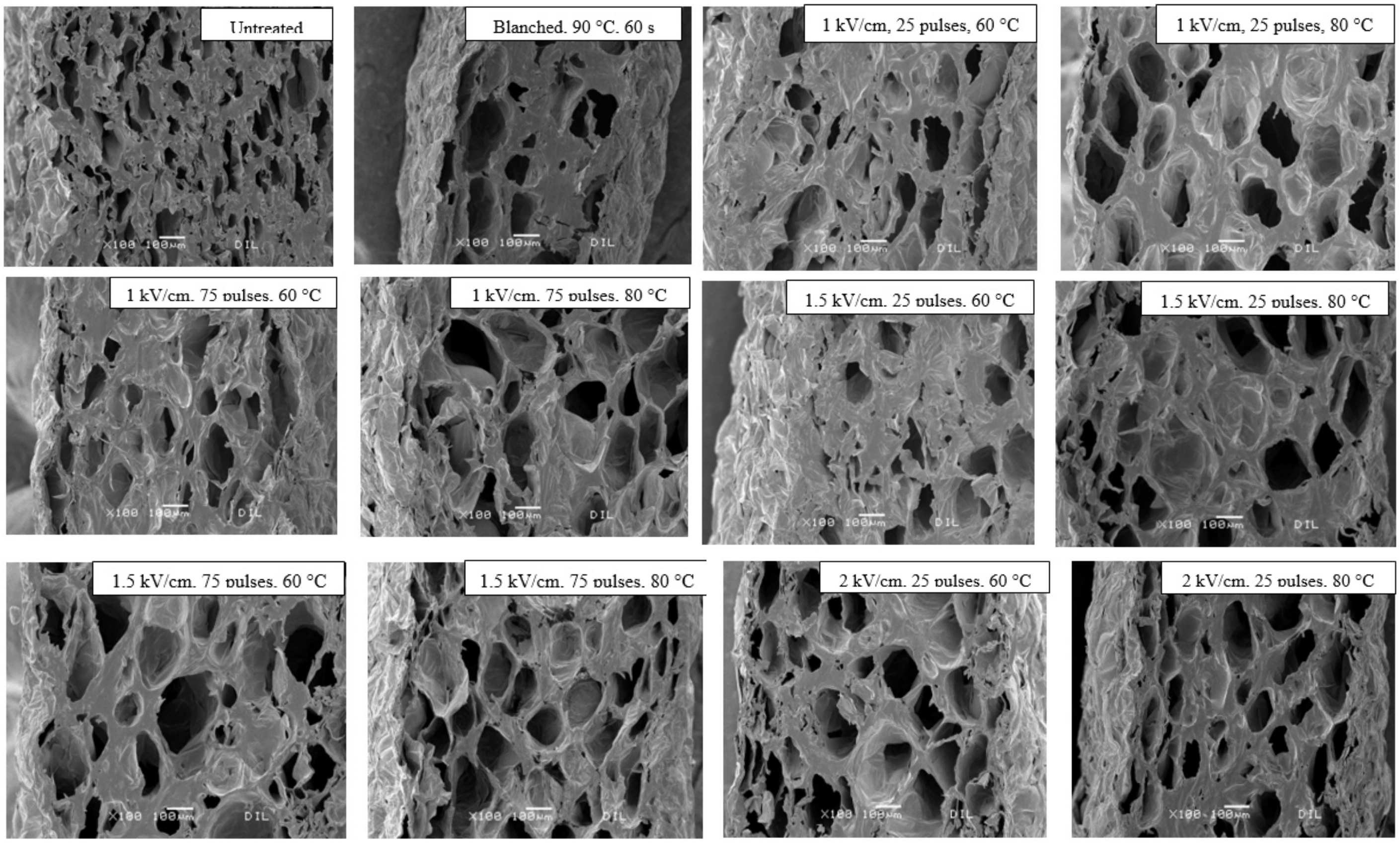
Table 2. Effect of electric field strength, number of pulses and treatment temperature on CIE color values (n=6)

Electric field strength (kV/cm)	Number of pulses	Temperature (°C)	L^* value	a^* value	b^* value
Untreated	-	-	79.99^{g}	6.06^{b}	26.70^{gh}
1.0	25	60	62.76^{a}	14.06^{n}	29.76^{k}
		80	71.73^{cd}	8.38^{d}	23.31^{d}
	75	60	62.16^{a}	13.39^{l}	28.31^{i}
		80	72.60^{d}	13.01^{k}	25.95^{e}
1.5	25	60	64.86^{b}	11.21^{g}	26.16^{f}
		80	64.42^{b}	12.28^{i}	26.92^{h}
	75	60	70.26^{c}	12.08^{h}	26.63^{g}
		80	73.98^{e}	6.49^{c}	21.41^{b}
2.0	25	60	63.62^{a}	13.93^{m}	29.29^{j}
		80	70.72^{c}	8.62^{f}	23.21^{d}
	75	60	63.91^{ab}	12.53^{j}	28.25^{i}
		80	72.08^{d}	8.51^{e}	22.38^{c}
Blanched	-	90	77.96^{f}	2.75^{a}	19.70^{a}
CD ($p<0.05$)			1.49	0.05	0.24
±SEM			0.51	0.02	0.08

Values with different superscript in the same column differ significantly ($p<0.05$).
CD: Critical difference. SEM: Standard error of mean.

3.3 CIE Colour Values

The data pertaining to CIE color values has been summarised in Table 2. It is evident from the colour data that PEF treatment has significant ($p<0.05$) effect on the CIE colour values. The L^* values which indicate lightness of the product was higher in the case of blanched sample as compared to PEF treated ones indicating darkening of slices due to PEF treatment. However, the difference was not much between the samples treated at 1.5 kV/cm, 75 pulses at 80 °C and blanched ones. The CIE a^* value which indicate redness of the product was also much higher in PEF treated samples compared to blanched one showing more redness in PEF treated samples. However, the CIE b^* values were significantly ($p <0.05$) higher in PEF treated samples indicating more yellowness in the product compared to blanched samples. Among the PEF treated samples the maximum lightness and minimum redness was observed in the one treated at 1.5 kV/cm, 75 pulses at 80 °C and this sample looks closer to blanched sample in terms of instrumental color values. Fruit tissues change colour due to enzymatic browning[32]. Further, heat treatment and application of anti-browning agents can inactivate the browning causing enzymes leading to colour



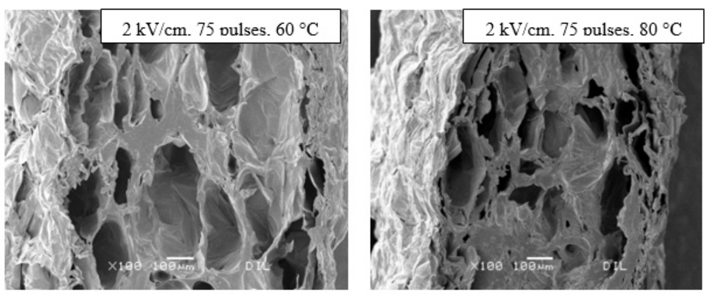


Figure 2. Scanning electron microscope photographs of dehydrated apple slices.

stabilisation in dehydrated products[33]. Amami[34], *et al.* reported increase in a^* and decrease in L^* values in PEF treated osmotically dehydrated apple, banana and carrot slices which is in agreement to the results shown by Wiktor[35], *et al.*

3.4 Microstructure

The microstructure SEM pictures show breakdown of tissues in untreated samples after dehydration as shown in Fig. 2. The tissues of untreated samples were seen to lose their structural integrity after dehydration. Whereas, in the case of blanched samples high breakdown of tissues were observed which may be due to higher temperature as well as higher treatment time used during blanching. However, in the case of PEF treated samples the cellular integrity was found to be maintained in all the electric field strengths used in the study. The cellular integrity was best maintained in the samples treated at 1.5 kV/cm for 75 pulse durations at 80 °C. It is evident from the SEM photographs that a judicious combination of electric field strength, treatment time and temperature used during treatment should be optimised for achieving better cellular integrity of the dehydrated tissues. Jalte[14], *et al.* reported improvements in the quality of PEF pre-treatment freeze-dried potato tissue showing lesser extent of shrinkage with less deformed cells and distorted polyhedral shape. Disruption of structural barriers, i.e. cellular membranes by various pre-treatment methods has been reported for enhancement of water as well as solute diffusivities within the products causing faster movement of water and solutes and selective membrane permeabilisation may cause a structural alteration that favours faster transfer of water across the membranes and maintaining the structural integrity of the tissues[23].

3.5 Sensory Attributes

The sensory attributes of the apple slices were recorded in terms of color, texture and overall acceptability as shown in Table 3. It was observed that the PEF treated slices tend to develop brown colour immediately after treatment whereas in the case of blanched ones there was no incidence of browning which is due to the inactivation of oxidative enzymes during blanching operation. PEF application has been found to enhance the activity of oxidative enzymes leading to more browning in several studies. Therefore, in the present investigation PEF was used in combination with hot water so that the enzymes can be inactivated and at the same time there should be sufficient cell disintegration to make the product more porous for faster drying during hot air dehydration. The samples treated at 1.5 kV/cm electric field strength for 75 pulses at 80 °C showed maximum sensory overall acceptability which was close to sensory scores for blanched ones. The blanched samples showed greater sensory score for color but the textural quality was found to be superior in the case of PEF treated ones. Lower levels of electric field strength led to decrease in the sensory scores, which increased with increase in the electric field strength and temperature of treatment. The PEF pre-treatment has been found to decrease the dehydration time and resulting in more uniform shape, lower shrinkage, lesser browning with better quality in the case of the freeze-dried potato samples[14]. Similar results have been observed in the present study where PEF treated samples had yielded better sensory attributes as compared to control ones.

Table 3. Effect of electric field strength, number of pulses and treatment temperature on sensory attributes* (n=10)

Electric field strength (kV/cm)	Number of pulses	Temperature (°C)	Color	Texture	Overall acceptability
Untreated	-	-	7.0[d]	6.0[a]	6.2[a]
1.0	25	60	6.2[a]	6.5[b]	6.3[b]
		80	6.7[c]	6.8[c]	6.4[c]
	75	60	6.3[ab]	6.6[b]	6.3[b]
		80	6.8[c]	7.0[d]	6.7[e]
1.5	25	60	6.4[b]	6.5[b]	6.5[d]
		80	6.8[c]	6.9[cd]	6.9[f]
	75	60	6.4[b]	6.5[b]	6.5[d]
		80	7.2[e]	7.2[e]	7.5[i]
2.0	25	60	6.3[ab]	6.5[b]	6.4[c]
		80	6.5[b]	7.0[d]	6.9[f]
	75	60	6.8[c]	6.6[b]	6.5[d]
		80	7.0[d]	7.5[f]	7.3[g]
Blanched	-	90	7.5[f]	7.5[f]	7.4[h]
CD ($p < 0.05$)			0.16	0.15	0.09
±SEM			0.05	0.05	0.03

*On 9 point hedonic scale. Values with different superscript in the same column differ significantly ($p<0.05$). CD: Critical difference. SEM: Standard error of mean.

4. CONCLUSIONS

Pulsed electric field as a blanching pretreatment affected significantly the quality of dehydrated apple slices. The PEF treated slices showed higher level of cell disintegration indicating higher membrane permeability leading to shorter drying time as compared to control. Though application of PEF led to slight browning in the product, judicious combination of pulsed electric field along with water blanching could be optimised for the development of good quality dehydrated products. This will further help in reduction in drying time as well as in maintaining the cellular integrity of the tissues after dehydration.

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