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Abstract

Electroporated and dried apples were characterised in terms of physical properties, such as sorption profile and mechanical test, in order to understand the treatment effects. Thermodynamic relationship between water activity and equilibrium moisture content at constant temperature and pressure has been mathematically expressed by sorption isotherm. High coefficient of determination of BET equation fitting were obtained, ranging from 0.974 to 0.990 (RMSE 0.07-0.08). The highest electric field strength (1.5 kV/cm) reveals a change in sorption isotherm shape. The III type of isotherm observed indicates a major presence of crystalline molecules increasing a water surface interaction.

Compression test were applied to the samples at different hydration level and PEF intensity applied. The plasticizing effect was observed as a function of hydration level raise and involve a reduction of the maximum force from about 14.85 (±2.44) N to 8.45 (±0.59) N. The opposite anti-plasticizing effect was a consequence of electric filed strength increase. Particularly, at low hydration level, where plasticizing effect did not produce softening of structures, PEF treatment induced a toughness and stiffness increase, revealing the anti-plasticizing effect. Fermi equation showed high coefficient of determination (0.902-0.959) and Y_0 value change from 17 to 35, as a function of PEF treatment intensity increase, confirming the structural differences in the analysed samples.

Thus, main results revealed the modification of water holding capacity induced by PEF and drying. Sorption profile and mechanical properties well described plasticizing and anti-plasticizing induced effect, underling the enhanced mobility of the system promoted by treatments.

1. Introduction

Pulsed Electric Field (PEF) technology has been proposed as a non-thermal pre-treatment for enhancing processes like extraction (M. Bazhal & Vorobiev, 2000; Prabhu et al., 2019), decanter centrifugation (Knorr et al., 2001), osmotic dehydration (Tylewicz et al., 2019; Tylewicz et al., 2017) and drying (Barba et al., 2015; Wiktor et al., 2013, 2016). PEF promotes the modification of the membrane permeability by application of high voltage short time pulses (Barba et al., 2015). The phenomenon that produce this transient (reversible) or permanent (irreversible) permeabilisation of the cell membrane, when electric pulses are applied, is known as electroporation (Golberg & Rubinsky, 2010).

Dehydration is a common method used to extend the shelf-life of fresh fruit and to produce alternative healthy snacks (Prawiranto et al., 2018). Water activity (a_w) reduction during the dehydration process, considerably defines the final fruit quality, such as nutritional value, colour (browning), texture and rehydration capacity (Caballero-Cerón et al., 1972). Furthermore, several of the quality attributes are in direct relation with the physical properties of the product, such as porosity and bulk density (Prawiranto et al., 2018). Traditional drying techniques are expensive in terms of energy consumption and limited by undesirable changes induced in the final product. When PEF is applied as a pre-treatment, the drying time is reduced (Lebovka et al., 2007) and also colour and mechanical properties of the final product are affected (Alam et al. , 2018; Wiktor et al., 2016). The extent of the changes produced on the solid matrix could be deduced for example, by monitoring textural quality changes of the treated materials (Bazhal et al., 2003). The investigation of product changes can be assessed also by means of the combined data of moisture desorption isotherms and glass transition temperatures as it was observed in osmo-dehydrated pear and apple (Djendouby-Mrad et al., 2013).

At the end of the combined PEF and drying process, the product moisture content reaches a value that could not corresponds to the equilibrium with the surrounding atmosphere. This thermodynamic instability can be characterised by equilibrium sorption isotherms, and its determination is essential for the better understanding of modelling problems on processing operations. Knowledge of the sorption equilibrium is also important for predicting stability and quality changes during packaging and storage of dehydrated foods and formulations (Djendoubi Mrad et al., 2013). Sorption isotherm describes the water holding capacity of a matrix at different hydration levels, thus it is highly important in food science and technology for the design and optimisation of packages, predictions of quality, stability, shelf-life and for calculating moisture changes that may occur during storage (Labuza, 1972). The typical isotherm shape reflects the way in which the water binds the system or in other terms how much water is available for degradative reactions (Barbosa-Canovas, 2007). The classification of sorption isotherms of foods has been widely studied (Roskar and Kmetec, 2005;

Bolin, 1980;Lim et al., 1995; Vazquez et al., 1999; Konopacka et al., 2002; Proton and Ahrne, 2004; Gondek and Lewicki, 2005; Mayor et al., 2005 and Moraga et al., 2006). Particularly, absorption and desorption isotherms of apple are widely known (Klewicki et al., 2016; Liu, et al., 2019; Mbarek & Mihoubi, 2019; Prawiranto et al., 2018b; Roškar & Kmetec, 2005; Saravacos, 1967; Velickova, et al., 2014; Veltchev & Menkov, 2000).

The impact of water content and a_w on physical, chemical and biological processes is attributed to the complex multicomponent interactions, as concept of polymer science confirms (Rocculi et al., 2011). In particular, these mechanisms are mainly related to small particles, such as water, having a plasticizing effect on the structures. Plasticisers screen off the attractive force or enlarge the spaces between polymer chains (Pittia & Sacchetti, 2008). Concerning the role of water among spaces between polymer chains, an opposite effect could be observed, the anti-plasticizing effect. Anti-plasticization is induced by inclusion of compounds of lower molecular weight inside an amorphous matrix, consisting of molecules of higher molecular weight (Job, 2018; Pittia & Sacchetti, 2008). These low molecular weight compounds, denoted here as diluents, should be compatible with the matrix constituents and are thought to have strong interaction with them (Iaccheri et al., 2019). For foodstuff recognized as multi component systems, typical diluents showing anti-plasticization behaviour are water, polyols, and various monosaccharide and disaccharide (Job, 2018). All of these diluents behave as anti-plasticizers only under specific conditions of temperature and concentration.

To our knowledge, no studies have been focused on the investigation of sorption profile, plasticizing and anti-plasticizing effect of water in dried apples pre-treated with PEF.

In this direction, the main focus of this research was to increase the understanding of the physical modification induced by the combination of PEF pre-treatment and further drying in apple tissues, with particular attention to sorption mechanism and mechanical properties issues.

2. Material and methods

2.1. Material and samples preparation

Apples (*Malus domestica* var. Granny Smith) were acquired from a local market in Cesena, Italy and stored in a refrigerated chamber at 10ºC until use. A constant geometry of the samples was obtained by sampling cylinders of 9 mm diameter with a manual cork borer, and then reducing their length to 10 mm with a manual cutter. The average moisture content of raw apples was 9.66 ± 0.32 kg water/kg dry matter (AOAC official method n. 934.06, oven method 70°C until steady weight).

2.2. Pulsed electric field treatment

Twenty samples per treatment were placed in a 5 x 5 x 5 cm treatment chamber, filled up with tap water, which conductivity was 515 ± 20 μs cm^{-1}, measured using an electrical conductivity meter mod. Basic 30 (Crison Instrument, Spain). Near-rectangular shaped pulses, with a fixed pulse width of 10 μs and frequency of 100 Hz were applied in the present work. Three field strength of 0.5, 1 and 1.5 kV/cm were tested at 25ºC. A non-PEF treated sample (control) was also prepared. The PEF treatments were applied using a pulse generator S-P7500 60A 8kV (Alintel srl., Bologna). The total treatment time was set to 1s. The energy specific intake (W_T) for the samples treated at 0.5, 1 and 1.5 kV/cm was 0.121, 0.483 and 1.086 kJ/kg respectively. The W_T was calculated using the equation proposed by Raso et al. (2016). for rectangular pulse:

$$W_T = \frac{n}{m}\int_0^\infty U(t).I(t)dt \qquad \text{eq. 1}$$

where: n is the number of pulses applied, m is the mass of treated sample, U(t) is the voltage across the treatment chamber and I(t) is the current through the treatment chamber.

The effectiveness of PEF treatment has been evaluated by texture disintegration index (Zt), which was calculated as follows:

$$Z_t = \frac{F-F_i}{F_d-F_i} \qquad \text{eq. 2}$$

where F is the maximum force at yield point (hardness, N) measured, and subscripts i and d refers to the values of intact and completely damaged tissue (obtained by subjecting samples to a freezing-thawing cycle), respectively as suggested by Tylewicz et al. (2019). The texture disintegration index (Zt) for the samples treated at 0.5, 1 and 1.5 kV/cm was 0.127, 0.253 and 0.459, respectively.

Immediately after PEF pre-treatment, apple samples were air dried in a tray drier mod. CLW 750 TOP+ (Pol-Eko- Aparatura SP.J., Poland) at 60 ºC, with transverse airflow, air velocity 2 m/s, an air renewal fee of 50% and a sieve load of 54 kg/m^2 until a water activity target of 0.2 was reached for control and treated samples. The water activity was measured by mean of a a dewpoint hygrometer, mod. Aqualab (Decagon Devices Inc., Pullman, WA), while the drying time was of about 6 hours.

After dehydration, all the samples were put in hygrostats for sorption isotherm assessment, as explained in the following section.

2.3. Sorption isotherm

The saturated salt slurry method (DES) was used for sorption isotherms assessment. The traditional DES method consists of reaching a define hydration level maintaining samples in sealed containers with a specific relative humidity, through the use of saturated salt solutions, soon after P_2O_5 dehydration. Apple samples were positioned in the hermetically closed desiccator containing, on the bottom, different saturated salt solutions at the required a_w (Table1). Each hydration experiment comprised reference samples and samples treated at a selected voltage. The thermodynamic equilibrium was evaluated gravimetrically at 20 ± 1°C, considering a steadiness at $\Delta w < 0.0005$ g (Lang et al., 1981).

The water activity of the equilibrated samples was measured by a dewpoint hygrometer, mod. Aqualab (Decagon Devices Inc., Pullman, WA) and the water content, evaluated using oven drying at 70°C until reaching steady weight, has been expressed on dry matter basis (AOAC 934.06).

The BET equation was used to fit experimental data and to determine the monolayer moisture contents:

$$X = \frac{V_m\, C\, a_w}{(1-a_w)(1+(C-1)a_w)} \qquad \text{eq. 3}$$

where X is the water content (g water/100 g solids), a_w is the water activity, V_m is the water content of the monolayer (g water/100g solids) and C is the constant related to monolayer sorption heat. According to the literature, the BET isotherm is one of the most successful ways to determine the monolayer moisture content in low moisture matrixes, as shown a good fitting in the low a_w region (Activity, 2007; Al-Muhtaseb et al., 2002; Correa et al., 2015; Roos & Karel, 1991; Wollny & Peleg, 1994)

2.4. Mechanical properties

The mechanical properties of the equilibrated samples at each water activity were analysed by a texture analyser mod. TA.XT2 (Stable Microsystems, Godalming UK) equipped with a 25 kg load cell and a craft knife blade of 0.5 mm thick. The test speed was 0.25 mm/s. Data were acquired with a resolution of 200 points/s. All samples were cut to 100% distance, and at least 10 measurements were carried out for each sample. The cutting/shearing test using a craft knife applies compression and shear forces (Nunak & Schleining, 2011). From the force-distance experimental data the following parameters were extrapolated: maximum force at yield point (hardness, N), toughness (mJ) and stiffness (N/mm). Toughness is defined as the area under the force-deformation curve until the yield point was reached and it is a measure of the total energy required to cut/shear the sample (Jain,

Pathare, & Manikantan, 2007; Sajeev, Manikantan, Kingsly, Moorthy, & Sreekumar, 2006). Stiffness is the resistance of a visco-elastic body to deflection. It is determined from the gradient of the force-distance curve (Jain et al., 2007). All the textural parameters were calculated by mean of a macro configured in Exponent Stable Micro Systems software (v. 6.1.16.0).

2.5. Modelling the texture properties

Mechanical parameters can be related to a_w assuming various shape, describing different structural behaviour. Sigmoidal data distribution was generally fitted with Fermi distribution function (Wollny & Peleg, 1994):

$$Y_{(aw)} = \frac{Y_0}{1+\exp\left(\frac{aw-aw_c}{b}\right)} \qquad \text{eq. 4}$$

where Y (a_w) is the magnitude of the mechanical parameter, Y_0 is its magnitude in the dry state, a_{wc} is a characteristic a_w where Y (a_{wc}) = Y/2 and b is a constant accounting for the steepness of the relationships around a_{wc}.

In some cases, particularly at low a_w values, Y would increase with a_w following a power relation before a further decrease. In this situation, the modified Peleg-Fermi model (Harris & Peleg, 1996) is more suitable for data interpolation (Eq. (5)):

$$Y_{(aw)} = \frac{Y_0 - Y_r + ka_w^n}{1+\exp\left(\frac{aw-aw_c}{b}\right)} + Y_r \qquad \text{eq. 5}$$

where, k is a constant that roughly represents the slope of the linear region and (Y_r) a term which accounts for the residual magnitude of x (a_w).

2.6. Statistical analysis

The sorption isotherms data acquired in triplicate for each measurement were fitted with BET equation (Statsoft 7.0, eq.1) to experimental data by nonlinear regression using the Marquardt algorithm (Mathematics, 1977). The algorithm calculates the set of parameters and their 95% confidence interval. The goodness of the fit was checked by the estimation of the determination coefficient R^2 and the associated averaged Root Mean Square Error (RMSE) values (Eq. (6)):

$$RMSE = \sqrt{\sum_{i=1}^{n} \frac{(\hat{y}_i - y_i)^2}{n}} \quad \text{eq. 6}$$

$\widehat{y_1}, \widehat{y_2}, \widehat{y_n}$ are predicted values, y_1, y_2, y_n are observed values and *n* is the number of observations. Significant differences among means of mechanical parameters (hardness, stiffness and toughness) at different PEF treatment were analyzed by means of ANOVA (analysis of variance, p-level < 0.05, post-hoc Tukey, Statgraphics Centurion XVI software).

3. Results and discussion

The physical behaviour of vegetable tissues is dependent on the structure constituents that influence the final product quality, storage stability and shelf-life. The properties of solid and fluid constituents have to be considered following a complex system approach, because different structures interact and behave mechanically, chemically and physically in a different way. Particularly, the relationship between moisture and a_w is essential to describe the state of water in the matrix. The thermodynamic equilibrium at the end of drying process becomes a sensible point of metastability, that can be understood by equilibrium sorption isotherms. The adsorption isotherms PEF pre-treated and dried apple are shown in figure 1.

As it can be observed, control and 0.5-1 kV/cm treated samples show a II type shape isotherm, typical of dried apples, while the higher voltage treatment (1.5 kV/cm) clearly turns the isotherm shape into a III type one. More in general it is worth noting that the typical shape of the major part of processed food is of II type. The sigmoidal curve, in general, well describes low a_w product, resultant of the additive effect of Raoult's law, capillary effects and surface interactions (Labuza, McNally, Gallagher, Hawkes, & Hurtado, 1972). Several regions can be noted for this type of isotherm, the first one in the 0.2 and 0.4 a_w range and the second one at about 0.6-0.7 a_w. The two regions are usually considered corresponding to the separation of physical and chemical effects, particularly the build-up of multilayers and filling of small pores in the lower region, followed by swelling, filling the large pores, and solute dissolution in the upper region (Labuza et al., 1972). The modification of the isotherm shape from II to III type, as a consequence of the highest PEF pre-treatment, suggests an increase in the matrix of the crystalline components such as sugars and salt, that are small particles usually available for moisture surface interactions. Previous work underlined a crystallinity increase of PEF treated apple tissue (Lammerskitten et al., 2019), evidencing that PEF pre-treatment on vacuum freeze and freeze dried apple tissue reduced processing time and increased water diffusion compared to untreated samples. Moreover, as a result of PEF application, water activity of

electroporated samples increased as a consequence of crystallinity raise, as proved also by thermal properties.

According to Caballero-Cerón, Serment-Moreno, Velazquez, Torres, & Welti-Chanes (2018b) the water holding capacity of apple is mainly dependent on the presence of reducing sugars, while non-reducing sugars have lower sorption capacity. Considering this hypothesis, PEF could be responsible of a sort of 'spreading' of reducing sugar, increasing in this way the h-bonding available for water interactions.

According to the literature, PEF application on plant cell material results in an improved mass transfer of intracellular substances (Janositz et al., 2011), probably due to the breaking in intracellular bond, creating a high free volume for physical and chemical reaction aid.

The fitting with BET equation showed a good agreement with experimental data, considering the high coefficient of determination and the low RMSE values evidenced for all the considered samples (Table 2).

BET equation was also chosen for its physical sense. In general, the BET model describes the isotherms well up to a relative humidity of 50%, depending on the material and the type of sorption behaviour. Furthermore, the monolayer value is a fundamental parameter for food stability description, especially around the typical a_w of a dried product. Monolayer can define the quantity of water un-available for physical structure modification. On account of this, monolayer cannot be exploited to described differences among control and PEF treated samples structural changes. Conversely, the BET constant C is related to the affinity of a solid to the adsorbate, so to the sorption heat. Therefore, higher C value corresponds to higher interaction of the solid matrix. As reported in table 2, treated samples were characterized by higher C values, confirming the hypothesis that PEF treatment promotes physical modification.

Mechanical properties were affected by moisture sorption behaviour. Averaged force-deformation curves of the control sample at the various a_w levels are shown in Figure 2.

The mechanical changes of different foodstuffs as a function of the hydration level have been reported by various authors; these modifications have been mainly attributed to the plastification effect of water (Kalichevsky et al., 1993; Mcnulty et al., 1978). On the contrary, no previous researches have been performed focused on the PEF pre-treatment effect on these aspects. In Figure 3, the force-distance curves for each considered a_w level are reported for the different investigated samples.

At the lowest a_w levels (0.11 and 0.22), a considerable force level increase, as a consequence of PEF pre-treatment, was observed. This kind of mechanical behaviour suggests an induced anti-plasticizing effect promoted by small amounts of adsorbed water. At higher a_w levels, a drop of the maximum force level together with a considerable smoothing of the force-deformation relationship has been

found, corresponding to the plasticising effect of the water on the structure, with a correspondent increase of structure cohesion and toughness, as reported in table 3.

Mechanical parameters, such as maximum force at yield point (Fmax, N) and stiffness (F/d, N/mm) were influenced by a_w values of about 0.11 up to 0.44, as shown Figure 4.

As reported in Table 3, for the lowest a_w levels, the detected highest values of maximum force and stiffness showed a greater resistance or hardening of the product, and a consequent loss of crispness of the structure. In this conditions the water molecules present in the matrix are not uniformly distributed, but rearranged within the structure. Even if not mobilised, in this condition, water could reduce the volume existing between the structures, creating a greater resistance to collapse. As reported above, this phenomenon is usually defined as 'anti-plasticizing' effect of water (Job, 2018; Pittia & Sacchetti, 2008; Rocculi et al., 2011). According to ANOVA, the lower a_w levels showed control samples significantly different compared to treated one, confirming that the anti-plasticizing effect was detectable as a consequence of PEF treatment (mainly for the higher field strength 1 and 1.5 kV/cm). Considering the highest a_w levels 0.57 and 0.75, significant differences were not revealed between control and PEF treated samples. At these high a_w levels, PEF treatment induced modification could be probably covered up by water plasticization.

The parameters of Fermi equation indicate at which a_w level the textural attributes are lost, and what is the a_w span at which the loss occurs. As can be seen in table 4, the estimated parameters of Fermi's model were different among the tested samples; particularly the differences in the Y_0 estimated value is a clear signal of structural differences. Good coefficients of determination were assessed applying Fermi's equation, ranging from 0.902 to 0.959. As expected, the model based on the mathematical structure of Fermi's distribution function was found appropriate for describing both aspects of plasticisation and anti-plasticization by moisture sorption of the force-deformation curves.

4. Conclusion

The sorption isotherm and mechanical approach applied in this study allowed to highlight the effect of PEF pre-treatment on the physical sorption behaviour of dried apple samples.

The highest PEF pre-treatment (1.5 kV/cm) promoted a modification of the isotherm shape, from type II to type III, while control, 0.5 and 1 kV/cm samples showed a II type isotherm, typical of the majority of complex food matrixes. The III type shape indicates a major presence of small crystalline particles, suggesting that, at the highest tested level, PEF pre-treatment could be responsible of the increase of the availability of reducing sugars to chemical interactions with water molecules.

This hypothesis was confirmed by the evaluation of mechanical properties and sorption profile.

BET constant C was calculated and higher values were observed for PEF treated samples resulting in physical modification enhancement.

Actually, at the lowest a_w values (0.11 and 0.22), PEF pre-treated samples seemed to be subjected to water anti-plasticizing effect, as confirmed by Fermi equation fitting.

Physical modifications promoted by PEF pre-treatment has to be taken carefully into account in product process and storage management. In this direction, microstructural assessment and the confirmation of the observed phenomena with different multi-analytical approach are in due course in our lab.

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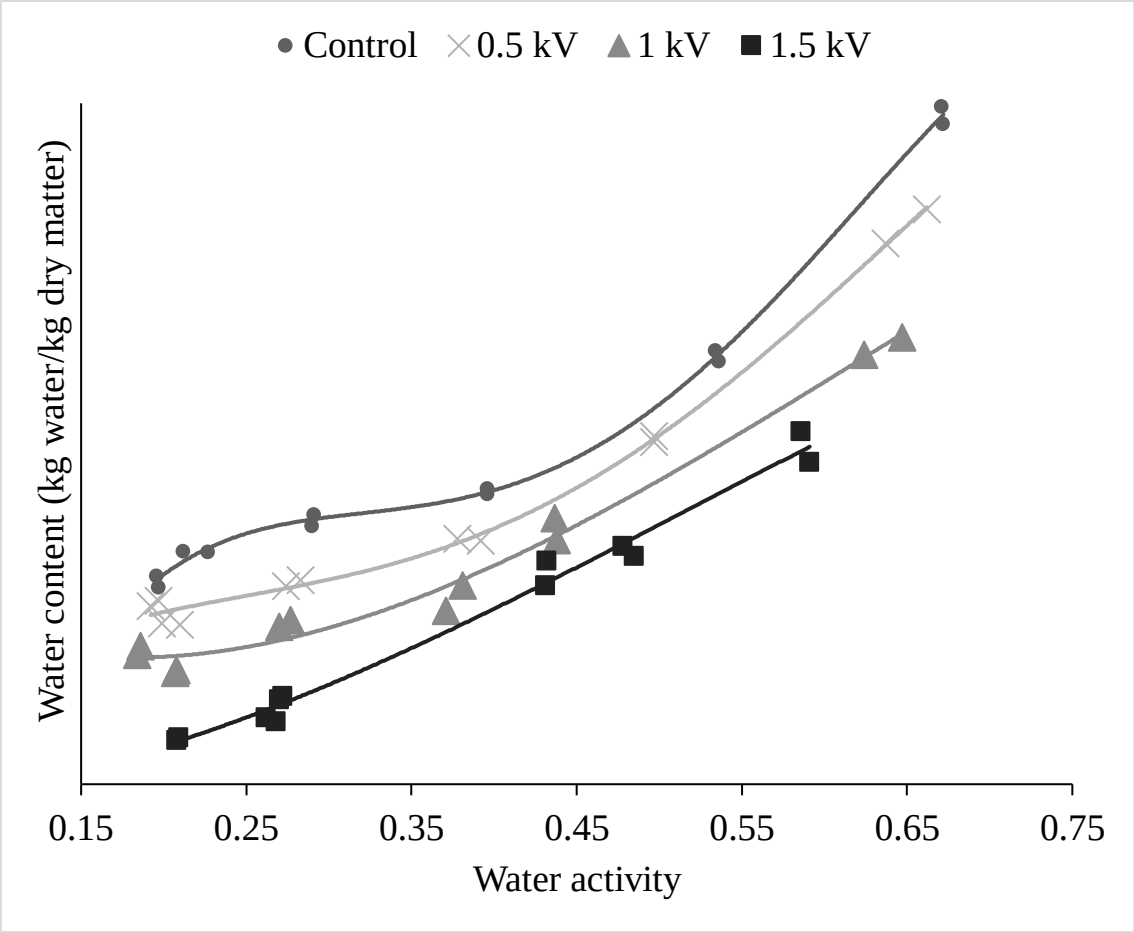

Figure 1. Sorption isotherms of control (point), 0.5 (X in light grey), 1 (triangle in gray) and 1.5 (square in black) kV/cm dried and PEF treated apple.

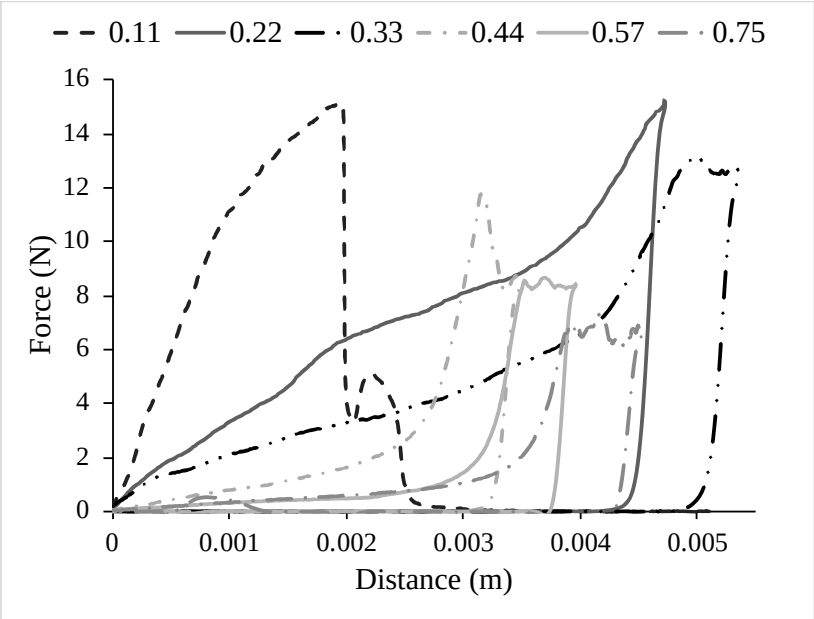

Figure 2. Force-distance curve of apple (control treatment) at the different a_w.

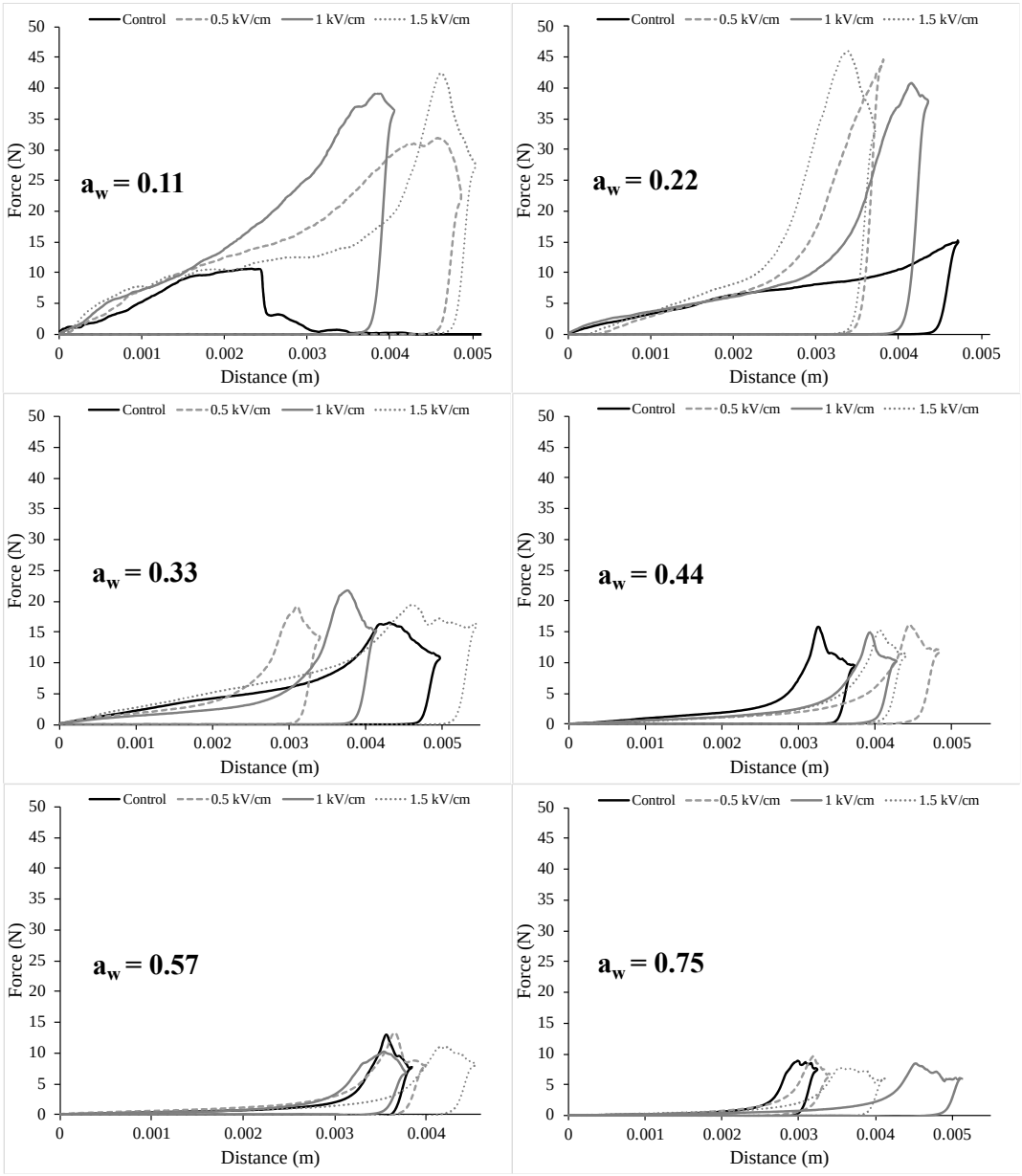

Figure 3. Force vs. Distance curve of apple samples untreated and treated with PEF equilibrated at the six different water activities.

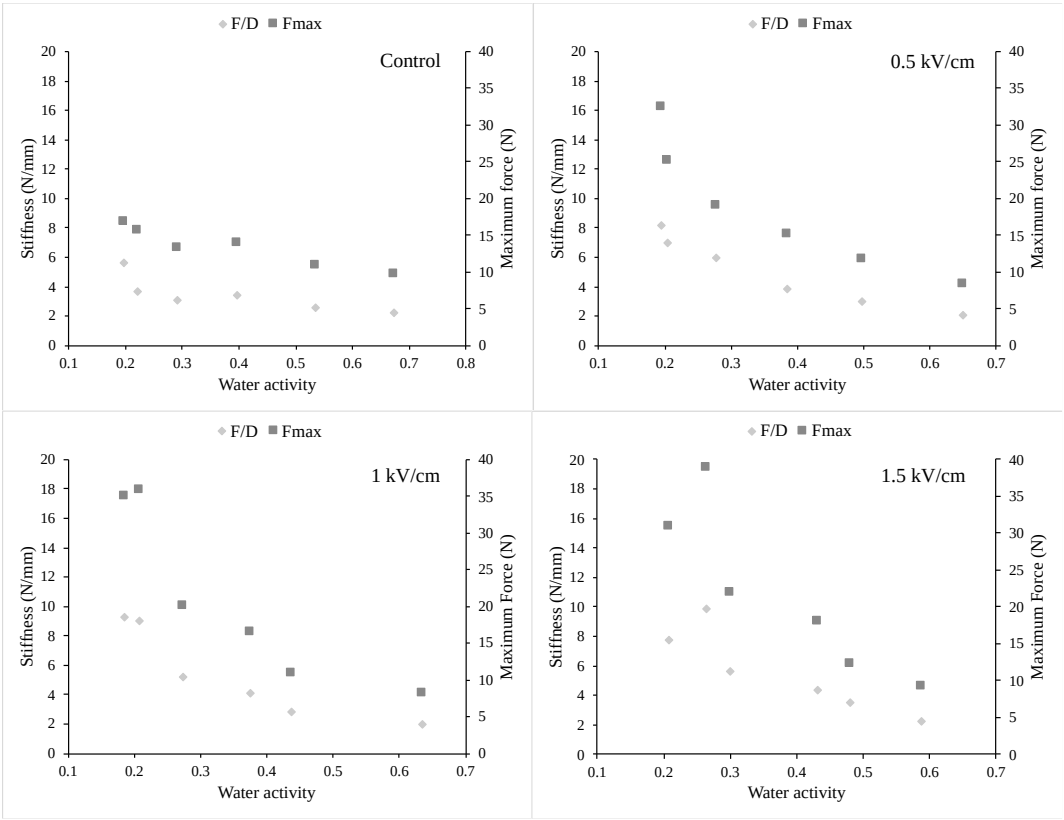

Figure 4. Relation among water activity, maximum force (F max) and stiffness (F/d).

Table 1. Water activities of selected saturated salt solutions at 20ºC

Salt	**Water activity**
Lithium chloride (LiCl)	0.113
Potassium acetate ($KC_2H_3O_2$)	0.231
Magnesium chloride ($MgCl_2$)	0.331
Potassium carbonate (K_2CO_3)	0.432
Sodium bromide (NaBr)	0.591
Sodium chloride (NaCl)	0.755

Table 2. Regression parameters of BET equation applied to sorption isotherm experimental data.

Samples	**V_m[a]** (g H_2O/100 g dm)	**C[a]**	**R^2**	**RMSE**
Control	0.10 ± 0.01	1.79 ± 0.64	0.988	0.008
0.5 kVcm^{-1}	0.09 ± 0.01	2.06 ± 0.64	0.990	0.007
1 kVcm^{-1}	0.08 ± 0.01	3.17 ± 1.23	0.980	0.007
1.5 kVcm^{-1}	0.09 ± 0.01	2.13 ± 0.95	0.974	0.008

[a] Average ± SD

Table 3. Values of maximum force, stiffness and toughness of the different apple samples.

		Maximum force (N)	Stiffness (N/mm)	Toughness (mJ)
0.11	Control	14.85 ± 2.44^{a}	5.66 ± 1.47^{a}	29.80 ± 17.52^{a}
	0.5kV/cm	25.20 ± 9.74^{b}	7.05 ± 2.54^{ab}	43.37 ± 15.44^{b}
	1kV/cm	33.70 ± 10.03^{c}	8.49 ± 1.09^{b}	46.96 ± 9.60^{bc}
	1.5kV/cm	30.97 ± 6.86^{bc}	7.70 ± 1.60^{b}	57.65 ± 16.03^{c}
0.22	Control	13.35 ± 2.02^{a}	2.90 ± 0.52^{a}	30.71 ± 4.84^{a}
	0.5kV/cm	31.28 ± 2.83^{b}	7.17 ± 0.69^{b}	39.22 ± 7.34^{b}
	1kV/cm	35.89 ± 4.85^{c}	8.66 ± 1.30^{bc}	43.04 ± 9.23^{bc}
	1.5kV/cm	38.84 ± 6.65^{c}	9.85 ± 3.41^{c}	48.23 ± 9.14^{c}
0.33	Control	13.39 ± 2.54^{a}	3.15 ± 0.72^{a}	21.09 ± 3.91^{b}
	0.5kV/cm	19.82 ± 1.26^{b}	6.02 ± 1.68^{b}	14.27 ± 3.60^{a}
	1kV/cm	20.05 ± 2.10^{b}	5.23 ± 0.69^{b}	15.60 ± 2.20^{a}
	1.5kV/cm	21.98 ± 1.81^{c}	5.65 ± 0.81^{b}	20.24 ± 5.13^{b}
0.44	Control	14.33 ± 1.68^{a}	3.56 ± 0.40^{a}	9.43 ± 2.07^{a}
	0.5kV/cm	16.06 ± 0.68^{b}	3.92 ± 0.47^{ab}	9.89 ± 2.07^{a}
	1kV/cm	16.60 ± 2.21^{bc}	4.16 ± 0.54^{b}	10.05 ± 1.45^{ab}
	1.5kV/cm	18.00 ± 1.55^{c}	4.34 ± 0.52^{b}	11.75 ± 2.11^{b}
0.57	Control	11.05 ± 2.25^{a}	2.47 ± 0.54^{a}	5.94 ± 1.46^{a}
	0.5kV/cm	11.72 ± 2.97^{a}	2.98 ± 0.96^{ab}	6.83 ± 1.57^{a}
	1kV/cm	11.20 ± 0.44^{a}	2.56 ± 0.11^{ab}	6.70 ± 1.89^{a}
	1.5kV/cm	12.51 ± 0.99^{a}	3.22 ± 0.43^{b}	7.20 ± 1.43^{a}
0.75	Control	8.45 ± 0.59^{a}	2.24 ± 0.45^{a}	4.14 ± 1.09^{a}
	0.5kV/cm	8.37 ± 0.97^{a}	2.03 ± 0.22^{a}	4.53 ± 0.76^{ab}
	1kV/cm	8.18 ± 0.74^{a}	2.06 ± 0.14^{a}	4.64 ± 1.02^{ab}
	1.5kV/cm	8.72 ± 0.20^{a}	2.21 ± 0.24^{a}	5.32 ± 1.39^{b}

Table 4. Estimated Parameters of Fermi and Fermi modified equation on a_w and maximum force.

Equation	**Samples**	**Setted parameters**				**Model estimation**	
		Y_0	**Xc**	**C**	**Yr**	**b**	**R^2**
Fermi	**Control**	17	0.670	nd	nd	0.30	0.913
	0.5kVcm^{-1}	33	0.378	nd	nd	0.15	0.902
Modified Fermi	**1.0 kVcm^{-1}**	35	0.500	3.747	8.2	0.09	0.959
	1.5 kVcm^{-1}	35	0.550	3.674	8.7	0.10	0.926