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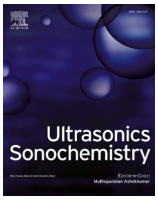

Enhancing ultrasonic-assisted drying of low-porosity products through pulsed electric field (PEF) pretreatment: The case of butternut squash

B. Llavata [a], A. Quiles [b], C. Rosselló [c], J.A. Cárcel [a,*]

[a] *Analysis and Simulation of Agro-food Processes Group, Food Engineering Research Institute – FoodUPV, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain*

[b] *Research Group of Food Microstructure and Chemistry, Food Engineering Research Institute – FoodUPV, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain*

[c] *Department of Chemistry, University of the Balearic Islands, Ctra. Valldemossa, km. 7.5, 07122 Palma de Mallorca, Spain*

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ABSTRACT

Ultrasonic-assisted drying is an effective technique for accelerating drying processes, particularly for products with high porosity. The structural changes induced by pulsed electric field (PEF) treatment can make low-porosity products more susceptible to the effects of ultrasound during drying. This study aimed to investigate the influence of PEF treatment on the structure of low-porosity products, such as butternut squash, and to evaluate its effect on ultrasonic-assisted drying. PEF pretreatment altered the physicochemical and microstructural properties of butternut squash. Thus, the higher the energy input, the higher the cell disintegration rate, the lower the shearing force and the lower the water holding capacity. For the same energy input applied, no influence was observed from the different combinations of pulse number and electric field intensity used. The microstructural analysis also showed greater effects with increasing intensity of PEF treatments. All these changes affected the subsequent drying, increasing the drying rate of conventional drying. Moreover, PEF pretreatment enhanced the ultrasound effects when applied during drying, reducing drying time by up to 47% when moderate PEF intensity was used. Therefore, PEF pretreatment under the appropriate conditions could make ultrasound-assisted drying of low-porosity products, such as butternut squash, more feasible.

1. Introduction

Butternut squash (*Cucurbita moschata*) is a widely cultivated vegetable, especially in warm climates [1]. It contains significant amounts of antioxidant compounds, vitamins [2], as well as carbohydrates, making it a good source of fiber with a positive effect on intestinal microbiota. One of its main uses is as an ingredient of soups and creams. However, the production seasonality is an important drawback. In this sense, the drying allows for a reduction in the moisture content, thus extending the shelf life of the products. Therefore, the application of this operation could broaden the possibilities of the use of butternut squash, making also possible the valorization of by-products and surpluses. Conventional drying is the most widespread industrial technique [3]. However, it entails high energy and environmental costs because of the long processing time, which is particularly important for dense products such as butternut squash. For this reason, there is great interest to enhance this operation and reduce its impact. Among the different strategies followed to this end, it can be found the application of emergent technologies such as ultrasound or pulsed electric fields.

Power ultrasound (US) are acoustic waves with a frequency higher than 20 kHz that can generate a series of mechanical effects in food systems that improve mass and heat transfer. These effects can range from the well-known sponge effect to the microchannels formation due to the stress caused in the samples or the creation of microturbulences on

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Abbreviations: % Var, percentage of explained variance; ANOVA, analysis of variance; Cryo-FESEM, Cryo-Field Emission Scanning Electron Microscopy; dm, dried matter; E, total energy input (kJ/kg); I, current intensity (A); LSD, Least Significance Difference; m, mass (kg); m_i, initial mass (kg); M_i, initial moisture content (kg w/kg dm); MR, moisture ratio; m_w, released water mass (kg); n_p, number of pulses; PEF, Pulsed Electric Field; S^2_{cal}, standard deviation of the calculated data; S^2_{exp}, Standard deviation of the experimental data; t, time (s); US, ultrasound; V, voltage (kV); WHC, Water Holding Capacity; X, moisture content (kg w/kg dm); X_0, initial Moisture Content (kg w/kg dm); X_{eq}, moisture content at the equilibrium (kg w/kg dm); Z, cell disintegration index; β, parameter of velocity of the process (min^{-1}); σ, electrical conductivity (S/m); α, parameter of behavior index.

* Corresponding author.

E-mail address: jcarcel@tal.upv.es (J.A. Cárcel).

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the samples surface [4]. The application of airborne ultrasound during the drying has been widely reported to be a good way to reduce drying time [5] while preserving the final quality of the products [6]. The effectiveness of ultrasound-assisted drying depends on the internal structure of the material. Thus, the technique is especially efficient in porous products, such as eggplant or mushroom, but less effective in dense products, such as potato or cassava [4].

Pulsed electric fields (PEF) represent another interesting technology used to improve mass transfer operations. It consists of the application of a high-intensity electric field in pulses of short duration. These treatments can modify the food matrix structures and induce the formation of pores (permanent or reversible) in cell membranes, a phenomenon known as electropermeabilization. PEF has been used for different applications in the food industry such as pasteurization [7], meat tenderization [8] or the enhancement of mass transfer processes such as extraction [9], frying [10], brining [11] or drying [12].

Moreover, the structural changes induced by PEF treatments could make the structure of products more prone to the effects induced by ultrasound, which would be very interesting in dense products, such as butternut squash. Thus, the combined application of a PEF pretreatment and ultrasound-assisted drying could have a synergistic effect, intensifying the drying process. In fact, recent studies about the combination of both technologies have found a significant enhancement of the drying rates in low density products such as orange peel and kiwifruit [13–15]. However, it is necessary to determine how the intensity of PEF treatments affects low-porosity products and identify the most adequate conditions to enhance ultrasound effects when applied during drying. In this sense, the aim of this work was to characterize the changes induced by PEF treatments on the physicochemical properties (cell disintegration index, shearing force, and water holding capacity) and microstructure of butternut squash samples and establish their influence on subsequent conventional and ultrasound-assisted drying.

2. Materials and methods

2.1. Sample preparation

The butternut squash (*Cucurbita moschata*) was acquired in a local supermarket in Valencia (Spain). The samples, with an initial moisture content of 91.1 ± 0.6 kg water/ 100 kg sample, were stored at 4 °C until required. Before PEF treatments, the samples were tempered at room temperature and cut into cubes of 30 mm side with a sharp knife to standardize the following PEF treatments.

2.2. PEF treatments

The PEF treatments of butternut squash were carried out in a batch-scale PEF system (EPLUSUS-PM1-10, Energy Pulse System, Lisbon, Portugal). To this end, each cubic sample was individually treated in a chamber provided with two electrodes separated by a distance of 5 cm. A standard KCl (laboratory grade, Sigma-Aldrich, Switzerland) solution (400 mL) of 0.3 mS/cm was used as electricity conductor medium [16]. The PEF treatments were tested by applying a different number (0, 2, 5, 10, 20, 30, 40, 50, 75, 100, 150, 200, 300, 500 and 1000) of pulses (20 µS; 50 Hz) at three different electric field strengths (0.67, 1.34 and 2.00 kV/cm). Each condition was tested at least in triplicate.

The total energy input (E; kJ/kg) applied in each PEF treatment tested was calculated from Eq. (1) [17].

$$E = \frac{V \cdot I \cdot t \cdot n_p}{m} \tag{1}$$

where m is the mass treated (kg), V is the voltage applied (kV), I is the current intensity (A), t is the pulse time duration (s) and n_p the number of pulses applied.

2.3. Influence of PEF treatments on sample structure

The level of structural changes induced by the PEF treatment was characterized both quantitatively and qualitatively.

2.3.1. Cell disintegration index

The level of electropermeabilization induced by the PEF treatments was estimated through the determination of the cell disintegration index (Z). For this purpose, after PEF treatments, the chamber containing the treated samples was connected to a waveform generator (33120a, Hewlett Packard, USA), which provided an electrical current of 1 V at 1000 Hz [18] The electrical intensity generated was measured with a digital power meter (WT210, Yokogawa, Japan). Subsequently, the electrical conductivity of the sample was estimated according to the equation derived from Ohm's Law (Eq. (2).

$$\sigma = \frac{I}{V} \tag{2}$$

where σ was the electrical conductivity of the sample (S/m); I, the electrical intensity measured (A) and V, the voltage applied (1 V).

The Z index was determined according to Eq. (3).

$$Z = \frac{\sigma - \sigma_i}{\sigma_d - \sigma_i} \tag{3}$$

where σ_i the electrical conductivity of fresh non-pretreated samples and σ_d the maximum electrical conductivity that a sample can reach, which will occur when the cell structure is completely damaged. This state was standardized by freezing (−18 °C) and thawing (room temperature) the fresh samples twice, as described by Ostermeier et al. [19].

2.3.2. Shearing force

Shearing force tests were carried out just after the different PEF treatments of butternut squash samples (0, 20, 100, 300 and 1000 pulses; 0.67, 1.34 and 2.00 kV/cm of electric field strength) with a texture analyzer (TA-XT2i, Stable Micro Systems, Surrey, England) provided with a Warner Bratzler Blade (A/BS, Aname, Spain). The parameters used in the tests were fixed after a set of preliminary experiments and were a descent speed of 1.00 m/s, a trigger force of 0.049 N and a total displacement distance of 20 mm. The maximum shearing force (N) was measured in each sample at least fourteen times.

2.3.3. Water holding capacity

The Water Holding Capacity (WHC) was evaluated according to Llavata et al. [20]. For this purpose, PEF treated cubic samples (30 mm side) were cut into parallelepipeds (15 x 15 x 7.5 mm) and placed in special centrifuge tubes developed by the research group. They contained a metal mesh in the equatorial plane which held the sample. This mesh permitted the separation of the water extracted from samples by centrifugal force, which flowed to the bottom of the tube. Afterward, the tubes containing the samples were centrifuged (Mixtasel, JP-Selecta, Barcelona, Spain) at 5000 rpm for 10 min at 4 °C. The WHC was estimated as indicated in Eq. (4).

$$\mathrm{WHC}\,(\%) = \frac{(m_i \cdot M_i) - (m_w)}{(m_i \cdot M_i)} \times 100 \tag{4}$$

where m_i is the initial mass of the sample (kg), M_i is the initial moisture content (kg water/kg sample), and m_w is the released water (kg) after applying centrifugal force. At least 6 replicates were carried out for each PEF treatment applied (0, 20, 100, 300 and 1000 pulses; 0.67, 1.34 and 2.00 kV/cm of electric field strength).

2.4. Microstructural analysis

The microstructure of butternut squash samples was studied by cryo-Field Emission Scanning Electron Microscopy (cryo-FESEM). The cell disintegration index (Z), previously determined, was used to select samples with different levels of PEF treatments. Specifically, micrographs of control (non-PEF treated, Z_0) and PEF-treated samples were obtained to achieve a Z index of 0.25 ($Z_{0.25}$), 0.75 ($Z_{0.75}$) and 0.98 ($Z_{0.98}$). This last Z level corresponded to the most intense PEF treatment tested, 1000 pulses and an electric field strength of 2.00 kV/cm. Thus, the different samples were placed in the holder, fixed with slush nitrogen at −210 °C and transferred frozen to the cryo unit, where they were fractured, etched (−90 °C) for 10 min, and platinum-coated (20 s of coating at 10.0 mA). Finally, they were observed with a field emission scanning electron microscope (Ultra 55 FESEM, Zeiss, Oberkochen, Germany) using a voltage of 3.0 kV and at a working distance of 10 mm.

2.5. *Drying experiments*

As in the case of the microstructural study, samples with different Z index values were selected to study the influence of the PEF treatment on conventional and ultrasound-assisted drying; specifically, what was considered was Z_0 (non-PEF pretreated as a control), $Z_{0.25}$ (73 pulses at 0.67 kV/cm) and $Z_{0.75}$ (330 pulses at 2.00 kV/cm).

Immediately after the application of the PEF treatment, the samples were cut into 3 mm thick slices simulating a snack shape sample, randomly distributed in a sample holder and placed into a lab-scale airborne ultrasound-assisted dryer (developed by the Simulation of Agro-food Processes Group of the Universitat Politècnica de València, Spain), as described by Polachini et al. [21]. Drying experiments were carried out at 40 ± 0.1 °C with an air velocity of 1 m/s. The sample weight was recorded automatically every 5 min with a balance attached to the system. In the case of ultrasound-assisted drying (50 W and 21.9 kHz) experiments (Z_0 + US, $Z_{0.25}$ + US and $Z_{0.75}$ + US), a piezoelectric transducer excited the cylindrical drying chamber generating a high intensity airborne ultrasonic field within it. There was no contact between the walls of the drying chamber and the samples, and no significant overheating was observed. All the experiments were performed at least 5 times.

2.6. *Modeling*

The modeling of the drying kinetics was performed to quantify and compare the influence of the PEF treatments and the application of US during drying. For this purpose, the Weibull empirical model (Eq. (5), widely used to describe drying processes, was considered [22].

$$MR = \frac{X - X_{eq}}{X_0 - X_{eq}} = e^{\left(-\frac{t}{\beta}\right)^{\alpha}} \tag{5}$$

where MR is the dimensionless moisture content, X is the moisture content of the sample after a certain drying time (t) (kg water/kg dry matter), X_0 is the sample moisture content before drying (kg w/kg dm) and X_{eq} is the moisture content at equilibrium (kg w/kg dm). This equilibrium was considered to have been reached when the weight of samples did not change over the last 2 h of drying. The fitting parameters of the model were α and β. The first represents a behavior index of the butternut squash during drying, and β is the kinetic parameter, which is inversely proportional to the drying velocity. Both parameters, α and β, were identified by minimizing the squared differences between experimental and calculated moisture contents using the Generalized Reduced Gradient optimization method from the Excel 2016 Solver tool (Microsoft, USA). The percentage of explained variance (% Var) was calculated as Eq. (6) in order to evaluate the goodness of the model fit.

$$\%Var = \left(1 - \frac{S^2_{cal}}{S^2_{exp}}\right) \cdot 100 \tag{6}$$

where S^2_{cal} is the standard deviation of the calculated data and S^2_{exp} is the standard deviation of the experimental data.

2.7. *Statistical analysis*

Analyses of variance (one-way ANOVA) were conducted, and the Least Significant Difference (LSD) intervals were performed with Statgraphics Centurion XVI (Statpoint Technologies Inc., Warrenton, USA) to evaluate the significance ($p < 0.05$) of differences between the treatments tested.

3. Results and discussion

3.1. *Cell disintegration index*

The cell disintegration index (Z) constitutes a measurement of the degree of electroporation generated by PEF treatments. This phenomenon permits the electrolytes to flow more easily from intra to intercellular space, increasing the electrical conductivity of the material. Thus, the Z value could indicate the level of cell membrane alteration due to pore generation [23]. In the case of butternut squash, the results obtained showed that the higher the number of pulses applied, the greater the Z value, the relationship not being linear but exponential (Fig. 1a). Thus, an increase in the number of pulses applied −up to 150- led to a sharp increase in Z. Above this number, the effect of increasing the number of pulses on the Z value was milder. Moreover, at the same number of pulses applied, the greater the electric field strength, the higher the Z. For example, for 150 pulses, the Z value of samples treated at 2.00 kV/cm (0.61 ± 0.06), the greatest electric field strength tested, was 24.5 % higher than those treated at 1.34 kV/cm (0.49 ± 0.06) and 69.4 % more than those treated at 0.67 kV/cm (0.36 ± 0.04). The increase in both the number of pulses and the electric field strength led to the increase in the intensity of the PEF treatment and, therefore, the intensification of the electroporation generated by the treatment. Likewise, the exponential relation shown in Fig. 1a could indicate the existence of a limit in cell alteration. This has been previously described by Fauster et al. [24] in the PEF treatments of potatoes or by Ammelt et al. [25] studying red beets and pineapples.

The maximum value of Z induced in this study was 0.98 ± 0.09 and was reached at the maximum number of pulses and the highest electric field strength tested (1000 pulses at 2.00 kV/cm). This value was close to 1, which corresponds to samples with a completely damaged cell structure that, as stated before, was standardized by frozen-thawing twice fresh samples [19]. In a previous study carried out by these authors on yellow turnip PEF treated under the same conditions, it was observed that the maximum cell disintegration index was 0.73 ± 0.08 [20]. These differences indicate that the extension of the effects of the PEF treatment depends not only on the conditions applied but also on the food matrix. In this sense, Alam et al. [16] found that the PEF treatment time needed to completely destroy the cell structure of parsnips samples was longer than that needed for carrots. These authors attributed this result to the structural differences between the matrices, and it means that it is necessary to study the behavior of each product to select the most appropriate processing conditions.

The total energy input (E) involved in each PEF treatment studied was calculated through Eq. (1). It was observed that the greater the E, the higher the Z, following a clear exponential relationship (Fig. 1b). Namely, at low energy values there was a fast increase in Z in line with the increase in E, followed by a more gradual increase beyond E values of over 20 kJ/kg. The increase in the number of pulses applied, as well as the higher electric field strength, led to an increase in the total energy applied during the treatment and then, a greater cellular degradation [26,27]. In this vein, in the range of values studied, the relationship between E and Z was independent of the specific combination of electric field strength and the number of pulses considered to reach a particular E value.

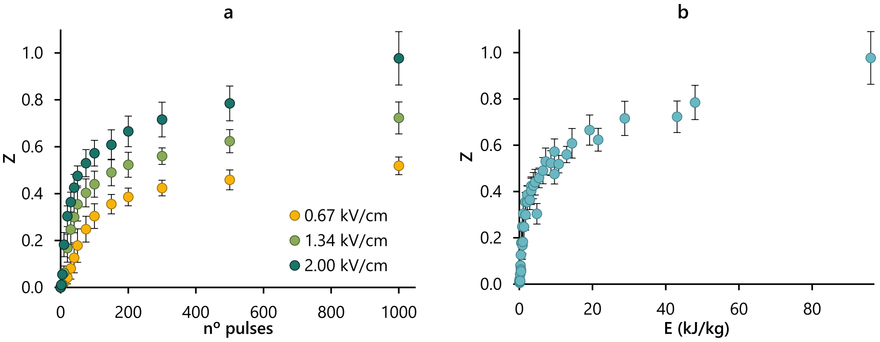

Fig. 1. Evolution of cell disintegration index (Z) of butternut squash samples after PEF treatment according to the electric field strength (0.67, 1.34 and 2.00 kV/cm) and the number of pulses applied (**a**) and the total energy input (E) of the different treatments (**b**).

3.2. *Shearing force*

The influence of the PEF treatment on sample texture was characterized through shearing force tests (Table 1). Thus, the maximum force required to cut the fresh butternut squash samples was 111 ± 27 N. This value significantly ($p < 0.05$) decreased after the PEF treatment whichever conditions applied. These textural changes can be explained by the loss of cell turgor after the PEF treatment [28]. When an electric field is applied, the electroporated membranes release part of their intracellular content, losing their structural integrity. Furthermore, PEF can cause structural changes in specific food components [29] which could also lead to tissue softening. The increase in the number of pulses applied resulted in lower shearing force values, which indicated a greater disintegration of the tissues when using the longer PEF treatments. The effect of the number of pulses applied was more significant at lower electric field strengths. In fact, at the maximum number of pulses tested (1000), no significant differences ($p < 0.05$) of the shearing force were observed in samples treated at the three electric field strengths tested (Table 1). These results are directly linked to the E applied and could indicate that above a certain E threshold, there was no increase in the effect of PEF on sample hardness. In this sense, Moens et al. [30] reported a decrease in the hardness of potatoes when increasing the electric energy applied until a plateau value is reached.

Table 1
Total energy input (E), cell disintegration index (Z), shearing force and water holding capacity (WHC) of butternut squash samples PEF treated at different electric field strengths (0.67, 1.34 and 2.00 kV/cm) and a different number of pulses (0, 20, 100, 300 and 1000).

Electric field strength (kV/cm)	Number of pulses	E (kJ/kg)	Z	Shearing force (N)	WHC (%)
0	0	0	0 ± 0^{a}	111 ± 27^{e}	94 ± 1^{mn}
0.67	20	0.22	0.04 ± 0.03^{a}	90 ± 20^{d}	93 ± 2^{m}
	100	1.08	0.30 ± 0.05^{c}	68 ± 16^{bc}	75 ± 4^{f}
	300	3.32	0.42 ± 0.03^{d}	54 ± 12^{ab}	74 ± 5^{f}
	1000	10.77	0.52 ± 0.04^{e}	55 ± 17^{ab}	74 ± 5^{f}
1.34	20	0.86	0.17 ± 0.06^{b}	77 ± 14^{cd}	89 ± 2^{kl}
	100	4.31	0.44 ± 0.06^{d}	56 ± 14^{ab}	70 ± 5^{e}
	300	12.39	0.56 ± 0.04^{e}	52 ± 12^{a}	70 ± 5^{de}
	1000	43.09	0.72 ± 0.07^{f}	49 ± 15^{a}	67 ± 7^{cd}
2.00	20	4.8	0.30 ± 0.04^{c}	63 ± 12^{abc}	78 ± 3^{g}
	100	9.6	0.57 ± 0.05^{e}	50 ± 11^{ab}	65 ± 7^{bc}
	300	28.8	0.72 ± 0.07^{f}	48 ± 18^{a}	64 ± 4^{b}
	1000	96	0.98 ± 0.09^{g}	47 ± 18^{a}	58 ± 5^{a}

Means ± standard deviation. $^{a\text{-}m}$Different superscripts in the same columns represent significant differences ($p < 0.05$) among the samples established from LSD intervals.

Therefore, both Z and shearing force of treated samples depended on the E applied. However, the relationship among them was not linear, as can be observed in Fig. 2a, which indicated that the influence of E on each parameter was different. Thus, at Z values below 0.5, the increase in Z was followed by a significant decrease in the shearing force. However, above this value, the increase in Z did not correspond with a decrease in the shearing force, which oscillated around a plateau value of 50 N. This fact will indicate that the main effects of the PEF treatment on the texture of the butternut squash samples took place at lower levels of E than in the case of Z. In other words, low intense PEF treatments can induce low Z levels but significant effects on shearing force, and therefore, on texture of samples.

3.3. *Water holding capacity*

The integrity of vegetable cells is mainly maintained by polysaccharides that contribute to their water holding capacity (WHC) [31]. The application of pulsed electric fields can cause changes in these macromolecules, thereby impacting their ability to hold water. In the case of butternut squash, the PEF treatments affected the WHC significantly ($p < 0.05$), as shown in Table 1. Thus, the WHC of fresh samples (94 %) decreased whichever PEF treatment was considered. Genovese et al. [32] stated that the permeabilization of the cell membrane modifies the structure of the cells, causing changes in the intra and extracellular volume. In this way, the pores formed in the membrane would lead to the release of cytoplasm from the cell and the expulsion of water from the sample when applying a centrifugal force. Thus, the greater the degree of electropermeabilization caused by the electrical treatment, the greater the loss of cell turgor and the weaker the WHC. In this sense, the increase in both the electric field strength and the number of pulses induced a decrease in WHC. However, it must be highlighted that for each electric field strength tested, the main reduction in WHC was observed when the number of pulses increased from 20 to 100. Above 100 pulses, this reduction was almost negligible ($p < 0.05$). This was linked to the total energy input (E), and, similarly to the shearing force, it was observed that the decrease in WHC was significant at low levels of E. Later, when applying greater values of E, it reached a value close to 65 % and slightly changed (Table 1). These findings agree with those of Redondo et al. [33] for peach samples, where PEF treatment increased the release of intracellular content until it reached a certain level of electric intensity applied; above it, no significant changes in the liquid

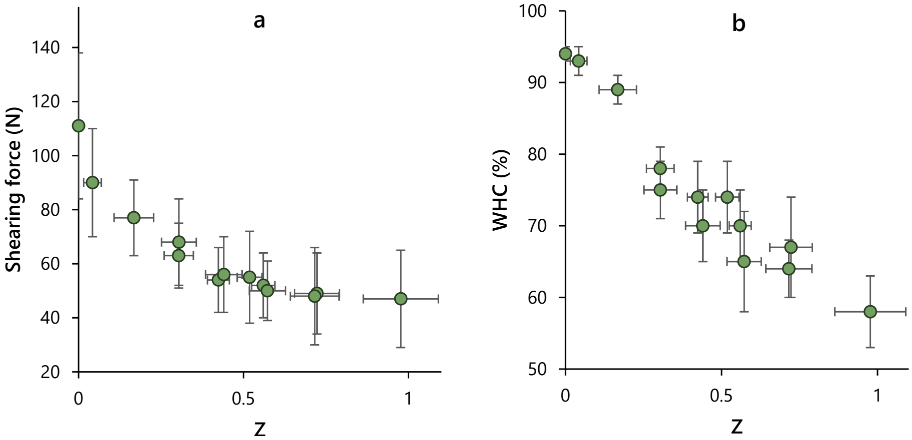

Fig. 2. Relationship between Z index and shearing force (a) and water holding capacity (WHC) (b).

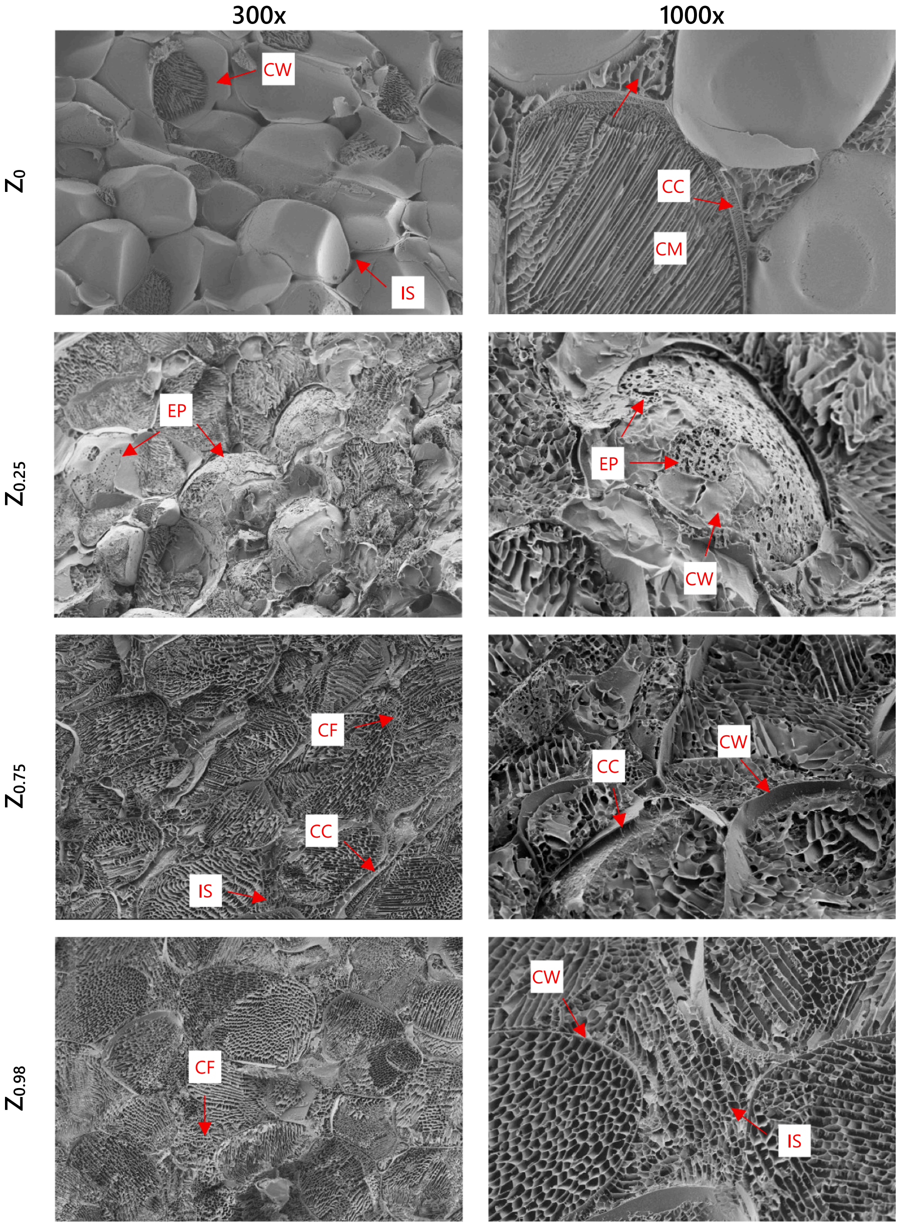

Fig. 3. Field Emission Scanning Electron Microscopy micrographs of control (Z_0) and PEF-treated (Z indices of 0.25, 0.75, and 0.98; $Z_{0.25}$, $Z_{0.75}$ and $Z_{0.98}$, respectively) butternut squash samples. IS: intercellular space; CC: cellulosic cements; CM: cell membrane; CW: cell wall; EP: electroporation; CF: cell fusion.

released were observed.

Therefore, as in the case of shearing force and Z, the influence of PEF treatment on WHC depended on the E applied. In this case, the relationship between Z and WHC was almost linear, as can be observed in Fig. 2b. This indicated that the influence of energy input in both parameters was similar. Thus, the level of electroporation and cell membrane damage induced by PEF was directly related to the capacity of the matrix to hold water. This relationship between the Z and WHC has also been previously described by other authors when studying the electrical treatment of mushroom stalks [34] or orange peels [35].

3.4. Microstructure

Butternut squash is characterized by having parenchymatic tissue composed of cells with small intercellular spaces [36]. As shown in the micrographs obtained by cryo-FESEM (Fig. 3), the fresh untreated samples (Z_0) presented swollen and turgid cells, closely united to each other and of homogeneous shape and size. Inside the cell, the observed cytoplasm was surrounded by the cell membrane that was perfectly adhered to the cell wall by cellulosic cements. In general, the parenchyma of untreated samples exhibited a high level of integrity of the membranes, cell wall, and middle lamella.

PEF application ($Z_{0.25}$, $Z_{0.75}$ and $Z_{0.98}$) modified this original structure of the butternut squash (Fig. 3). The PEF treatment induced a loss of cell turgor due to chemical imbalances generated in the cell membrane. This caused the cell walls to warp, losing their original shape. In $Z_{0.25}$ samples, a partial dissolution of the cellulosic cement occurred, leading to the separation of the cell wall from the plasmalemma in some regions. At this level of PEF treatment, it was possible to appreciate the electroporation generated on the cell membrane surface (Fig. 3), which appeared perforated, indicating the presence of numerous pores. Under more intense PEF conditions ($Z_{0.75}$ and $Z_{0.98}$) it was not possible to identify this electroporation since the cell membrane was notably degraded. The cytoplasm was only surrounded by cell walls that became very thin. This would explain the decrease in the shearing force values found when the intensity of the PEF treatments increased. Li et al. [37] stated that the changes produced in the texture are closely related to microstructural tissue damage.

In addition, it could also be observed that the intercellular spaces grew in size as the rate of cell disintegration increased. This phenomenon may stem from the breakdown of the peptide components of the middle lamellae that favored the separation of the walls of adjacent cells [38] and the liberation of intracellular contents [11]. This release of electrolytes increased the electrical conductivity which corresponded with the highest Z values obtained. Consequently, cell fusion occurred in localized regions, which lost their individuality, generating a disordered and stacked tissue. Similarly, when analyzing cryo-FESEM micrographs of PEF-treated potatoes, Zhang et al. [39] reported that an increase in the electric field strength applied produced a greater modification of the matrix. Samples treated at the highest electric field strengths tested (5–20 kV/cm) exhibited a complete collapse of the structure. When treating apple samples with PEF (125–300 V/cm), Trusinska et al. [40] also observed that the samples treated with the higher electric field strengths exhibited significantly greater tissue damage.

Finally, the micrographs of the samples treated at $Z_{0.75}$ and $Z_{0.98}$ were quite similar, which agrees with the similar results previously found in the shearing force test and WHC analysis. PEF had a significant effect on the butternut squash matrix under mild treatment conditions, but after a certain threshold, once the cell membrane had been disintegrated and the cellulosic cements had dissolved, no major changes were observed.

3.5. Drying kinetics

To evaluate the influence of PEF application on the drying of butternut squash, two PEF pretreatments of differing intensities were selected based on the previously obtained results (Z, index, shearing force, WHC and microstructure); specifically, a low intensity treatment resulting in a Z value of 0.25 ($Z_{0.25}$) and a high intensity treatment resulting in a Z value of 0.75 ($Z_{0.75}$). Thus, $Z_{0.25}$ samples registered a shearing force of 71 ± 7N and a WHC of 86 ± 4 %, while $Z_{0.75}$ registered 48 ± 10 N and 58 ± 5 %, respectively. Then, conventional and ultrasound-assisted drying experiments were carried out on control and PEF-treated butternut squash samples (Fig. 4). In every case, the drying kinetics presented a fast, almost linear decrease in the moisture content in the early stages of the process. This could be attributed to the presence of free water at the sample surface due to the high initial moisture content of the samples (10.21 ± 0.06 kg water/kg dm) and the low air velocity applied (1 m/s). The velocity of the moisture content reduction slowed down the longer the processing lasted, and the length of the process varied according to the conditions used. Thus, compared with the control sample (Z_0), the application of PEF pretreatment shortened the drying time by 15.7 and 16.9 % in $Z_{0.75}$ and $Z_{0.25}$ samples, respectively. The cell membrane alterations induced by the application of PEF to the plant matrix could potentially enhance water diffusion and, thus, increase the drying rate [41]. Some studies reported that an increase in the cell disintegration index, which indicates greater electropermeabilization, can increase the drying rate [42–44]. However, this was not observed when drying butternut squash since the drying time differences between $Z_{0.25}$ and $Z_{0.75}$ samples were not significant ($p < 0.05$). As the cryo-FESEM micrographs demonstrated (Fig. 3), the level of electropermeabilization of the cell membranes of the $Z_{0.75}$ samples was greater than those of the $Z_{0.25}$. This could produce a greater release of the intracellular content and make moisture diffusion easier. However, the more intense treatment could also induce a greater structural disorder, hindering the movement of water within the matrix. In this sense, some authors have reported no significantly different drying rates in samples pretreated using different intensities of PEF [45,46], or even slower drying rates when applying more severe PEF treatments [47]. Therefore, the enhancement of the drying process when applying PEF would not be directly related to the degree of electropermeabilization induced. Indeed, the results seemed to indicate the existence of an electropermeabilization level above it, at which level the moisture transport process would not be improved but even partially prevented.

On the other hand, the application of ultrasound (Z_0 + US) reduced the drying time by 41.4 % compared with conventional butternut squash drying. The US generated a series of continuous compressions and expansions (sponge effect) of samples that could facilitate the internal movement of water molecules. At the same time, the microturbulences

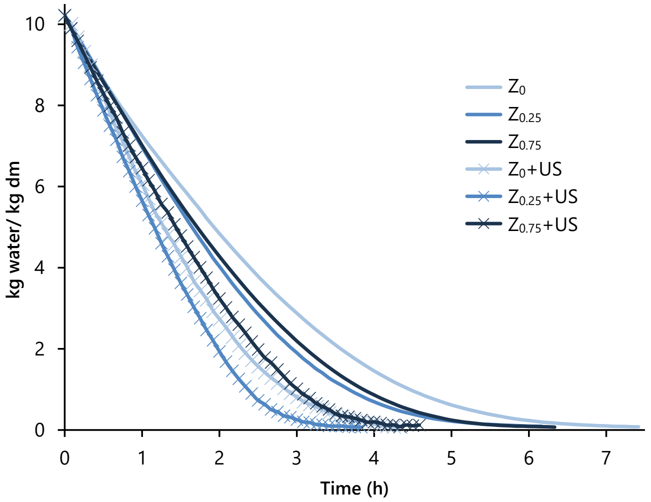

Fig. 4. Experimental drying kinetics of butternut squash samples PEF treated at different Z indices (0, 0.25 and 0.75) during conventional and ultrasound-assisted drying (US).

generated by ultrasound at the sample-air interface would permit the transport of water to the environment to be faster [48].

Regarding the combination of PEF pretreatment and ultrasound-assisted drying, the influence on the drying time depended on the PEF intensity considered. In the case of the $Z_{0.75}$ + US samples, a time reduction of 38.2 % was achieved compared to the control sample, a reduction which was more limited than that achieved by the ultrasound-assisted drying of non-PEF pretreated samples (Z_0 + US). The likely explanation for this could be an excessive PEF effect in the structural matrix. As observed in the shearing force tests, the more intense PEF treatments could lead to a softer cell matrix and a partially collapsed cellular structure, which may lead to a highly disorganized microstructure that did not enhance but partially hindered the ultrasound effects on water release. On the contrary, the maximum drying time reduction compared to the control sample (47.2 %) was obtained when milder PEF conditions were applied ($Z_{0.25}$ + US). Under these conditions, the moderate changes induced by PEF in the matrix structure could enhance the ultrasound effects during drying. Water molecules would not only move more easily through the pores generated during the electrical pretreatment but also through the microchannels generated by ultrasound mechanical stress. Therefore, ultrasonic drying might benefit from a mild PEF treatment.

3.6. Modeling

The Weibull model provided an adequate description of the drying kinetics of butternut squash slices, with the %Var values obtained in every case being over 99.7 %. As can be observed in Table 2, the identified α values were higher than 1.0 in every condition under study, which would indicate process downtime. No significant ($p < 0.05$) differences were found between the α values identified for the conventional drying of Z_0, $Z_{0.25}$, and $Z_{0.75}$ samples. This suggests that the PEF treatment did not affect the behavior of butternut squash during drying. However, the α values were significantly ($p < 0.05$) higher when combining PEF pretreatments and ultrasound-assisted drying. This could indicate that the changes induced in the samples by the PEF treatment could generate a structure that is more prone to US effects. Indeed, previous studies have reported that PEF can alter some mechanical and acoustic properties of plant matrices (Wiktor et al., 2016, 2018), which can facilitate US propagation.

The other parameter of the Weibull model, β, as stated in the material and methods section, is a kinetic-related parameter that has a reverse relationship with the drying rate; so, a decrease in this parameter indicates faster drying. In this sense, the values identified in the case of the PEF pretreated samples ($Z_{0.25}$ and $Z_{0.75}$) were significantly ($p < 0.05$) lower than those of the Z_0 samples (Table 2), meaning faster drying kinetics. As stated before, the electropermeabilization of the cells would make the movement of free water easier, increasing the drying rate [41]. As regards the samples dried with ultrasound assistance, the β values identified were significantly ($p < 0.05$) lower than those previously described (Z_0, $Z_{0.25}$ and $Z_{0.75}$). The mechanical effects of ultrasound were able to decrease the internal and external resistance to mass transfer, increasing the drying rate and thereby decreasing the β values [49]. Furthermore, it was the combination of the moderate PEF treatment and ultrasound application during drying ($Z_{0.25}$ + US) that provided easily the lowest β value (37.1 % lower than the control sample), indicating, once again, that the combination of both technologies in these conditions produced a synergistic effect on the drying rate. The application of the highest intensity PEF treatment ($Z_{0.75}$ + US) provided a significantly ($p < 0.05$) greater value of β, which meant a significantly lower drying rate. Therefore, as a whole, it can be stated that the application of a PEF pretreatment at adequate intensity can intensify the ultrasound effects when applied during drying.

Table 2
Weibull model parameters (α and β) identified for the conventional and ultrasound-assisted (US) drying of butternut squash treated under different PEF conditions (Z index of 0, 0.25 and 0.75). Percentage of explained variance (% Var) reached by the model.

Drying conditions	α	β	% Var
Z_0	1.32(0.03)^a	8457(313)^d	0,9985
$Z_{0.25}$	1.33(0.07)^a	7432(74)^c	0,9989
$Z_{0.75}$	1.34(0.03)^a	7589(171)^c	0,9980
Z_0 + US	1.38(0.03)^{ab}	6036(269)^b	0,9972
$Z_{0.25}$ + US	1.49(0.03)^c	5318(325)^a	0,9968
$Z_{0.75}$ + US	1.44(0.04)^{bc}	6308(267)^b	0,9975

Means ± standard deviation. $^{a\text{-}d}$Different superscripts in the same columns represent significant differences ($p < 0.05$) among the samples established from LSD intervals.

4. Conclusions

The energy input (E) applied during pulsed electric field (PEF) treatments affected physicochemical and microstructural characteristics of butternut squash. Thus, the greater the E, independently of the number of pulses or the electric field strength used, the greater the Z and the lower the shearing force and the WHC. The relationship between Z and WHC was almost linear, which indicated a similar influence of the E on both. On the contrary, the no linear relationship between Z and shearing force meant that the low levels of Z induced at low E levels could be linked to significant changes in the butternut squash texture. The analysis of the microstructure also showed a progressive effect of PEF on the cell structure until it reached a high membrane degradation level, above which no major changes were observed.

The independent application of PEF, as pretreatment, or ultrasound, during drying produced reductions in drying time, which were more significant in the case of ultrasound. However, combining both techniques was the alternative that shortened the drying process the most. In this case, the intensity of the PEF applied, and therefore, the extension of structural changes induced was significant. Thus, the shortest ultrasound-assisted drying was found at mild PEF pretreatments (Z of 0.25), reducing drying time by nearly half. Therefore, when the PEF pretreatment was applied at adequate conditions, it generated a product structure more prone to the ultrasound effects, intensifying the moisture removal and making ultrasound-assisted drying more viable for low-porosity products.

CRediT authorship contribution statement

B. Llavata: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **A. Quiles:** Writing – review & editing, Methodology, Investigation. **C. Rosselló:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Formal analysis. **J.A. Cárcel:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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