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**Observations on the water distribution and extractable sugar content in carrot slices after pulsed electric field treatment**

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**ABSTRACT**

The impact of pulsed electric field (PEF) processing conditions on the distribution of water in carrot tissue and extractability of soluble sugars from carrot slices was studied. Time domain NMR relaxometry was used to investigate the water proton mobility in PEF-treated carrot samples. Three distinct transverse relaxation peaks were observed in untreated carrots. After PEF treatment only two slightly-overlapping peaks were found; these were attributed to water present in the cytoplasm and vacuole of carrot xylem and phloem tissues. This post-treatment observation indicated an increase in water permeability of tissues and/or a loss of integrity in the tonoplast. In general, the stronger the electric field applied, the lower the area representing transverse relaxation ($T_2$) values irrespective of treatment duration. Moreover an increase in sucrose, J3- and a-glucose and fructose concentrations of carrot slice extracts after PEF treatment suggested increased both cell wall and vacuole permeability as a result of exposure to pulsed electric fields.

KEYWORDS: carrot, pulsed electric fields, TD-NMR relaxometry, soluble sugar

## 1. INTRODUCTION

The application of pulsed electric fields (PEFs) to intact plant tissues is receiving considerable attention for their potential in enhancing extractability of intracellular metabolites (Donsì, Ferrari, & Pataro, 2010), drying efficiency (Lebovka, Shynkaryk, & Vorobiev, 2007) or juice extraction yields (Grimi et al., 2007; Jaeger et al., 2012). PEF processing involves the application of short pulses of moderate electric fields (0.5-5 kV/cm) to food materials placed between the two electrodes. PEF effects are explained by the phenomenon of electroporation, i.e. a significant increase in the electrical conductivity and permeability of the cell plasma membrane caused by an externally applied electrical field (Toepfl, Heinz, & Knorr, 2005). In the presence of pulsed electric field biological membranes are electrically pierced and lose their semi-permeability either temporarily or permanently through the formation of induced pores. These pores permit the passage of molecules of various sizes from inside the cell to the surrounding extra-cellular liquid (Weaver & Chizmadzhev, 1996). Hence, the effects of PEF treatment depend mainly on the number and size of induced pores, which vary with the relevant electric parameters used, i.e. electrical field strength, number of pulses and pulse duration (Bouzrara & Vorobiev, 2003; López et al., 2009). Little information is available regarding the reversible or irreversible structural changes arising in plant tissues during and after the application of PEF. The degree of membrane disruption has been estimated by the cell disintegration index which measures the frequency-dependent passive electrical properties of biological cell systems (Toepfl et al., 2005; Angersbach, Heinz, & Knorr, 1999).

Both cell walls and membranes disruption can also be estimated by time-domain nuclear magnetic resonance (TD-NMR) water proton relaxometry. The measured proton spin-lattice ($T_1$) and spin-spin relaxation times ($T_2$) are related to water

content, physical properties of water and interaction of water with macromolecules (Van As, 1992). In plant cells proton relaxation time is often a multi-exponential process indicating the presence of multiple water compartments with different relaxation times (Belton & Ratcliffe, 1985). To date, only Ersus et al. (2010) have reported the use of NMR relaxometry to track changes in cell integrity in PEF-treated onion tissue. TD-NMR relaxometry has also been used to explore the effect of high pressure or freeze-drying treatments on the integrity of cells of strawberries, tomatoes and carrots (Hills & Remigereau, 1997; Marigheto et al., 2004; 2009; Voda et al., 2012). PEF has also been used in softening of the sugar beet tissues in order to enhance sugar extractability from the sugar beets (Bouzrara & Vorobiev, 2003; López et al., 2009; Eshtiaghi & Knorr, 2002). Eshtiaghi and Knorr (2002) indicated that a cell disintegration index increased with increasing field strengths (over the range from 1.2 to 3.6 kV/cm) when processing sugar beet. Aguiló-Aguayo et al. (2014) reported that processing carrot purees at 1 kV/cm for 2 ms increased concentrations of sucrose and J3-glucose in 43 and 48%, respectively. Thus, the observed enhancement in sugar extractability seems to arise from PEF processing conditions that induce electroporation.

Therefore, the objectives of this work were (a) to monitor PEF-induced changes in the integrity of carrot both cell walls using TD-NMR relaxation measurements and (b) to investigate the influence of such changes on soluble sugar extractability.

## 2. MATERIAL AND METHODS

### 2.1. Chemicals

Deuterium oxide ($D_2O$ 99%), 3-(trimethylsilyl)propoinic acid-$d_4$ (TSP) sodium salt, fructose, sucrose, J3-glucose, a-glucose, methanol and water of HPLC grade (99%) were obtained from Sigma-Aldrich (Arklow, Ireland).

**2.2. Preparation of carrot slices**

Carrots (*Daucus carota* cv. Nerac) were purchased in a local market (Tesco, Dublin, Ireland) in May of 2013Unpeeled carrots were washed with tap water and cut into slices of 1 cm thickness retaining the peel. Only slices of around 3.6 ± 0.2 cm diameter were used for processing under PEF conditions; slices with smaller diameter were discarded. Carrot slices selected for the experiment consisted of a central stele (mostly vascular tissue) and a peripheral cortex layer. The unprocessed carrot slices have an electrical conductivity of 0.015 ± 0.002 S/m.

**2.3. PEF conditions**

PEF equipment used in this investigation was an ELCRACK® HVP 5 unit (DIL, Quakenbrück, Germany) working in batch mode. A parallel-plate treatment chamber consisting of two stainless steel electrodes (total electrode area of 16 $cm^2$ and gap of 1 cm) was used. Samples were in contact with electrodes where the area of carrot-electrode contact point was 10.20 ± 1.13 $cm^2$. The apparatus was set to generate square waveform pulses at 20 Hz frequency in bipolar mode. Peak pulse voltage was varied between 1 and 4 kV resulting in electric field strengths of 1 to 4 kV/cm. A series of 100, 300 and 500 pulses (pulse width of 20 ts each) were applied at room temperature corresponding to specific energy inputs (W) from 0.20 to 15.09 kJ/kg (Table 1). The treatment time was calculated by multiplying the pulse width (τ) by the number of pulses applied which corresponded to values from 2 to 10 ms.

Temperature of carrot purees after each PEF treatment was recorded with fibre optic temperature probes (Lumasense technologies Fluoroptic® Temperature probe, USA). Measurement of temperature was taken directly on the sample before and after each treatment which did not exceed beyond 30ºC.

The specific energy (W) depends on the voltage applied, treatment time, and resistance of the treatment chamber that varies according to the geometry and

conductivity of the material treated (Heinz, Alvarez, Angersbach & Knorr, 2001). The specific energy per pulse (W´) was calculated by following equation 1:

$$W' = \frac{1}{t} \int_{0}^{t} K \cdot E_{(t)}^{2} dt \quad \text{.....(1)}$$

where $\rho$ (kg/m$^3$) is the density of the treated product; K is the electrical conductivity (S/m), E (V/m) is the electric field strength; and $t$ (s) is the pulse width. The total specific energy applied (W) was calculated by multiplying the energy per pulse (W´) by the number of pulses.

Three batches of fifteen carrot slices (n=15) were exposed to each PEF treatment level. From each batch, 5 carrot slices were kept separately for TD-NMR relaxation measurements. The rest of the samples were frozen in a blast freezer (Avon Refrigeration Co., Bristol, UK) operating at -30ºC at an air velocity of 8m/s until frozen (2 h) and then stored at -80ºC freezer prior to lyophilisation. Lyophilisation was achieved in a freeze-drier (D80 Leanne freeze drier, Cuddon, New Zealand) at 30°C and at 0.2 MPa for 18 hours. Untreated carrot slices were used as controls in all the experiments.

**2.4. Water proton mobility by time domain nuclear magnetic resonance (TD-NMR) relaxometry**

TD-NMR relaxometry measurements were carried out on a MQC23-benchtop instrument (Oxford Instruments, Abington, Oxfordshire, UK) with a resonance frequency for protons of 23.2 MHz. Cylindrical plugs from the inner regions of PEF-treated and untreated carrot slices were removed using a cork borer (10 mm diameter). Four such plugs per batch were neatly layered in an NMR tube for analysis and covered externally with stretched Parafilm-M to avoid dehydration of the samples. Samples were equilibrated to room temperature (~25 °C) prior to analysis. NMR data for a total of nine replicates per treatment were recorded. Transverse relaxation times ($T_2$) were measured using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence

and the resultant relaxation decays analysed by tri-exponential fitting in the RI Win-DXP software (V. 1.2.3 Oxford Instruments, Abington, Oxforshire, UK).

### 2.5. Quantitative determination of soluble sugars in carrot juice by proton nuclear magnetic resonance ($^1$H-NMR) spectroscopy

Soluble sugars were extracted from freeze-dried carrot slices (10 mg) using a methanol:water (4:1 v/v) mixture (Macedo & Peres, 2001). Extracts were centrifuged at 14,000 rpm for 2 min. The supernatant (0.5 mL) was taken and dried under $N_2$ at room temperature. The dried extract was then resuspended in 0.6 mL $D_2O$ phosphate buffer (400 mM salt concentration with a pD value of 6.0) and 5 µL 1 % solution of 3-(trimethylsilyl) propoinic acid-$d_4$ (TSP) as an internal standard (Cazor et al., 2006). Each sample was then vortexed and transferred into an NMR tube (5 mm internal diameter) for spectral data acquisition.

$^1$H-NMR spectra were recorded on a Bruker™ Avance Spectrometer (Coventry, UK) at 500.162 MHz and 300 K using a 5 mm PABBO Broad Band Observed probe. Spectra were acquired using a water suppression pulse sequence (NOESYGPPR1D: RD-30°-t1-30°-tm-30°-acquire) set with a 4 µs 30° pulse, 10 ms mixing time (tm) and 10 s relaxation delay (RD). Eight scans (n=8) of 64 k data points each were collected. The spectral width was 6009.615 Hz and acquisition time was 5.45 s. All the acquired spectra were line broadened (0.5 Hz), manually phased and baseline corrected using MestReNova v 6.0.2 (MestreLab Research, Santiago de Compostela, Spain) Baseline correction was carried out automatically using a procedure based on the Whittaker smoother algorithm. All spectra were then aligned with the TSP signal at δ 0.00 ppm. Each major constituent sugar was identified in control and PEF-treated samples by peak assignment using $^1$H-NMR spectra by comparison to published data (Cazor et al., 2006; Sobolev, Segre, & Lamanna, 2003; Sobolev et al., 2005) and by spiking the

sample with standard compounds. Proton chemical shifts used for identification of fructose, sucrose, J3-glucose and a-glucose in untreated and PEF-treated samples are shown in Table 2.

The peak area for each signal obtained was integrated using classical integration and normalised with respect to the $^{1}H$ contents of the internal standard TSP and the sugar signals respectively. Concentrations of fructose, sucrose, J3-glucose and a-glucose in the individual NMR spectrum were calculated using Equation 2:

$$Sugar_{NMR} = \frac{A_S}{TSP} \cdot \frac{100}{n_p} \quad \text{.....(2)}$$

where $A_S$ corresponds to the normalised area of the studied sugar; $n_p$ to the $^{1}H$ amount assigned for each sugar (Table 2); [TSP] to the concentration of TSP (mM); [$Sugar_{NMR}$] to the concentration of the specific sugar (mM). This value was then converted into concentration of the specific sugar per g of dry weight (DW) of carrots. Analyses were carried out in triplicate and average values are reported.

**2.6. Data analysis**

Statistical analysis was performed using IBM SPSS Statistics (version 19; IBM Corp., Sommers, New York). Data were analysed by analysis of variance (ANOVA) and means separation was achieved using the Duncan multiple range test. Principal component analysis (PCA) of the $^{1}H$-NMR data was conducted using The Unscramber X (v. 10.0; CAMO Software AS, Oslo, Norway). Spectral data binning for PCA was carried out at an interval of 0.04 ppm. Binned spectra were scaled using auto-scaling. Following this the spectra (signal intensity as a function of frequency) were imported into The Unscrambler. Regions of the NMR spectra that contained signal originating from the deuterated water ($D_2O$) and TSP were excluded from

subsequent analysis. Hence the regions between 0.5-6.00 ppm were submitted for principal component analysis.

## 3. RESULTS AND DISCUSSION

### 3.1. Water mobility by TD-NMR relaxometry in PEF-treated carrot slices

Water distribution in the inner carrot regions from untreated and PEF-treated samples was studied by TD-NMR. Inner regions of carrots included vascular and part of ground tissues while the vascular tissues may be further divided into two sections: xylem and phloem (Esay, 1940). Xylem is primarily made of thick and rigid vessels responsible for water and mineral transportation while phloem is primarily made of large, thin-walled, tightly-packed parenchyma cells which mainly transport nutrients. Ground tissue is composed of carrot cortex which is largely composed of parenchyma cells and may store sugars or starches (Esay, 1940). Three transverse relaxation times were identified in raw unprocessed carrot slices, i.e. $T_{2a}$, $T_{2b}$ and $T_{2c}$ (Table 3 and Figure 1A). The peak with the shortest $T_2$ value $(T_{2a})$ was observed at 10.3 ms suggesting that it may have arisen from the more rigid components of the cell walls in the carrot tissues. $T_{2b}$ occurred at the longer relaxation time of 54.2 ms and may be associated with water in the cytoplasm and extra-cellular compartments. In cytoplasm, some of the water is hydrogen-bonded to side chains of the proteins forming the framework of the protoplasm (Kramer, 1983). $T_{2c}$ was the third and largest peak centred on the considerably longer transverse relaxation time of 318 ms (Table 3 and Figure 1A). In plant cells, 50-80% or more of the water occurs in the vacuoles (Kramer, 1983). Thus peak $T_{2c}$ may be associated with water in cell vacuoles. $T_{2a}$ was the sharpest peak followed by $T_{2b}$ while $T_{2c}$ is a broad peak while the peak heights increased in the order $T_{2b}<T_{2a}<T_{2c}$. Previous studies on water mobility in rehydrated freeze-dried carrots also identified three transverse relaxation components at similar

relaxation times in the rehydrated cortical tissue of carrots (Voda et al., 2012). Marigheto, Venturi et al. (2008) reported four relaxation peaks between 67-1090 ms in apple parenchyma tissues which these authors interpreted as water located within different cell compartments while admitting that the assignment to particular sub-cellular compartments is somewhat problematic because of acknowledged biological diversity in cell sizes and shapes.

The sharp $T_{2a}$ peak in the relaxation spectrum of untreated raw carrot was reduced but broadened in the PEF-treated carrot slices as illustrated in Figure 1. In untreated samples, the sum of areas corresponding to transverse relaxation times $T_{2b}$ and $T_{2c}$ was considered as the $T_{2b'}$ peak area. Moreover the well-defined $T_{2b}$ and $T_{2c}$ peaks in the relaxation spectrum of untreated samples merged together as $T_{2b'}$ in the $T_2$ spectrum of PEF-treated carrot slices (Figure 1B). Overlapped peaks in the spectra of PEF-treated samples could be the result of a greater degree of diffusion resulting from an increase in the permeability and/or a loss of integrity of the tonoplast that forms the barrier between vacuole and cytoplasm (Zhang & McCarthy, 2012). Among PEF-treated samples, $T_{2b'}$ peaks were reduced and broadened after PEF application of the highest energy inputs in this study (4 kV/cm for 10 ms) (Figure 1B). This proton population might reflect essentially the dynamics of the water molecules proportionately to the pore size distribution in PEF-treated samples. In contrast, thicker (broader) and lower amplitude $T_{2c}$ peaks were observed from untreated carrot samples reflecting the lower water content and subsequently the lower water mobility (Figure 1A).

Figure 2 shows how the relative area under the water proton relaxation peaks at $T_{2a}$ and $T_{2b'}$ in untreated and PEF-treated carrot slices varied with applied field strength. Initially the relative area of $T_{2a}$ in unprocessed raw carrot tissues was 3.77%; and as a

general trend this percentage significantly decreased ($p<0.05$) with increasing applied electric fields irrespective of the treatment time used (Figure 2A). This trend suggests that the PEF conditions applied in this work have reduced the proportion of water-bound cell wall structures due to electroporation. The water in cell wall is present in very small pores with strong water-binding sites held by matrix and molecular forces (Dunn, 2001). With the exception of the 1 kV/cm treatments, the highest proportion of changes in $_{T2a}$ relative area occurred using the shortest (2 ms) treatment time. $T_{2a}$ relative areas were significantly ($p<0.05$) lower in samples treated at 4 kV/cm compared to samples treated at 1 kV/cm. Moreover relative areas observed in samples treated at 2, 3 and 4 kV/cm converged to a single data point at the longest treatment time (10 ms) since no significant differences ($p>0.05$) in $_{T2a}$ values of these samples were observed. Relative $_{T2b'}$ peak areas greater than 96% were observed in untreated and PEF-treated carrot slices (Figure 2B). In general these relative $_{T2b'}$ peak areas increased significantly ($p<0.05$) with increasing electric field reaching a maximum of 98.15% in carrot slices treated with 4 kV/cm for 2 ms. A similar pattern to that of the $_{T2a}$ peak areas was also observed for the $_{T2b'}$ peak areas where larger change in relative peak area occurred at the shortest treatment time except for the 1 kV/cm treatments. Peak area percentage values converged to a single point after 10 ms treatment time. These trends indicate that water moves from vacuoles to cytoplasm and extracellular space upon application of these PEF conditions. Cell membrane, tonoplast and plasma membrane are boundary layers that regulate water and solute movement in plant cells (Zhang & McCarthy, 2012). Previous studies have found that plant tissue disruption under PEF treatments can be achieved within 0.1 to 10 ms at electric field strengths between 0.5 to 5.0 kV/cm (Lebovka, Bazhal, & Vorobiev, 2001; 2002; Angersbach, Heinz, & Knorr, 2000). Significant membrane breakdown

in potato and apple tissues has been observed when electric field strengths applied increased from 0.4 to 0.8 kV/cm (El-Belghiti, Rabhi, & Vorobiev, 2005). Under the conditions applied in the present study, it is suggested that membranes may be considered to exist in either of the two states, i.e. normal or porous, with little suggestion that degrees of porosity occur with increasing specific PEF energy input. Values of the main $_{(T2c)}$ component of untreated carrot slices were generally higher than the corresponding $_{T2b'}$ peak in the PEF-treated samples (Table 3). This probably arises from the intact vacuole which will have greater amplitude in the relaxation spectrum. To date, only Ersus et al. (2010) have carried out relaxation time measurements in PEF-treated intact plant tissues. They observed a significant decay of $T_2$ signals in onion tissues treated at 0.5 kV/cm for both 0.5 and 5 ms in comparison to untreated samples indicating that cells were significantly damaged. These findings are consistent with the behaviour observed in this study for $_{T2b'}$ in PEF-treated carrot slices; this further supports the assumption that the vacuoles from vascular tissue of carrots were significantly affected by PEF treatments (Figures 2B). On the other hand, Ersus et al. (2010) have also reported that the presence of the same number of relaxation peaks in PEF-treated onions as in untreated samples but with shorter relaxation times which would show that there is slow exchange of water by molecular diffusion; this implies that there are still significant permeability barriers to water exchange (Hills, & Remigereau, 1997). In PEF-treated carrot slices, the compromised integrity of both cell membranes and vacuoles and probably the faster water diffusion seems likely to be the main reason for the new water redistribution. From these observations it could be drawn that each relaxation time could be proportional to the pore size and higher number of pores.

According to Voda et al. (2012), hydrogen exchange between water and sugar protons can also reduce the transverse relaxation time of the main $T_2$ population. Although diffusion of water between compartments in PEF-treated samples is notably enhanced, small differences between PEF treatments do exist and could be attributed to differences in sugar concentration. Vacuolar fluid contains a relatively high sugar content of around 15% $^{w}/_{w}$ and it is expected that relaxation time would show a relationship with sugar concentration. In fact model aqueous solutions revealed a reduction in $T_2$ of up to 1.5 s as compared to pure water for a native sugar concentration in carrots ranging from 5 to 20% $^{w}/_{w}$ (Voda et al., 2012). Therefore a study of soluble sugars has been performed with a view to clarify the impact of PEF-treated in possible sugar exchange between sub-cellular compartments.

### 3.2. Qualitative and quantitative determination of extractable sugars in carrot slices by $^{1}$H-NMR spectroscopy

A detailed overview of the complete set of $^{1}$H-NMR spectra of untreated and PEF-treated carrot slices (without $D_2O$ and TSP signals) was performed using principal component analysis (PCA). Principal components (PCs) 1 and 2 accounted for 95 and 3% respectively of the variance in the dataset; scores of the carrot samples in the space defined by these principal components are shown in Figure 3A. A separation of untreated and PEF-treated samples is suggested in this plot indicating that PEF processing significantly affected the content of extractable sugars in carrot slices. This is not surprising since PEF treatment has been shown to increase the permeability of both cell membranes and vacuoles as discussed above. Examination of individual principal component loadings can provide a way to uncover the identities of the molecular species responsible for the variability accounted for by the individual PCs. PC1 was sensitive to sucrose signals but other unidentified species were involved as

indicated in Figure 3B. PC2 was a more complex structure and although it reflected information on α- and J3- glucose contents, many other unidentified components also played important roles (Figure 3C). Close examination of the PCA data (Figure 3A) showed a general trend of increasing severity of process treatment from right to left although some samples did not appear to fit this observation fully, for e.g. 3 kV/cm for 2 ms. The complexity of carrot tissue response to PEF-treatment is suggested by the trend in $_{T2b'}$ values shown in Table 3 and this observation may at least partly explain the complexity of the PC scores plot (Figure 3A). In general no sample clustering in the PC1 and 2 space could be detected. This pattern remained unchanged when rerunning a new PCA without untreated samples indicating that more sugars are extracted by increasing the intensity of the PEF treatment. However, differences in the NMR spectral intensities of samples indicate that amounts of individual saccharides varied between PEF-treated and untreated samples. Therefore quantification of sucrose, α-glucose, J3-glucose and fructose at signals 4.22, 5.24, 4.65 and 4.11 ppm (Table 2) respectively with respect to the internal standard TSP was carried out (Figure 4). Sucrose was by far the most common of these sugars in unprocessed raw carrot slices with a concentration of 75.4 mmol/g of dry matter (DM), followed by J3-glucose (34.1 mmol/g DM), then a-glucose (18.4 mmol/g DM), and fructose (8.9 mmol/g DM). In general PEF processing (1 kV/cm) caused a significant increase ($p<0.05$) in the concentration of extractable sugars by approximately 125% on average at all field strengths and treatment times except sucrose which showed no significant differences ($p>0.05$) between PEF-treatments (Figure 4). Results presented above slightly contrast with those observed in previous studies in which less severe PEF conditions (from 0.15 to 0.94 kV/cm) were reported to be required to obtain measurable enhancement of soluble transfer when extracting sugar from sugar beets

(Jemai, & Vorobiev, 2002; Wouters, Bos, & Ueckert, 2001). A significant increment ($p<0.05$) in the α-glucose, β-glucose and fructose contents of carrot slices arose when treatment times increased from 2 to 6 ms at constant electric field strength of 1 kV/cm (Figures 4B, C and D). Hence increases in the initial extractability of carrot sugars ranging from 71 to 100% were achieved after applying this treatment condition. Application of longer and more intense treatments did not further enhance the α-glucose, β-glucose and fructose extraction yields as might have been expected.

## 4. CONCLUSIONS

TD-NMR relaxation spectral analysis of carrot slices gave an indication of the extent of disruption at the sub-cellular level caused by PEF processing conditions. PEF treatments induced alterations in the integrity of both cell membranes and vacuoles causing significant changes in the $T_2$ relaxation spectrum as a result of water redistribution between sub-cellular structures. Determination of soluble sugar contents by $^1$H-NMR spectroscopy confirmed higher extractability levels of sucrose, β-glucose, α-glucose and fructose in PEF-treated slices than in untreated samples. These results support the hypothesis that permeability changes occur in plant cell structures as a result of PEF treatments and suggest the feasibility of deploying this technology to enhance the extractability of valuable compounds from intact plant tissues.

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**Table 1.** Specific energy and Temperature increase associated with PEF treatments applied to carrot slices at different electric field strengths.

| Electric field (kV/cm) | Treatment time (ms) | Specific energy (kJ/kg) | Increase of temperature* |
|---|---|---|---|
| 1 | 2 | 0.20 | 0.30±0.14 |
| 1 | 6 | 0.57 | 0.40±0.10 |
| 1 | 10 | 0.94 | 1.05±0.10 |
| 2 | 2 | 0.75 | 0.55±0.21 |
| 2 | 6 | 2.26 | 0.40±0.00 |
| 2 | 10 | 3.77 | 2.25±0.21 |
| 3 | 2 | 1.70 | 1.05±0.10 |
| 3 | 6 | 5.09 | 2.20±0.14 |
| 3 | 10 | 8.49 | 3.40±0.25 |
| 4 | 2 | 3.02 | 1.50±0.14 |
| 4 | 6 | 9.05 | 3.60±0.30 |
| 4 | 10 | 15.09 | 6.40±0.50 |

Temperature of carrot slices before processing was 21.0 ± 0.50 °C. *mean of three replicates ± standard deviation.

**Table 2**. $^{1}$H NMR chemical shifts (ppm) and coupling constants (Hz) identified in untreated raw carrot slices using $^{1}$H-NMR spectra.

| No. | Compound* | $_{1}$H chemical shifts (Ö) and coupling constants (Hz) | Assignment |
|---|---|---|---|
| 1 | Sucrose | 5.44 (d, J=3.8), 4.22 (d, J=8.78), 3.79, 3.67 | CH,CH,CH,$CH_2$ |
| 2 | α-Glucose | 5.24 (d, J=3.7) | CH |
| 3 | J3-Glucose | 4.65 (d, J=8.0) | CH |
| 4 | Fructose | 4.1 1(α,J3, m) | J3(CH-3 + CH-4)+ ctCH-3 |

**Table 3.** Average (and standard deviation) values of the $T_2$ water proton relaxation times of untreated and PEF-treated carrot slices for different electric field strengths.

| E (kV/cm) | t (ms) | $T_{2a}$ (ms) | $T_{2b}$ (ms) | $T_{2c}$ (ms) | $T_{2b'}$ (ms) |
|---|---|---|---|---|---|
| **0** | 0 | 10.3 ±0.3 | 54.2 ±0.1 | 318±5 | nd* |
| **1** | 2 | 12.5±0.7 | nd | nd | 173±4 |
| | 6 | 19.2 ±0.5 | nd | nd | 204±6 |
| | 10 | 17.2±0.8 | nd | nd | 197±9 |
| **2** | 2 | 20.2 ±1.5 | nd | nd | 171±5 |
| | 6 | 23.3± 1.3 | nd | nd | 224±6 |
| | 10 | 22.5 ±0.6 | nd | nd | 217±6 |
| **3** | 2 | 25.2± 1.2 | nd | nd | 221±5 |
| | 6 | 20.5± 1.1 | nd | nd | 243 ±6 |
| | 10 | 18.9±1.0 | nd | nd | 182±6 |
| **4** | 2 | 20.3±2.0 | nd | nd | 217±5 |
| | 6 | 24.4±0.6 | nd | nd | 207 ±5 |
| | 10 | 21.2±1.1 | nd | nd | 201±5 |

*nd: not determined

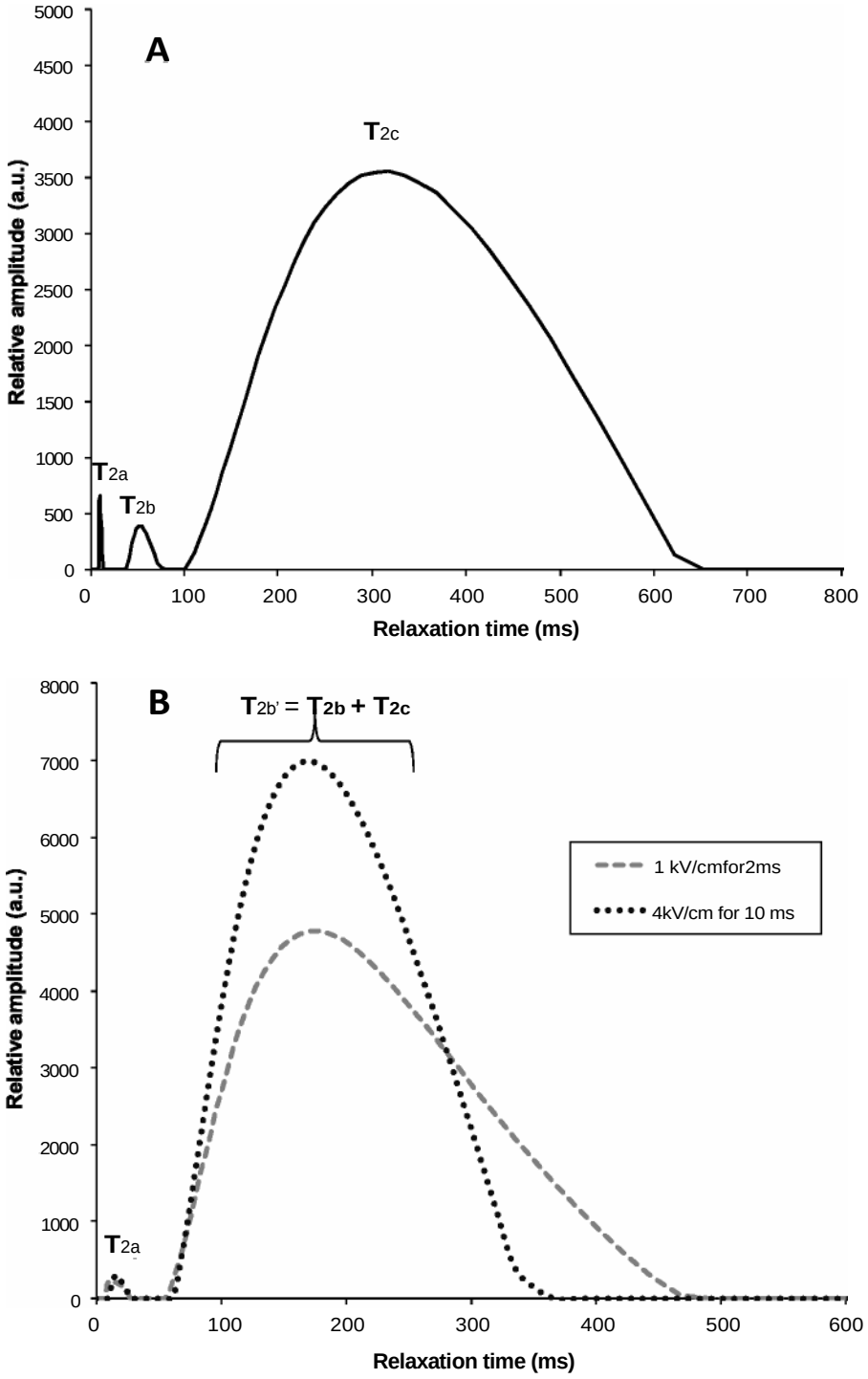


**Figure 1**. Representative distribution of $T_2$ relaxation times for untreated (A) and PEF-treated (B) (1 kV/cm for 2 ms and 4 kV/cm for 10 ms) raw carrot slices. Numbers refer to relaxation peaks of water protons in the different cell compartments: T2a, cell wall; T2b, cytoplasm and extra-cellular compartment; T2c, the cell vacuole.

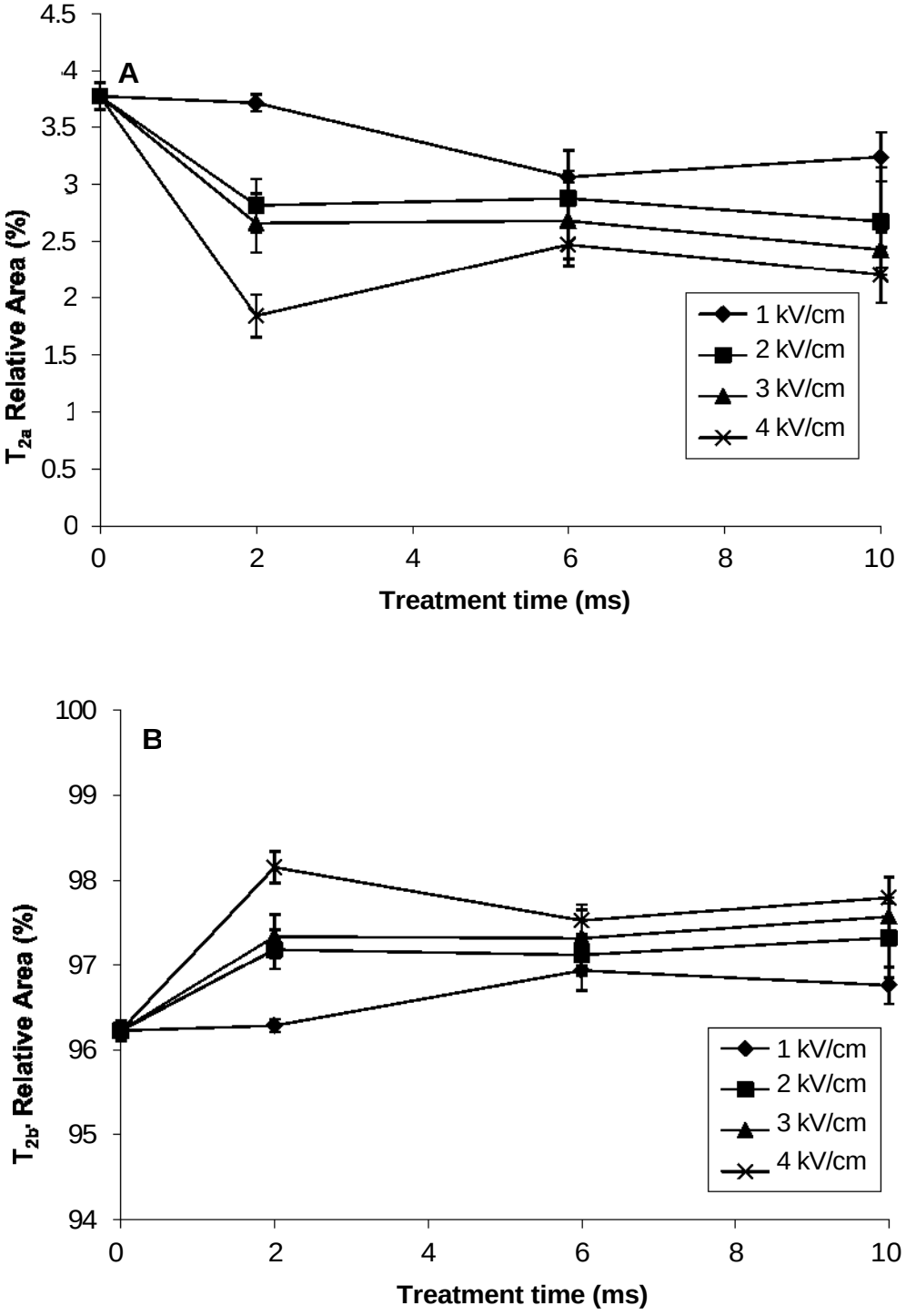


**Figure 2**. Relative area representing percentage values of the water proton relaxation times of $T_{2a}$ and $T_{2b'}$ peaks in PEF-treated carrot slices (mean of nine replicates ± standard deviation). $T_{2b'}$ area of untreated samples was considered as the sum of areas corresponding to relaxation times $T_{2b}$ and $T_{2c}$.

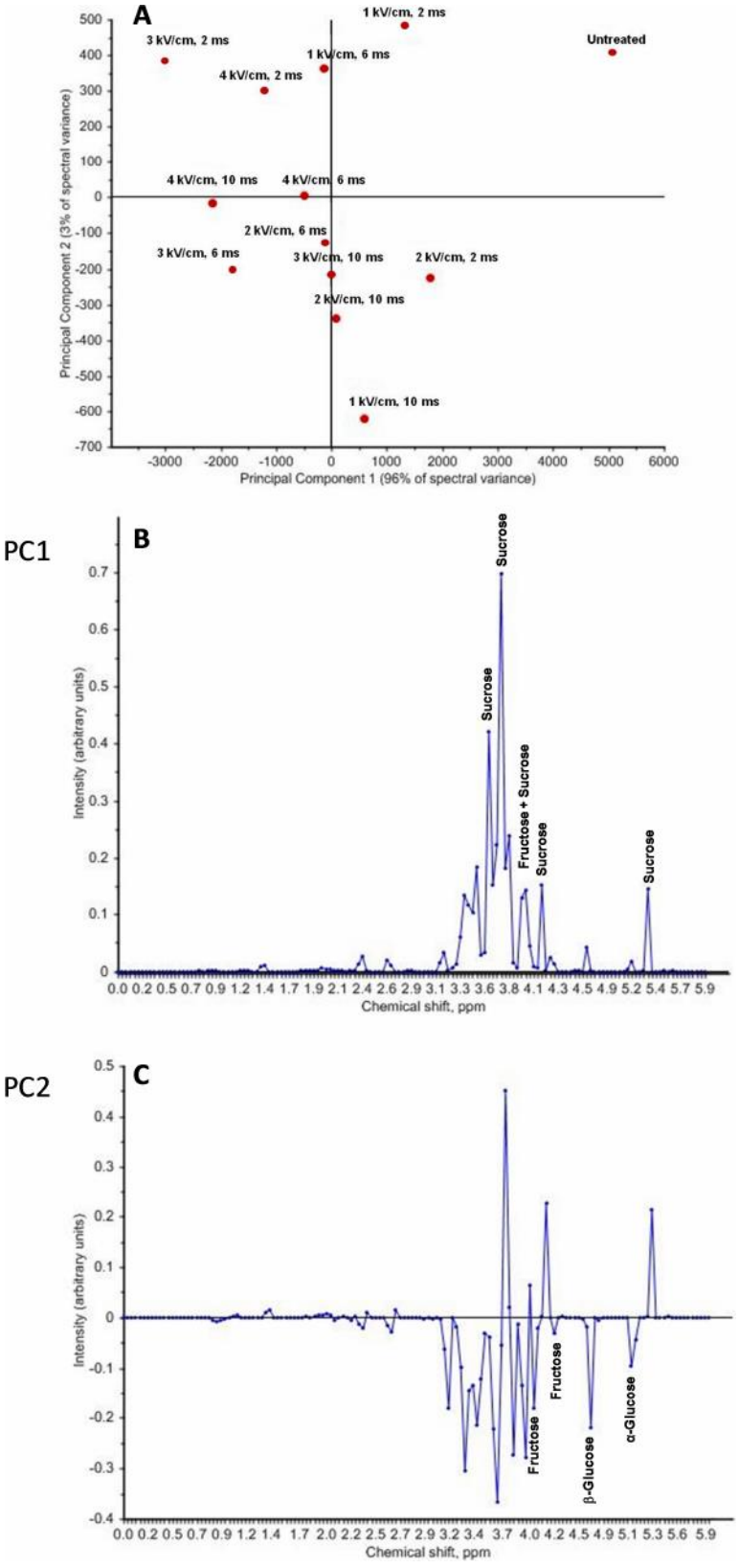


**Figure 3**. (A) PC1 *vs* PC2 score plots for $^{1}$11 NMR spectra; (B) PC1 and (C) PC2 loading plots for $^{1}$11 NMR spectra (data are the mean of three replicates).

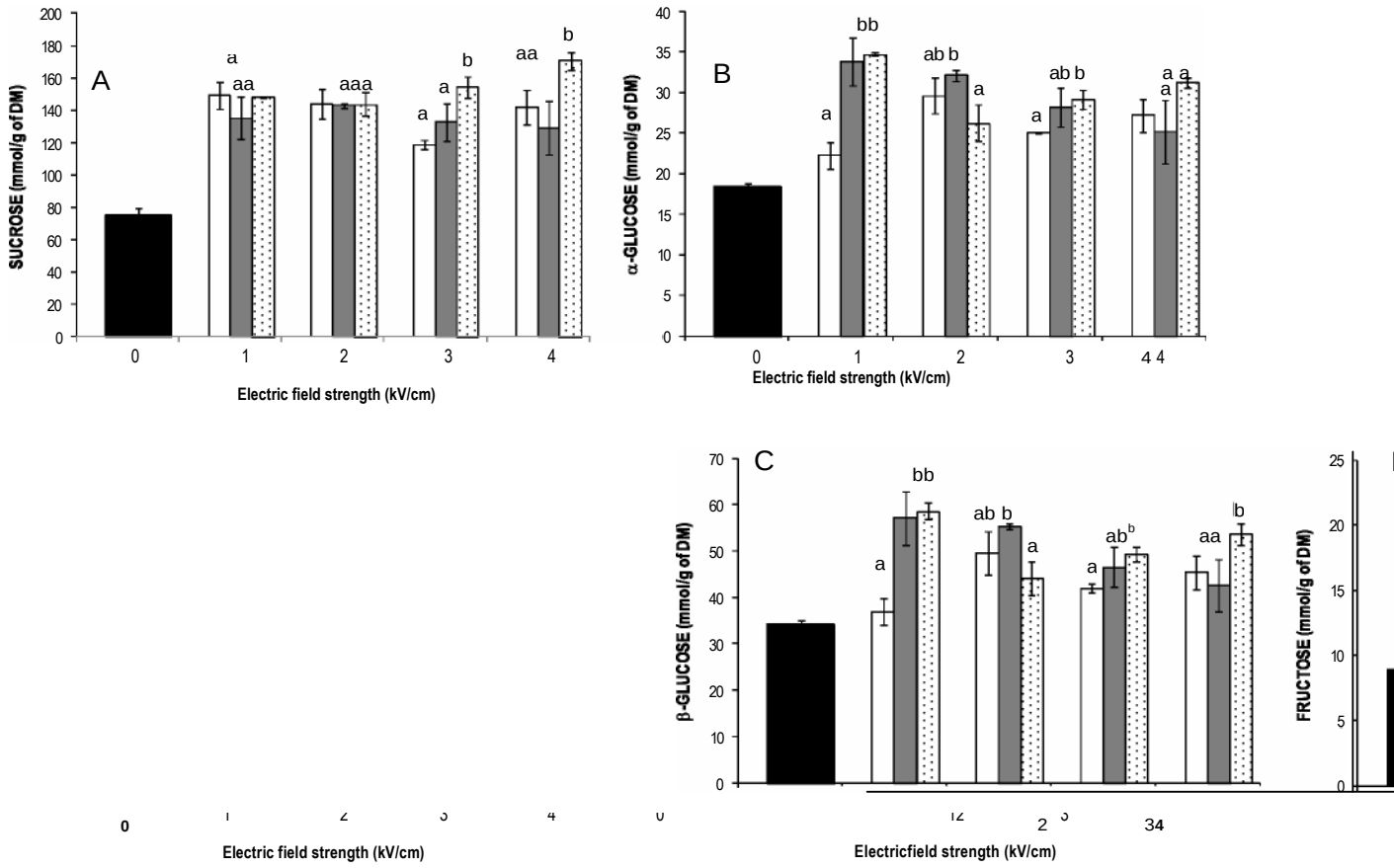


**Figure 4**. Effect of electric field and treatment time on extractable sugar concentration of sucrose (A), a-Glucose (B), 1 -Glucose (C) and Fructose (D) of carrot slices (mean of three replicates ± standard deviation). (■) untreated control; (□) 2 ms; (■) 6 ms; ⊡ ( ) 10 ms. Different letters (a, b) mean significant differences ($p < 0.05$) between sugar content in various samples based on Duncan's test.