Effects of high-voltage electrostatic field on the freezing behavior and quality of pork tenderloin

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Running title: Maintaining the quality of frozen pork using HVEF

Abstract: This study investigated the effects of a high-voltage electrostatic field (HVEF) on the freezing behavior and quality maintenance of pork tenderloin. Based on the freezing behavior of deionized water (DW) under both AC and DC conditions, the freezing of pork samples using DC treatment was examined. It was found that the freezing point (T_m) and phase-transition time (crystallization time, t_c) of the meat varied with increasing voltage. The water-holding capacity of samples treated by 10 kV exceeded that of the control group, which was proven using low-field nuclear calorimetry (LF-NMR) and differential scanning calorimetry (DSC). The total area of ice crystal for the cross sections of pork tenderloin treated with 10 kV HVEF was much smaller than that without HVEF. Microstructural observations indicated that freezing assisted by 10 kV HVEF was superior to freezing without HVEF in maintaining the quality of frozen pork.

Keywords: high-voltage electrostatic field; pork tenderloin; freezing behavior; meat quality; ice crystal

Chemical compounds studied in this article:

Sodium iodoacetate (PubChem CID: 5239); potassium chloride (PubChem CID: 4873)

1. Introduction

As one of the most efficient methods for food preservation, freezing plays a vital role in ensuring the safety of exported meat products, which are worth at least US\$13 billion globally (Leygonie et al., 2012). However, the ultrastructure of meat can be easily damaged by the formation of large ice crystals; these affect certain biochemical reactions as well as quality parameters, such as color and moisture content. Many large ice crystals form when a sample's temperature is maintained at the solid-liquid equilibrium (T_m) for an extended period; thus, the T_m and phase-transition time are factors that determine product quality in the frozen food industry. A large number of small ice crystals and a short phase-transition time for the food matrix in meat, vegetables, and fruit—as well as for pharmaceutical products, including drugs and vaccines—are necessary to satisfy quality demands. High-pressure freezing is used to reduce the formation of ice I and increase the formation of ice II–IX; at these stages, ice does not increase in volume, thereby reducing tissue damage (Li & Sun, 2002). In high-pressure freezing experiments, the phase-transition time has been found to be shorter than at atmospheric pressure (Otero & Sanz, 2006). However, high-pressure freezing is expensive. In addition, radiofrequency was exploited by Anese et al. (2012) to assist pork freezing. Fewer intercellular voids were found by using radiofrequency assisted cryogenic freezing than by cryogenic freezing. Xanthakis et al. (2014) found that microwave field assisted freezing of pork decreased the average ice crystal size by 62%.

A high-voltage electrostatic field (HVEF) has been used to control ice formation

when freezing foods (Orlowska et al., 2009; Wei et al., 2008; Xanthakis et al., 2013). The plate electrode has been designed to control water ice nucleation and reduce ice crystal size of pork by the Alain Le-Bail' team (Orlowska et al., 2009; Xanthakis et al., 2013). The nucleation and crystallization process occurs when the Gibbs free energy for a system is negative ($\Delta G < 0$). For a spherical crystallite with no applied electrostatic field, $\Delta G_0 = \Delta G_s + \Delta G_v = 4\pi r^2 \gamma - \frac{4}{3}\pi r^3 \Delta G_v$; for a spherical crystallite in an electrostatic field, $\Delta G_E = 4\pi r^2 \gamma - \frac{4}{3}\pi r^3 (\Delta G_v + PE)$. This indicates that a system will freeze more easily with the application of an electric field (Orlowska et al., 2009). Further, Wei et al. (2008) determined that water molecules are induced to align in an electrostatic field; under that condition, the direction of water's permanent dipole moment is the same as E (the angle between the permanent dipole moment and E is 0 or 2π). Shevkunov and Vegiri (2002) calculated that the water cluster converged into an almost aligned state at the electric field strength of 1.5×10^7 V/m.

For a considerable time, researchers have been investigating HVEF technology as one of the most promising approaches to food processing (Xanthakis et al., 2013; He et al., 2013). One study involved reducing the thawing time of pork tenderloin under HVEF treatment (He et al., 2013). In those previous studies, DC HVEF was applied for electric thawing. Hitherto, however, no comparison has been made of the effects of DC and AC HVEF in electro-freezing. Accordingly, in the present study, these two types of HVEF were analyzed in electro-freezing. The aim of this research was to make a systematic investigation of the optimal HVEF and voltage conditions for freezing pork.

2. Materials and methods

2.1 Materials

Fresh pork tenderloin was purchased from a MerryMart supermarket in Beijing, China. The selected meat was light cherry red in color. The moisture content was 71.54 ± 0.47 % according to direct drying method. The protein content was 20.3 ± 0.7 % based on Kjeldahl method. After purchase, the meat was sealed in plastic bags to prevent the oxidation of proteins, such as sarcoplasmic proteins (which are associated with meat color) and myofibrillar proteins (which have an impact on water-holding capacity ([WHC])). They were then transported in a polystyrene box containing ice bags so as to avoid temperature fluctuations. The fresh meat was cut into cuboids ($50 \times 50 \times 10 \text{ mm}^3$) and stored in a 4°C refrigerator (BCD-285WNMVS; Samsung, Suzhou, China). The storage time of pork was less than 24 h. All the chemicals used were of analytic grade.

2.2 HVEF freezing experiment and temperature-monitoring system

The experimental system used was designed for this study (Fig. 1). Briefly, it comprised a miniature refrigerator, a DC voltage generator (DW-P303-1ACCC, Tianjin Dongwen High Voltage Power Supply Company, Tianjin, China), an AC voltage generator, a treatment chamber, a multiple point-to-plate electrode, and a real-time temperature- and humidity-monitoring system. The electrode used in this study was different from the one used in the study by the Alain Le-Bail' team

(Orlowska et al., 2009; Xanthakis et al., 2013). The refrigerator consisted of a condensing unit (2HES-1, Bitzer Compressors [Beijing] Ltd., Beijing, China), an evaporator (DD15, Beijing Kaidi Refrigeration Equipment Co., Ltd., Beijing, China), a solenoid valve (Danfoss [China] Co., Ltd., Tianjin, China), and a pressure controller (Danfoss). The refrigerator was 1700 mm long, 1200 mm wide, and 2200 mm high. The refrigerator body was made of polyurethane with a thickness of 100 mm, which conforms to Chinese industrial standards (JBT 6527-2006). The temperature could be adjusted from $+8^{\circ}$ to -25°C with an accuracy of $\pm 2^{\circ}\text{C}$. In a preliminary experiment, it was determined that the refrigerator cooled at a rate of $1.1^{\circ}\text{C}/\text{min}$ when the temperature was set to -20°C . This rate is similar to one reported previously (Xanthakis et al., 2013).

The output voltage could be adjusted in the range of 0–10 kV using the DC voltage generator. The virtual voltage output in the range of 0–10 kV could be adjusted using an AC voltage-regulation system, which consisted of a transformer (TDM2.5/60; Beijing Transformer Company, Beijing, China) and a voltmeter (JGM-100; Beijing Transformer Company). To avoid electrical conduction, the treatment chamber was constructed of wood and acrylic board. The upper electrode contained 81 needles (70 mm long, 1 mm in diameter; 20 mm between any two needles). The temperature- and humidity-monitoring system contained a midi logger (GL220, Graphtec, Yokohama, Japan), a T-type thermocouple, and a humidity-voltage conversion module (B-530, Graphtec). Thermocouples were also used in the electromagnetic field environment by Lyng et al. (2005) and Yarmand et al. (2013). During measurements, the temperature

change of the samples was recorded per minute using the data logger. The whole experimental system was set up in a basement with almost constant temperature ($17.63^{\circ} \pm 0.43^{\circ}\text{C}$) and humidity ($15.25\% \pm 0.63\%$).

2.3 HVEF freezing process

Two systems were studied in this work, the first of which was used to compare the different freezing processes of almost pure substances both with and without the application of a DC or AC HVEF. With system 2, the experiments used a single type of HVEF selected from the optimal results of the system 1 experiments. With system 1, 10 mL DW was placed in an aluminum crucible (30 mm long, 50 mm in diameter). Temperature sensors were immersed in the water for each trial. With system 2, cuboid samples ($50 \times 50 \times 10$ mm) of fresh pork tenderloin were put on aluminum foil. The temperature sensors were placed at the geometric center of the samples. Since nucleation is a stochastic, spontaneous process, at least five samples were measured for both systems under the two types of voltage conditions. In addition, new samples of DW and meat were used in each experiment.

The initial temperatures of the DW and meat samples were maintained at almost the same level among each group in each experiment. The AC or DC voltage was applied at the same time upon initiation of cooling the samples to less than -15°C . The voltages studied in the two systems were as follows: 0, 4, 6, 8, and 10 kV. For AC, the voltage's true value was equal to the DC voltage that could produce the same power consumption at the same resistance. After freezing the frozen samples were thawed

under ambient conditions ($20^{\circ} \pm 1^{\circ}\text{C}$ for nearly 60 minutes) before analysis.

2.4 Nuclear magnetic resonance spectroscopy

The nuclear magnetic resonance (NMR) proton relaxation measurements of the meat samples were performed at low-field NMR (LF-NMR). The meat was cut into cuboids ($15 \times 15 \times 10$ mm; 2.617 ± 0.170 g). Then, the samples were individually wrapped in parafilm membrane and put into a flat-bottomed cylindrical glass tube (200 mm long, 25 mm in diameter). The LF-NMR experiments were carried out on a low-resolution spectrometer (PQ001, Newman, Shanghai, China). The Carr-Purcell-Meiboom-Gill pulse sequence was adopted to measure the transverse relaxation time (T_2) between 0 and 10,000 ms. The separations between the 90° and 180° pulses were 4.00 and 9.00 μs , respectively, and 247,200 data points were collected. Eight scans were made with a 3000-ms waiting time. The peak areas that represented the distribution of different water populations were transformed into percentages. The moisture content was calculated using the peak area as a percentage of the sum of all peaks. The NMR measurements were performed at ambient temperature.

2.5 Differential scanning calorimetry

The meat samples were examined using differential scanning calorimetry (DSC) with no extraction or purification. The DSC analyses were performed on a DSC-60 Differential Scanning Calorimeter (Shimadzu, Kyoto, Japan). Pork weighing 3–5 mg

were used for the DSC. The heating rate was 10°C/min, and the final temperature for the DSC scans was 110°C. An empty crucible was used as a reference. The three maximum temperatures of the peaks (T_{peak}) and denaturation enthalpy (DH) were used to determine the characteristics of the denaturation of the pork proteins. The levels of DH of peak 1 ($DH_{\text{peak 1}}$), peak 2 ($DH_{\text{peak 2}}$), peak 3 ($DH_{\text{peak 3}}$), and peak 1 + peak 2 ($DH_{\text{peak 1+2}}$) were estimated.

2.6 Quality parameters of pork tenderloin

2.6.1 Color measurement

The color of the frozen-thawed pork tenderloin samples was determined using a CR-300 chromameter (Konica Minolta Sensing, Inc., Tokyo, Japan). CIE L^* (lightness), a^* (redness), and b^* (yellowness) values were recorded. The chroma (C), total color difference (ΔE), and whiteness were calculated using the following equations (Dai et al., 2013; Liu et al., 2013):

$$C = \sqrt{a^{*2} + b^{*2}}$$

$$\Delta E = \left[(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2 \right]^{\frac{1}{2}} = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right]^{\frac{1}{2}}$$

$$\text{Whiteness} = 100 - \left[(100 - L^*)^2 + a^{*2} + b^{*2} \right]^{\frac{1}{2}}$$

2.6.2 pH and expressible moisture

The buffer (pH 7.0) used for pH measurement consisted of 5 mM sodium iodoacetate and 150 mM potassium chloride. Minced pork tenderloin (2.5 g) was homogenized with 25 mL cold buffer using a ceramic mortar. Then, the pH was

measured using a pH meter (Orion 5-star, Thermo Electron Corporation, Lexington, Kentucky, USA) (Liu et al., 2013).

The expressible moisture (*EM*) was determined by the method of Dai et al. (2013), with some modifications. Briefly, the pork tenderloin samples used here were $50 \times 10 \times 10$ mm in size and weighed 9.24 ± 0.92 g. They were centrifuged at 2500 g for 20 min at 4°C. *EM* was calculated as the percentage of moisture loss after centrifugation relative to the initial weight of meat.

2.7 Microscopic analysis

2.7.1 Scanning electron microscopy

Meat samples that were frozen with or without being subjected to a 10-kV DC HVEF were prepared for microstructural analysis. A scanning electron microscope (SEM; Quanta 200F, FEI, Hillsboro, OR, USA) at Tsinghua University was used. The frozen samples were lyophilized, pasted onto the conducting resin, and coated with gold for 90 s. The high-vacuum mode was chosen with 130-Pa chamber pressure. Unlike the 150× magnification used for observing the microstructural change of duck meat (Li et al., 2013), the samples were observed at 100× magnification by means of an accelerating voltage of 15 kV. In addition, the micro-graphs were also taken at magnification of 4500× to observe the arrangement of myofibril.

2.7.2 Light microscopy

The fixation method for light microscopy was in accordance with the method

reported by Liu et al. (2013). Fresh and frozen meat samples ($10 \times 10 \times 10$ mm), which had been cut perpendicular and parallel to the muscle fibers, were fixed with 3% glutaraldehyde in sodium phosphate buffer (0.1 M, pH 7.4) for 48 h. The samples were then dehydrated at 4°C with a graded series of ethanol solutions (70%–100%, v/v) using a dehydrator (Asp200s, Leica, Wetzlar, Germany). Thereupon, the tissues were immersed in xylene to make them transparent. Wax (39601095, Leica) was used to soak the meat to ensure fixation of the meat tissue. The sample was then embedded in paraffin by means of an embedding station (EG1150H, Leica) to facilitate slicing. Slices were cut using a microtome (CM3050S, Leica) and subjected to hematoxylin and eosin (HE; ST5020, Leica), followed by observation at 200× magnification. The imageJ 1.46r (Wayne Rasband, National Institutes of Health, USA) was used to calculate the area of both extra- and intracellular spaces which were not stained by HE. For the extra- and intracellular spaces, they contained the voids caused by ice crystal. So the total area of ice crystal could be calculated by the following equation:

$$\text{Total area of ice crystal (\%)} = S_{\text{frozen}} - S_{\text{fresh}}$$

Where S_{frozen} is the area of both extra- and intracellular spaces which were not stained by HE in frozen pork tenderloin, while the S_{fresh} is the area of both extra- and intracellular spaces which were not stained by HE in fresh pork tenderloin.

2.8 Data analysis

The results were statistically analyzed using SPSS 20.0 (IBM Corporation, Armonk, NY, USA). The least significant difference procedure and Duncan's test were used to compare mean values, with significance set at $P < 0.05$.

3. Results and Discussion

3.1 Freezing behavior of DW and pork tenderloin

The processes of freezing DW and pork tenderloin samples are shown in Fig. 2. The DW frozen in the aluminum crucible exhibited supercooling before starting to freeze, as has been widely reported elsewhere (Kiani et al., 2011). The T_n of DW is consistent with a previously published finding of water samples being nucleated at $-7.4^\circ \pm 2.4^\circ\text{C}$ (Kiani et al., 2011). The T_m of pork tenderloin was similar to those of Farouk et al (2013) (Table 2). The range of initial freezing points of beef measured by them was -0.9°C to -1.5°C . Moreover, the T_m of pork tenderloin measured in this study is in the range of the freezing points introduced by Stonehouse & Evans (2015). From a comparison of the two typical curves during the course of the freezing experiment, it was difficult to identify a T_n point for the pork tenderloin. The curve of pork tenderloin internal temperature was similar with that of lamb (Muela et al., 2012). The phase transition time (crystallization time, t_c) was calculated in this paper as the time span between initial freezing point and reaching a temperature 1.5°C below the corresponding initial freezing point. Meat is a complex matrix of water, fat, and protein, whereby the water molecules bind to the proteins and other components. Water is a major component of food, and so studying the freezing of DW is vital toward understanding the freezing process in meat (Kiani & Sun, 2011).

3.2 Freezing behavior of DW with AC and DC

It was found that AC HVEF freezing and DC HVEF freezing led to notable shifts in T_n of DW, respectively. For AC, the T_n of DW treated with freezing at 0, 4, 6, 8, and 10 kV was $-5.3^\circ \pm 0.6^\circ\text{C}$, $-3.7^\circ \pm 1.4^\circ\text{C}$, $-4.7^\circ \pm 1.6^\circ\text{C}$, $-5.3^\circ \pm 0.8^\circ\text{C}$ and $-3.7^\circ \pm 0.8^\circ\text{C}$. There were significant differences of T_n between the DW treated without AC HVEF and the ones treated with 4 kV or 10 kV AC HVEF ($P < 0.05$). Orłowska et al. (2009) also reported the phenomenon of T_n becoming almost 1.5 times higher as a result of electro-freezing. The extent of supercooling of the DC HVEF-assisted freezing group in Table 1 was smaller than that of the AC HVEF-assisted one. Moreover, Pearson correlation coefficient was calculated to quantify the association between t_c and the treatment voltage. The correlation coefficients were 0.627** (a 2-tailed significant correlation at the 0.01 level is indicated by **). So the time required for complete solidification of DW increased with the treatment voltage, which was also consistent with the result by Orłowska et al. (2009). DC HVEF has a more beneficial effect on nucleus formation at relatively high temperatures than AC HVEF, and so DC treatment was selected for further study using the meat model.

3.3 Freezing behavior of meat with DC

The temperature profiles of pork tenderloin showed a region of supercooling in a very few cases. In the averaged data sets, the effect of supercooling for the meat samples was minimal. As shown in Table 2, there were not significant differences of the crystallization temperature for each group. It was also determined in this study that the application of a DC current at 4, 6, 8 and 10 kV did not prolong the

phase-transition time. As the moisture condensation was very serious in the miniature refrigerator, the highest voltage used in this study was 10 kV to protect the voltage generator. Although there are several studies regarding on freezing pork (Anese et al., 2012; Xanthakis et al., 2013, 2014; Choi et al., 2016), unfortunately, few studies have examined the process of freezing pork using HVEF with prick electrode. This type of electrode was effective in shortening the thawing time of pork tenderloin with a positive effect to decrease the time in the zone of maximum ice crystal formation (He et al., 2013).

According to the power equation, the DC HVEF energy consumption levels at 10 kV for thawing pork tenderloin were 0.4 ± 0.1 W; that level compares favorably with the power consumption of a 3 kW refrigerator. DC HVEF-assisted freezing is therefore an energy-efficient method. Freezing of meat are related with many important qualities alteration, such as WHC and meat colour. Consequently, the indicators will be discussed in the following sections.

3.4 Relaxation of water in meat

Low-resolution NMR spectroscopy has been considered suitable for measuring the mobility of water molecules in a complex matrix (Huang et al., 2011). It is used to study the water distribution and mobility in meat and meat products (Zheng et al., 2015). In addition, it has been demonstrated that NMR parameters are connected with the quality of meat in terms of drip loss and WHC (Gudjónsdóttir et al., 2011; Li et al., 2012). It has been reported that there are three water populations in pork: water

closely associated with macromolecules (e.g., proteins), at 1–3 ms; water in myofibrils, at 40–80 ms; and water outside myofibrils, at 200–400 ms (Bertram & Andersen, 2007). The various groups of water molecules are evident in the T_2 distribution (Fig. 3). The peaks of relaxation times represent the three components of water: bound water, immobilized water, and free water, labeled “*peak 1*,” “*peak 2*,” “*peak 3*,” respectively. Among them, immobilized water and free water, have been studied in many LF-NMR studies (Aursand, Veliyulin, Böcker, Ofstad, & Erikson, 2008; Zheng et al., 2015). The classification of the T_2 relaxation times of water into these populations in fresh pork tenderloin is shown in Fig. 3A. Our T_2 relaxation time data are consistent with those of Bertram and Andersen (2007). In our preliminary experiment, it was demonstrated that freezing led to shorter T_2 relaxation times; the relative amount of water in each population also changed markedly compared with that in fresh pork tenderloin. Thus, the frozen meat was thawed and used for NMR data acquisition to compare the differences between fresh and frozen-thawed samples (Fig. 3). In addition, the relaxation times and relaxation populations were both significantly affected by the treatment method, e.g., brine salting and pressure/thermal treatments. Hence, both of these characteristics should be studied.

The degree of freedom of molecular water is associated with T_2 . Specifically, an increase in water mobility is associated with a shift toward longer T_2 relaxation times. The T_2 relaxation times of molecular water in pork tenderloin subjected to different DC HVEF-assisted freezing treatments are presented in Fig. 3 and Table 3. There were significant differences in the T_2 of *peak 1* between fresh and defrosted meat ($P <$

0.05). Briefly, for the defrosted meat, the shifts in T_2 indicated that the bound water became freer. In addition, the decrease in T_2 of *peak 3* suggested that the degree of freedom of free water was reduced. The change in T_2 would appear to have been caused by the freezing and thawing process. HVEF had little impact on the T_2 value.

The peaks' area, or the proton population (PP), refers to the relative quantity of the water population in pork tenderloin (Table 3). It was believed that the lower population of *peak 3*, the lower the centrifugation loss (Zheng et al., 2015). Except for the PP (%) of *peak 3* in fresh meat, the PP (%) of *peak 3* in meat subjected to 10 kV was the smallest. Moreover, Sánchez-Alonso et al (2012) reported that the WHC decreased with an increase in the PP (%) of *peak 3* and a decrease in the PP (%) of *peak 2* during storage. So the WHC of pork tenderloin treated with 10 kV HVEF freezing was better than other groups. As we know, the centrifugation loss is related with WHC. Moreover, there were significant differences for the population of *peak 2* ($P < 0.05$). Since the PP (%) of *peak 2* in meat subjected to 10 kV freezing was the closest to that of *peak 2* in fresh meat, meat quality (e.g., WHC) with 10 kV freezing should be superior to that of the other groups. In contrast, the PP (%) of *peak 2* and *peak 3* for defrosted meat that had not been frozen under HVEF conditions was the smallest and largest among the groups, respectively, which illustrates that the freezing process significantly influenced the content of the three moisture components in meat and would have negatively affected its quality. Except with 8 kV freezing, the PP (%) of *peak 3* gradually decreased with increasing voltage. The water molecules were reoriented by the HVEF in the freezing process, which would have affected the peaks'

area. In addition, the electrostatic repulsion of the myofibrillar proteins as a result of the HVEF could have changed the water mobility and PP (%) of *peak 2* and *peak 3*.

3.5 Denaturation of myosin related to WHC

The thermograms of pork tenderloin appear in Fig. 4. The denaturation of myosin heads, myosin tails/sarcoplasmic proteins, and actin are represented by the three endothermal peaks. There were significant differences in T_{peak1} between fresh and frozen-thawed pork tenderloin ($P < 0.05$; Table 4). This indicates that structural changes in myosin occurred during the freezing and thawing of the meat. However, the myosin denaturation of meat frozen without being subjected to a 10 kV HVEF was more severe than that of HVEF-assisted frozen meat. Myofibrillar proteins, including myosin, are known to be associated with WHC in muscle or meat. Meat quality is poor when myosin ATPase activity has been suppressed. Therefore, meat treated by 10 kV HVEF had superior quality to that not subjected to HVEF. In addition, no significant differences in T_{peak2} and T_{peak3} were observed among the samples in this study. Thus, myosin was shown to be more sensitive to a low-freezing-temperature environment than sarcoplasmic proteins or actin.

The DH of fresh meat was more pronounced than that of the control group (Ck) and groups by 10 kV, which mainly indicated that fresh pork proteins were the most stable; the DH of the Ck was the smallest (Table 4). In addition, sarcoplasmic proteins also play an important role in determining WHC of meat (Marcos et al., 2010). There were significant differences between the DH_{peak2} of Ck and 10 kV HVEF assisted freezing

groups ($P < 0.05$), which indicated better WHC of groups assisted by 10 kV than that of Ck.

3.6 Color, pH, and EM

Color parameters including L^* , a^* , b^* , C , and total color difference (ΔE) are important indicators of meat quality and market value; they appear in Table 5. Fresh pork exhibited higher L^* values than meat subjected to freezing and thawing ($P < 0.05$; Table 5). In addition, the meat samples became red during the freezing and thawing process, especially in the Ck. As well as single color parameters, meat color can be estimated using ΔE . The appearance of meat is considered to have modified when the value of ΔE is at least 10 (Marcos et al., 2010). In the present study, the values of ΔE of the Ck and meat samples treated by 10 kV were both less than 10; thus, meat color was not considered to have changed significantly. Pearson correlation coefficient was calculated to quantify the association between EM and L^* values. The correlation coefficients were -0.79^* (a 2-tailed significant correlation at the 0.05 level is indicated by $*$). So the WHC is closely correlated with the colour of pork tenderloin. The flesh colour of pork tenderloin should be associated with myoglobin, heme-based pigments and tissue structure (Mancini & Hunt, 2005).

There were no significant differences in pH between the fresh and the one treated by 10 kV HVEF (Table 5). The pH in the Ck was smaller than in the other groups, which may have been caused by oxidative rancidity. Finally, although the mean EM of electro-freezing group by 10 kV HVEF did not show a significant decrease compared

with the one of Ck, at the level of 10 kV the *EM* showed around 6 % decrease of their average *EM* compared to the Ck, which is consistent with our NMR and DSC data. The degradation of parameters (e.g., *EM* and texture) for pork tenderloin is also closely connected with the ice crystal in the meat. So the destructive effects caused by ice crystal were observed in the following section.

3.7 Microscopic observation

The surface and cuts perpendicular to the direction of the fibers in the samples are illustrated in Fig. 5. At the surface of meat subjected to 10 kV DC and freezing (Fig. 5B), the voids were smaller than upon freezing without electrical treatment (Fig. 5A). The surfaces of frozen and electrically treated samples were thus smoother than those of ordinary frozen samples. Regarding the interior of the meat samples, the voids were smaller in the group treated by 10 kV DC (Fig. 5B) than in the group without electrical treatment (Fig. 5A). It is assumed that the size of the ice crystals that develop during the freezing process reflects the size of the voids. Therefore, our results are consistent with the conclusion of Xanthakis et al. (2013), whereby morphological changes could be caused by ice crystals. The size of ice crystal is an important factor related to muscle deterioration, because the formation of large ice crystals should lead to an extensive mechanical damage; as a consequence, interaction of cellular components (e.g., lipids and proteins) with enzymes leads to protein denaturation and lipid degradation. Moreover, the myofibril could be seen clearly for the 4500× magnification. Compared to freezing without electrical treatment, the

freezing assisted by 10 kV HVEF could lead to dense arrangement of myofibril (Fig. 5B). Thus, the destructive effects of freezing could be minimized by combination with 10 kV DC treatment.

The cross and longitudinal sections of the samples stained with HE are shown in Fig. 6. For fresh meat (Fig. 6A), the typical shape of the cells was well preserved, and the muscle fibers were tightly attached to one another. With the frozen-thawed meat (Fig. 6B, C), the space between the muscle fibers had become larger than with fresh meat. Moreover, there was greater damage to the cells in frozen meat, which may have been caused by ice crystals (Fig. 6B). In a comparison of cross sections of frozen-thawed meat, the cells in the freezing group assisted by 10 kV DC were more intact than those not subjected to electrical treatment. The microstructural damage of electro-frozen pork tenderloin was thus minimal compared with meat subjected to ordinary freezing. The area of both extra- and intracellular spaces which were not stained by HE is shown in Fig. 7. Based on the area results, the total area of ice crystal for the cross sections of pork tenderloin by ordinary freezing and freezing with 10 kV HVEF was $13.77 \pm 0.93 \%$ and $1.37 \pm 0.61 \%$, respectively. There were significant differences of total area of ice crystal for the two freezing method ($P < 0.05$). Hence, freezing pork tenderloin with 10 kV DC should offer advantages over conventional freezing.

4. Conclusions

In this study, supercooling was not evident in the pork tenderloin samples; however,

it did occur during the freezing of DW. The T_n of DW was affected by both AC and DC HVEF; however, T_n changed more with DC than with AC treatment. It was also observed that the phase-transition time of DW increased with the treatment voltage, while the t_c of pork tenderloin did not increase with the treatment voltage. Moreover, the power consumption of DC HVEF was little, suggesting that HVEF is a promising application with frozen food.

Freezing in combination with exposure to a 10 kV DC environment maintained the water properties at levels similar to those in fresh pork tenderloin; it also led to smaller ice crystals than ordinary freezing treatments. Furthermore, this approach decreased protein denaturation. The meat quality indicators, including color, pH, and WHC, were closer to those of fresh pork tenderloin after the freezing assisted by 10 kV treatment than after freezing without HVEF. It is therefore concluded that 10 kV DC assisted freezing of pork tenderloin offers quality advantages over conventional air freezing.

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Fig. 1. Scheme of the experimental DC/AC HVEF refrigerator system used in the studies. 1. Treatment chamber. 2. Bottom plate. 3. Prick electrode. 4. Refrigerator body. 5. Cover. 6. Power supply. Sample (deionized water and pork tenderloin) were placed on the stainless steel plate. The voltage was applied between the upper needle-shaped electrode and the lower metallic surface.

Fig. 2. Freezing curves of deionized water (A) and pork tenderloin (B).

Fig. 3. CPMG spectra of pork tenderloin. Capital letters A, B, C, D, E, F denote fresh meat and the thawed meat which was freezed with 0 kV, 4 kV, 6 kV, 8 kV, 10 kV DC HVEF before.

Fig. 4. Typical DSC thermograms from analysis of pork tenderloin. a) frozen pork with 10 kV HVEF treatment, b) fresh pork, c) frozen pork. The arrow represents endothermic peaks.

Figure

Fig. 5 Scanning electron micrograph of surface and transversal cuts to the fiber direction of pork tenderloin. A, frozen pork; B, frozen pork with 10 kV HVEF treatment.

Fig. 6 Light microscopy observations on the cross and longitudinal sections of pork tenderloin. A, fresh pork; B, frozen pork; C, frozen pork with 10 kV HVEF treatment. The arrow represents the voids in the cells caused by ice crystals.

Fig. 7. The area of both extra- and intracellular spaces which were not stained by HE for pork tenderloin.

**Table 1 Effect of DC HVEF treatment on the nucleation temperature (T_n) and
phase transition time (crystallization time) of DW.**

Voltage (kV)	Electrostatic field (V/m)	T_n (°C)	t_c (s)
0	0	-5.3 ± 0.6 ^c	588 ± 50 ^b
4	1.0×10^5	-4.7 ± 0.7 ^c	720 ± 112 ^b
6	1.5×10^5	-2.4 ± 0.9 ^a	675 ± 150 ^b
8	2.0×10^5	-3.6 ± 0.6 ^b	732 ± 143 ^b
10	2.5×10^5	-1.8 ± 1.0 ^a	900 ± 49 ^a

Results are presented as mean values ± SD of at least 3 replicates. Different letters in each row indicate significant difference ($P < 0.05$) in T_n (°C) and t_c (s).

Table 2 Effect of DC HVEF treatment on the freezing point (T_m) and **phase transition time (crystallization time) of pork tenderloin.**

Voltage (kV)	Electrostatic field (V/m)	T_m (°C)	t_c (s)
0	0	-1.1 $\pm$ 0.1 ^a	3480 $\pm$ 433a
4	1.0×10^5	-1.2 $\pm$ 0.1 ^a	2840 $\pm$ 35b
6	1.5×10^5	-0.9 $\pm$ 0.2 ^a	3740 $\pm$ 362a
8	2.0×10^5	-1.1 $\pm$ 0.1 ^a	3240 $\pm$ 365ab
10	2.5×10^5	-1.0 $\pm$ 0 ^a	3680 $\pm$ 242a

Results are presented as mean values $\pm$ SD of at least 3 replicates. Different letters in each row indicate significant difference ($P < 0.05$) in T_m (°C) and t_c (s).

Table 3 Effect of HVEF treatment on the T_2 relaxation time and peak area fraction of water in the pork tenderloin.

	T_2^c (ms)			PP ^c (%)		
	<i>Peak 1</i>	<i>Peak 2</i>	<i>Peak 3</i>	<i>Peak 1</i>	<i>Peak 2</i>	<i>Peak 3</i>
Fresh	1.16 ± 0.16 ^b	41.41 ± 3.26 ^{ab}	307.37 ± 42.79 ^a	2.65 ± 0.44 ^b	97.13 ± 0.39 ^a	0.25 ± 0.10 ^d
0 kV	1.92 ± 0.15 ^a	37.65 ^b	231.01 ^b	3.23 ± 0.32 ^{ab}	82.78 ± 1.26 ^d	14.00 ± 1.12 ^a
4 kV	1.84 ± 0.15 ^a	39.53 ± 3.26 ^{ab}	200.92 ^{bc}	2.93 ± 0.31 ^{ab}	84.03 ± 2.15 ^{cd}	13.03 ± 2.31 ^{ab}
6 kV	1.92 ± 0.15 ^a	37.65 ^b	231.01 ^{bc}	2.55 ± 0.31 ^b	86.70 ± 2.19 ^c	10.73 ± 2.17 ^b
8 kV	1.92 ± 0.15 ^a	39.53 ± 3.26 ^{ab}	183.47 ± 15.11 ^{bc}	2.65 ± 0.52 ^b	83.50 ± 3.13 ^d	13.80 ± 2.74 ^a
10 kV	1.89 ± 0.20 ^a	43.29 ^a	220.98 ± 17.37 ^c	3.53 ± 0.51 ^a	91.83 ± 1.11 ^b	4.63 ± 0.81 ^c

^c spin-spin relaxation time.

Results are presented as mean values ± SD of 3 replicates. Different letters in each row indicate significant difference ($P < 0.05$) in T_2 (ms) and PP (%), respectively.

Table 4 Effect of HVEF treatment on the T_{peak} and DH of the pork tenderloin from DSC analysis.

	$T_{\text{peak1}}^{\text{a}}$ (°C)	$T_{\text{peak2}}^{\text{b}}$ (°C)	$T_{\text{peak3}}^{\text{c}}$ (°C)	$DH_{\text{peak 1}}^{\text{A}}$ (J/g)	$DH_{\text{peak 2}}^{\text{B}}$ (J/g)	$DH_{\text{peak 1+2}}^{\text{D}}$ (J/g)	$DH_{\text{peak 3}}^{\text{C}}$ (J/g)
Fresh meat	$59.61 \pm 1.83^{\text{a}}$	$67.70 \pm 3.49^{\text{a}}$	$80.27 \pm 1.56^{\text{a}}$	$-0.16 \pm 0.02^{\text{a}}$	$-0.13 \pm 0.00^{\text{a}}$	$-0.28 \pm 0.02^{\text{a}}$	$-0.32 \pm 0.03^{\text{a}}$
0 kV	$56.23 \pm 0.87^{\text{b}}$	$65.89 \pm 0.86^{\text{a}}$	$81.27 \pm 0.22^{\text{a}}$	$-0.05 \pm 0.01^{\text{b}}$	$-0.07 \pm 0.01^{\text{b}}$	$-0.12 \pm 0.02^{\text{c}}$	$-0.12 \pm 0.02^{\text{b}}$
10 kV	$57.11 \pm 0.20^{\text{b}}$	$65.74 \pm 0.19^{\text{a}}$	$82.04 \pm 0.49^{\text{a}}$	$-0.05 \pm 0.00^{\text{b}}$	$-0.12 \pm 0.01^{\text{a}}$	$-0.17 \pm 0.006^{\text{b}}$	$-0.13 \pm 0.03^{\text{b}}$

^{a, b, c} Temperature which represented 3 thermal transition temperature in the DSC process.

^{A, B, C} Denaturation enthalpies per gram wet weight. ^D Sum of $DH_{\text{peak 1}}$ and $DH_{\text{peak 2}}$.

Results are presented as mean values $\pm$ SD of 3 replicates. Different letters in each row indicate significant difference ($P < 0.05$) in T_{peak} (°C) and DH (J/g), respectively.

Table 5 Colour parameters, pH and expressible moisture of pork tenderloin.

	<i>L*</i>	<i>a*</i>	<i>b*</i>	<i>C</i>	ΔE	<i>Whiteness</i>	pH	EM (%)
Fresh	45.01 $\pm$ 0.38 ^a	5.92 $\pm$ 0.28 ^c	4.76 $\pm$ 0.73 ^b	7.53 $\pm$ 0.21 ^c	---	44.48 $\pm$ 0.34 ^a	5.72 $\pm$ 0.083 ^a	22.83 $\pm$ 1.97 ^b
0 kV	42.83 $\pm$ 0.34 ^b	9.70 $\pm$ 0.16 ^a	7.39 $\pm$ 0.87 ^a	12.21 $\pm$ 0.42 ^a	5.15 $\pm$ 0.18 ^a	41.54 $\pm$ 0.25 ^b	5.55 $\pm$ 0.0058 ^b	32.28 $\pm$ 2.25 ^a
10 kV	42.28 $\pm$ 0.34 ^b	7.32 $\pm$ 0.50 ^b	2.83 $\pm$ 0.45 ^c	7.86 $\pm$ 0.41 ^b	3.67 $\pm$ 0.33 ^b	41.75 $\pm$ 0.28 ^b	5.71 $\pm$ 0.0794 ^a	30.35 $\pm$ 0.31 ^a

Different letters in each row indicate significant difference ($P < 0.05$).