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**Effects of Pulsed Electric Field (PEF) and Oscillating Magnetic Field (OMF) Combination
Technology on the Extension of Supercooling for Chicken Breasts**

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Abstract

In this study, a pulsed electric field (PEF) and an oscillating magnetic field (OMF) were used to achieve an extension of a supercooled state within chicken breasts. The specifications of protocol are: (i) PEF with duty cycle sequence of 0.8, 0.5, and 0.2 were applied for 300 sec, 120 sec, and 90 sec, respectively and (ii) during PEF with duty cycle of 0.2, OMF with 1 Hz was applied to vibrate water molecules and inhibit sudden ice nucleation. At the freezer temperature of -7°C (± 0.5), temperature of chicken breast samples under PEF and OMF treatment decreased down to -6.5°C in a supercooling state during the whole testing period (approximately 12 h); while the control samples were partially frozen down to -6.5°C . The impacts of the supercooling on microstructure, drip loss, color, texture, pH and lipid oxidation were evaluated as compared with samples stored at refrigeration (4°C) and at freezing (-7°C). It was found that the PEF + OMF supercooling was highly effective in the maintenance of original meat qualities without significant physical damages or chemical changes. The supercooling of meat samples using the developed PEF and OMF combination technique led to the advanced designing of innovative food freezers for long term preservation of ‘fresh-like’ foods at subzero temperature.

Keywords Supercooling; pulsed electric field; oscillating magnetic field; chicken breast; preservation; freshness

1. Introduction

Chicken meat is one of the most popular food commodities and its consumption is increasing globally, because of relatively low price, low fat content, health benefits and the high nutritional values (Cantalejo et al., 2016; Yang et al., 2016). Unlike beef or pork, there are no religious taboos against its consumption (Anang et al., 2010). Due to its increased consumption, microbial safety of chicken breasts during its processing, and distribution is becoming a growing concern (De Barcellos et al., 2010). Meat safety is considered to be a prerequisite by consumers (Van Wezemaelet al., 2010). Poultry meats are highly perishable to bacterial contaminants due to large amounts of variable nutrients, a high water activity (a_w) and a higher final pH limiting the shelf-life of the product (Lawrie, 1998). Especially, raw poultry meat is the most common reservoir of *Salmonella*, *Campylobacter*, and *Yersinia enterocolitica* (Yang et al., 2016; Olaimat and Holley, 2015). Decreasing microbial activity and delaying enzymatic and chemical reactions such as lipid and protein oxidation during storage can increase the shelf-life of meat and meat products (Jayasena et al., 2015).

Freezing technology has been used as a preservative method for meat and meat products. In addition, freezing halts the activities of spoilage microorganisms that cause foodborne diseases in and on foods and can preserve some microorganisms for long periods of time (Archer, 2004). However, freezing procedure and freezer storage irreversibly influence meat quality attributes such as drip loss, color, and tenderness. The series of changes such as protein denaturation, lipid oxidation, and change in moisture take place in food products when subjected to excessively prolonged frozen storage (Grunert et al., 2016). The degree of structural damages by formation, growth, and distribution of ice crystals are directly related to the **serious** quality degradation of the frozen foods. To decrease the damage of freezing, a large number of investigations have been

carried out using various freezing and thawing methods with different animal meats and dimensions (Anese et al., 2012; Molina-García et al., 2004; Ngapo et al., 1999). However, the damage caused by the formation of ice crystals could not be totally eliminated from the frozen materials.

Supercooling is a metastable state of water and the process of lowering the temperature of a product below its usual freezing point without the formation of ice crystals occurring (Stonehouse and Evans, 2015). Supercooled food products can be processed or consumed at freezing storage condition without quality deterioration by ice crystallization occurring even after long term. Previously, a number of studies have examined the supercooling phenomenon under different conditions and treatments such as pressure shift (Kalichevsky et al., 1995; Martino et al., 1998), irradiation of ultrasonic waves (Inada et al., 2001; Zhang et al., 2001), addition of polyvinyl alcohol (Kumano et al., 2011) and antifreeze proteins (Feeney and Yeh, 1998; Li and Lee, 1998). Recently, according to Wowk (2012) and Mok et al. (2015) the electric field and magnetic field combination treatments could affect the mobility of water molecules. A number of studies were carried out using magnetic fields to generate tiny ice crystals throughout the frozen product, prevent cell destruction, and preserve the quality of the fresh product intact after thawing process (Otero et al., 2016). In addition, the effects of materials and shapes of the electrodes on supercooled solutions under electric field have also been investigated to characterize the degree of supercooling (Hozumi et al., 2003, 2005). According to previous studies, PEF technology is a valuable tool that can improve functionality, extractability, and recovery of nutritionally valuable compounds. Additionally, PEF is a promising technology to improve drying and freezing processes (Carbonell-Capella et al., 2016; Barba et al., 2015; Puértolas and Barba, 2016; Koubaa et al., 2015). Even though various researches on the

supercooling phenomenon have been carried out, still more remains to be done in order to maintain foods in their supercooled state throughout cold storage. Additionally, there are a limited number of studies on the impact of the supercooling phenomenon on food qualities.

In the present study, to extend the supercooling in chicken breasts, the combination of pulsed electric field (PEF) and oscillating magnetic field (OMF) was used. Specially, to achieve a supercooled state quicker and prevent the sudden ice nucleation, a combined PEF and OMF treatment protocol was developed based on the results of electric current. In addition, quality assessment factors such as microstructure, drip loss, pH, color and texture for supercooled chicken breast samples were evaluated and compared with refrigerated (at 4°C), partially frozen (at -7°C) and, fresh samples.

2. Material and methods

2.1. Meat preparation

Fresh chicken breast samples were purchased from a local market in Hawaii and immediately transported to the laboratory under temperature-controlled condition. On arrivals, samples were trimmed of all visible connective tissue and excess fat and cut to fit into cubic blocks ($3.81 \times 3.81 \times 1.91 \text{ cm}^3$). All samples were weighed and wrapped in polyethylene film to avoid superficial dehydration before experiments. The quality assessments for the fresh chicken breast sample were immediately evaluated. In addition, each treatment was carried out on the day of purchase.

2.2. Freezing equipment

A freezing system was designed and fabricated consisting of oscillating magnetic field (OMF) and pulsed electric field (PEF) modules and real-time temperature, current and voltage measurement units. A freezing cube ($3.81 \times 3.81 \times 3.81 \text{ cm}^3$), equipped with one pair of titanium plate electrodes in parallel for applying PEF, was placed on the top of the spacer block between the permanent magnet and electromagnet (Figure 1). For applying OMF, a block of NdFeB permanent magnet (N52, DX88-N52, K & J Magnetics, Inc., Jamison, PA; size: $5 \times 5 \times 2.5 \text{ cm}^3$) and an electromagnet were fixed on the fabricated freezing device. The electromagnet contained an iron core (VIMVAR, Ed Fagan, Inc., Franklin Lakes, NJ) with coiled magnet wire (22 AWG, EIS, Inc., Atlanta, GA) and an alternating current was applied to achieve the charge and discharge system. The fabricated cell device with electrodes and magnets was placed in the chest freezer (FCM7SUWW, GE, Inc, Fairfield, CT). For efficient cold air circulation, freezing cube was designed with plates with multiple holes. K-type thermocouple wire (K-type, PP-K-24S, Omega Engineering, Inc., Stamford, CT) aligned at the center of the cell was used to measure sample temperature. A data acquisition unit (DAQ, Agilent 39704A, Agilent Technologies, Inc., Palo Alto, CA) was used to monitor and collect the applied voltage and current, and temperature values of the sample and air in the freezer. The data were scanned and transmitted at the interval of 1 sec. The output signal was monitored in a digital oscilloscope (Model TDS2014; Tektronix, Beaverton, OR) and the magnetic field intensity between two different magnets was measured using a handheld teslameter (4060.50 AE Teslameter, Frederiksen, Inc., Ølgod, Denmark). Freezer temperatures were controlled using a digital temperature controller (A419, Johnson Controls, Inc., Milwaukee, WI).

2.3. OMF and PEF treatments

In order to achieve an extension of the supercooled state within chicken breast samples, pulsed electric field (PEF) and oscillating magnetic field (OMF) were applied. The PEF and OMF were generated using an integrated-gate-bipolar-transistor (IGBT, IRAMX20UP60A, International Rectifier, El Segundo, CA) based power supply. Function generators (33220A, Agilent Technologies, Santa Clara, CA) were used to control the square wave forms with various duty cycles (D) and working frequencies. A repeating sequence of three different duty cycles, 0.8, 0.5 and 0.2 were used for the PEF treatment. The input voltage ($10 V_{p-p}$, peak-to-peak voltage) at the frequency of 20 kHz was determined to avoid electro-conducting heating even at the maximum duty cycle (0.8). The maximum electric current were estimated to 0.032 A. To initiate the supercooling state in the sample, the constant input voltage with duty cycle of 0.5 was applied to the sample until the electric current reached its minimum value in the supercooling state. From our preliminary tests, the use of 0.5 (on/off) duty ratio was closely associated with stabilization of the supercooling state and minimization of ice nucleation. Duty cycle sequences were initiated after the electric current of chicken breast samples reached its minimum value for supercooling. The applied treatment times associated with three different duty cycles, i.e. 0.8, 0.5 and 0.2 were 300 sec, 120 sec, and 90 sec, respectively. The lower electric current showed the higher cooling rate and it caused sudden ice nucleation frequently. To suppress ice nucleation, the OMF was applied only with PEF duty cycle of 0.2. The repeating sequence of three different duty cycles was repeated until the temperature of samples reached to the freezer temperature, $-7 \pm 0.5^{\circ}\text{C}$. Four experiments were carried out to validate the reproducibility. The oscillating magnet field was generated by pulsed magnetic field with an intensity ranging from -150 to 150 mT. The applied voltage and pulse duty cycle was 25 V and 0.01, respectively and the applied frequency

was as low as 1 Hz. In present study, the combined magnetic field intensity by permanent magnet and electromagnet was oscillated between 50 to 100 mT at the center of freezing cube.

2.4. Electrical conductivities of chicken breast during supercooling and freezing

To measure electrical conductivities of chicken breasts during treatments, samples were placed and contacted between two electrodes in the freezer (at $-7 \pm 0.5^\circ\text{C}$) (Choi *et al.*, 2011). To determine input PEF voltage based on the changes in electrical conductivities with temperature, the applied voltage and current were determined and tested for the desired cooling temperature profile. Acquired electrical conductivities of the samples were calculated by the following equation (Palaniappan and Sastry, 1991):

$$\sigma = \frac{LI}{AV} \quad (1)$$

where L is the distance between two electrodes (m), A is the internal cross-sectional area (m^2), V is the applied voltage (V) and I is the measured electric current (A). From the obtained data which were collected from 0°C to temperature where begins to phase transition, the electrical conductivities of tested food materials were plotted against temperature and the temperature dependence of the measured electrical conductivity was depicted by a linear equation as given by:

$$\sigma = \sigma_{\text{ref}} + mT \quad (2)$$

2.5. Microstructure analysis

In this study, a fixation method similar to those proposed by Fischer *et al.* (2006) was used to observe the microstructures of the chicken breast samples. The chicken breast samples were cut

using 4 mm slicer. The partially frozen sample was cut after thawing at 4°C for 1 h. The cut samples were fixed with 4% paraformaldehyde in 0.1 M sodium cacodylate (pH 7.4). The fixed samples were processed in a graded ethanol series (70%, 80%, 95% and 100%), xylene, paraffin, then embedded in paraffin. The embedded samples were cut using a microtome (Leica, SM 2000R, Germany) into 5 µm thick section. The sliced samples were then stained with hematoxylin and eosin (Fischer et al., 2006). All the prepared slides were observed with a microscope (Olympus BX51, Japan) fitted with an Optronics Macrofire SP CCD camera. The microstructures of both surface and middle parts for chicken breast samples were measured at two different magnifications (10× and 20×). The optical microscope images were analyzed using picture analysis software (ImageJ, National Institute of Health). The percentage area of intercellular space was calculated from three micrographs of every treatment at each magnification used. To evaluate average area of the muscle fiber cells, one hundred cells were randomly selected from each image, and measured by using NIH ImageJ Magic Wand tool.

2.6. Drip loss

In this study, drip has been used to describe exudates both from frozen thawed meat (drip) and from refrigerated or supercooled meat (weep). The drip loss was measured according to the method previously described (Ngapo et al., 1999). Fresh meat samples were measured within 30 min after being cut from chicken breast chunk; drip loss of partially frozen samples were measured after thawing for 4 h at 4°C. Five samples were used for each combination of freezing, thawing and storage. The samples were cut into six cubes of approximately 0.5 inch length. Samples were suspended using nylon mesh and centrifuged at $40 \times g$ for 90 min. Drip loss was measured as:

$$Drip\ loss(\%) = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100 \quad (3)$$

2.7. Color measurement

To determine **whether** the effects of treatments had any negative effects on the appearance of chicken breasts, instrumental color analysis was conducted. The Hunter L^* (lightness), a^* (redness-greenness), and b^* (yellowness-blueness) values were measured using a color meter (ColorTec PCM, Clinton, NJ). The net color difference (ΔE) was calculated with the equation:

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \quad (4)$$

where the subscripts 1 and 2 are referred to as color components before and after treatment, respectively.

2.8. Texture analysis

After cooking in a 70°C water bath for 30 min, texture was evaluated by shear force using a TA-XT2 texture analyzer (Stable Micro Systems, Godalming, UK) as described by Barbanti and Pasquini (2005) with a slight modification. The peak shear force (N) for 50% compression was measured using a 25 kg maximum cell load at a cross-head speed of 5 mm/s. Four measurements were performed on each sample.

2.9. pH measurement

The pH value of chicken meat was determined using 5 g samples, homogenized with 5 mL of water. The pH was measured using a digital pH-meter (Mettler Toledo, Columbus, OH) with

direct insertion of the probe electrode after calibration. Measurements of pH were calculated from the average of four replicates.

2.10. Lipid oxidation measurement

Thiobarbituric acid reactive substances (TBARS) indicate the oxidative changes in muscle foods during storage. The amounts of TBARS in raw chicken breast samples were determined by using the procedure of McDonald and Hultin (1987). Sample (1 g) was weighed in plastic bags (ZipLoc, SC Johnson, USA) and homogenized with 10 mL of deionized water. Aliquot of the sample (1 mL) was added to 2 mL of trichloroacetic acid/thiobarbituric acid (TCA/TBA), consisting of 15% TCA (w/v) and 0.375% TBA (w/v) in 0.25 M HCl and 3 mL of 2% butylated hydroxytoluene (BHT) (w/v) prepared in ethanol and mixed thoroughly. The mixture was vortexed and incubated for 15 min in 90°C of water bath. The sample was cooled at room temperature for 10 min and centrifuged for 10 min at 1000 × g. The absorbance of the resulting supernatant solution was determined at 532 nm on a visible spectrophotometer (Thermo Scientific GENESYS20, Thermo Fisher Scientific, Inc., Rochester, NY). The TBARS values were calculated using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$ and expressed as mg malondialdehyde (MDA) per kg of meat sample. For the measurement of lipid oxidation, four replicates were performed.

2.11. Statistical analysis

In this study, data are expressed as means ± SD. Differences between groups were analyzed using an ANOVA (IBM SPSS Statistics 20). A *p*-value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. PEF and OMF combination

The measured electrical current passing through a non-treated chicken breast sample stored at -7°C was observed to change during the freezing process. As shown Fig. 2a, the flow of electric current decreased linearly with decreasing temperatures of the non-treated sample. In addition, the spontaneous deflection of electric current was observed around -3°C . The nucleation of water inside the non-treated chicken breast sample led to the significant change in the electric current. As shown Fig. 2b, the electrical conductivities of the sample in its **supercooling** state presented high linear correlation ($R^2 = 0.969$) which has been investigated as one of electrical properties of unfrozen samples during freezing (Palaniappan and Sastry, 1991). In contrast, no significant correlation between electrical conductivities and temperature was demonstrated after nucleation. The value of electric conductivities at 0°C was 0.64 S/m , which was similar to findings from Zell et al. (2009). The electrical conductivity and current prior to freezing during the supercooled state of the non-treated chicken breast samples were measured to be 0.58 S/m and 0.024 Amps , respectively. The **The spontaneous deflection (around -3°C)** in the electrical conductivity and current of the non-treated chicken breast sample are caused by the protonic defects of ice during ice formation and growth. The defects act to cancel out the applied external electric field, in turn lowering both conductivity and current within food samples (Petrenko and Whitworth, 1999).

PEF with duty cycles of 0.8, 0.5 and 0.2 was optimized for sequential application in durations of 300, 120 and 90 sec (t_1 , t_2 , and t_3 , respectively, Figure 3a). The different duty cycles and durations facilitated an efficient cooling rate to achieve a supercooled state quicker. It was observed that a decrease in duty cycles from 0.8 to 0.2 caused an increase in cooling rates of

chicken breast samples of up to $-0.12^{\circ}\text{C}/\text{min}$. In this manner the cooling rate of chicken breast samples were controlled with the use of three different duty cycle sequences.

Although the cooling rate of the chicken breast sample during the supercooling state was controlled, ice nucleation occurred frequently **during or in turn of** the 0.2 duty phase (data not shown). The weak electron distribution inside the sample might lead to electrically polarized water molecules to return to its native random states, **resulting in inconsistent freezing time as well (Table 1)**. To prevent the sudden ice nucleation during the 0.2 duty phase, a combined PEF and OMF treatment was applied. According to previous studies, pulsed magnet field could increase the vibration of water molecules (Iwasaka et al., 2011) and be utilized for suppression of ice formation (Kiani and Sun, 2011). The use of this combination technology successfully prevented the ice nucleation and extended the supercooled state within the chicken breast sample for 12 h (**Table 1** and Fig. 4).

3.2. Effects of developed PEF and OMF combination on extension of supercooling

Fig. 4 show the results of the chicken breast sample temperature profile under the newly developed, combined PEF and OMF treatment protocol. The temperature profile of the chicken breasts without treatments (non-treated sample) is also displayed. Note there is no difference in the cooling rates between treated and non-treated chicken breast samples prior to ice nucleation of the non-treated sample. When the minimum current threshold as determined prior was detected during the cooling phase (0.024 A), PEF with OMF treatment was initiated to maintain the supercooled state. The similar cooling rates of treated vs. non-treated samples suggest no thermal processes dominate, particularly with the PEF treatment. This suggests interaction

between water molecules with PEF and OMF are responsible for ice nucleation prevention rather than thermal effects.

During the supercooling period, the temperatures of the treated samples were maintained at -6.5°C (near freezer ambient temperature) and temperatures of non-treated samples reached -6.5°C after the phase transition period. The treated sample was maintained in an extended supercooled state without ice crystals formation, while the controls became partially frozen during the 12-hour test period. The degree of supercooling of treated chicken samples was estimated approximatively by the temperature difference between the freezer temperature and freezing point of non-treated samples. The degree of supercooling for treated chicken breasts sample ($5.6 \pm 0.2^{\circ}\text{C}$) was significantly different from the degree of supercooling of the non-treated sample ($1.6 \pm 1.4^{\circ}\text{C}$) ($P < 0.05$). For all treated samples there was no sudden ice nucleation until the end of experiments. The developed PEF and OMF protocol was shown effective in suppressing ice formation during our 12 h test period.

3.3. Effects of developed PEF and OMF combination on the microstructure of chicken breasts

The cross-sectional microscopic images of chicken breast samples after 12 h test period are shown in Fig. 5. The measurements of microstructure for the chicken breast surface and middle parts were carried out at two different magnifications. The optical microscopic images displayed the muscle fibers within the muscle fascicle. The endomysium or space between muscle fibers, in the refrigerated and supercooled samples was uniform, whereas the endomysium of the partially frozen sample had shrunk which indicated large ice crystal growth and microstructure damage.

For the middle part of the chicken breast samples, the average percent of the muscle fiber space for the refrigerated, supercooled, and partially frozen samples were 65.34 ± 3.11 , $67.71 \pm$

0.80, and $72.98 \pm 1.18\%$, respectively. The muscle fiber space of the partially frozen sample was significantly higher than supercooled and refrigerated samples ($P < 0.05$), this is so because the endomysium of the partially frozen sample had shrunk, indicating large ice crystal growth and microstructure damage. In addition, the average area of the muscle fiber for the refrigerated, supercooled, and partially frozen samples were 2900.0 ± 1157.6 , 2725.8 ± 705.2 and $1408.7 \pm 775.1 \mu\text{m}^2$, respectively.

For the surface part of the chicken breast samples, the average percent of the muscle fiber space for the refrigerated, supercooled, and partially frozen samples were 73.69 ± 2.26 , 75.44 ± 1.92 , and $85.38 \pm 2.38\%$, respectively. The muscle fiber space of the partially frozen sample was the highest, compared with that of supercooled and refrigerated samples ($P < 0.05$) because of drip loss caused by freezing injury. In addition, the average values of the muscle fiber area for the refrigerated and supercooled samples were 2617.3 ± 1084.1 and $2034.5 \pm 544.0 \mu\text{m}^2$, respectively. The muscle fibers of the partially frozen sample in the surface part could not be measured because the endomysium of the partially frozen sample had shrunk (Fig. 5e and f).

3.4. Effects of developed PEF and OMF combination on the qualities of supercooled chicken breasts

As shown in **Table 2**, quality assessment factors such as drip loss, color, texture, pH and lipid oxidation for supercooled chicken breast samples were evaluated and compared with those of refrigerated (at 4°C), partially frozen (at -7°C) and fresh samples.

The drip loss of four different samples such as fresh, refrigerated, partially frozen and supercooled samples were 0.83, 0.84, 1.74 and 0.80%, respectively. There's no significant difference in the drip loss of chicken breast samples after each treatments ($P > 0.05$), except

partially frozen sample which indicated endomysium shrinkage and muscle cell damages by the formation of ice crystals (Lee et al., 2008). As seen in the microstructure of breast after frozen storage (Fig. 5e and f), the structural damage in the muscle fibers led to a decrease in water holding capacity of the chicken breast sample, resulting in an increase of drip loss (Lee et al., 2008; Yoon, 2002). In addition, the decrease in water holding capacity promotes protein aggregation reactions which lead to toughening of the muscle (Mackie, 1993).

The shear force of fresh, refrigerated, partially frozen and supercooled samples were measured as 27.24, 27.17, 25.03 and 27.80 Newtons, respectively. The significant increase in tenderness was observed only in partially frozen chicken breasts ($P < 0.05$). The tenderization is caused by the breakdown of the muscle fibers and the loss of structural integrity. The formation of extracellular ice crystals disrupts the physical structure. Both refrigerated and supercooled chicken breasts were as tender as original samples ($P > 0.05$).

The measurement of thiobarbituric acid reactive substances (TBARS) is a well-established method for screening and monitoring lipid peroxidation (Tang et al., 2001). The TBARS value of four different samples such as fresh, refrigerated, partially frozen and supercooled samples were 0.26, 0.29, 0.27 and 0.26 malondialdehyde (MDA) kg^{-1} sample, respectively. The TBARS value of the refrigerated chicken breast was significantly higher than other chicken breast samples ($P < 0.05$). Malondialdehyde, a secondary product of lipid peroxidation, is the major substrate in the TBARS test. The compound is thought to be toxic, carcinogenic and mutagenic (Tang et al., 2001). The subzero temperature storages such as freezing and supercooling could delay lipid oxidation significantly and reduce the potential risk induced by lipid oxidation products.

For the pH difference measurements, there were no significant differences in all of values among four different conditions ($P > 0.05$). In color changes studies, regardless of storage

methods, L^* - and b^* -value (degree of lightness and yellowness, respectively) decreased during storage. However, there were no overall significant difference ($P > 0.05$) between treatments after 12 hours of storage. In contrast, all other treatments gave similar a^* values, beyond which, a small but statistically significant ($P < 0.05$), increase in redness was recorded from partially frozen samples. Even though a^* parameter values have known to correlate to accumulation of pigment content and redox state of myoglobin (Mancini and Hunt, 2005), such a trend requires further investigation. Although pH and color are known to cause significant effects on food qualities, there were no significant changes on a practical level in present study due to relatively short storage time. For the most part of quality tests, the supercooled chicken breast samples maintained the original fresh qualities. Overall, the findings of the current study demonstrate that the strategy of PEF and OMF combination for extension of supercooling state is applicable to maintain the original qualities while achieving satisfactory long term storage of raw chicken breasts. Furthermore, the optimized supercooling conditions under developed PEF and OMF combination technologies could deliver the premium food qualities to consumers.

Conclusions

This study presents a novel method for supercooled storage of chicken breast using a combination pulsed electric field and oscillating magnet treatment at temperatures below the equilibrium freezing point. To achieve a supercooled state quicker and prevent the sudden ice nucleation, a combined PEF and OMF treatment protocol was developed based on the results of electric current. PEF with duty cycles of 0.8, 0.5 and 0.2 was optimized for sequential application in durations of 300, 120 and 90 sec. During the 0.2 duty phase of PEF, a combined PEF and OMF treatment was applied to prevent the sudden ice nucleation. At a freezer

temperature of $-7 \pm 0.5^{\circ}\text{C}$, the internal temperature of the chicken breast under PEF and OMF treatments was -6.5°C during the entire testing period (approximately 12 h). In addition, the results of quality assessments such as microstructure, drip loss, pH, color and texture showed that the supercooled chicken breasts maintained the original qualities without any quality deteriorations. Future studies may include designing of a commercial scale unit with larger sample scales that can guarantee premium quality of foods for the longer term preservation.

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Table 1. Occurrence and degree of supercooling in individual chicken breast samples at -7°C under different condition (conventional and PEF with and without OMF treatments) for 12 h

Trials	Condition	Parameters		Freezing (Time to freeze, min)	Freezing point (°C)	Degree of supercooling (°C)
		PEF (10 V _{p-p})	OMF (Oscillated from 50 to 100 mT per second, periodically*)			
1.1	Conventional	-	-	Yes (47.1)	-1.4	1.6 ± 1.4 ^a
1.2		-	-	Yes (52.7)	-1.7	
1.3		-	-	Yes (46.8)	-1.3	
1.4		-	-	Yes (54.5)	-1.2	
2.1	PEF only	0.5	-	Yes (67.3)	-1.8	3.7 ± 0.6 ^b
2.2		0.5	-	Yes (70.5)	-1.3	
2.3		0.5	-	Yes (68.1)	-2.0	
2.4		0.5	-	Yes (66.4)	-1.4	
3.1		0.8-0.5-0.2**	-	Yes (260.8)	-1.7	5.1 ± 0.5 ^c
3.2		0.8-0.5-0.2	-	Yes (106.4)	-1.5	
3.3		0.8-0.5-0.2	-	Yes (158.3)	-1.4	
3.4		0.8-0.5-0.2	-	Yes (306.6)	-1.3	
4.1	PEF + OMF	0.8-0.5- <u>0.2</u>	Applied	No		5.6 ± 0.2 ^{c, ***}
4.2		0.8-0.5- <u>0.2</u>	Applied	No		
4.3		0.8-0.5- <u>0.2</u>	Applied	No		
4.4		0.8-0.5- <u>0.2</u>	Applied	No		

*OMF was applied when PEF duty cycle of 0.2 was applied.

**Applied sequential PEF duty cycles set as 0.8, 0.5 and 0.2 for 300, 120, and 90 sec, respectively and repeated for 12 hours.

***Estimated from mean freezing point from Trials 1-3.

Dissimilar small letters in same column indicate significant differences at 0.05 levels.

Table 2. Mean values ($\pm$ S.D.) of physical and chemical changes over initial (at 4°C, Control), refrigerated (at 4°C for 12 h, Control-), partially frozen (at -7°C for 12 h and thawed at 4°C for 4 hours, respectively, Control+) and supercooled (at -7°C for 12 h) chicken breast samples

Parameter	Initial	Refrigerated	Frozen	Supercooled
Drip loss (%)	0.83 ± 0.14^a	0.84 ± 0.05^a	1.74 ± 0.23^b	0.80 ± 0.13^a
<i>L*</i>	55.62 ± 0.01^a	55.29 ± 0.02^b	55.30 ± 0.01^b	55.31 ± 0.01^b
<i>a*</i>	3.24 ± 0.02^a	3.29 ± 0.02^b	3.32 ± 0.01^c	3.28 ± 0.02^b
<i>b*</i>	3.33 ± 0.01^a	3.21 ± 0.01^b	3.23 ± 0.01^b	3.20 ± 0.02^b
ΔE	-	0.35 ± 0.02^a	0.34 ± 0.01^a	0.33 ± 0.01^a
Texture (N)	$27.24 \pm 1.68^{a,b}$	27.17 ± 1.45^a	25.03 ± 2.24^c	27.80 ± 1.36^b
pH	6.40 ± 0.01^a	6.41 ± 0.01^a	6.40 ± 0.01^a	6.40 ± 0.01^a
TBARS (mg MDA/kg meat)	$0.26 \pm 0.03^{a,b}$	0.29 ± 0.02^b	0.27 ± 0.01^a	0.26 ± 0.02^a

In each rows, dissimilar small letters in each column indicate significant difference at 0.05 levels.

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