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Effect of pulsed electric field on structural modification and thermal properties of whey protein isolate

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Abstract

This study evaluated structural modification and thermal properties of whey protein isolate (WPI) treated by pulsed electric fields (PEF). Structural modification and thermal properties of WPI were evaluated by using fluorescence spectroscopy and differential scanning calorimetry (DSC) techniques. The effect of PEF treatments (electric field intensity ranging from 12, 16 and 20 kV cm^{-1} and pulses ranging from 10-30) on intrinsic tryptophan fluorescence and extrinsic 1-anilino-naphthalene-8-sulfonate (ANS) fluorescence of WPI were studied. PEF treatments of WPI resulted in an increase in intrinsic tryptophan fluorescence intensity and 2-4 nm red shifts. There was an increase in extrinsic ANS fluorescence intensity which showed increases in the surface hydrophobicity of WPI. The apparent enthalpy and denaturation temperatures of PEF-treated WPI samples were modified by PEF treatments. Whey protein was denatured by approximately 43.7% when WPI was treated by PEF at the electric field intensity of 20 kV cm^{-1} and 30 pulses. The results of this study indicated that PEF treatments affected structural modification and thermal properties of whey protein isolates.

Keywords: Pulsed electric field (PEF), whey protein isolate (WPI), relative fluorescence intensity, surface hydrophobicity, differential scanning calorimetry (DSC), protein structure, thermal properties.

Introduction

Whey protein isolate (WPI) is the purest form of whey protein and contains more than 90% β-lactoglobulin and α-lactalbumin. It is an excellent source of nutrients (high amino acids) and an important source of functional ingredients used in many formulated foods, such as processed meat, bakery, and dairy products (Kinsella and Whitehead, 1989). Whey proteins possess varying functional properties because of their dynamic structures and amphiphilic nature. The dynamic structure and flexibility of whey proteins indicate the possibility of further enhancing their functional properties, such as foaming and emulsifying properties. The functional properties of whey proteins are influenced by structural modification or denaturation of whey proteins (Kinsella et al., 1994). The protein denaturation may be affected by many factors including heat treatment, pH, ionic strength and solvent, high pressure and pulsed electric field (PEF) (Rhim et al., 1990; Phillips et al., 1990; Phillips et al., 1991; Boye et al., 1995; Hayakawa et al., 1992; Hinrichsa and Rademacher, 2005; Barsotti et al., 2002; Perez and Pilosof, 2004). Knowledge of the denaturation behavior of whey protein in a particular medium is the basic requirement in setting operating conditions to produce a desired effect. Measurements of structural modification and thermal properties are very useful in predicting the extent of whey protein denaturation and functional properties.

PEF processing presents unique advantages over conventional thermal processing methods, simultaneously providing consumers with microbiologically safe, minimally processed, nutritious and freshlike foods (Qin et al., 1996). Most studies have been carried out on the effects of PEF on the structural modification and inactivation of enzymes (Ho et al., 1997; Loey et al., 2002; Espachs-Barroso et al., 2003; Zhong et al., 2005a; Zhong et al., 2005b; Zhang et al., 2006; Zhang et al., 2007). The literature available regarding whey protein structural modification and thermal properties induced by PEF treatment is however limited (Fernández-Díaz et al., 2000; Perez and Pilosof, 2004). The current work was undertaken to evaluate the effects of PEF on structural modification and thermal properties of WPI.

Fluorescence spectroscopy is a sensitive, rapid, and non-invasive analytical method that provides information on the presence of fluorescent molecules and their environment in biological and food samples (Herbert et al., 1999; Christensen et al., 2003). The fluorescence properties of aromatic amino acids of proteins have been used to study protein structure or protein-hydrophobic molecule interactions (Defour et al., 1994; Defour and Riaublanc, 1997). On the other hand, differential scanning calorimetry (DSC) is a powerful technique to study the thermodynamics of protein stability that can provide a basic understanding of protein denaturation (Thorarinsdottir et al., 2002; Kurganov et al., 1997).

The objective of this study was to investigate the effects of PEF treatments on structural modification, surface hydrophobicity and thermal properties of WPI using fluorescence spectroscopy and DSC techniques.

Materials and methods

Materials

BiPRO whey protein isolate (WPI) powder (lot no. JE348-6-440) containing approximately 97.7% β-lactoglobulin and α-lactalbumin and was provided by Davisco Foods International, Inc. (Le Sueur, MN). Fluorescence spectroscopy probe, 1-anilino-8-naphthalenesulfonate (ANS) was purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). All of the chemicals (such as hydrochloric acid) used was purchased from Fisher Chemicals (Fairlawn, NJ).

Preparation of samples

WPI solutions of 30 mg/mL were prepared by using the dissolving distilled water. The pH of WPI solution was adjusted to 7.0 using 1 M hydrochloric acid (HCl). The prepared WPI solution was allowed to stand for about 2 h prior to the PEF treatments.

Pulsed electric field treatment

PEF treatment was performed using a high voltage decay pulse generator TG2 and a batch parallel plate treatment chamber (TC-01001) (Food Process Engineering Laboratory, Macdonald Campus of McGill University) described by Xiang et al. (2007). Figure 1 shows the experimental set-up used for the PEF treatment of WPI. The samples were treated with the electric field intensity ranging from 12, 16 and 20 kV cm^{-1}, pulses ranging from 10 to 30, capacitance of discharge capacitor of 0.33 μF and pulse frequency of 0.5 Hz. The procedures of the PEF treatments of WPI were described by Xiang et al. (2008).

Intrinsic fluorescence measurements

The intrinsic tryptophan fluorescence of the WPI was monitored by using an excitation wavelength of 290 nm and the emission spectra monitored using emission wavelength ranging from 305 to 400 nm according to the method of Kulmyrzaey and Defour (2002). The diluted whey protein solutions (0.3 mg/mL) were used in fluorescence measurements and made the absorbance of the diluted samples below 0.1 in order to avoid a screening effect or a decrease in the fluorescence intensity and a distortion of the excitation spectra (Genot et al., 1992). The intrinsic fluorescence intensity of WPI was measured at room temperature (25±1.0^{o}C) using the Hitachi F-2000 fluorospectrophotometer (Hitachi, Ltd., Tokyo, Japan) with slit for excitation of 2.0 nm and scanning speed of 60 nm/min. The measurements were taken at an excitation wavelength of 290 nm, with 2.0 nm excitation slit, 305 to 400 nm emission spectrum wavelengths and a scanning speed of 60 nm/min.

Surface hydrophobicity of WPI using ANS probe

Surface hydrophobicity of WPI solutions (30 mg/mL) was measured according to Kato and Nakai (1980) and Nakai (1983) using fluorescence probe ANS. The PEF-

treated and non-treated (control) WPI solutions were diluted by using distilled water to the final protein concentration of 0.3 mg/mL. Then 0.1 mL of ANS (50 mM in distill water) was added to 9.9 mL of the diluted WPI sample and mixed. The extrinsic ANS fluorescence intensity of WPI was assayed at an excitation wavelength of 375 nm and an emission spectrum wavelength of 420-560 nm in a Hitachi F-2000 fluorospectrophotometer (Hitachi, Ltd., Tokyo, Japan). The relative fluorescence intensity was defined as the ratio of the extrinsic ANS fluorescence intensity to the whey protein concentration (0.3 mg/mL). Surface hydrophobicity of WPI solutions was expressed as the relative fluorescence intensity (RFI) with the whey protein concentration (0.3 mg/mL) described by Li et al. (2007).

Differential scanning calorimetry (DSC) measurement

The thermal properties of whey protein in WPI solution (30 mg/mL) were examined by using a differential scanning calorimeter (Q100, TA Instruments, New Castle, DE, USA). The DSC was calibrated by using an indium standard. About 10 mg of WPI solution was accurately weighed and put into the aluminum pan. The pan was hermetically sealed. WPI samples were equilibrated at 20^{o}C for 2 h. During the temperature increases from 20 to 90°C at a programmed heating rate of 5°C/min and the samples ware scanned. An empty pan was used in the treatment cell of the DSC as a reference for each run. All DSC measurements were done in at least triplicate.

The peak transition temperature or denaturation temperature (T_d) and the apparent enthalpy of denaturation (ΔH) were computed from each thermogram by the TA instruments software (Universal Analysis, TA version 4.10). Enthalpy values were based on the total weight of WPI. The percent denaturation of WPI was obtained by comparison of denaturation enthalpies (ΔH) of native WPI and the PEF-treated WPI:

$$Denaturation\% = \frac{\Delta H1 - \Delta H2}{\Delta H1} \times 100 \quad (1)$$

Where $\Delta H1$ is the apparent enthalpy of the native WPI sample (J/g), $\Delta H2$ is the apparent enthalpy of the PEF-treated WPI sample (J/g).

Statistic analysis

All experiments and analyses were done in triplicate (3 independent runs). The analysis of variance test for significant effects of treatments and assay samples were determined using the SAS software (SAS Version 9.1, SAS Institute Inc., Cary, NC, USA). Main effect differences were considered significant at $P<0.05$ level.

Results and discussion

Intrinsic tryptophan fluorescence of WPI

The intrinsic tryptophan fluorescence spectra of WPI solution (30 mg/mL) with the PEF treatments (20 kV cm^{-1} and pulses ranging from 10 to 30) are shown in Figure 2. For control WPI sample, the emission wavelength of the maximum fluorescence intensity was 333 nm and the excitation wavelength was at 290 nm indicating the characteristic peak of tryptophan residue in the fluorescence emission spectra. The increases in tryptophan fluorescence intensity of WPI and red shifts of the emission wavelength of the maximum tryptophan fluorescence intensity were observed after PEF treatments. PEF treatments of 20 kV cm^{-1} and 10 or 20 pulses resulted in a 2 nm red shift of the emission wavelength from 333 to 335 nm. There was a sharp increase in tryptophan fluorescence intensity and a 4 nm red shift of the emission wavelength from 333 to 337 nm after PEF treatment of 20 kV cm^{-1} and 30 pulses. The increases in the intrinsic fluorescence intensity and red shifts observed after the PEF treatments indicated changes in the polarity of tryptophan residues microenvironment of whey proteins from a less polar to a more polar environment. This result was similar to the results of whey protein concentrate treated with high pressure (Liu et al., 2005) and whey protein unfolding due to exposition of the tryptophan residues to the aqueous solvent (Moro et al., 2001).

The tryptophan fluorescence emission spectra of WPI exposed to PEF treatments are shown in Figure 3. Compared to the native WPI, PEF treatments increased the tryptophan fluorescence intensities of WPI. As the electric field intensity increased, the tryptophan fluorescence intensity of WPI increased correspondingly. The fluorescence intensity of WPI increased from 26.91 to 29.16 a.u when electric field intensity increased from 12 to 20 kV cm^{-1}. The changes in the fluorescence intensity of WPI were significantly different ($P<0.05$) with increasing the electric field intensity. The results obtained in this study were consistent with other studies reported in the literature (Zhong et al., 2005b; Zhang et al., 2007). PEF caused an increase in the intrinsic fluorescence intensity of horseradish peroxidase and this was attributed to modification of the tertiary structure of the horseradish peroxidase (Zhong et al., 2005b). The increase in the fluorescence intensity of WPI at 333 nm reflected the alteration of the tertiary structure of WPI.

Surface hydrophobicity modification of WPI with PEF treatment

Surface hydrophobicity of proteins is one of the structural characteristics to evaluate the change in protein structure (Nakai, 1983). The modification of surface hydrophobility of WPI with PEF treatments of 16 kV cm^{-1} are shown in Figure 4. The surface hydrophobility of WPI increased with increasing pulses and it reached the maximum (115% of the control) at 16 kV cm^{-1} and 30 pulses. The results were similar to the results of Li et al. (2007) with PEF treatment with soy protein isolate (30 kV cm^{-1} and treatment time of 288 μs). Figure 5 shows the modification of surface hydrophobility of WPI with PEF treatments of 30 pulses. A significant increase in the surface hydrophobicity was observed with increasing electric field intensity as compared with the

control. The surface hydrophobility reached the maximum (123% of the control) at 20 kV cm^{-1} and 30 pulses. The results of this study (Figures 4 and 5) suggested that more pulses or higher electric field intensity induced molecular unfolding of WPI, destroyed hydrophobic interactions of protein molecules, caused more hydrophobic groups and regions inside the molecules to expose outside, thus increased the surface hydrophobility of WPI. The results were similar to those that heat-induced whey protein aggregates had quite a high hydrophobicity (Jean et al., 2006) and high pressure-induced whey protein increased surface hydrophobicity due to unfolding of whey proteins (Lee et al., 2006). The results indicated that PEF-treated WPI structure became more loose thereby allowing ANS molecules to enter to the hydrophobic core of the protein. Alvarez (2004) also obtained similar results showing that high pressure treated WPI changed its three-dimensional structure to expose small hydrophobic pockets previously inaccessible to solvent.

Thermal properties of WPI with PEF treatments

The DSC thermograms of the control and the PEF-treated WPI (electric field intensity of 20 kV cm^{-1} and 30 pulses) are shown in Figure 6. Both the denaturation temperatures (T_d) and apparent enthalpy (ΔH) were modified by PEF treatments. The apparent enthalpy changes correspond to dimmer dissociation and denaturation processes (endothermic) which are superimposed to an aggregation process (exothermic) during the time scale of DSC measurements (Relkin et al., 1998). The DSC thermogram of the control sample had two peak denaturation temperatures namely ~68 and ~78^{o}C. With the PEF treatments, the denaturation temperatures of WPI had changed. The denaturation temperatures of WPI involved in the denaturation process were changed by PEF treatments (Table 1). The denaturation temperatures (T_{d1} and T_{d2}) were modified by PEF treatments. The denatured WPI after PEF treatment (electric field intensity of 20 kV cm^{-1} and 30 pulses) was approximately 43.7%. WPI was partially denatured by PEF treatments. Perez and Pilosof (2004) reported that PEF treatment (electric field intensity of 12.5 kV cm^{-1} and capacitance 40 μF) changed the thermal stability of β-lactoglobulin by shifting the temperature by 4~5^{o}C and the denatured β-lactoglobulin after 10 pulses was approximately 40%.

The residual denaturation enthalpy provides a net value from a combination of endothermic reactions like the disruption of hydrogen bonds, and exothermic processes, including the break-up of hydrophobic interactions and protein aggregation (Ma and Harwalkar, 1991). Table 2 shows the changes of the denaturation temperatures and the apparent enthalpy of WPI with PEF treatments at 30 pulses and different electric field intensities of 12, 16 and 20 kV cm^{-1}. An increase in electric field intensity, the apparent enthalpy decreased more and it showed that higher electric field intensity had more effect on WPI and made WPI more structural modification. As a result, DSC thermograms of the PEF-treated WPI exhibited subsequent thermal denaturation of the proteins remaining in a native-like WPI structure. The results were similar to other researchers (Hayakawa et al., 1996; Van der Planchen et al., 2007) and they also explained that a reduction in residual enthalpy could be an indication for a partial loss of protein structure during high pressure treatment.

Conclusion

The effects of PEF on structural modification and thermal properties of WPI were studied. The increases in the tryptophan fluorescence intensity of WPI and red shifts of 2-4 nm of the emission wavelength of the maximum fluorescence intensity were observed when the applied electric field intensity or the pulse increased during PEF treatment. Extrinsic ANS fluorescence intensity changes of WPI were related to the structural modification of WPI. This confirmed that the main parameters influencing structural modification of WPI were electric field intensity and pulse. This suggested that the electric field intensity and pulse were closely related to their effects on structural modification of WPI. Comparison of DSC thermal parameters of whey protein showed that the thermal stability of whey protein was greatly reduced by PEF treatments. The whey protein was denatured about 43.7% with PEF treatment at 20 kV cm^{-1} and 30 pulses. In the electric field intensity, an increase of pulses also improved protein denaturation of WPI. Further studies on the effect of PEF on the whey protein are needed to understand its denaturation mechanism.

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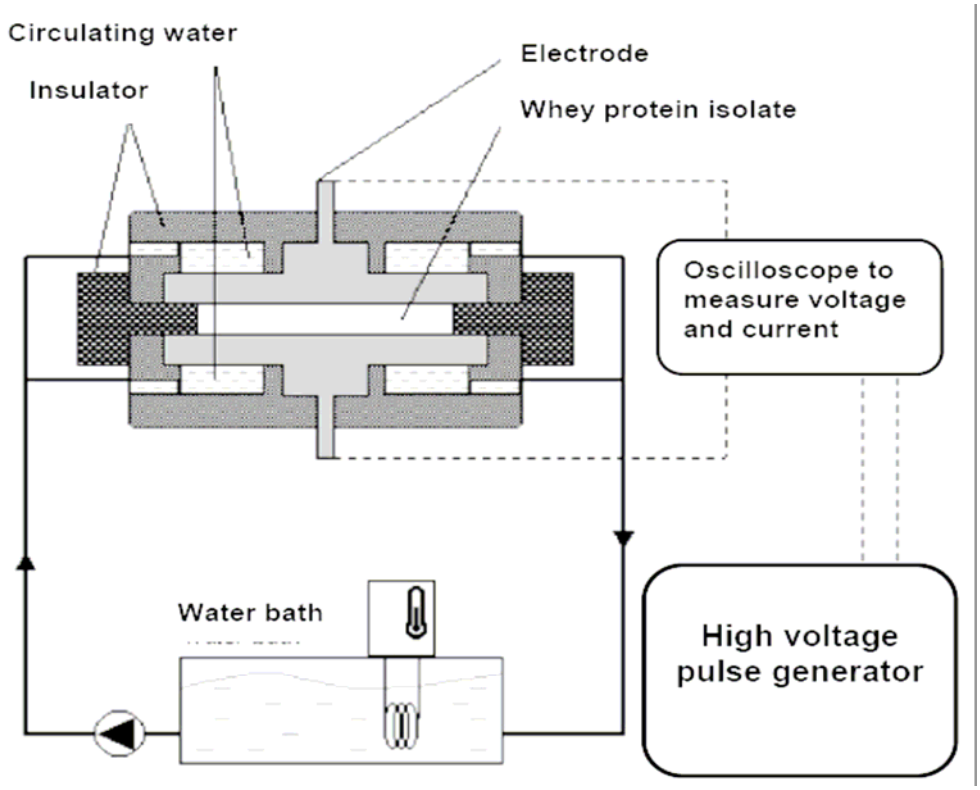

Figure 1. Experimental set-up used for the PEF treatment of whey protein isolate

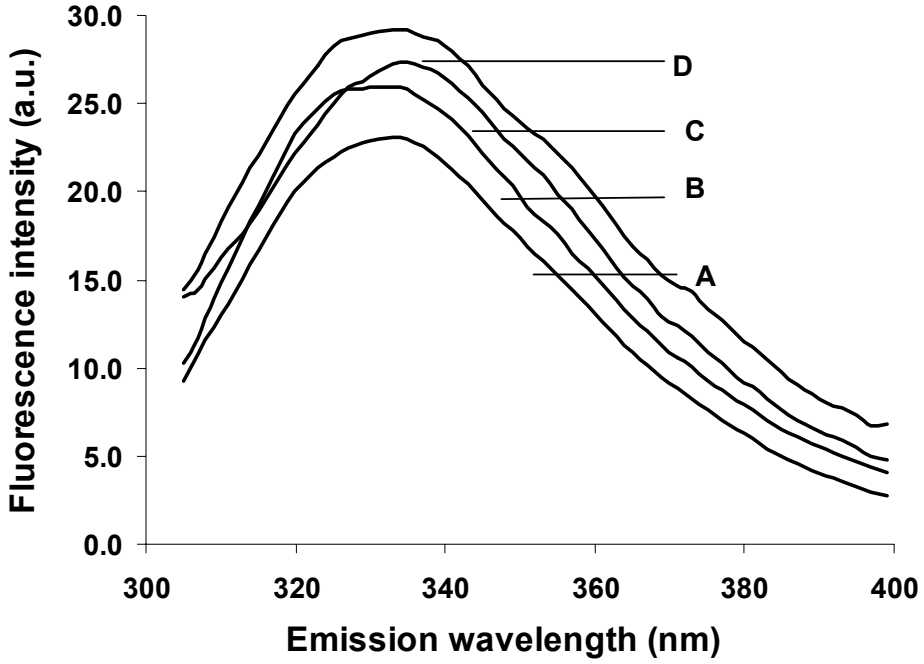

Figure 2. Tryptophan fluorescence emission spectra of WPI applied to 20 kV cm^{-1}. (A) Control; (B) 10 pulses; (C) 20 pulses; (D) 30 pulses.

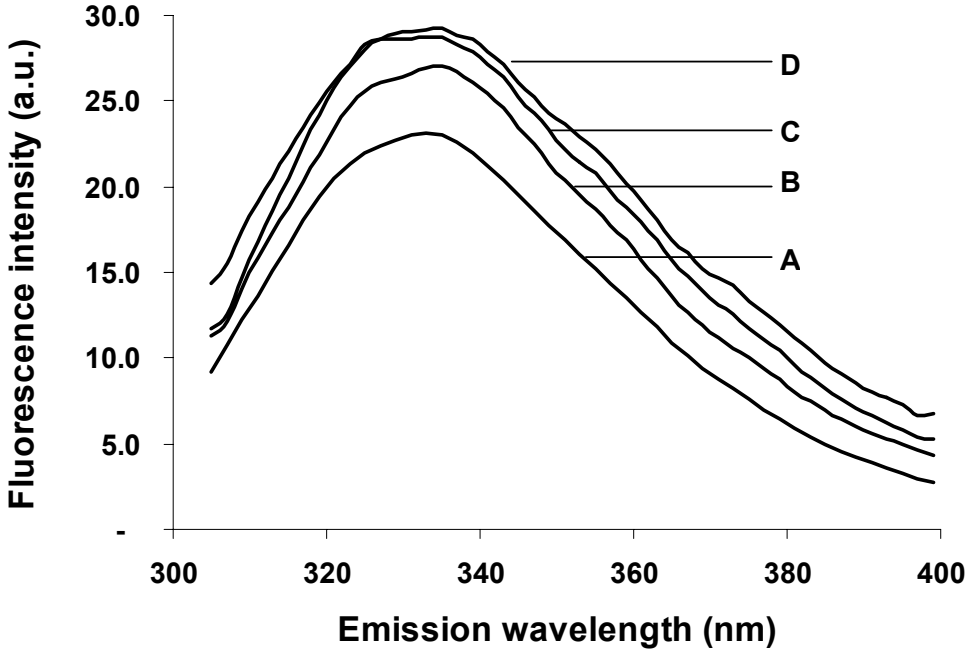

Figure 3. Tryptophan fluorescence emission spectra of WPI with PEF treatments. (A) Control; (B) 12 kV cm^{-1}; (C) 16 kV cm^{-1}; (D) 20 kV cm^{-1}. All treatments are at 30 pulses.

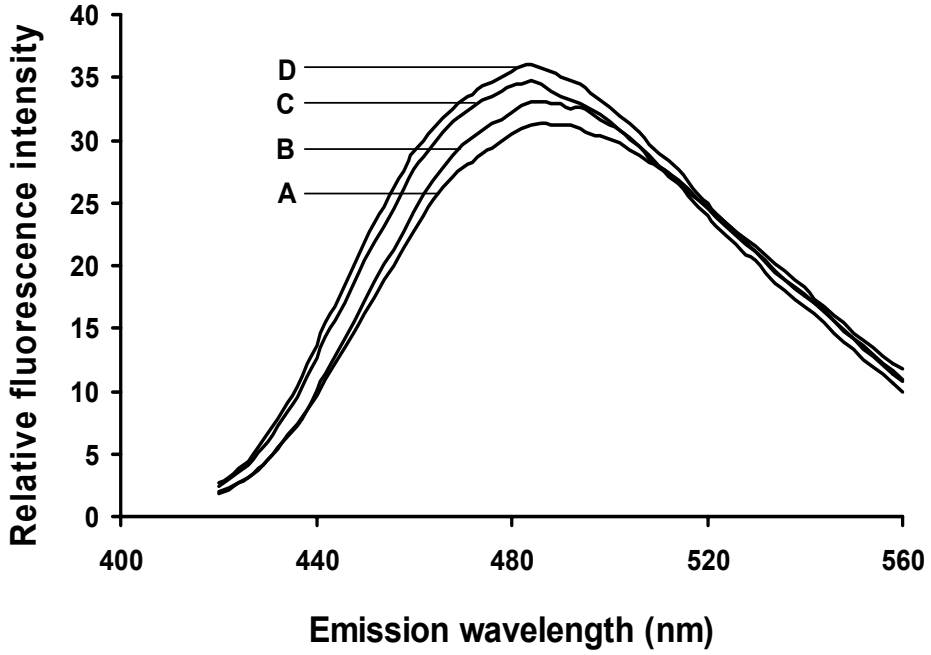

Figure 4. The surface hydrophobility modification of WPI with PEF treatments of 16 kV cm^{-1}. (A) Control; (B) 10 pulses; (C) 20 pulses and (D) 30 pulses.

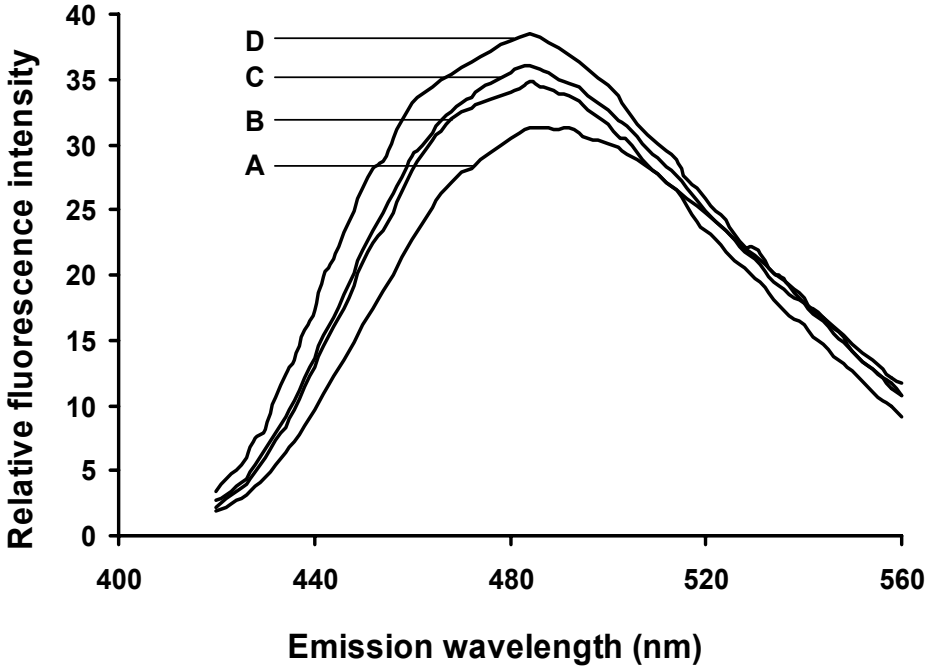

Figure 5. The surface hydrophobility modification of WPI with PEF treatment of 30 pulses. (A) Control; (B) 12 kV cm^{-1}; (C) 16 kV cm^{-1} and (D) 20 kV cm^{-1}.

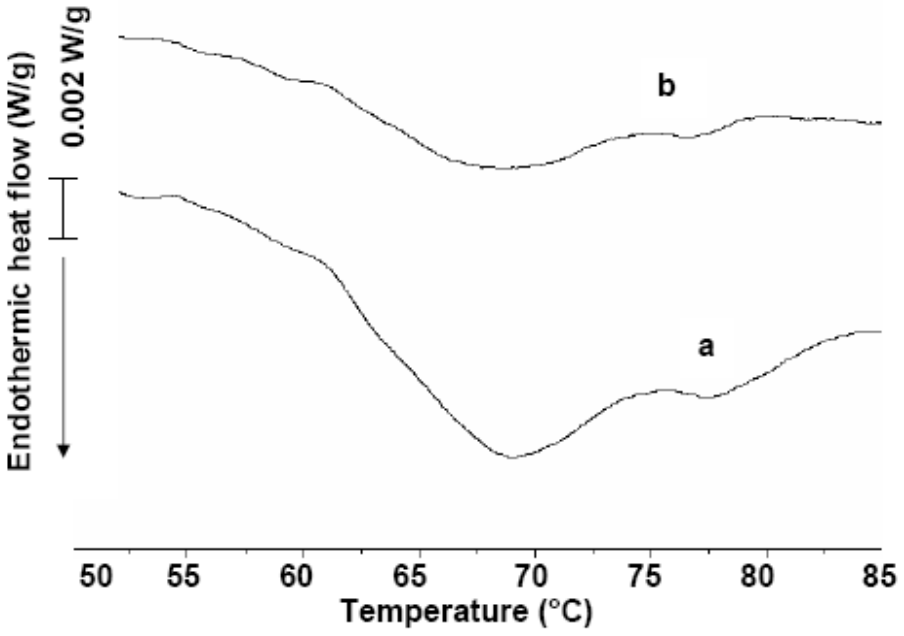

Figure 6. DSC thermograms of WPI. (a) Control; (b) PEF-treated at electric field intensity of 20 kV cm^{-1} and 30 pulses

Table 1. Calorimetric parameters of WPI with PEF treatments (electric field intensity of 16 kV cm^{-1} and pulses ranging from 10 to 30)

Treatment	T_{d1}(^{o}C)	T_{d2} (^{o}C)	$\Delta H2$ (J/g)	Denaturation %
Control	68.35 ± 0.21^{a}	77.91 ± 0.12^{a}	0.071 ± 0.001^{a}	-----
10 pulses	66.77 ± 0.19^{b}	77.74 ± 0.13^{a}	0.053 ± 0.001^{b}	35.4 ± 0.4^{a}
20 pulses	66.56 ± 0.17^{c}	77.49 ± 0.06^{b}	0.047 ± 0.001^{c}	33.8 ± 0.2^{b}
30 pulses	66.94 ± 0.13^{b}	78.57 ± 0.11^{c}	0.042 ± 0.001^{d}	40.8 ± 0.3^{c}

*Means ± SD (n=3). Means in the column with different superscripts are significantly different ($P < 0.05$).

Table 2. Calorimetric parameters of WPI with PEF treatments (electric field intensities of 12, 16 and 20 kV cm^{-1} and 30 pulses)

Treatment	T_{d1}(^{o}C)	T_{d2} (^{o}C)	$\Delta H2$ (J/g)	Denaturation %
Control	68.35 ± 0.21^{a}	77.91 ± 0.12a	0.071 ± 0.001^{a}	-----
12 kV cm^{-1}	67.65 ± 0.13^{b}	76.73 ± 0.16^{b}	0.046 ± 0.001^{b}	35.2 ± 0.3^{a}
16 kV cm^{-1}	66.94 ± 0.14^{c}	78.57 ± 0.13^{c}	0.042 ± 0.001^{c}	$40.8.0 \pm 0.4^{b}$
20 kV cm^{-1}	67.49 ± 0.13^{b}	77.49 ± 0.07^{d}	0.040 ± 0.001^{d}	43.7 ± 0.5^{c}

*Means ± SD (n=3). Means in the column with different superscripts are significantly different ($P<0.05$).