



Research article

Effects of ohmic heating on structural and physicochemical changes of whey proteins

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Abstract

Ohmic heating has received attention as a novel thermal process for foods containing protein. However, there is limited information on the effects of electric fields in addition to thermal effects on the physicochemical changes of proteins. This study investigated the electric field effects of ohmic heating on conformational changes, aggregation and the surface charge of whey proteins. Whey protein dispersion was heated at 60°C, 70°C and 80°C for 15 min under identical thermal histories for ohmic and conventional heating to study the electric field effects. The tertiary structure, aggregation and surface charge of the whey proteins were determined using intrinsic fluorescence spectroscopy, particle size distribution and the ζ -potential, respectively. Ohmic heating at $\geq 70^\circ\text{C}$ induced conformational changes to the whey protein molecules (intrinsic fluorescence intensity of $23,690 \pm 1,164$ A.U. and $29,814 \pm 1,149$ A.U. at 70°C and 80°C, respectively compared to $27,457 \pm 1,262$ A.U. and $32,988 \pm 1,213$ A.U. after conventional heating), dissociated submicron-sized protein clusters and re-assembled the proteins to nano-sized particles, which were smaller than the nano-sized particles found in conventional-heated whey protein dispersions. Ohmic-heated whey protein dispersions retained greater clarity than conventional heat-treated dispersions at the respective temperature, although the ζ -potential values of the whey protein aggregates in the suspensions treated using conventional heating and ohmic heating were not different ($p > 0.05$). In addition to heat-induced effects, the electric fields might cause alignment, stretch and relaxation of whey protein molecules. The electric field effects and uniform heating probably accounted for the reduced tertiary structural changes and smaller particle sizes of aggregates of whey proteins. This study suggested that ohmic heating could be used as an alternative thermal process to modify the protein conformation and physicochemical properties for specific protein functionalities.

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Introduction

Whey protein isolate (WPI) and whey protein concentrate (WPC) have been used as functional ingredients due to their excellent functional and nutritional properties in various food products (Dybowska, 2011; Rodrigues et al., 2015). Whey proteins generally consist of β -lactoglobulin (β -lg), α -lactalbumin (α -la), bovine serum albumin and immunoglobulins (Kinsella and Whitehead, 1989). β -lg is a major globular protein, which constitutes approximately 54% of total whey proteins (Schmitt et al., 2011) and is primarily responsible for WPI and WPC functionalities (Lefèvre et al., 2005).

There has been extensive investigation of whey protein denaturation and aggregation due to extrinsic factors, such as heat, pressure, pH and ionic strength (Raikos, 2010; Nicolai et al., 2011). Changes in the physicochemical properties of whey proteins are related to specific functional properties. Several researchers have studied the effect of thermal treatments on the transition of conformation and subsequent aggregation of whey proteins to facilitate the modification or improvement of their functional properties associated with emulsifying, foaming and gelling (Schmitt et al., 2007; Nicorescu et al., 2008; Dybowska, 2011; Rodrigues et al., 2015).

Thermal denaturation of β -lg at a temperature above 60°C partially unfolds β -lg (De Wit, 1990), accounting for the exposure of free sulfhydryl (SH) groups and hydrophobic moieties previously buried in the native β -lg molecules (Zúñiga et al., 2010). The free reactive SH groups further interact with disulfide bonds through the sulfhydryl (SH)-disulfide (SS) interchange, leading to intra- and intermolecular disulfide bonds, polymerization and subsequent protein aggregation. The availability and reactivity of the free SH groups directly affect the denaturation kinetics of whey proteins (Kroslak et al., 2007). Besides SS bonds, noncovalent forces (hydrophobic interactions, ionic bonding and van der Waals forces) are also involved in the aggregation as inter-molecular interactions among unfolded and polymerized β -lg (Tolkach and Kulozik, 2007). Excessive aggregation of whey proteins induced by heat treatment could increase the opacity of heated whey protein suspensions and limit their uses if clarity of the protein suspension is required.

Ohmic heating has increasingly received attention because of its ability to provide uniform heating and a rapidly controllable heating rate with high energy efficiency, as the passage of electric current through foods allows the direct conversion of electrical energy to volumetric heat generation (Ramaswamy

et al., 2014). This technology achieves high temperatures with a reduction of excessive denaturation of proteins (Parrott, 1992) and provides high-quality products with less destruction of thermally labile compounds (Castro et al., 2003). The non-thermal (electric field) effect of ohmic heating has been widely used for microbial inactivation (Kanogchaipramot et al., 2016) and extraction (Sastri and Barach, 2000; Ramaswamy et al., 2014), since the build-up charges interfere with membrane structure and function. However, information is limited on its effect on protein structure and consequent functionalities and this merits further investigation.

The electric field effect following ohmic heating might contribute to less denaturation of whey proteins and consequently less aggregation (Pereira et al., 2010, 2011; Rodrigues et al., 2015). Furthermore, the existence of an electric field probably alters conformational disturbances of protein structure, resulting in different interactions among denatured proteins compared to conventional heat treatment. Differences in the patterns of denaturation and aggregation due to electric fields might produce unique functional properties of whey proteins, such as, film-forming and gelling properties (Pereira et al., 2010; Rodrigues et al., 2015).

There has been only limited publication related to the additional electric field effects following ohmic heating on the conformational changes of proteins. The current study hypothesized that an oscillating electric field enables the periodic alignment, stretch and relaxation of proteins (Bekard and Dunstan, 2014). The electric field effect possibly causes the destabilizing of the protein tertiary structure leading to different denaturation and aggregation patterns and physicochemical properties.

The objective of this study was to investigate the impact of heating in the absence of an electric field (conventional heating) and in the presence of an electric field (ohmic heating) under identical thermal histories on tertiary structural alteration and the physicochemical properties of whey proteins, especially regarding emulsifying properties (size, ζ -potential and surface hydrophobicity), at different heating temperatures and electric field intensities.

Materials and Methods

Materials and chemicals

Commercial whey protein isolate (WPI), Provon 292, was purchased from Glanbia Nutritionals, Inc. (Fitchburg, MA, USA). The composition of WPI was 93 wt% protein (dry weight

basis), 4.6 wt% moisture, 0.7 wt% fat, < 1.5 wt% lactose and 1.5 wt% minerals, as described in the manufacturer's datasheet. NaH_2PO_4 and Na_2HPO_4 of analytical grade were procured from QReC (Auckland, New Zealand). The phosphate buffer solution was prepared in distilled water. NaN_3 that was added into phosphate buffer at 0.04% w/v as a preservative agent was purchased from Thermo Fisher Scientific Inc. Ajax Finechem (Taren Point, NSW, Australia).

Preparation of whey protein dispersions

Whey protein dispersions were prepared by dissolving WPI powder into 0.1 M phosphate buffer (pH 7) at 1% weight per volume (w/v) concentration. The dispersions were further stirred at 400 rpm for 2 hr using a magnetic stirrer (Systematic Multi-Hotplate Stirrer SMHS-6; Daihan Scientific Co., Ltd.; Wonju, South Korea) and stored overnight for complete hydration. The pH of the whey dispersions was constantly controlled at 7.0 ± 0.1 measured using a pH meter (S220 SevenCompact; Mettler-Toledo International Inc.; Greifensee, Switzerland). The determination of the electrical conductivity σ of the whey protein dispersions was conducted in the temperature range 25–80°C using the method described by Shynkaryk et al. (2010). The electrical conductivity as a function of the temperature of whey protein dispersions was calculated using Equation 1 (Kanogchaipramot et al., 2016):

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

where σ is the electrical conductivity (S/m), T is the temperature (°C), L is the inter-electrode gap (cm), I is the electric current (A), V is the voltage (V), and A is the cross-section electrode surface area (cm²).

Thermal treatments for whey protein dispersions

Whey protein dispersion was separated into different groups for unheated (control) and thermal treatments as: conventional heating (CONV) or ohmic (OHM) heating at the target temperatures of 60°C, 70°C and 80°C for 15 min. The come-up-time (CUT) was defined as the time in minutes to increase the temperature of a sample from the initial to the target temperature. After the CUT period, the target temperature was maintained for 15 min. The heated whey dispersions were collected for further characterization. The whey protein dispersions were subjected to conventional heating or ohmic heating under identical thermal histories to

investigate the non-thermal effects of the electric fields on their structural and physicochemical properties (Pereira et al., 2010, 2011; Rodrigues et al., 2015). According to Moakes et al. (2015), heating under steady shear could affect the particle size and the particle-particle interaction of whey protein. Therefore, heating without stirring was conducted in this study.

Conventional heating

Conventional heating for the whey protein dispersions was performed using a temperature-controlled water bath (WiseBath WHB-6; Daihan Scientific Co., Ltd.; Wonju, South Korea). A sample (200 mL) of whey protein dispersion was poured into a cylindrical bottle made of borosilicate glass with a 6 cm inner diameter and 14 cm height, enclosed with a cap and placed in a water bath. The temperature of dispersion was measured at the geometrical center of the dispersion using a type K thermocouple (TP882; FLIR Commercial Systems, Inc.; Nashua, NH, USA) and recorded using a digital multimeter (Gwinstek GDM-8261A; Good Will Instrument Co., Ltd.; Tucheng, Taiwan).

Ohmic heating

The overall ohmic heating system comprised: 1) an open T-tube cylindrical chamber made of borosilicate glass with a 5 cm inner diameter and an inter-electrode gap of 8.5 cm (length = 8.5 cm), containing 200 mL of whey protein dispersion; 2) a pair of titanium electrodes connected to cylindrical Teflon rods and electrical cables; and 3) a variable voltage transformer (model TDGC2-5K; Silicon, China) with electric power of 5 kW, a maximum voltage of 250 V and a maximum current of 20 A at a constant frequency of 50 Hz (Fig. 1). The applied voltage and current were simultaneously monitored and recorded using a digital multimeter. The heat generation rate depended on the electrical conductivity of the sample and the electric field intensity ($E = V/L$; Ramaswamy et al., 2014). E was adjusted by varying the voltage to match the thermal history of conventional and ohmic heating.

Structural characterization of heated whey proteins

The intrinsic fluorescence intensity was analyzed using a Tecan microplate reader (Infinite 200 PRO; Grödig, Austria), with an ultraviolet xenon flashlamp as the light source. The intrinsic fluorescence characteristics of proteins contributed from tryptophan (Trp), tyrosine and phenylalanine were monitored due to their high quantum yield or fluorescence efficiency (Pain, 2005). The higher the quantum yield, the

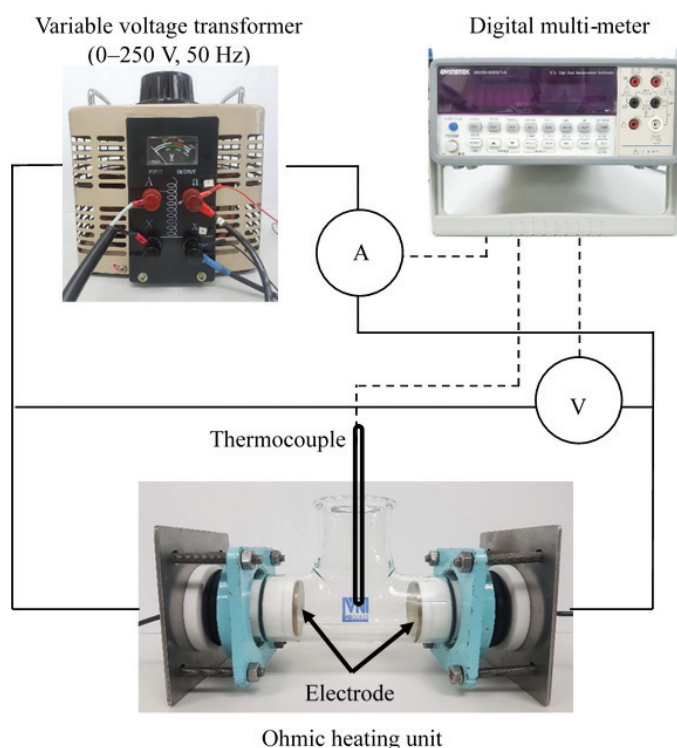


Fig. 1 Overall diagram of ohmic heating system

higher the sensitivity of detection to monitor protein structural change. The intrinsic fluorescence properties (maximum emission wavelength and emission intensity) were examined to monitor the conformational alteration in the tertiary structure of whey proteins. The maximum emission wavelength of the whey dispersions was determined from the fluorescence spectrum using an excitation wavelength of 280 nm. The fluorescence emission spectrum within the wavelength range 325–500 nm was recorded. In addition, the fluorescence intensity was measured at the maximum emission wavelength of each unheated and heated whey protein sample. Whey protein dispersions were diluted to 0.05% w/v concentration (20 folds) with 0.1 M phosphate buffer (pH 7) to exclude the effect of light scattering (Birlouez-Aragon et al., 2002). The intrinsic fluorescence spectroscopy of all dispersions was examined at least in triplicate at 25°C.

Physicochemical properties of heated whey proteins

Particle size distribution

The particle size of the whey proteins was determined using a dynamic light scattering (DLS) technique with a Zetasizer Nano ZS90 unit (Malvern Panalytical Ltd.; Malvern, UK) equipped with an He-Ne laser emitting at 633 nm. The

detector was positioned at 90° to collect the light scattering pattern. The whey protein dispersions were diluted with 0.1 M phosphate buffer (pH 7) at a ratio of 1:10 to reduce the effects of multiple scattering; 1 mL of diluted dispersions was filled into disposable polystyrene cuvettes. The measurements (at least duplicates) were performed at 25°C. The fluctuation in the light scattered intensity with time was plotted using an autocorrelation function and analyzed using a cumulative fit to determine the diffusion coefficient, hydrodynamic diameter and polydispersity index of particles (Mehalebi et al., 2008; Donato et al., 2009) using the Zetasizer software version 7.12 (Malvern Panalytical Ltd.; Malvern, UK). The refractive index (RI) was measured using a digital refractometer (DR6000; Krüss Optronic GmbH; Hamburg, Germany). The RI was 1.33 for the dispersing medium and 1.43 for whey proteins. The absorbance value was 0.001 for proteins (Dybowska, 2011).

Surface charge

The ζ -potential values of unheated and heated whey proteins were examined based on a combination of techniques involving electrophoresis and laser doppler velocimetry using a Zetasizer Nano ZS90 unit (Malvern Panalytical Ltd; Malvern, UK). A sample (1 mL) of whey protein dispersions was loaded into the folded capillary cell (DTS1070; Malvern Panalytical Ltd; Malvern, UK) and measured at least in duplicate at 25°C.

All experiments were performed in duplicate unless otherwise stated.

Statistical analysis

The results were presented as mean $\pm$ SD. Data were analyzed using analysis of variance and the significant difference between means was determined using Fisher pairwise comparisons at the 95% confidence interval. All statistical analysis was conducted using the MINITAB software (v. 16; Minitab Inc.; State College, PA, USA) with significance tested at $p < 0.05$.

Results and Discussion

Thermal history determination

The identical thermal histories for both conventional heating and ohmic heating are illustrated in Fig. 2. The CUT periods for conventional heating to achieve the target temperatures of 60°C, 70°C and 80°C were 28.9 ± 0.3 min, 30.9 ± 1.3 min and 31.4 ± 0.7 min, respectively. To match the thermal histories of ohmic heating to those of conventional heating, electric field

intensities were: initially applied at 5.59 V/cm, 6.25 V/cm and 8.05 V/cm; reduced to 2.74–2.04 V/cm, 3.92–2.20 V/cm and 4.27–2.35 V/cm during CUT; and maintained at 2.04 V/cm, 2.20 V/cm and 2.47 V/cm during the holding periods at 60°C, 70°C and 80°C, respectively, for 15 min (Fig. 3). The electrical conductivity of the whey dispersions linearly increased as the temperature increased in the range 1.20–2.65 S/m between 25°C and 80°C (Fig. 4) and met the conditions to be classified as a good conductivity medium (Goullieux and Pain, 2005). From Equation 1 and Fig. 4, electrical conductivity depends on the current, and the heating rate relies on the electrical conductivity and the electric field. Therefore, the higher the electric field and current, the higher the temperature. The amperage sharply increased at the beginning of the process and then decreased and remained constant relative to the applied electric field (Fig. 3).

Conformational changes of whey proteins after ohmic or conventional heating

The maximum emission wavelengths of unheated and heated whey protein particles (60°C, 70°C and 80°C for 15 min) are presented in Table 1. The unheated whey proteins emitted the maximum fluorescence intensity at a wavelength of 326.8 nm. The proteins heated under conventional or ohmic heating at 60°C exhibited no significant shift of the maximum emission wavelength compared to the unheated protein suspension. However, higher heating temperatures (at and above 70°C) induced a significant red-shift, indicating an increase in the surface hydrophobicity of the whey proteins (Moro et al., 2001), the exposure of Trp residues previously buried in a more hydrophilic environment (Lakowicz, 2006)

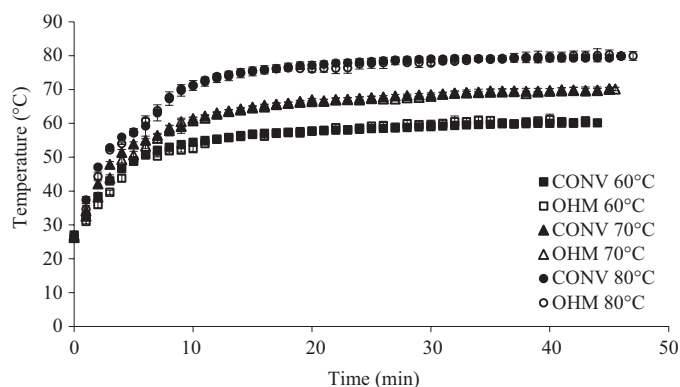


Fig. 2 Temperature profiles resulting from conventional (CONV) or ohmic (OHM) heating at temperatures of 60°C, 70°C and 80°C for 15 min, where data are averages of three replicates and error bars show $\pm$ SD

and/or the dissociation of macromolecular aggregates such as those found in soy proteins (Keerati-u-rai et al., 2012).

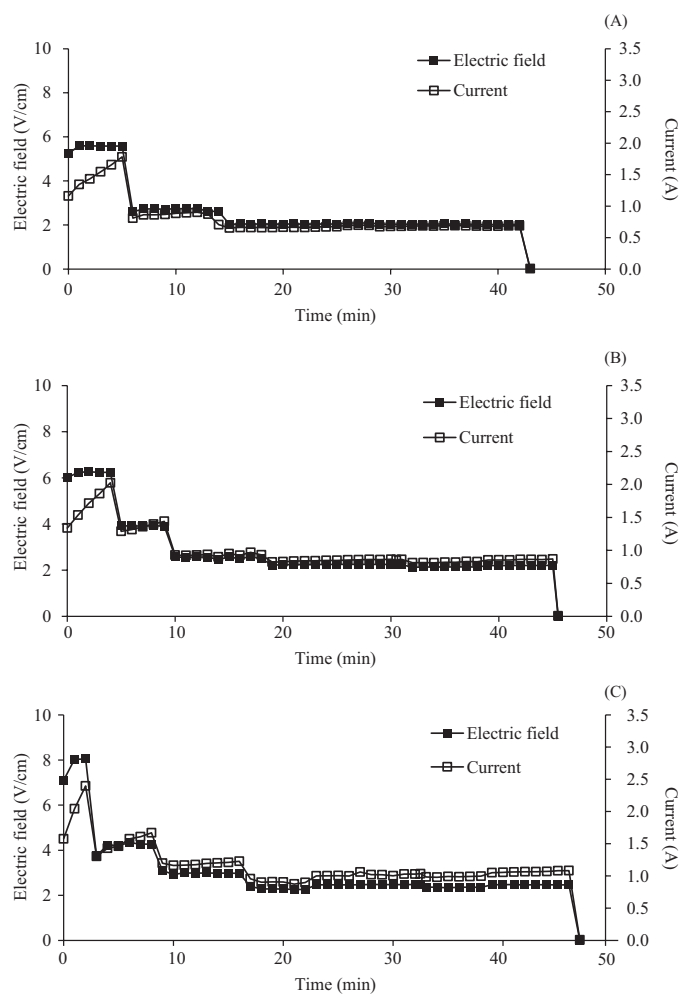


Fig. 3 Electric field strength and current from ohmic heating for 15 min under identical thermal profiles for conventional heating at: (A) 60°C; (B) 70°C; (C) 80°C

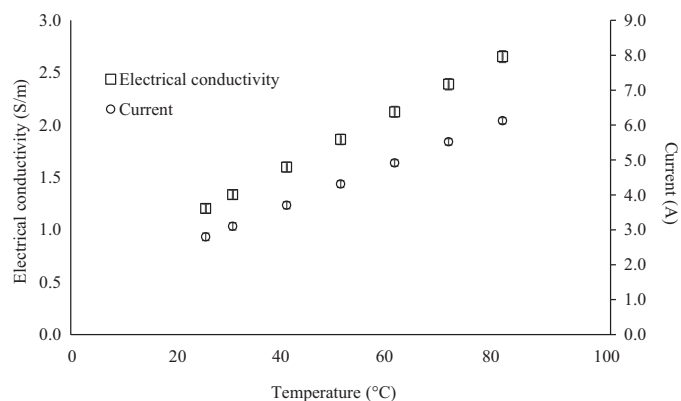


Fig. 4 Electrical conductivity and current of whey protein dispersions, where data are averages of three replicates and error bars show $\pm$ SD

Table 1 Maximum emission wavelength determined using intrinsic fluorescence spectroscopy (at excitation wavelength of 280 nm) of unheated and heated whey proteins resulting from conventional or ohmic heating for 15 min under identical thermal profiles at 60°C, 70°C and 80°C

Heating method	Heating temperature (°C)	Maximum emission wavelength (nm)
Unheated		326.8±0.7 ^a
Conventional heating	60	327.3±1.5 ^{ab}
	70	329.5±0.2 ^{bc}
	80	331.0±1.7 ^c
Ohmic heating	60	326.7±1.5 ^a
	70	329.7±0.6 ^c
	80	329.7±0.6 ^c

Mean ± SD with different lowercase superscripts indicate significant ($p < 0.05$) differences.

Fig. 5 illustrates the intrinsic fluorescence intensity at the maximum emission wavelength. Conventional heating at 60°C increased the fluorescence intensity of the whey protein solution and exposed more hydrophobic residues, particularly Trp, indicating an alteration of the tertiary structure of the whey proteins, while ohmic heating at 60°C did not significantly change the protein conformation. On the other hand, heat treatments at 70°C and 80°C significantly increased the fluorescence intensity, indicating extensive structural changes in the proteins (Jiang et al., 2018). The magnitude of the fluorescence intensity of the whey proteins subjected to ohmic heating at 70°C ($23,690 \pm 1,164$ A.U.) and 80°C ($29,814 \pm 1,149$ A.U.) was significantly less than that from conventional heating ($27,457 \pm 1,262$ A.U. at 70°C and $32,988 \pm 1,213$ A.U. at 80°C). The presence of the electric field during ohmic heating at and above 70°C might have affected the motion of

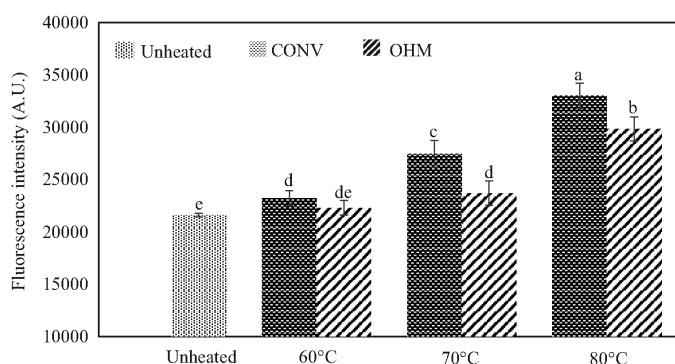


Fig. 5 Intrinsic fluorescence intensity emitted at maximum emission wavelength (excitation at wavelength of 280 nm) of unheated and heated whey proteins obtained from conventional (CONV) or ohmic (OHM) heating under identical thermal profiles at 60°C, 70°C and 80°C for 15 min, where data are averages of two replicates and error bars show ± SD and different letters above bars indicate significant ($p < 0.05$) differences.

ionic molecules, which interfered with unfolding mechanisms or, at least, interactions (Pereira et al. 2010). In addition, the result was in agreement with the study of Rodrigues et al. (2015) who reported the lower availability of free sulfhydryl groups of unfolded protein during electric heating.

Physicochemical changes of whey proteins after ohmic or conventional heating

The particle size distributions of the whey proteins are shown in Fig. 6. The unheated whey protein suspensions and protein suspensions heated at 60°C had bimodal distributions of the protein molecules and aggregates. The size of small particles was less than 10 nm and the larger particles were in the range 40–460 nm, respectively. The first peak indicated whey protein monomers and dimers. For example, the β -lg monomer had a hydrodynamic radius of approximately 2 nm (Aymard et al., 1996). Native β -lg at neutral pH existed in equilibrium between the monomer and dimer stabilized by hydrogen bonds (De Wit, 1998). The second peak involved native whey aggregates typical of hydrated commercial WPI (Mahmoudi et al., 2007; Nicorescu et al., 2008; Rodrigues et al., 2015).

Above 60°C, the β -lg monomer partially unfolded (Zúñiga et al., 2010) and then formed inter-molecular disulfide bonds through SH-SS interchange that might result in irreversible aggregates or polymers (Surroca et al., 2002; Tolkach and Kulozik, 2007). The size distribution curves for the whey proteins heated by conventional or ohmic heating at 70°C and 80°C had monomodal distributions in the size range 10–70 nm (Figs. 6B and 6C).

The dissociation of submicron-sized, unheated whey protein aggregates and the increase in the intensity of the nanometer-sized particles after heating was due to the unfolding and aggregation of dissociated whey protein molecules induced by thermal effects (Dybowska, 2011; Rodrigues et al., 2015). However, there was also a difference in the average sizes of the heated whey protein aggregates obtained from conventional heating compared to ohmic heating. The formation of smaller whey aggregates might have been related to the lower reactivity and availability of free sulfhydryl groups of the unfolded protein upon ohmic heating. Furthermore, the non-covalent interactions among whey proteins treated with ohmic heating were possibly retarded, resulting in the change in aggregation pattern (Rodrigues et al. 2015).

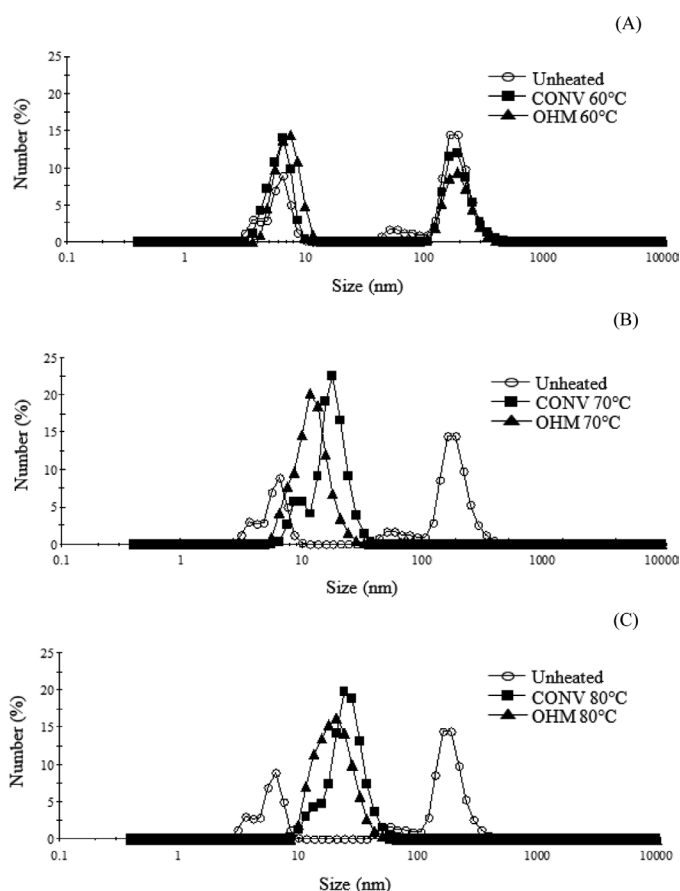


Fig. 6 Particle size distribution of unheated and heated whey protein particles resulting from conventional (CONV) or ohmic (OHM) heating for 15 min under identical thermal profiles at: (A) 60°C; (B) 70°C; (C) 80°C

Fig. 7 illustrates the appearance of unheated and heated whey protein dispersions following conventional or ohmic heating for 15 min at 60°C, 70°C and 80°C. Whey protein suspensions heated at higher than 70°C were more turbid than the unheated suspensions. The ohmic-heated suspensions were less turbid than the conventional-heated suspensions. The greater clarity of the whey protein dispersions heated by ohmic heating compared to conventional heating could have resulted from the smaller particle sizes of aggregates.

The ζ -potential values for the unheated and heated whey protein particles from conventional or ohmic heating for 15 min at 60°C, 70°C and 80°C are shown in Fig. 8. The whey proteins were negatively charged before and after heating. The unheated whey proteins had a ζ -potential value of -13.4 mV. Both the conventional heat treatment and the ohmic heat treatment at 80°C slightly but significantly increased the ζ -potentials of the whey proteins to -15.2 mV and -16.7 mV, respectively, compared to those of the native whey proteins.

However, the influences of heating on the surface charge of the protein molecules by conventional treatment or ohmic treatment were not significantly different.

An oscillating electric field enables the periodic alignment, stretch and relaxation of proteins according to their charged and dipole properties (Bekard and Dunstan, 2014). The result might account for the destabilizing of the protein tertiary structure. In addition to the heat-induced effect, the electric field effect possibly interfered with hydrogen bonds and electrostatic interactions, leading to the different denaturation and aggregation patterns and physicochemical properties of ohmically heated whey proteins. Furthermore, during the passage of electric current through whey protein dispersions, the electrical energy was volumetrically dissipated into heat. The uniform heat generation of ohmic heating resulted in the absence of overheated surfaces (Goullieux and Pain, 2005; Pereira et al., 2011). Therefore, the effect of the electric field

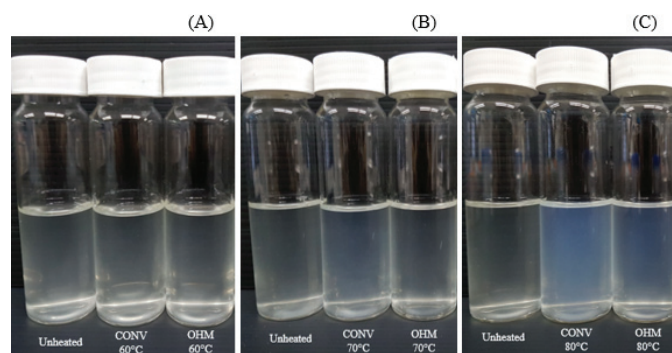


Fig. 7 Visual appearance of unheated and heated whey protein dispersions following conventional (CONV) or ohmic (OHM) heating for 15 min under identical thermal profiles at: (A) 60°C; (B) 70°C; (C) 80°C

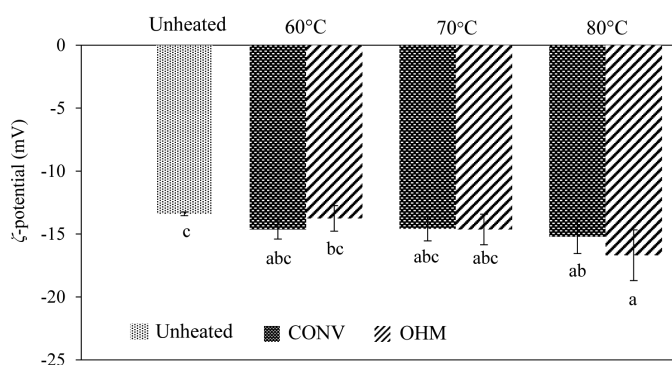


Fig. 8 ζ -Potential of unheated and heated whey protein particles subjected to conventional (CONV) or ohmic (OHM) heating for 15 min under identical thermal profiles at 60°C, 70°C and 80°C, where data are averages of two replicates and error bars show $\pm$ SD and different letters below bars indicate significant ($p < 0.05$) differences.

and the uniform heating possibly reduced tertiary structural changes and produced smaller particle sizes of aggregates of whey proteins. Further investigations are needed to elucidate the possible mechanisms.

Ohmic heating changed the conformation and physicochemical properties of whey proteins based on the heating temperature and the electric field at $\geq 70^{\circ}\text{C}$. The electric field and uniform heating were responsible for the reduction in the changes in the tertiary structure of whey proteins, the smaller size of the whey protein aggregates and the greater clarity of the whey protein dispersions compared with conventional heating using identical temperature histories at 70°C and 80°C . Therefore, ohmic heating offers great potential as an alternative modification process for specific physicochemical and functional properties of whey proteins.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

- Aymard, P., Durand, D., Nicolai, T. 1996. The effect of temperature and ionic strength on the dimerization of β -lactoglobulin. *Int. J. Biol. Macromol.* 19: 213–221. doi.org/10.1016/0141-8130(96)01130-0
- Bekard, I., Dunstan, D.E. 2014. Electric field induced changes in protein conformation. *Soft Matter* 10: 431–437. doi.org/10.1039/C3SM52653D
- Birlouez-Aragon, I., Sabat, P., Gouti, N. 2002. A new method for discriminating milk heat treatment. *Int. Dairy J.* 12: 59–67. doi.org/10.1016/S0958-6946(01)00131-5
- Castro, I., Teixeira, J.A., Salengke, S., Sastry, S.K., Vicente, A.A. 2003. The influence of field strength, sugar and solid content on electrical conductivity of strawberry products. *J. Food Process Eng.* 26: 17–29. doi.org/10.1111/j.17454530.2003.tb00587.x
- De Wit, J.N. 1990. Thermal stability and functionality of whey proteins. *J. Dairy Sci.* 73: 3602–3612. doi.org/10.3168/jds.S0022-0302(90)79063-7
- De Wit, J.N. 1998. Nutritional and functional characteristics of whey proteins in food products. *J. Dairy Sci.* 81: 597–608. doi.org/10.3168/jds.S0022-0302(98)75613-9
- Donato, L., Schmitt, C., Bovetto, L., Rouvet, M. 2009. Mechanism of formation of stable heat-induced β -lactoglobulin microgels. *Int. Dairy J.* 19: 295–306. doi.org/10.1016/j.idairyj.2008.11.005
- Dybowska, B.E. 2011. Whey protein-stabilized emulsion properties in relation to thermal modification of the continuous phase. *J. Food Eng.* 104: 81–88. doi.org/10.1016/j.jfoodeng.2010.11.030
- Goullieux, A., Pain, J.P. 2005. Ohmic heating. In: Sun, D.W. (Ed.). *Emerging Technologies for Food Processing*. Elsevier. London, UK, pp. 469–505.
- Jiang, S., Altaf Hussain, M., Cheng, J., Jiang, Z., Geng, H., Sun, Y., Sun, C., Hou, J. 2018. Effect of heat treatment on physicochemical and emulsifying properties of polymerized whey protein concentrate and polymerized whey protein isolate. *LWT—Food Sci. Technol.* 98: 134–140. doi.org/10.1016/j.lwt.2018.08.028
- Kanogchaipramot, K., Tongkhao, K., Sajjaanantakul, T., Kamonpatana, P. 2016. Ohmic heating of an electrically conductive food package. *J. Food Sci.* 81: E2966–E2976. doi.org/10.1111/1750-3841.13542
- Keerati-u-rai, M., Miriani, M., Iametti, S., Bonomi, F., Corredig, M. 2012. Structural changes of soy proteins at the oil-water interface studied by fluorescence spectroscopy. *Colloids Surf. B.* 93: 41–48. doi.org/10.1016/j.colsurfb.2011.12.002
- Kinsella, J.E., Whitehead, D.M. 1989. Proteins in whey: Chemical, physical, and functional properties. *Adv. Food Nutr. Res.* 33: 343–438. doi.org/10.1016/S1043-4526(08)60130-8
- Kroslak, M., Sefcik, J., Morbidelli, M. 2007. Effects of temperature, pH, and salt concentration on β -lactoglobulin deposition kinetics studied by optical waveguide lightmode spectroscopy. *Biomacromolecules* 8: 963–970. doi.org/10.1021/bm060293+
- Lakowicz, J.R. 2006. Protein fluorescence. In: Lakowicz, J.R. (Ed.). *Principles of Fluorescence Spectroscopy*. Springer. Singapore, pp. 529–575.
- Lefèvre, T., Subirade, M., Pézolet, M. 2005. Molecular description of the formation and structure of plasticized globular protein films. *Biomacromolecules* 6: 3209–3219. doi.org/10.1021/bm050540u
- Mahmoudi, N., Mehalebi, S., Nicolai, T., Durand, D., Riaublanc, A. 2007. Light-scattering study of the structure of aggregates and gels formed by heat-denatured whey protein isolate and β -lactoglobulin at neutral pH. *J. Agr. Food Chem.* 55: 3104–3111. doi.org/10.1021/jf063029g
- Mehalebi, S., Nicolai, T., Durand, D. 2008. Light scattering study of heat-denatured globular protein aggregates. *Int. J. Biol. Macromol.* 43: 129–135. doi.org/10.1016/j.ijbiomac.2008.04.002
- Moakes, R., Sullo, A., Norton, I. 2015. Preparation and characterisation of whey protein fluid gels: The effects of shear and thermal history. *Food Hydrocoll.* 45: 227–235. doi.org/10.1016/j.foodhyd.2014.11.024
- Moro, A., Gatti, C., Delorenzi, N. 2001. Hydrophobicity of whey protein concentrates measured by fluorescence quenching and its relation with surface functional properties. *J. Agr. Food Chem.* 49: 4784–4789. doi.org/10.1021/jf001132e
- Nicolai, T., Britten, M., Schmitt, C. 2011. β -Lactoglobulin and WPI aggregates: Formation, structure and applications. *Food Hydrocoll.* 25: 1945–1962. doi.org/10.1016/j.foodhyd.2011.02.006
- Nicorescu, I., Loisel, C., Vial, C., Riaublanc, A., Djelveh, G., Cuvelier, G., Legrand, J. 2008. Combined effect of dynamic heat treatment and ionic strength on denaturation and aggregation of whey proteins-Part I. *Food Res. Int.* 41: 707–713. doi.org/10.1016/j.foodres.2008.05.003

- Pain, R.H. 2005. Determining the fluorescence spectrum of a protein. In: Wrolstad, R.E., Acree, T.E., Decker, E.A., et al. (Eds.). *Handbook of Food Analytical Chemistry*, Vol 1: Water, Proteins, Enzymes, Lipids, and Carbohydrates. John Wiley & Sons. New Jersey, NJ, USA, pp. 245–266.
- Parrott, D.L. 1992. Use of ohmic heating for aseptic processing of food particulates. *Food Technol.* 46: 68–72.
- Pereira, R.N., Souza, B.W.S., Cerqueira, M.A., Teixeira, J.A., Vicente, A.A. 2010. Effects of electric fields on protein unfolding and aggregation: Influence on edible films formation. *Biomacromolecules* 11: 2912–2918. doi.org/10.1021/bm100681a
- Pereira, R.N., Teixeira, J.A., Vicente, A.A. 2011. Exploring the denaturation of whey proteins upon application of moderate electric fields: A kinetic and thermodynamic study. *J. Agr. Food Chem.* 59: 11589–11597. doi.org/10.1021/jf201727s
- Raikos, V. 2010. Effect of heat treatment on milk protein functionality at emulsion interfaces. A review. *Food Hydrocoll.* 24: 259–265. doi.org/10.1016/j.foodhyd.2009.10.014
- Ramaswamy, H.S., Marcotte, M., Sastry, S., Abdelrahim, K. 2014. *Ohmic Heating in Food Processing*. CRC Press. Florida, FL, USA.
- Rodrigues, R.M., Martins, A.J., Ramos, O.L., Malcata, F.X., Teixeira, J.A., Vicente, A.A., Pereira, R.N. 2015. Influence of moderate electric fields on gelation of whey protein isolate. *Food Hydrocoll.* 43: 329–339. doi.org/10.1016/j.foodhyd.2014.06.002
- Sastry, S.K., Barach, J.T. 2000. Ohmic and inductive heating. *J. Food Sci.* 65: 42–46. doi.org/10.1111/j.1750-3841.2000.tb00617.x
- Schmitt, C., Bovay, C., Rouvet, M., Shojaei-Rami, S., Kolodziejczyk, E. 2007. Whey protein soluble aggregates from heating with NaCl: Physicochemical, interfacial, and foaming properties. *Langmuir* 23: 4155–4166. doi.org/10.1021/la0632575
- Schmitt, C., Bovay, C., Vuillomenet, A.M., Rouvet, M., Bovetto, L. 2011. Influence of protein and mineral composition on the formation of whey protein heat-induced microgels. *Food Hydrocoll.* 25: 558–567. doi.org/10.1016/j.foodhyd.2010.05.010
- Shynkaryk, M.V., Ji, T., Alvarez, V.B., Sastry, S.K. 2010. Ohmic heating of peaches in the wide range of frequencies (50 Hz to 1 MHz). *J. Food Sci.* 75: E493–E500. doi.org/10.1111/j.1750-3841.2010.01778.x
- Surroca, Y., Haverkamp, J., Heck, A.J.R. 2002. Towards the understanding of molecular mechanisms in the early stages of heat-induced aggregation of β -lactoglobulin AB. *J. Chromatogr. A.* 970: 275–285. doi.org/10.1016/S0021-9673(02)00884-1
- Tolkach, A., Kulozik, U. 2007. Reaction kinetic pathway of reversible and irreversible thermal denaturation of β -lactoglobulin. *Dairy Sci. Technol.* 87: 301–315. doi.org/10.1051/lait:2007012
- Zúñiga, R.N., Tolkach, A., Kulozik, U., Aguilera, J.M. 2010. Kinetics of formation and physicochemical characterization of thermally-induced β -lactoglobulin aggregates. *J. Food Sci.* 75: E261–E268. doi.org/10.1111/j.1750-3841.2010.01617.x