Effect of pH on heat-induced interactions in high-protein milk dispersions and application of fluorescence spectroscopy in characterizing these changes

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ABSTRACT

This study investigated casein-whey protein interactions in high-protein milk dispersions (5% protein wt/wt) during heating at 90°C for 1.5 to 7.5 min at 3 different pH of 6.5, 6.8, and 7.0, using both conventional methods (gel electrophoresis, physicochemical properties) and fluorescence spectroscopy. Conventional methods confirmed the presence of milk protein aggregates during heating, similar to skim milk. These methods were able to help in understanding the denaturation and aggregation of milk proteins as a function of heat treatment. However, the results from the conventional methods were greatly affected by batch-to-batch variations and, therefore, differentiation could be drawn only in nonheated samples and samples heated for a longer duration. The front-face fluorescence spectroscopy was found to be a useful tool that provided additional information to conventional methods and helped in understanding differences between nonheated, low-, and high-heated samples, along with the type of sample used (derived from liquid or powder milk protein concentrates). At all pH values, tryptophan maxima in nonheated samples derived from powdered milk protein concentrates presented a blue shift in comparison to samples derived from liquid milk protein concentrates, and tryptophan maxima in heated samples presented a red shift. With the heating of the sample, Maillard emission and excitation spectra also showed increases in the peak intensities from 408 to 432 and 260 to 290 nm, respectively. As the level of denaturation increased with heating, a marked differentiation can be seen in the principal component analysis plots of tryptophan, Maillard emission, and excitation spectra, indicating that the front-face fluorescence technique has a potential to monitor and classify samples according to

milk protein interactions as a function of pH and heat exposure. Overall, it can be said that the pattern of protein-protein interactions in high-protein dispersions was similar to the observation reported in skim milk systems, and fluorescence spectroscopy with chemometrics can be used as a rapid, nondestructive, and complementary method to conventional methods for following heat-induced changes.
Key words: milk protein concentrate, high-protein dispersions, protein interactions, fluorescence spectroscopy

INTRODUCTION

Caseins and whey proteins are the major proteins in milk. In contrast to caseins, whey proteins are heat labile, and their structure opens upon heating and consequently exposes the reactive groups (sulfhydryl group, –SH) that can interact and form aggregates with caseins (mainly κ-casein) or with other whey proteins (Anema and Li, 2003a; Donato and Dalgleish, 2006). The denaturation and subsequent interactions are highly dependent on the pH of milk. At low pH, there is a preferential interaction between denatured whey proteins and casein (about 70%). On the other hand, at neutral and high pH, only 30% of the denatured whey protein associates with casein, and the rest exist as whey protein–whey protein aggregates (Anema and Li, 2003b; Vasbinder and de Kruif, 2003). Caseins, whey protein, and their selective interactions can effectively be used to modify functionality of high-protein dairy ingredients. The milk proteins-based ingredients are added to foods to impart functional attributes such as water binding, viscosity, emulsifying, and foaming abilities. Among different milk protein ingredients, milk protein concentrates (**MPC**) are the preferred choice because of their high protein (50–85%) and low lactose content (Huppertz and Patel, 2012).

From the literature (Anema and Li, 2003a,b; Vasbinder and de Kruif, 2003; Donato et al., 2007; Kethireddipalli et al., 2010; Ozcan et al., 2015; Taterka and

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Castillo, 2015, 2018), it has been reported that the denaturation of whey protein and its aggregation are complex phenomena and highly dependent on pH and heating time and temperature. All these studies related to thermal behavior of milk proteins at different pH were carried out either in milk or reconstituted milk at ≤3.5% protein concentrations. However, the denaturation and aggregation become more complex at high-protein dispersions, especially protein concentrations above 70 g/L (Nair et al., 2013). Denaturation of whey protein increased with increase in protein concentration during heating at 80°C (Law and Leaver, 1997), which may affect the aggregation behavior of milk proteins. Therefore, during the manufacturing of high-protein milk foods, the denaturation and aggregation of milk proteins may be different than low-protein foods. However, the denaturation and aggregation behavior of milk proteins at high-protein concentration using MPC as a function of pH has not been studied in detail. Most studies related to MPC were limited to heat stability and solubility of reconstituted MPC powder (Havea, 2006; Crowley et al., 2014, 2015; Eshpari et al., 2014; Gazi and Huppertz, 2015; Babu et al., 2018; Renhe et al., 2019). Therefore, this work will improve the current understanding of denaturation of whey protein and its aggregation in high-protein MPC dispersions using fluorescence spectroscopy and chemometrics as a tool.

Fluorescence spectroscopy is a very sensitive and powerful analytical technique with potential to measure heat-induced changes in milk proteins. Tryptophan fluorescence is an indicator of milk protein denaturation (Schamberger and Labuza, 2006) because its fluorescence is highly sensitive to conformation changes in proteins and interaction with other molecules (Pan et al., 2011). In addition to protein denaturation, fluorescence spectroscopy can be used as a tool to follow Maillard browning in dairy and food products. Maillard reaction is a nonenzymatic reaction that occurs mainly through the reaction between amino acids and lactose. The early (Van Boekel, 2001; Bastos et al., 2012) and advanced Maillard products (Tessier et al., 2002) corresponding to Amadori products and advanced glycosylation end products are known to exhibit fluorescence. Therefore, fluorescence spectroscopy could be an important tool for evaluating heat-induced changes in protein dispersions containing lactose. Ayala et al. (2020) applied front-face fluorescence spectroscopy on reconstituted skim milk exposed to heat (70–100°C for 10–60 min) and observed differences in fluorescence spectra of tryptophan, including reduced fluorescence intensity and a red shift. All the above-mentioned studies were conducted on milk or reconstituted milk samples with a protein content of ≤3.5[AU1: Should this be 3.5%?].

As the denaturation and protein-protein aggregations of milk proteins are considerably influenced by intrinsic (pH and protein concentration) and extrinsic (heating time and temperature) variables, the objective of the present study was to understand heat-induced interactions in high-protein dispersions (at 5% wt/wt) prepared from liquid MPC and reconstituted MPC during heating at 90°C for up to 7.5 min at 3 pH levels, using fluorescence spectroscopy as one of the analytical tools.

MATERIALS AND METHODS

Experimental Design

Two lots of MPC 80 were obtained from a commercial manufacturer as liquid ultrafiltration retentates (collected before spray drying) and their powders (collected after spray drying). A protein dispersion (~5% wt/wt) was prepared using both liquid retentate and powdered MPC. For simplicity, the protein dispersion prepared using the liquid retentate of MPC is referred to as the sample derived from liquid MPC, and the dispersion prepared using spray-dried MPC is referred to as the sample derived from powder MPC. Each sample was divided into 3 equal parts, and subsequently, pH was adjusted to 6.5, 6.8, and 7.0. The pH-adjusted samples were heated at 90°C for 1.5, 3, 4.5, 6, and 7.5 min. Nonheated samples at each pH value served as respective controls. After heating, heat-induced changes were analyzed by conventional methods such as gel electrophoresis, physicochemical properties (mean particle size, zeta potential, and apparent viscosity), and front-face fluorescence spectroscopy. Additionally, the soluble phase was separated by lowering the pH to 4.6 and was analyzed for protein content, native gel electrophoresis, and right-angle spectrofluorimetric methods for understanding heat-induced changes.

Sample Preparation. The protein content of liquid-derived and powder-derived MPC were adjusted to 5% (wt/wt) with deionized water (40 ± 1°C) under constant stirring using a magnetic stirrer for 30 min. The protein dispersion was allowed to hydrate overnight at 4 ± 1°C. The next day, samples were divided into 3 equal parts to adjust the pH 6.50 ± 0.01, 6.80 ± 0.01, and 7.00 ± 0.01 by gentle addition of 0.5 M hydrochloric acid or 0.5 M sodium hydroxide. The systems were allowed to equilibrate for an additional 3 h, and minor modifications (if required) were made to attain the desired pH. The deionized water was used to make up the difference in volume of HCl or NaOH used. Subsequently, 5 mL of sample was transferred into a glass vial (8 mL) and heated at 90 ± 1°C for 1.5, 3, 4.5, 6, and 7.5 min in a temperature-controlled water

bath (Isotemp 2340, Fisher Scientific). To perform the heating, each tube was immersed in a water bath maintained at 100°C (first step) to heat MPC dispersions from room temperature to 90°C (approximate time: 5 s) and immediately transferred into a second water bath at maintained 90°C (second step); after every 1.5 min, the tubes were withdrawn and immediately placed in an ice-water bath to cool down to room temperature. Temperature of the MPC dispersions were monitored during heating and cooling using thermocouples (Omega Corp.). The nonheated samples at each pH served as the control at each pH.

SDS-PAGE. The SDS-PAGE analysis (Mao et al., 2012) was carried out using precast gels (4–15% Tris-glycine gels) in the Mini-Protean II dual slab cell system (Bio-Rad Laboratories). All samples were diluted with deionized water to a protein concentration of 5 µg/µL and analyzed for SDS-PAGE under reducing and nonreducing conditions. The difference between reducing SDS (**SDS-R**) and nonreducing SDS (**SDS-NR**) was the presence of 2-mercaptoethanol (0.05%) in the sample buffer (Laemmli 2×, Sigma-Aldrich) that was present in SDS-R. All samples were mixed with sample buffer (SDS-R and SDS-NR) in 1:1 ratio and heated for 5 min in a boiling water bath. Then 15 µL of each sample was loaded onto the precast gel and run at 100 V until band reached the bottom of gel (approximately 1.5–2 h). All the gels were stained with the 0.025% solution (in 10% acetic acid) of Coomassie Blue G-250 (Bio-Rad) for 12 h and destained in the 10% acetic acid solution for 2 h.

Mean Particle Size and Zeta Potential of Casein Micelle. Mean particle size and zeta potential of protein dispersions were measured using a dynamic light scattering analyzer (DelsaMax Assist, Beckman Coulter) using the method described by Anema and Li (2003a). For the measurement, samples were diluted to 1/100, and the diluted samples were injected into the flow cell using a syringe at 20 ± 1°C to obtain mean particle size and zeta potential.

Apparent Viscosity. The apparent viscosity was measured at 20°C at a shear rate of 100 s^{-1} using a rheometer (MCR-92 Anton Paar) with a 50-mm diameter stainless steel cone with an angle of 1° and a 0.101-mm gap (Loveday et al., 2012). On the center of the base plate, 0.575 mL of sample was transferred, and the probe was lowered so that the gap between the probe and base plate remained 0.101 mm. Subsequently, the relative apparent viscosity (V_t/V_0), which is the ratio of apparent viscosity at time t to the apparent viscosity at time 0, was calculated.

Front-Face Fluorescence Spectroscopy. Front-face fluorescence spectra of the high-protein dispersions were collected using a LS50B Luminescence spectrometer (PerkinElmer) equipped with 15 W Xenon lamp and a front-face geometry accessory, adjusted to an angle of incidence at 56°. A quartz rectangular fluorimeter cell (Starna Cells Inc.) was used for analysis. The spectra were acquired for fluorescence emission of tryptophan (excitation: 290 nm; emission range: 305–450 nm), Maillard products (emission range: 380–480 nm at excitation 360nm, and excitation range: 260–350 nm at emission 410 nm). Seven scans were performed on each sample at room temperature (23°C) and averaged to improve signal-to-noise ratio. The slit widths were set at 10.0 and 7.0 nm for excitation and emission, respectively, and 1% attenuation filter was used. The collected spectral data were smoothed (Savitzky–Golay) and normalized using Unscrambler X 10.4.1 (CAMO Software Inc.).

Separation of Soluble (Undenatured) Whey Proteins

To determine the level of undenatured whey proteins in the nonheated and heated samples derived from both liquid and powder MPC, 1 g of sample was diluted to 15 mL with deionized water and acidified to pH 4.6 with 1 mL of acetate buffer (0.1 M, pH 4.6). After 10 min, the solution was filtered with Whatman No. 42 filter paper and filtrate was collected. All the casein and denatured whey protein were precipitated, and undenatured whey proteins remained in the soluble phase (Rowland, 1933). The soluble phase was analyzed for soluble whey protein, native PAGE, and right-angle spectrofluorimetric analysis.

Estimation of Soluble (Undenatured) Whey Proteins. The Bradford method (Kruger, 2002) was used to quantify protein content in the soluble phase. A representative sample from the collected soluble phase (100 µL) was mixed with 5 mL of reagent (0.01% Coomassie blue G 250: 100 mg of G-250 was dissolved in 50 mL of 95% ethanol, mixed with 100 mL of 85% phosphoric acid and volume made up to 1 L with deionized water), kept for 15 min at 22 ± 1°C, and absorbance was measured at 595 nm against reagent blank. The protein concentration was calculated from the standard curve prepared from known concentrations native whey proteins (permeate fraction from microfiltration of skim milk) from 0.05 to 0.25 mg of protein concentration.

Native Polyacrylamide Gel Electrophoresis. On the soluble protein filtrate, native PAGE analysis was performed following the method described by Allen (2010) in precast 12% Tris-glycine gels. All the samples were mixed with native sample buffer (Bio-Rad) in 1:1 ratio. The native sample buffer contained 62.5 mM Tris-HCl at pH 6.8 and 0.01% bromophenol blue as

tracking dye. Subsequently, 25 µL of each sample was loaded, and gel was run at 100 V until the band reached the bottom of gel (approximately 1.5–2 h). Gels were stained with the 0.025% solution (in 10% acetic acid) of Coomassie Blue G-250 (Sigma-Aldrich) for 12 h and destained in the 10% acetic acid solution for 2 h.

Right-Angle Fluorescence Spectroscopy

Right-angle fluorescence spectra of all the soluble phase protein samples were collected using a Perkin-nElmer LS50B Luminescence spectrometer equipped with 15 W Xenon lamp and right-angle accessory. A rectangular quartz fluorometer cell with 10-mm path length (Starna Cells, Inc.) was used for analysis. Five scans were performed on each sample at room temperature (23°C) to record the fluorescence emission spectra. Tryptophan emission spectra (305–450 nm) were acquired at an excitation wavelength of 290 nm with slit widths of 3.0 and 3.0 nm, respectively. Maillard products emission spectra (380–480 nm) was acquired at an excitation wavelength of 360 nm, and the Maillard excitation spectra in the 260 to 350 nm range at an emission of 410 nm with slit widths of 6.0 and 6.0 nm, respectively.

The spectral data generated were also used to determine fluorescence of advanced Maillard products and soluble tryptophan (**FAST**) index (Damjanovic Desic and Birlouez-Aragon, 2011). Tryptophan fluorescence of the soluble fraction at 290/340 nm and fluorescence of advanced Maillard products measured at 360/420 nm were used to calculate FAST index using Equation 1:

$$\text{FAST index} = 100 \times \frac{\text{fluorescence of advanced Maillard products}}{\text{tryptophan fluorescence}}.$$

[1]

Statistical Analysis

Statistical analysis was performed using SPSS software**[AU2: Please provide version for software]** (IBM Corp.) and Tukey's test was used to determine difference between treatments which were declared significant when $P \leq 0.05$. The acquired fluorescence spectra were analyzed by multivariate statistical analysis using Unscrambler X 10.4.1 software (CAMO Software Inc.). Principal component analysis (**PCA**) was applied on normalized spectra to examine the differences between control and heated samples derived from liquid and powder MPC.

RESULTS AND DISCUSSION

Understanding Protein Interactions by Conventional Methods

Gel Electrophoresis. Figure 1 shows the electrophoretic mobility patterns of nonheated and heated to 90°C for 1.5, 3, 4.5, 6, and 7.5 min samples derived from liquid MPC at pH 6.8 under nonreducing (**NR**)-SDS-PAGE. The electrophoretic mobility of milk proteins in nonheated sample (lane 1) on NR-SDS-PAGE resulted in separation of bands as α_{S2}-casein < α_{S1}-casein < β-casein < κ-casein < β-LG < α-LA. The κ-casein also appeared as κ-casein polymers (as a band on top of stacking gel) and κ-casein monomer in NR-SDS-PAGE. The κ-casein can exist as polymer (dimer to decamer) due to the presence of 2 intermolecular disulfide bonds. However, under NR conditions, polymeric κ-casein only partly dissociated into monomeric κ-casein (Groves et al., 1998). As the time of heating increased from 1.5 to 7.5 min at 90°C (lanes 2–6, Figure 1), there was a clear decrease in band intensity of β-LG that completely disappeared after 6 min of heating. In heated samples, there were number of bands observed between κ-casein and β-LG bands, with molecular weight ranged from 15 to 25 kDa identified from the molecular weight ladder. These could be aggregates of whey protein-whey protein as the molecular weight was greater than whey protein but less than caseins. Some bands were observed on top of stacking gel that could correspond to aggregates of casein-whey protein. The results NR-SDS-PAGE also suggested that out of 2 major whey proteins α-LA and β-LG, the β-LG was preferably interacted with caseins.

When the pH was adjusted to 6.5 and 7.0, there were no differences in NR-SDS-PAGE profile of nonheated sample (lane 1, Figures 2a and 2b). During heating at pH 7.0, the NR-SDS-PAGE profile was similar (lanes 2–6 Figure 2b, as intensity of β-LG decrease and number of bands with molecular weights between 15 and 25 kDa increase) to NR-SDS-PAGE profile of samples heated at pH 6.8. At pH 6.5, the band intensity of β-LG decreased with increased heating time (1.5–7.5 min), and the intensity of bands observed at the top of the stacking gel increased (could be due to interaction between κ-casein and β-LG), but the bands of MW between 15 and 25 kDa were not observed (lanes 2–6, Figure 2a); however, the bands were observed at pH 6.8 and 7.0, suggesting that at pH 6.5, whey protein-whey protein interactions were less. Similar results were obtained for samples derived from powder MPC heated at pH 6.8, 6.5, and 7.0 (figures not shown).

To further understand the nature of aggregates, SDS-PAGE was run under reducing conditions. Figure 3
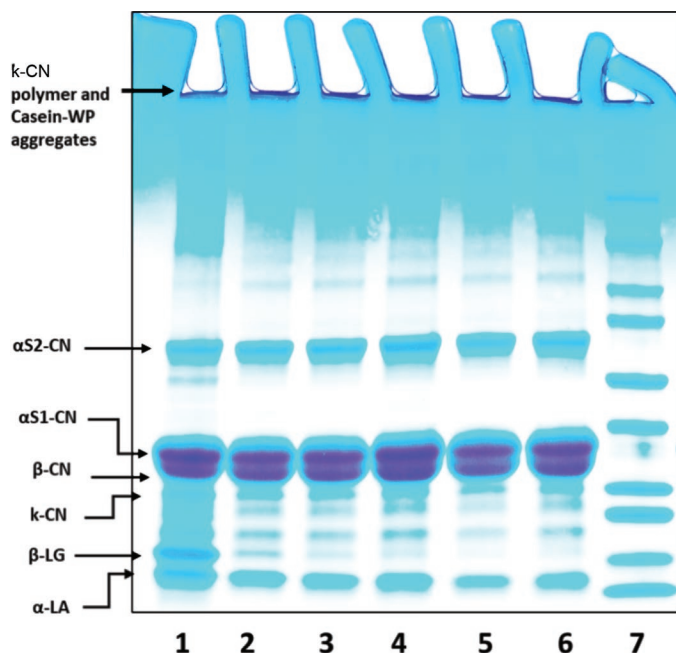
Figure 1. The nonreducing SDS-PAGE pattern of a sample derived from liquid milk protein concentrate at pH 6.8 on 4 to 15% gel. Lane 1 = control (nonheated); lane 2 = heated (1.5 min); lane 3 = heated (3 min); lane 4 = heated (4.5 min); lane 5 = heated (6 min); lane 6 = heated (7.5 min); lane 7 = molecular weight ladder. All heating was performed at 90°C.

shows the electrophoretic mobility patterns of samples derived from liquid MPC at pH 6.8 heated at 90°C for 1.5, 3, 4.5, 6, and 7.5 min in reducing (**R**)-SDS-PAGE. The R-SDS-PAGE patterns in nonheated and heated samples at pH 6.8 have shown negligible differences except for small amounts of protein aggregates that appeared on top of the stacking gel (not entered into separating gel) in the heated sample (lanes 2–6, Figure 3). The comparison between R- and NR-SDS-PAGE suggest that whey protein-whey protein aggregates and casein-whey protein complex were predominantly linked by disulfide bonds that were dissociable under reducing conditions (β-mercaptoethanol) of R-SDS-PAGE; however, the aggregates observed in stacking gel in R-SDS-PAGE also showed the presence of aggregates formed by hydrophobic interactions.

From the above-mentioned results, the heat-induced interactions of milk proteins in high-protein dispersions were similar to the skim milk system (∼3.5% protein), as reported for the heated model system containing redispersed casein micelles with added α-LA and β-LG (Smits and Brouwershaven, 1980), heated skim milk (Vasbinder and de Kruif, 2003), heated reconstituted skim milk (Anema and Li, 2003a), and concentrated heated milk (Nair et al., 2013). These researchers agreed that heating of skim milk systems at pH above 6.6 led to

more whey protein-whey protein interactions, whereas heating carried out at pH below 6.6 led to interaction of whey proteins with caseins, depending on heating temperature and time. The presence of disulfide bonds (Nair et al., 2013) and hydrophobic interactions (Smits and Brouwershaven, 1980; Singh and Myhr, 1998) was reported between denatured WP**[AU3: Define WP (whey protein?)]** or casein and whey protein.

Mean Particle Size, Zeta Potential, and Relative Apparent Viscosity. At pH 6.8, the mean particle size of casein micelle in nonheated sample derived from liquid MPC was 184.35 ± 27.79 nm. Moreover, an increase in heating time from 1.5 to 7.5 min did not significantly ($P \geq 0.05$) affect size of casein micelle as provided in Table 1. There were no significant differences ($P > 0.05$) in particle size of casein micelle in nonheated samples when pH was adjusted to 6.5 and 7.0. During heating at pH 7.0, a decrease in particle size of casein micelle ($P \geq 0.05$) was observed; however, during heating at pH 6.5, an increase (not significant $P \geq 0.05$) in particle size of casein micelle was observed (Table 1). For samples derived from powdered MPC, a similar trend in particle size of casein micelle was observed (Table 2). In this present study, at pH 6.5, mean particle size of casein micelle in the heated samples was larger than the nonheated samples, whereas at pH 6.8 and pH 7.0, the particle size of casein micelle in the heated samples was smaller than those in the nonheated milk samples. However, this difference was not significant ($P > 0.05$). About 80% of denatured whey protein interact with caseins at lower pH (<6.5; Anema and Li, 2003a; Kethireddipalli et al., 2010) and resulted in an increased casein micelle size (Anema et al., 2004) and a higher voluminosity (Jeurnink and Kruif, 1993). When pH of the heating medium was 6.8 and 7.0, more whey protein-whey protein aggregates were observed, which could be due to the fact that at pH 6.7 and 7.1, only 30 and 10% of denatured whey proteins interact with caseins, respectively (Anema and Li, 2003a, b). However, a decrease in particle size of casein micelle at pH 6.8 and 7.0 may be due to dissociation of κ-casein from casein micelle. At pH 7.1, about 20% of the κ-casein is in the serum phase at 20°C, whereas this increases to about 70% after heat treatment at 90°C (Singh and Creamer, 1991; Anema and Klostermeyer, 1997; Donato and Dalgleish, 2006). The decrease in particle size of micelle-bound complex was also observed with increasing pH from 6.3 to 7.3; (Anema and Li, 2003a; Vasbinder and de Kruif, 2003; Anema et al., 2004; del Angel and Dalgleish, 2006; Renan et al., 2006; Guyomarc'h et al., 2009; Taterka and Castillo, 2015).

In all the samples derived from liquid and powder MPC, zeta potential was found to be between -18.54
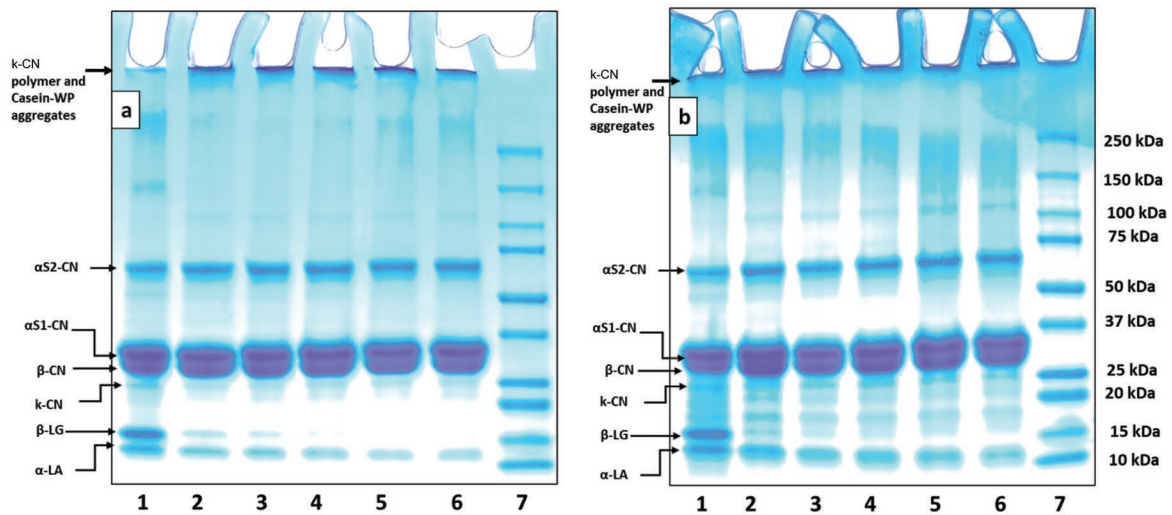
Figure 2. The nonreducing SDS-PAGE patterns of a sample derived from liquid milk protein concentrate at pH 6.5 (a) and 7.0 (b) on 4 to 15% gel. Lane 1 = control (nonheated); lane 2 = heated (1.5 min); lane 3 = heated (3 min); lane 4 = heated (4.5 min); lane 5 = heated (6 min); lane 6 = heated (7.5 min); lane 7 = molecular weight ladder. All heating was performed at 90°C.
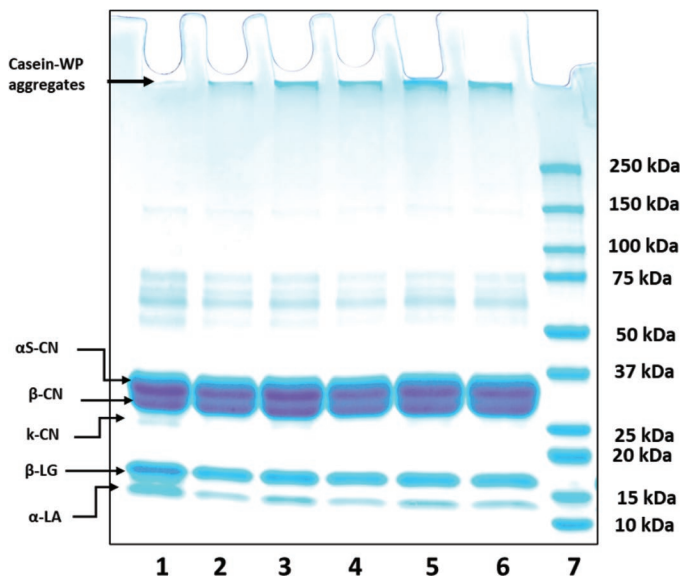

Figure 3. The reducing SDS-PAGE pattern of sample derived from liquid milk protein concentrate at pH 6.8 on 4 to 15% gel. Lane 1 = control (nonheated); lane 2 = heated (1.5 min); lane 3 = heated (3 min); lane 4 = heated (4.5 min); lane 5 = heated (6 min); lane 6 = heated (7.5 min); lane 7 = molecular weight ladder. All heating was performed at 90°C.

and −21.97, and −19.61 and −22.11 mV, respectively, and was not affected by pH or heating ($P > 0.05$). Similar results were also reported for skim milk at pH values 6.6 to 7.5 during heating at 95°C for 2 min (Lin et al., 2018**[AU4: Add to refs]**).

Figures 4a and 4b show the relative apparent viscosity (V_t/V_o) with increase in heating time (t) at different pH of samples derived from liquid and powder MPC, respectively. First of all, not much appreciable increase in viscosity (in all samples the viscosity was <4 mPa·s at 20°C) was observed with increased heating time, and this may be due to the fact that all heated samples were stable colloidal dispersions (Jeurnink and Kruif, 1993). However, the increase in relative apparent viscosity was greater at pH 6.5 and 6.8 than pH 7.0 ($P > 0.05$). Higher increase in apparent viscosity in the present study at pH 6.5 may be attributed to the casein-whey protein interaction at this pH. Anema et al. (2004) also reported an increase in relative viscosity of milk samples heated for 30 min at 90°C at pH 6.5 compared with pH 6.7. The increase in viscosity due to interaction of denatured whey protein with casein micelle was also reported (Jeurnink and Kruif, 1993).

Interpretations from the conventional methods provided a broad picture of heat-induced interactions and indicate that protein-protein interactions in high-protein dispersions within the pH range (6.5–7.0) in

Table 1. Size of casein micelles (nm) and fluorescence of advanced Maillard products and soluble tryptophan (FAST) index in sample derived from liquid milk protein concentrate at different pH (6.5, 6.8, and 7.0) and heating times at 90°C[1]

Heating time (min) at 90°C	Size of casein micelle (nm)			FAST index		
	pH 6.5	pH 6.8	pH 7.0	pH 6.5	pH 6.8	pH 7.0
0	$176.5 \pm 6.2^{a,y}$	$184.4 \pm 27.8^{a,x}$	$174.7 \pm 16.2^{a,x}$	$11.8 \pm 0.12^{a,y}$	$10.3 \pm 0.1^{a,x}$	$11.1 \pm 0.6^{a,x}$
1.5	$190.5 \pm 16.3^{a,y}$	$156.3 \pm 3.8^{a,x}$	$153.9 \pm 4.3^{a,x}$	$43.2 \pm 5.8^{b,y}$	$33.8 \pm 8.3^{b,x}$	$31.6 \pm 5.8^{b,x}$
3.0	$186.9 \pm 2.0^{a,y}$	$162.1 \pm 8.6^{a,x}$	$155.4 \pm 10.2^{a,x}$	$54.8 \pm 9.5^{bc,y}$	$42.0 \pm 8.0^{bc,x}$	$38.1 \pm 5.0^{bc,x}$
4.5	$182.6 \pm 5.0^{a,y}$	$162.5 \pm 12.9^{a,x}$	$160.6 \pm 0.4^{a,x}$	$58.8 \pm 6.1^{cd,y}$	$45.9 \pm 6.9^{cd,x}$	$44.2 \pm 4.9^{cd,x}$
6.0	$185.4 \pm 5.3^{a,y}$	$159.9 \pm 11.2^{a,x}$	$160.7 \pm 1.4^{a,x}$	$61.4 \pm 3.1^{cd,y}$	$49.9 \pm 5.3^{cd,x}$	$46.2 \pm 5.4^{cd,x}$
7.5	$183.4 \pm 1.3^{a,y}$	$162.6 \pm 9.2^{a,x}$	$159.5 \pm 2.8^{a,x}$	$65.6 \pm 5.0^{d,y}$	$52.7 \pm 6.7^{d,x}$	$49.6 \pm 6.5^{d,x}$

[a-d]Different superscripts within a row differ significantly ($P < 0.05$).**[AU13: Should this read "within casein micelle size or FAST index" (because these values can't be compared across the whole row). Are the a-d footnotes supposed to be within a column and the x,y footnotes within a row and trait? Please address all "letter" footnotes in both tables and make sure the comparisons are clear.]**

[x,y]Different superscripts within a column differ significantly ($P < 0.05$).**[AU14: Please check this footnote. No column has both x and y values. Please address this in both tables.]**

[1]Values are means ± SD, n = 2.

the current study also followed a similar pattern as in skim milk. The conventional methods did not provide any conclusive differentiation between the samples derived from liquid versus powder MPC and any possible changes caused by spray drying. To further understand the difference between the liquid- and powder-derived MPC, front-face fluorescence spectroscopy was explored to understand the changes in pH, time-temperature combination, and type of sample (derived from liquid and powder).

Front-Face Fluorescence Spectroscopy. Tryptophan emission, Maillard products emission, and Maillard products excitation fluorescence spectra were acquired using the front-face accessory on heated and nonheated liquid- and powder-derived samples to explore its potential as a rapid and nondestructive method for monitoring the heat-induced changes in high-protein dairy dispersions. Overlaid plot of tryptophan emission, Maillard emission, and Maillard excitation spectra before and after heating at 90°C and pH 6.8 for samples derived from liquid and powder MPC are presented in Figures 5, 6, and 7.

Tryptophan Fluorescence Spectra. The tryptophan emission spectra of nonheated sample derived from liquid MPC at pH 6.8 exhibited a peak at 342 nm. In the nonheated sample, no wavelength shift was observed in the maxima of tryptophan on changing the pH to 6.5 and 7.0 (Figure 5a). Changes were observed in tryptophan emission spectra as heating progressed at pH 6.8 (Figure 5b). Upon heating at 90°C, the tryptophan emission peaks presented a red shift in maxima and decrease in intensity was observed between 310 and 345 nm. After 7.5 min of heating, the tryptophan emission spectra exhibited a tryptophan maximum at around 343.5 nm. During heating at pH 6.5 and 7.0, the tryptophan emission spectra followed a similar trend as at pH 6.8 (data not shown).

In nonheated sample derived from powdered MPC at pH 6.8, tryptophan exhibited maxima around 340.5 nm that presented a blue shift in comparison to the sample derived from liquid MPC. The blue shift observed in the emission spectrum of tryptophan in spray-dried samples could be due to an increased hydrophobic environment (Ghisaidoobe and Chung, 2014). Dur-

Table 2. Size of casein micelles (nm) and fluorescence of advanced Maillard products and soluble tryptophan (FAST) index in samples derived from powder milk protein concentrate at different pH (6.5, 6.8, and 7.0) and heating times at 90°C[1]

Heating time (min) at 90°C	Size of casein micelle (nm)			FAST index		
	pH 6.5	pH 6.8	pH 7.0	pH 6.5	pH 6.8	pH 7.0
0	$153.7 \pm 4.1^{a,y}$	$150.9 \pm 8.9^{a,x}$	$150.2 \pm 12.2^{a,x}$	$13.0 \pm 0.4^{a,y}$	$14.6 \pm 3.0^{a,x}$	$14.5 \pm 1.1^{a,x}$
1.5	$243.5 \pm 60.7^{a,y}$	$146.3 \pm 6.4^{a,x}$	$145.0 \pm 10.5^{a,x}$	$47.42 \pm 11.7^{ab,y}$	$33.3 \pm 12.2^{ab,x}$	$31.9 \pm 16.1^{ab,x}$
3.0	$264.7 \pm 11.5^{a,y}$	$147.8 \pm 8.5^{a,x}$	$143.4 \pm 10.8^{a,x}$	$52.9 \pm 13.9^{b,y}$	$42.7 \pm 20.2^{b,x}$	$35.5 \pm 17.8^{b,x}$
4.5	$231.8 \pm 37.3^{a,y}$	$144.6 \pm 7.9^{a,x}$	$141.9 \pm 7.5^{a,x}$	$65.0 \pm 9.5^{b,y}$	$45.2 \pm 20.0^{b,x}$	$37.1 \pm 19.8^{b,x}$
6.0	$208 \pm 15.3^{a,y}$	$144.9 \pm 5.5^{a,x}$	$138.5 \pm 4.7^{a,x}$	$72.2 \pm 8.3^{b,y}$	$51.2 \pm 11.1^{b,x}$	$40.6 \pm 18.9^{b,x}$
7.5	$214.1 \pm 39.2^{a,y}$	$149.4 \pm 9.9^{a,x}$	$138.7 \pm 7.8^{a,x}$	$77.0 \pm 11.8^{b,y}$	$57.2 \pm 13.6^{b,x}$	$49.3 \pm 18.7^{b,x}$

[a,b]Different superscripts within a row differ significantly ($P < 0.05$).

[x,y]Different superscripts within a column differ significantly ($P < 0.05$).

[1]Values are means ± SD, n = 2.

ing storage (1–12 wk) losses of moisture occurred in MPC 70 and 90 at 25°C, which presented a blue shift in tryptophan emission peaks (Babu and Amamcharla, 2018). In nonheated samples, no wavelength shift was observed in the maxima of tryptophan on changing the pH to 6.5 and 7.0 (Figure 5c). During heating at pH 6.8, the tryptophan emission spectra followed a similar trend as in samples derived from liquid MPC and after 7.5 min of heating, the maxima shifted to around 343.5 nm (Figure 5d). During heating at pH 6.5 and 7.0, the tryptophan emission spectra followed a similar trend as at pH 6.8 (data not shown).

It can be said that emission of tryptophan is highly sensitive to its local environment, and the spectral peak shifts in both liquid and powder MPC demonstrated the protein conformational changes during heating (unfolding of whey protein due to denaturation) and further due to protein-protein association (Lakowicz and Masters, 2008). The red shift in tryptophan maxima upon heating was reported to be due to unfolding whey proteins during denaturation, which caused change in the hydrophobicity (Ayala et al., 2020). The decrease

Figure 4. Relative increase in viscosity (V_t/V_o) of a sample derived from liquid milk protein concentrate (a) and powder milk protein concentrate (b) at different pH (6.5, 6.8, and 7.0) and heating time (1.5–7.5 min) at 90°C.**[AU10: Please define error bars.]**

in tryptophan fluorescence intensity is attributed to protein-protein interactions between denatured whey proteins with casein micelles (Kulmyrzaev et al., 2005). The similar observations of red shift in spectral peak and decrease in tryptophan intensity were reported during front-face spectrofluorometric analysis of heated milk (Kulmyrzaev et al., 2005; Schamberger and Labuza, 2006; Alvarado Mamani, 2016; Ayala et al., 2020). In all the previous reported studies, the shift in fluorescence spectra and decrease in tryptophan intensity of milk was observed on a higher heat load, 90°C for more than 5 min (Alvarado Mamani, 2016; Ayala et al., 2020) or treatments at 100°C (Schamberger and Labuza, 2006; Ayala et al., 2020). However, in the present study, the shift in fluorescence spectra and decrease in tryptophan intensity was observed after 1.5 min of heating at 90°C that may be attributed to higher protein content (5%) in samples.

Maillard Emission Spectra. No wavelength shift or change in intensity was observed in the Maillard emission spectra of the nonheated samples derived from liquid and powder MPC at pH 6.8, 6.5, and 7.0 (Figure 6a). The Maillard emission spectra of heated samples derived from liquid MPC showed an increase in the peak intensities between 408 to 432 in comparison to nonheated samples (Figure 6b). Babu and Amamcharla (2018) also reported increase in Maillard emission intensity between 420 and 450 nm in front-face spectrofluorometric analysis during storage (1–12 wk) of MPC 70 and 90 at 40°C. Maillard emission spectrofluorometric spectra between 394 and 447 nm wavelength was also found to be sensitive in distinguishing heat treatment in milk (Schamberger and Labuza, 2006). The increase in intensity corresponded to Amadori products and advanced glycosylation end products that has an emission wavelength in the range of 420 to 450 nm (Matiacevich and Buera, 2006). The heated sample derived from powder MPC at pH 6.8 followed a similar trend as in samples derived from liquid MPC (Figure 6c). During heating at pH 6.5 and 7.0, the Maillard emission spectra followed the similar trend as at pH 6.8 (data not shown).

Maillard Excitation Spectra. In the Maillard excitation spectra of nonheated samples derived from liquid and powder MPC at pH 6.8, the peak maxima were found about 300 nm. On changing the pH (6.5 and 7.0), the maxima did not shift but the intensity of Maillard excitation was found to be higher between 260 to 290 nm and lower between 300 to 320 nm at lower pH (Figure 7a). Changes were also observed in Maillard excitation spectra as heating progressed at pH 6.8. For samples derived from liquid MPC, an increase in the peak intensities between 260 to 300 nm and decrease in intensity between 315 to 335 nm was observed in Mail-
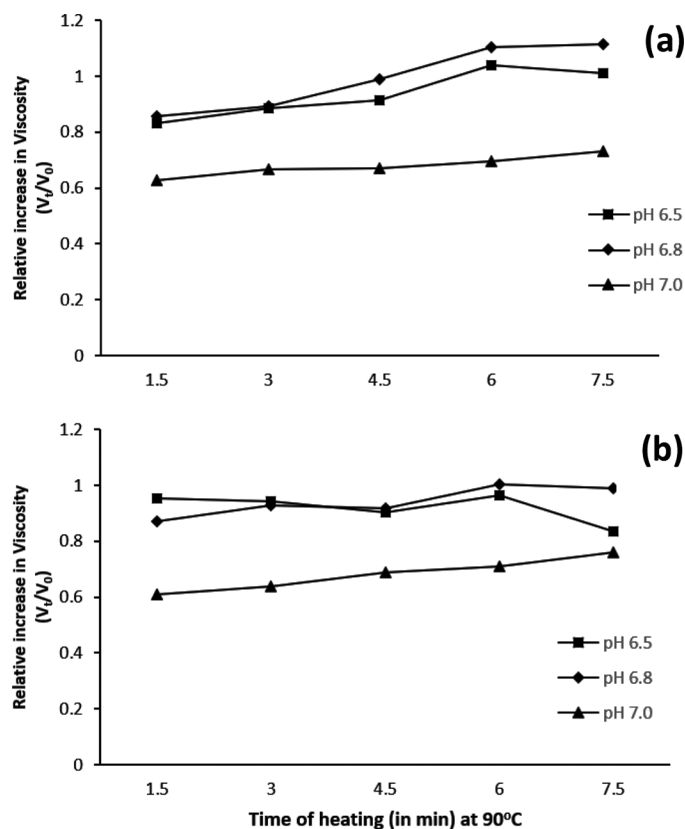
lard excitation spectra (Figure 7b). For samples derived from powder MPC, increase in the peak intensities was between 280 to 300 nm and decrease in intensity between 315 to 335 nm was observed in Maillard excitation spectra (Figure 7c). During heating at pH 6.5 and 7.0, the Maillard excitation spectra followed the similar trend as at pH 6.8 (data not shown). Babu and Amamcharla (2018) also reported Maillard excitation peak between 300 and 335 nm for MPC in front-face spectrofluorometric analysis during storage (1–12 wk) of MPC 70 and 90 at 40°C.

Multivariate Analysis of Front-Face Fluorescence Spectra. The PCA was applied to collected spectral data. The distribution of samples (liquid and powder MPC, heated and nonheated) can be observed in similarity plot according to 2 principal components

(**PC**) of tryptophan emission (Figure 8a), Maillard emission (Figure 8b), and Maillard excitation spectra (Figure 8c), representing the tendencies in the fluorescent signal. The first 2 PC (PC1 and PC2) accounted for 96, 92, and 85% of total variability in tryptophan emission, Maillard emission, and Maillard excitation spectra, respectively.

Spray drying induced changes were clearly evident from all spectra as 2 distinct groups were formed from the samples derived from liquid and powder MPC (Figure 8). From the PCA plots obtained from tryptophan spectra (Figure 8a), the samples derived from liquid MPC samples (control and heated) have higher PC1 and PC2 scores than samples derived from powder MPC samples. Liquid MPC control has a similar PC2 score as that of heated powdered MPC. As the time of
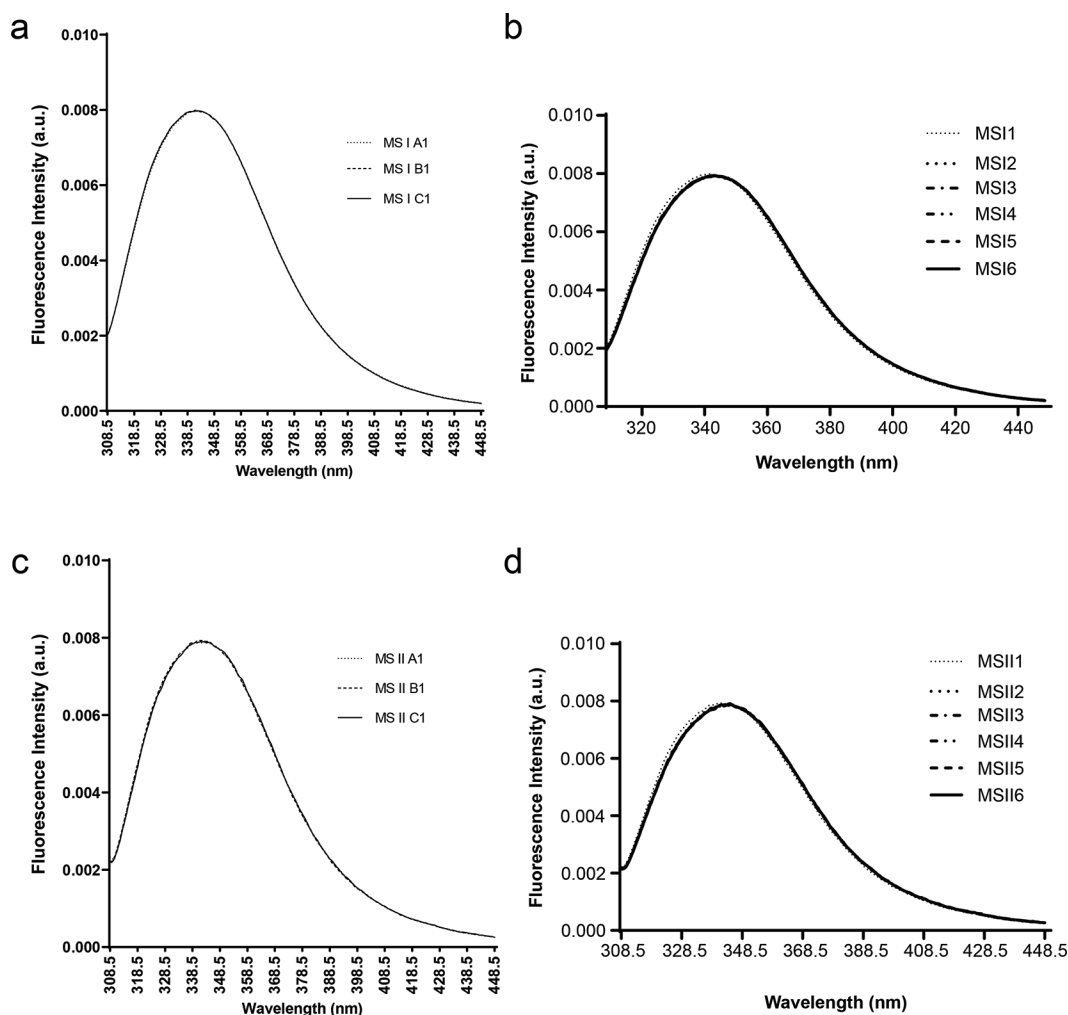

Figure 5. Front-face spectrofluorometric spectra for tryptophan emission for (a) control (nonheated) sample derived from liquid milk protein concentrate (MS I) at different pH, (b) sample derived from liquid milk protein concentrate of pH 6.8 during heating at 90°C, (c) control (nonheated) sample derived from powder milk protein concentrate (MS II) at different pH, (d) sample derived from powder milk protein concentrate of pH 6.8 during heating at 90°C. Labels A, B, and C indicate pH 6.5, 6.8, and 7.0, respectively; labels 1, 2, 3, 4, 5, and 6 indicate heating times of 0, 1.5, 3, 4.5, 6, and 7.5 min, respectively.
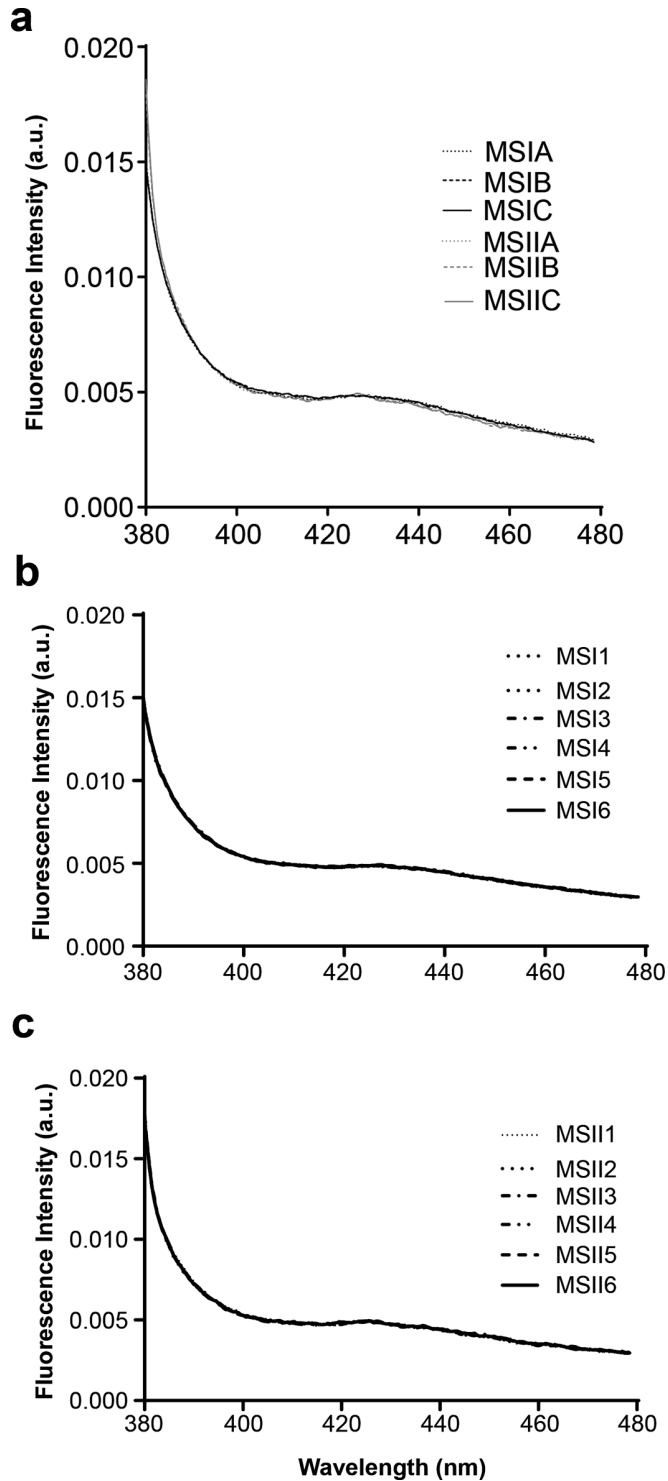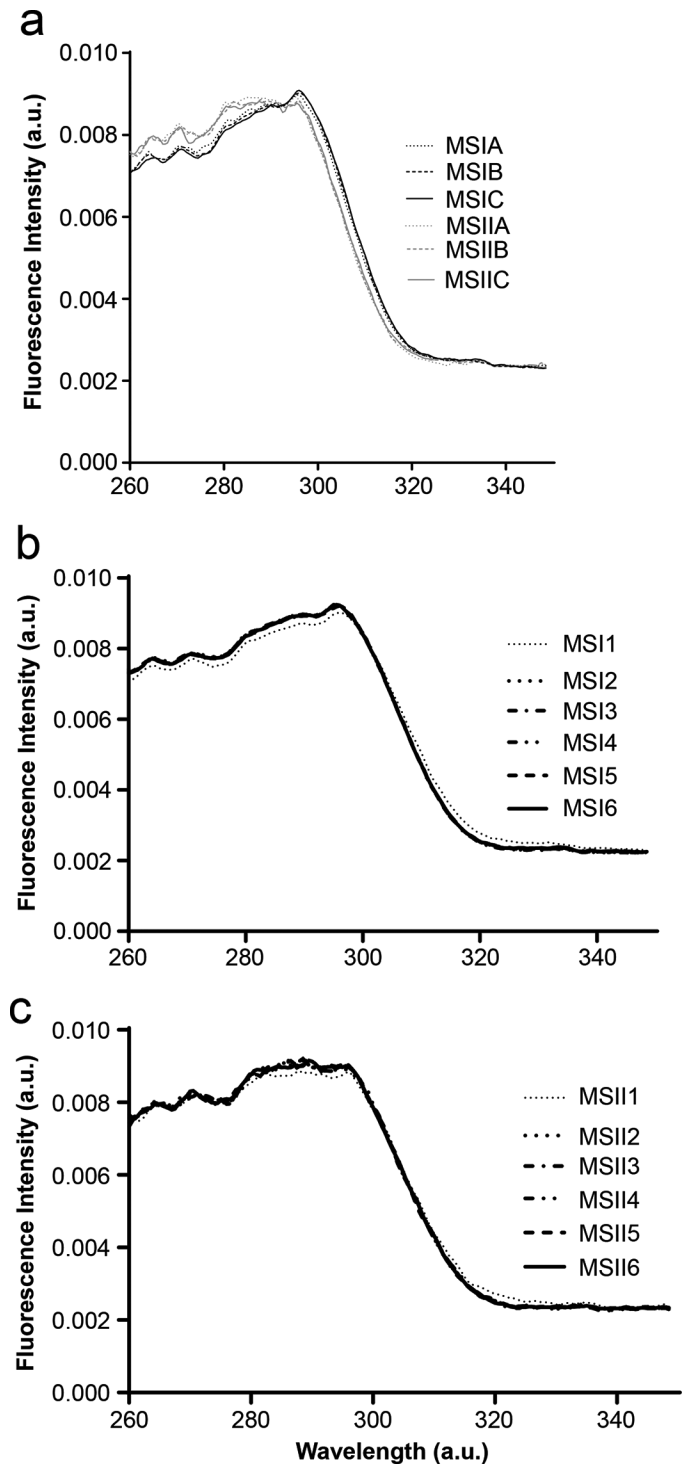
Figure 6. Front-face spectrofluorometric spectra for Maillard emission for, (a) a control (nonheated) sample derived from liquid milk protein concentrate (MS I) and powder milk protein concentrate (MS II) at different pH, (b) a sample derived from liquid milk protein concentrate of pH 6.8 during heating at 90°C, and (c) a sample derived from powder milk protein concentrate of pH 6.8 during heating at 90°C. Labels A, B, and C indicate pH 6.5, 6.8, and 7.0, respectively; labels 1, 2, 3, 4, 5, and 6 indicate heating times of 0, 1.5, 3, 4.5, 6, and 7.5 min, respectively.

Figure 7. Front-face spectrofluorometric spectra for Maillard excitation for (a) a control (nonheated) sample derived from liquid milk protein concentrate (MS I) and powder milk protein concentrate (MS II) at different pH, (b) a sample derived from liquid milk protein concentrate of pH 6.8 during heating at 90°C, and (c) a sample derived from powder milk protein concentrate of pH 6.8 during heating at 90°C. Labels A, B, and C indicate pH 6.5, 6.8, and 7.0, respectively; labels 1, 2, 3, 4, 5, and 6 indicate heating times of 0, 1.5, 3, 4.5, 6, and 7.5 min, respectively.

heating increased and consequently the level of protein denaturation increased, a trend can be observed in the PCA plots of tryptophan spectra where, nonheated (control) samples were given larger PC1 and lower PC2 scores than their respective heated samples. Within heated samples (derived from both liquid and powder MPC), as the heating time increased (from 1.5–7.5 min), there was a decrease in PC1 score and increase in PC2 score.

From the PCA plots obtained from Maillard emission spectra (Figure 8b), the samples derived from liquid MPC (both nonheated and heated) were given higher PC1 score than samples derived from powder MPC

(both nonheated and heated). The effect of heating can also be observed in samples derived from powder MPC, where heated samples were given higher PC1 and lower PC2 scores than their respective nonheated samples. In contrast to powder MPC, the heating effect cannot be verified in samples derived from liquid MPC.

From the PCA plots obtained from Maillard excitation spectra (Figure 8c), the samples derived from liquid MPC (both nonheated and heated) were given higher PC1 score than samples derived from powder MPC (both nonheated and heated). The effect of heating can also be observed, where heated samples were given lower PC1 and higher PC2 scores than their respective
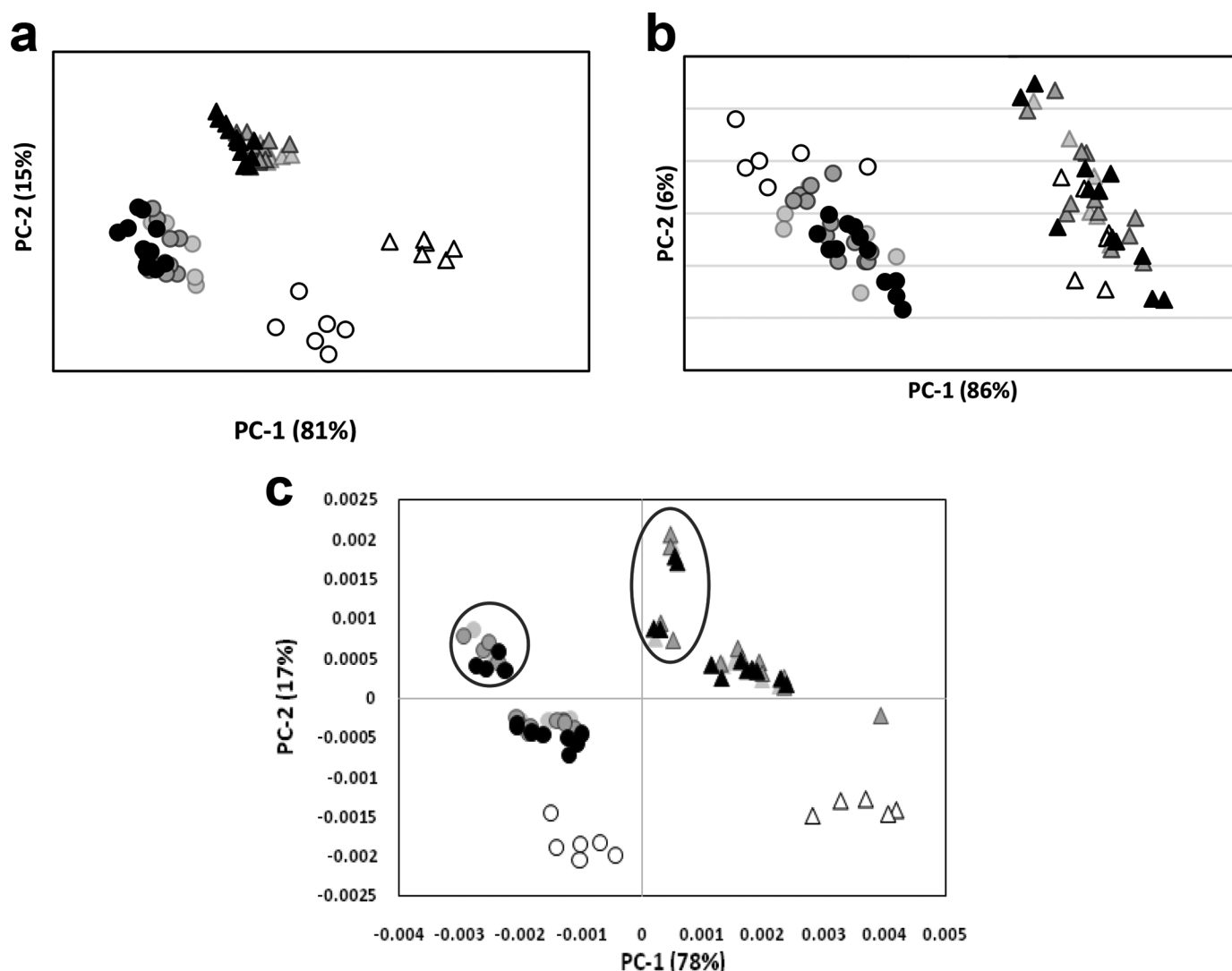

Figure 8. Similarity map of principal component (PC) analysis made on the tryptophan emission spectra (a), Maillard emission spectra (b), and Maillard excitation spectra (c), obtained from front-face spectrofluorometric analysis of samples derived from liquid and powder milk protein concentrate (MPC) at different pH (6.5, 6.8, and 7.0) and heating time at 90°C. Open triangles indicate control samples from liquid MPC; filled triangles indicate heated samples from liquid MPC; open circles indicate control samples from powder MPC; filled circles indicate heated samples from powder MPC. As the heating time increased (from 1.5–7.5 min), the intensity of the filled color increased. In figure (c), the data placed in circle represent the samples heated at pH 6.5**[AU11: Caption was edited for clarity. Please review carefully to confirm it is accurate.]**.

nonheated samples for both liquid and powder MPC. The effect of pH of heating medium (6.5, 6.8, and 7.0) were clearly evident from Maillard excitation spectra as distinct groups formed from the heated samples at lower pH (6.5), which were given lower PC1 and higher PC2 than samples heated at higher pH (6.8 and 7.0).

Therefore, from the comparative analysis of all 3 spectra (tryptophan emission, Maillard emission, and Maillard excitation) generated on the front-face fluorescence spectroscopy with chemometrics, has potential to differentiate a small change in denaturation with increase in heating time (from 1.5–7.5 min) at 90°C or adjustment in pH (6.5 to 7.0) or the heating prehistory, in this case derived from liquid or powder MPC. Moreover, the front-face fluorescence of tryptophan, and Maillard emission and excitation spectra can also be able to predict heat-induced changes using appropriate prediction models such as partial least square regression analysis. In addition to the complete milk protein system, soluble phases separated from all samples were also analyzed by gel electrophoresis and compared with spectrofluorometric data.

Soluble (Undenatured) Whey Proteins. Analyzing the soluble phase of high-protein dispersions heated to different times showed changes in the amounts of undenatured whey proteins. As more insoluble material formed (denatured whey protein complexed with casein), the protein concentration in the soluble phase depleted. Thus, the decrease in soluble protein content (undenatured whey protein) occurred with heating at all pH values (6.5, 6.8, and 7.0) for both samples derived from liquid and powder MPC and presented in Figure 9. The decrease in soluble protein content was very fast during initial stages of heating (up to 3 min), and then it reached a plateau value for both samples derived from liquid and powder MPC. Similar observations were reported by Anema (2017). The soluble protein content in the nonheated sample derived from liquid MPC at pH 6.8 was 0.76 ± 0.05%. After heating for 1.5 min at 90°C, there was about 33% reduction (0.51 ± 0.11%) in soluble protein content and it continued to decrease as heating time increased to 7.5 min. After 7.5 min heating at 90°C, about 53% reduction (0.35 ± 0.03%) was observed. The sample derived from liquid MPC at pH 6.5 has shown higher reductions (34 to 56%) in soluble protein content compared with pH 6.8 (32–53%) and 7.0 (32–50%), during heating from 1.5 to 7.5 min at 90°C. However, the effect of pH was less pronounced with increasing heating time and these findings are in accordance to Law and Leaver (1997), who reported similar results for the effect of pH on the thermal denaturation of whey proteins in milk. The reduction of soluble protein content in samples derived

from powder MPC (Figure 9b) with heating time were similar to sample derived from liquid MPC. The initial soluble protein content in samples both derived from liquid and powder MPC were lower (0.7%) than theoretically expected values (~1%), which may be due to the loss of proteins (mainly α-lactalbumin) in membrane processing during manufacturing of MPC (de la Fuente et al., 2002).

Native PAGE. The native PAGE profile of soluble phase derived from liquid and powder MPC samples at different pH (6.5, 6.8, and 7.0) and heating time interval (0, 3, and 7.5 min) were also observed and presented in Figure 10. In the native PAGE analysis, the band of whey proteins appeared as BSA < α-LA < β-LG B < β-LG A, and the effect of heating was clearly visible with fading of bands of β-LG (B and A) that proves majorly β-LG and to some extent α-LA was involved in association with casein.
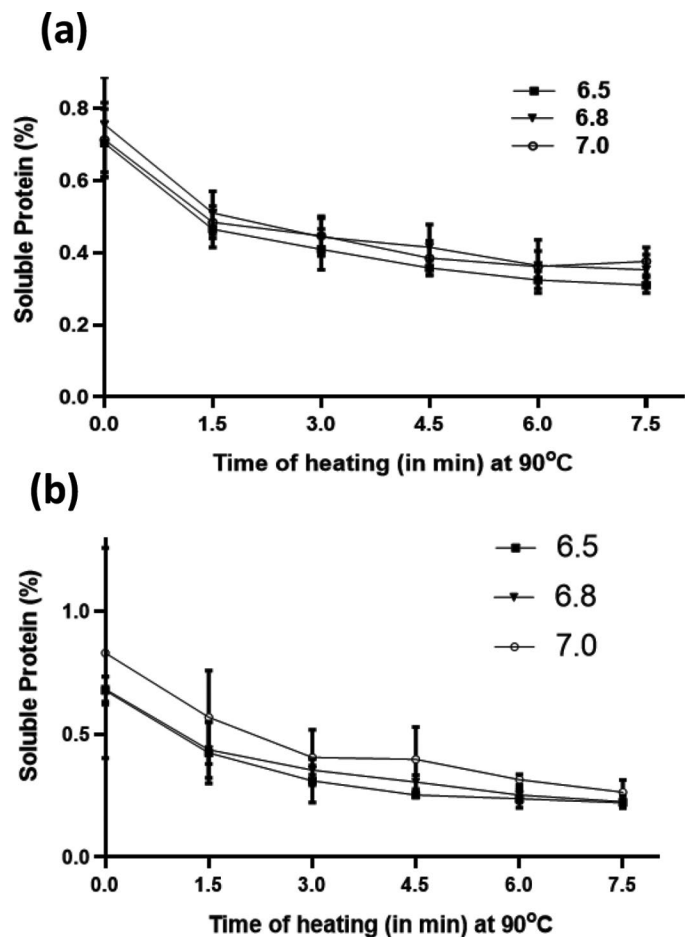

Figure 9. Soluble protein (%) of a sample derived from liquid milk protein concentrate (a) and powder milk protein concentrate (b) at different pH (6.5, 6.8, and 7.0) and heating times (1.5–7.5 min) at 90°C.**[AU12: Please define error bars.]**
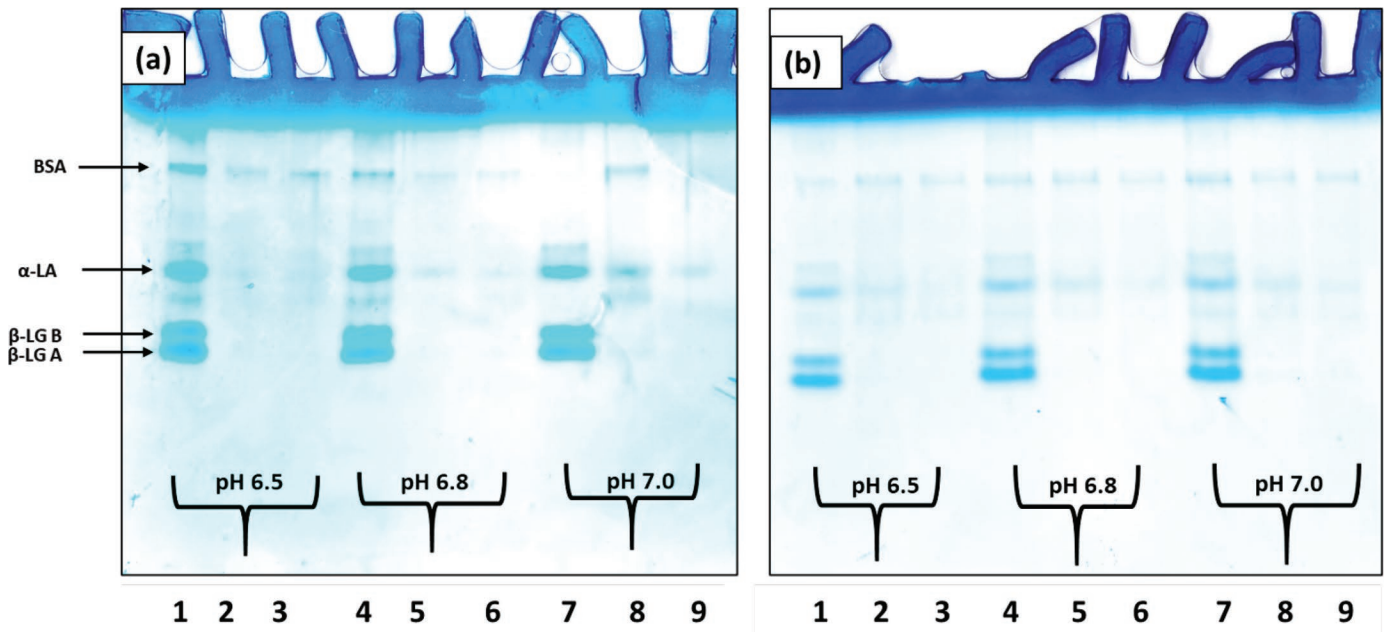
Figure 10. Native PAGE patterns of soluble phase in sample derived from (a) liquid milk protein concentrate (MPC) and (b) powder MPC at different pH (6.5, 6.8, and 7.0) on 12% gel. Lanes 1, 4, 7 are control (nonheated); lanes 2, 5, and 8 are heated (3 min); lanes 3, 6, 9 are heated (7.5 min). All heating was performed at 90°C.

The results of native PAGE analysis were in accordance with the amount of soluble protein present in sample (as presented in Figure 9) and were only able to differentiate the nonheated and heated samples. Results of both methods (measurement of soluble protein and gel electrophoresis) showed no appreciable differences between samples derived from liquid and powder MPC as well as the effect of pH was also not observed.

Right-Angle Fluorescence Spectroscopy. The right-angle fluorescence spectroscopy was conducted on soluble phase to acquire fluorescence spectra of tryptophan emission, Maillard product emission, and excitation spectra, and further to explore its potential to be used as a method for monitoring the heat-induced changes in high-protein dispersions. Overlaid plot of tryptophan emission, Maillard emission, and Maillard excitation spectra before and after heating at 90°C at pH 6.8 for samples derived from liquid and powder MPC are presented in Figure 11.

Tryptophan Spectra. The tryptophan emission spectra of a nonheated sample derived from liquid MPC at pH 6.8 exhibited tryptophan maxima at 339 nm, and evident changes were observed as the heating time increased. There was red shift in maxima and a decrease in tryptophan peak intensity between 300 to 350 nm after 1.5 min, and it continued to decrease with an increase in heating time (Figure 11a). The sample derived from powdered MPC at pH 6.8 exhib-

ited a tryptophan maximum around 337 nm (Figure 11a). Similar to whole system, this shift in wavelength maxima was observed in emission spectrum of liquid and powder MPC control samples may be attributed to hydrophobic environment in powdered samples (Ghisaidoobe and Chung, 2014). In nonheated sample (derived from both liquid and powder), on changing the pH (6.5 and 7.0), no wavelength shift was observed in the maxima of tryptophan; however, the intensity of tryptophan was found to be lower at pH 6.5 (data not shown). Samples at pH 6.5 and 7.0 displayed similar trends during heating (data not shown).

Maillard Emission Spectra. The Maillard emission spectra of heated samples derived from liquid and powder MPC at pH 6.8 showed decrease in the peak intensities between 350 to 405 nm and increase in intensity between 415 to 450 nm in comparison to nonheated samples (Figure 11b). On changing the pH (6.5 and 7.0), there was no effect on the intensity and during heating also at pH 6.5 and 7.0, the Maillard emission spectra followed the similar trend as at pH 6.8 (data not shown).

Maillard Excitation Spectra. The Maillard excitation spectra of heated samples derived from liquid MPC at pH 6.8 showed an increase in the peak intensities between 260 to 300 and 310 to 350 nm, and a decrease in intensity was observed between 315 and 350 compared with the nonheated sample (Figure 11c). The Maillard
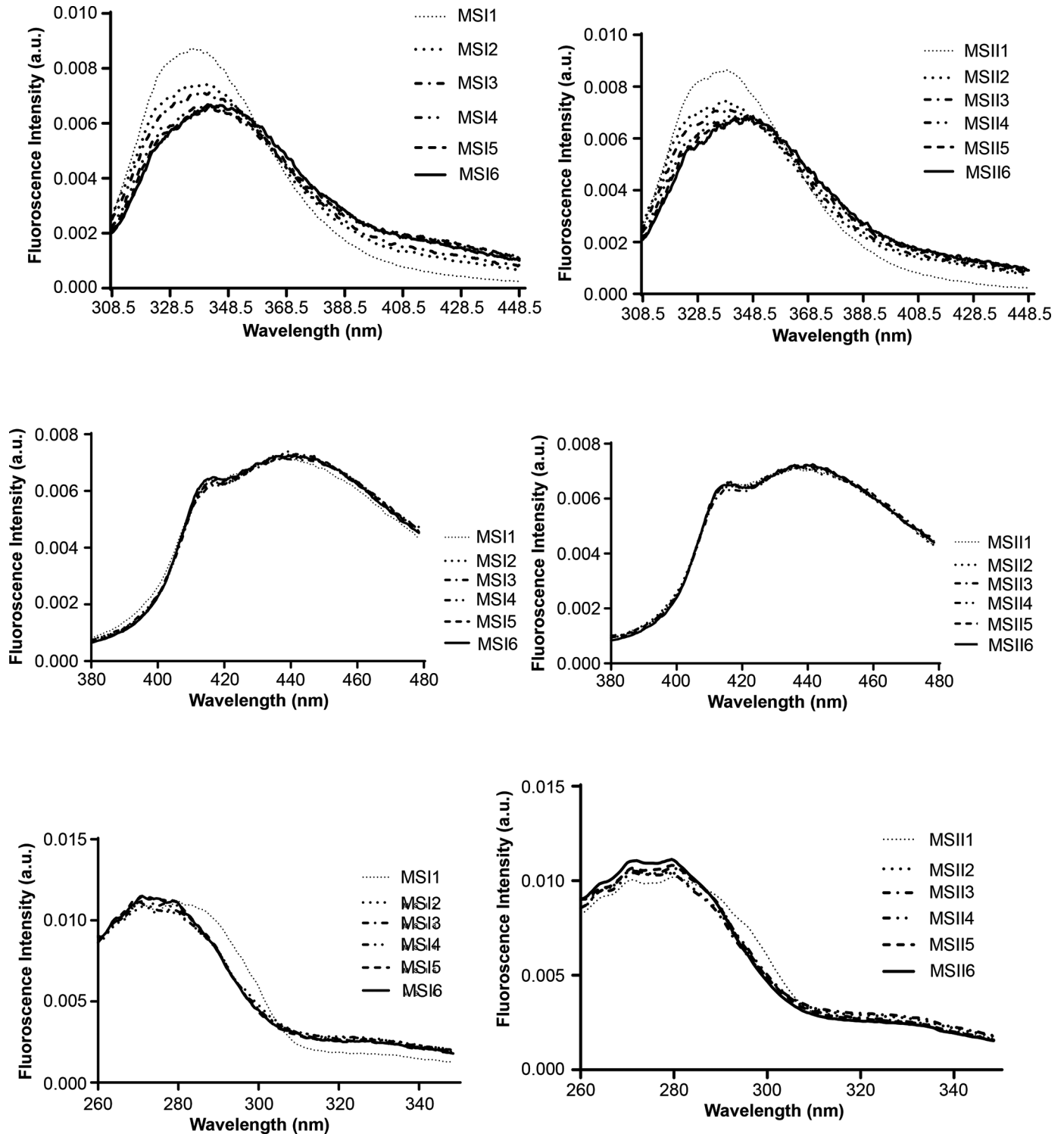
Figure 11. Tryptophan emission (a), Maillard emission (b), and Maillard excitation (c) spectra in the soluble phase analysis of sample derived from liquid and powder milk powder concentrate at pH 6.8 during heating at 90°C. The label MS I represents samples derived from liquid milk protein concentrate, and MS II represents samples derived from powder milk protein concentrate. Labels 1, 2, 3, 4, 5, and 6 represent heating times of 0, 1.5, 3, 4.5, 6, 7.5 min, respectively.

excitation spectra of heated samples derived from powder MPC at pH 6.8 were similar to sample derived from liquid MPC, except decrease in intensity between 310 to 350 nm. On changing the pH (6.5 and 7.0), there was no effect on excitation spectra in samples derived from liquid MPC. For samples derived from powder samples at pH 7.0, the Maillard excitation spectra followed the trend (decrease in intensity between 310 and 350 nm) similar to liquid MPC (data not shown).

Principal Component Analysis of Right-Angle Fluorescence Spectra. In a similarity map of PCA from tryptophan emission spectra (Figure 12) in soluble phase samples derived from liquid and powder MPC at different heating time at 90°C, PC1 accounted for 85% of total variability and PC2 accounted for 12% of total variability. In the similarity map, it is evident that the control sample derived from liquid and powder MPC were given larger PC1 scores than their respective heated samples. Within heated samples, as the time of heating increased (from 1.5 to 7.5 min), there was a decrease in PC1 and PC2 scores. However, the variability was not able to distinguish between the samples derived from liquid and powder MPC sample like in case of front-face fluorescence spectroscopy data.

The similarity map of PCA made on Maillard emission and excitation spectra in soluble phase of samples derived from liquid and powder MPC for different heating time at 90°C was not able to differentiate variability between heated and nonheated as well as between samples derived from liquid and powder sample (data not shown).

The results of fluorescence analysis were in correlation with results of native PAGE, where except for difference between heated and nonheated sample, no other difference (i.e., type of sample or change of pH) was visible.

The FAST index is a sensitive indicator of the advanced Maillard reaction (Birlouez-Aragon et al., 2002). As the heating time increased, there was an increase in FAST index, representing more denaturation. At pH 6.5, the increase in FAST index was significantly higher than at pH 6.8 and 7.0; however, no significant difference was observed between samples derived from liquid and powder MPC samples. The effect of heating medium pH on the FAST index was due to relatively more decrease in the tryptophan intensity at pH 6.5, due to more casein and denatured whey protein interactions (Kulmyrzaev et al., 2005) at this pH compared with pH 6.8 and 7.0. All the conventional methods (undenatured whey protein, gel electrophoresis) and fluorescence-based techniques conducted on the soluble phase of samples were mainly able to distinguish between nonheated and heated samples and failed to understand the changes induced by spray drying.
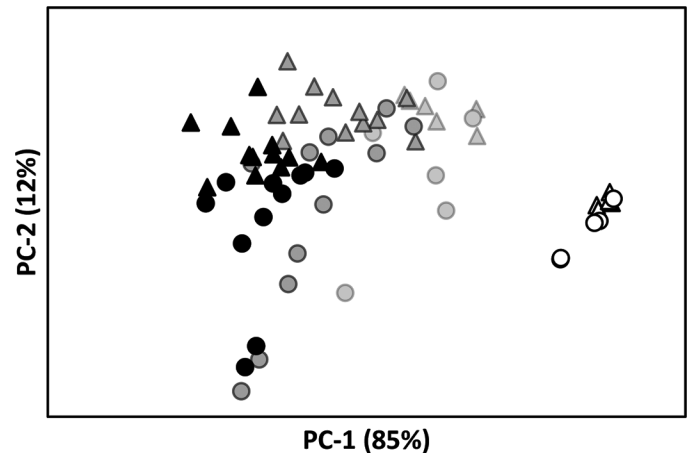

Figure 12. Similarity map of principal component analysis (PCA) made on tryptophan emission spectra obtained from soluble phase of sample derived from liquid and powder milk protein concentrate (MPC) at different heating time at 90°C. Open triangles indicate control samples from liquid MPC; filled triangles indicate heated samples from liquid MPC; open circles indicate control samples from powder MPC; filled circles indicate heated samples from powder MPC. As the heating time increased (from 1.5–7.5 min), the intensity of the filled color increased.

CONCLUSIONS

The results indicated that the heat-induced denaturation and interaction in high-protein dispersions are affected by pH and heating history. The routine methods were able to help in understanding the phenomena of denaturation (PAGE analysis, soluble protein content) and aggregation of milk proteins (PAGE analysis, particle size, and viscosity of medium) as a function of heat treatment. These methods were able to reflect differences between heated and nonheated samples, but clear differentiation can only be marked after 6 min of heating at 90°C; they are unable to help in understanding the changes in protein interaction caused by industrial spray drying. The front-face spectrofluorometric analysis with chemometrics was found to compliment the conventional methods in understanding casein-whey protein and whey protein-whey protein interactions in high-protein dispersion. This method was able to make out clear differentiation in samples even for a small change in time (from 1.5–7.5 min) of heating at 90°C, heating prehistory (liquid vs. powder MPC) of a sample and, to some extent, the change in pH of the heating medium (6.5 to 7.0). Therefore, front-face fluorescence spectroscopy along with conventional techniques will provide a more informative and broader picture of whey protein denaturation and its aggregation with milk proteins in high-protein dispersion, as a factor of pH of heating medium and heating load (in terms of time and temperature combination).

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