INTRODUCTION

Milk protein concentrate (MPC) is a powder manufactured from skim milk through membrane separation and spray drying. The protein content of MPC powders ranges from 40 to 90%, based on total solid content (Kelly, 2011; Sikand, Tong, Roy, Rodriguez-Saona, & Murray, 2011). MPCs are named according to their protein content. For example, MPC85 contains approximately 85% protein content. MPC powders are widely applied in cheese, yogurt, beverage, and confection manufacturing due to their desirable functional properties and nutritional qualities (Farkye & Yim, 2003; Francolino, Locci, Ghiglietti, Iezzi, & Mucchetti, 2010). The solubility of specific MPC powders is closely related to their various functional properties, such as emulsification, gelation, and foaming (Dybowska, 2008; Sandra & Corredig, 2013; Ye, 2011). Despite their widespread use, the solubility of MPC powders gradually decreases during storage, which limits their application (De Castro-Morel & Harper, 2002; Anema, Pinder, Hunter, & Hemar, 2006; Havea, 2006). For example, it was reported that the solubility of MPC85 was 53% and 32% after 2 days and 24 months of storage at 20°C, respectively (Havea, 2006). Thus, various approaches have been used to improve the solubility of MPC powders, such as raising the water temperature and extending hydration time (Fang, Selomulya, Ainsworth, Palmer, & Chen, 2011; Kuo & Harper, 2003). However, after extended storage, the solubility was still low, even at a high dissolution water temperature (McKenna, 2000). Generally, it is believed that the loss of solubility is linked to processing. Some studies have suggested that ultrasonication and the addition of salt could increase the solubility (Mao, Tong, Gualco, & Vink, 2012; McCarthy, Kelly, Maher, & Fenelon, 2014; Sikand, Tong, & Walker, 2013). For example, the
solubility of stored MPC80 was increased from 63 to 100% by the addition of 50–150 mM NaCl during the diafiltration step of manufacturing. However, these studies did not fundamentally solve the low solubility of stored MPC. Thus, determination of the mechanism underlying the decreased solubility of MPC powders during storage is very important for improving its functionality and expanding its applications in the food industry.

Protein cross-linking, the Maillard reaction, lipid oxidation, lipolysis, and proteolysis of MPC powders may occur during storage (Gaiani et al., 2007; Le, Holland, Bhandari, Alewood, & Deeth, 2013). Protein cross-linking and the Maillard reaction are the two main factors that contribute to the decreased solubility of MPC powders. The objective of this study was to investigate the changes in the chemical and physical properties of modified commercial MPC powders after storage for a certain time period at 50°C. The solubility, protein cross-linking, and Maillard reaction were examined in stored MMPC powders to clarify the mechanism underlying the solubility decrease.

### 2 MATERIALS AND METHODS

#### 2.1 Preparation of MMPC samples

Fresh MPC85 powder (MPC containing 85% protein) was purchased from Idaho Milk Products (Jerome, ID, USA). Fresh MPC70 powder (MPC containing 70% protein) was purchased from Dairy Farmers of America (Kansas City, KS, USA). MPC85 and MPC70 powders with no sugar (MPC85-NS and MPC70-NS, respectively) were obtained by dialyzing out the lactose and then freeze-drying. Using Kjeldahl Method and phenol-sulfuric acid method, the final protein and sugar contents of MPC85-NS were 92.76% and 0.1%, respectively, and the protein and sugar contents of MPC70-NS were 89.32% and 0.1%, respectively (Table 1). Modified MPC powders with different amounts of protein (MPC-P) were obtained by mixing MPC85-NS or MPC70-NS with sucrose, and the measured protein content of modified MPC85 (MMPC85), modified MPC70 (MMPC70), modified MPC55 (MMPC55), and modified MPC40 (MMPC40) was 85%, 70%, 55%, and 40%, respectively (Table 2). Modified MPC powders with different amounts of lactose (MMPC-L) were prepared from MPC70 through hydration, dialysis for various lengths of time, and vacuum freeze-drying. The measured protein content of the MMPC-L samples modified MPC75 (MMPC75), modified MPC77 (MMPC77), modified MPC78 (MMPC78), and modified MPC80 (MMPC80) was 75%, 77%, 78%, and 80%, respectively, and the measured lactose content was 14%, 7%, 1.4%, and 0.14%, respectively (Table 3). All powders were stored at 50 ± 1°C for up to 45 days. The control powders were stored at −20°C. The solubility, electrophoretic pattern, and color of each MMPC powder were examined after 15, 30, and 45 days of storage.

#### 2.2 Solubility assay

The rehydration procedure was carried out as described previously, with a minor modification (Arnaud Mimouni, Deeth, 2010). The solubility of MMPC powders was calculated using the equation below.

\[
\text{Solubility} = \frac{\text{Solids in the solution} - \text{Solids in the sediment}}{\text{Solids in the solution}} \times 100%
\]

### Table 1: Protein and sugar content of MPC-NS

| Samples        | MPC85-NS         | MPC70-NS         |
|----------------|------------------|------------------|
| Protein (%)    | 92.76 ± 0.48a    | 89.32 ± 1.78a    |
| Sugar (%)      | 0.10 ± 0.02      | 0.10 ± 0.01      |

MPC-NS, MPC powders with no sugars; MPC powders with no sugar (MPC85-NS and MPC70-NS) were obtained by dialyzing total lactose and vacuum freeze-drying.

Whittaker, Gidley, & Bhandari, 2009, MMPC solutions (4%) were reconstituted by stirring the powders in distilled water at 1500 rpm for 10 min at 30°C. The rehydrated solutions were then centrifuged at 2400 × g for 10 min at 10°C. The centrifugation sediments and rehydrated solutions were subjected to solid determination by oven-drying (at 105°C) to a constant weight. The solubility of MMPC powders was calculated using the equation below.

#### 2.3 Polyacrylamide gel electrophoresis

The protein composition of the supernatant was determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) technique under native (no β-mercaptoethanol) and reducing (with β-mercaptoethanol) conditions in a modified Laemmli system (Laemmli, 1970) with 5% acrylamide in the stacking gel and 12% acrylamide in the separating gel. Protein bands were visualized by staining with Coomassie R250 brilliant blue. Standard proteins (Thermo Scientific, Shanghai, China) were used for molecular mass determination as follows: β-galactosidase (116 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), lactate dehydrogenase (35 kDa), REase Bsp981 (25 kDa), β-lactoglobulin (18.4 kDa), and lysozyme (14.4 kDa). After staining and destaining, the gels were scanned using a Bio-Rad Gel Doc XR system and analyzed with Quantity One software.

#### 2.4 Changes in color

The surface color properties of the MMPC powders were measured using a Color Meter ZE6000 (Nippon Denshoku Industries, Tokyo, Japan), and the results were expressed as L*, a*, and b* values (Morales & Van Boekel, 1998). The color values reported are the means of three measurements.

#### 2.5 Statistical analysis

Data were analyzed by analysis of variance and the F test using the Statistical Product and Service Solutions (SPSS) 11.0 software package (SPSS Inc., Chicago, IL, USA). p values <0.05 were considered statistically significant.
3 | RESULTS AND DISCUSSION

3.1 | Effects of protein content on solubility

The solubility of MMPC-P samples stored at 50°C for up to 45 days is shown in Figure 1. MMPC40, MMPC55, and MMPC70 initially showed relatively good solubility. Then, the solubility decreased rapidly during storage. The MMPC85 sample showed lower solubility than others, even at the beginning of storage (day 0). As shown in Table 2, the MMPC85 was the mixture of MPC85-NS and sucrose, MMPC70, MMPC55, and MMPC40 were derived by mixing MPC70-NS and sucrose. It took much longer to rehydrate powders with high protein content; thus, MMPC85 showed lower solubility than MMPC70 under the same conditions. As for the stored MPC powders, the internal structure of the casein micelles remained unchanged, whereas the interface became porous and gel-like due to the cross-linking of α-caseins and β-caseins (Mata, Udabage, & Gilbert, 2011; Mimouni, Deeth, Whittaker, Gidley, & Bhandari, 2010). The porous gel-like structure limited the dispersion of micelles into water and contributed to the loss of solubility during storage (Mimouni et al., 2009).

After 15 days, the solubility of all the MMPC-P samples decreased significantly. The solubility continuously declined during the 30 days, and MMPC70 showed the greatest decline among the four groups. After 45 days, the solubility of MMPC40, MMPC55, MMPC70, and MMPC85 declined to 74.21%, 52.33%, 44.57%, and 42.29%, respectively, which indicates that the solubility of MMPC powders is closely related to the protein content. The possibility of protein-protein interactions increases at higher protein content; therefore, at the same storage time point, the MMPC85 sample had the lowest solubility. Protein unfolding and surface hydrophobicity were also increased when MPC powders were stored at high temperatures, which could lead to protein-protein interactions (Haque, Bhandari, Gidley, Deeth, & Whittaker, 2011; Haque et al., 2010).

3.2 | Effects of lactose content on solubility

The solubility changes of MMPC-L samples stored at 50°C for various time points are shown in Figure 2. Compared with the
MMPC-P samples, the solubility of the MMPC-L samples decreased significantly faster. In addition, MMPC75 had the lowest protein concentration, which also contributed to its higher solubility. After 15 days, the solubility of all MMPC-L powders decreased sharply, and the solubility of MMPC75 and MMPC80 was higher than the other samples. These results indicated that both the protein and lactose contents influenced the solubility of the powders. Over the next 30 days, the solubility of all the MMPC-L powders continued to decrease. At 45 days, the solubility of MMPC75, MMPC77, MMPC78, and MMPC80 was reduced to 36.24%, 13.93%, 11.36%, and 17.76% of the initial value, respectively. At this time point, the solubility of MMPC80 was higher than that of MMPC77 and MMPC78. These results suggest that the Maillard reaction may also influence the solubility of MMPC powders. Reconstitution may also influence the solubility of the powders due to protein denaturation. For example, the solubility of fresh powder MMPC75 was higher than that of the reconstituted powders MMPC77, MMPC78, and MMPC80.

We concluded that during storage, the casein in commercial MPC85 powder was lactosylated (Anema et al., 2006). The Maillard reaction can produce glyoxal and methylglyoxal, two substances that further promote protein cross-linking, which indirectly influence solubility. We detected a correlation between the Maillard reaction and the solubility of commercial MPC80, and we concluded that casein and α-lactalbumin were involved in the Maillard reaction (Le, Bhandari, & Deeth, 2011; Le, Deeth, Bhandari, Alewood, & Holland, 2012). However, further studies are required to investigate the relationship between protein cross-linking and the Maillard reaction in modified MPC powders.

### 3.3 SDS-PAGE analysis

The SDS-PAGE of MMPC-P samples after 0, 15, 30, and 45 days of storage is shown in Figure 3. The changes in the MMPC-P protein pattern increased with storage time. The molecular weights of bands observed in the SDS-PAGE are summarized in Table 4. There were reductions in the densities of the α-, β-, and κ-casein bands after 15, 30, and 45 days when compared to the densities of those of protein bands on day 0. Cross-linking between casein and other proteins could account for the insolubility of MPC powders with higher protein content. MMPC85 showed that most obvious changes among the MMPC-P samples, which was consistent with the changes in the solubility of the MMPC-P samples.

As the hydrophobic bonds and hydrogen bonding were disrupted by the dissociating agent SDS, and the disulfide bonds were reduced with β-mercaptoethanol, band I in (d) was generated through nondisulphide covalent bonding. The new bands, named X and Y in (e), (f), (g), and (h), contained disulfide bonds, and there were high molecular weight bands at position X and three clear bands at position Y. The density of the bands at position Y increased with increasing storage time. The number of the bands at position X changed with storage time. At 15 and 30 days, there were two bands at position X in MMPC70 and MMPC55. The native-PAGE showed that the protein at position 1 gradually aggregated into high molecular weight bands and then became trapped in the well; therefore, there was no protein band at position 1 on days 15–45. The protein at position 2 also formed large protein aggregates through nondisulphide covalent bonding. At 45 days, the density of the bands at position 2 increased, and the number of bands changed from 3 to 2 due to the formation of large protein aggregates through nondisulphide covalent bonding.

The gels of the MMPC-L samples after 0, 15, 30, and 45 days are shown in Figure 4. On the whole, the changes in the MMPC-L samples were greater than those observed for the MMPC-P samples. The molecular weights of the bands with the most remarkable changes are shown in Table 4. In addition, there were also reductions in the densities of the α-, β-, and κ-casein bands in the MMPC-L powders during storage. The SDS-PAGE of MMPC78 (1.4%) and MMPC80 (0.14%) after storage for 45 days also showed band I, which was generated through nondisulphide covalent bonding. These results showed that protein cross-linking generated this new band.

As shown in Figure 4e–h, there were high molecular weight bands at position X and three clear bands at position Y. The density of the bands at position Y increased, and the number of the bands at position X changed with increasing storage time. At 30 and 45 days, there were three clear bands in MMPC75 (14%) at position X. Thus, lactose content had an effect on protein cross-linking; high lactose content promoted the formation of high molecular weight proteins, whereas low content of lactose promoted the formation of new low molecular weight proteins. The gel of (i), (j), (k), and (l) in Figure 4 showed that the protein at position 1 gradually aggregated into larger molecules and then became
Figure 3. Electrophoretic separation of the proteins in the MMPC-P samples. M: Marker, 85: MMPC85, 70: MMPC70, 55: MMPC50, 40: MMPC40. (a, b, c and d): SDS-PAGE (with SDS, with β-mercaptoethanol) of MMPC-P samples stored for 0, 15, 30, and 45 days, respectively. (e, f, g and h): PAGE (with SDS, no β-mercaptoethanol) of MMPC-P samples stored for 0, 15, 30, and 45 days, respectively. (i, j, k, and l): Native-PAGE (no SDS, no β-mercaptoethanol) of MMPC-P samples stored for 0, 15, 30, and 45 days, respectively.

Table 4. The molecular weight of new bands

| Name | MMPC-P (kDa) | MMPC-L (kDa) |
|------|--------------|--------------|
|      | 0 day | 15 days | 30 days | 45 days | 0 day | 15 days | 30 days | 45 days |
| I    | 23.24 | 23.24 | 23.24 | 23.24 | 22.73 | 22.73 | – | 22.73 |
| X    | 200.28 | 101.57 | 101.57 | 200.28 | – | 94.34 | 94.34 | 94.34 |
|      | 200.28 | 200.28 | 200.28 | 200.28 | 136.45 | 136.45 | 136.45 | 136.45 |
| Y    | 19.80 | 19.80 | 19.80 | 19.80 | 19.68 | 19.68 | 19.68 | 19.68 |
|      | 22.39 | 22.39 | 22.39 | 22.39 | 22.12 | 22.12 | 22.12 | 22.12 |
|      | 26.31 | 26.31 | 26.31 | 26.31 | 26.17 | 26.17 | 26.17 | 26.17 |
| 1    | 67.27 | – | – | – | 70.88 | – | – | – |
| 2    | 18.76 | 18.76 | 18.76 | 18.81 | 17.03 | 17.03 | 17.03 | 19.19 |
|      | 21.8 | 21.8 | 21.8 | 22.9 | 20.33 | 20.33 | 20.33 | 23.32 |
|      | 25.5 | 25.5 | 25.5 | 25.5 | 24.3 | 24.3 | 24.3 | 24.3 |

–, without the band.
trapped in the well. Therefore, there was no band at position 1 at 15, 30, and 45 days. At 45 days, the density of the bands at position 2 increased and the number of bands changed from 3 to 2 due to the formation of large protein aggregates through nondisulphide covalent bonding.

As shown in Table 4, the molecular weights of the new bands in the MMPC-L samples were lower than those in the MMPC-P samples. In addition, at position X, one band (136.45 kDa) was absent in the MMPC-P powders but was present in MMPC77 (an MMPC-L sample), which indicated that there were different protein-crosslinks in the MMPC-P and MMPC-L samples, and the 136.45 kDa protein was probably produced via the Maillard reaction. The electrophoresis results revealed that hydrogen bonding, disulfide bonding, hydrophobic interactions, and nondisulphide covalent bonding were involved in the protein cross-linking of stored MPCs.

3.4 | Changes in color during storage

The L*, a*, and b* values were used to assess the changes in color of MMPC-L samples during storage. These color values, L*, a*, and b*, represent lightness (or whiteness), redness (or greenness), and yellowness (or blueness), respectively. Figure 5 shows the changes in the color of the MMPC-S samples during storage. The decrease in the L* value represents a decrease in lightness and a corresponding increase in darkness. The color of MMPC77 (7%) was the darkest, and the color of MMPC40 was the lightest. The increases in a* and b* represent increases in redness and yellowness, respectively. MMPC77 (7%) showed significant changes in L*, a*, and b* during storage, whereas the other samples only showed gradual changes. There was significant correspondence between the solubility change in MMPC77 (7%) during storage and the presence of Maillard reaction products, which caused the protein cross-linking
that was responsible for the decreased solubility (Le, Bhandari, Holland, & Deeth, 2011; Le et al., 2013). In the Maillard reaction, lactose binds to protein at lysine residues, resulting in the formation of the first-stage Maillard reaction product, lactulosyllysine. Moreover, advanced glycation end products can lead to browning and the formation of high molecular weight protein complexes (Mottram, Wedzicha, & Dodson, 2002; Singh, 1991).

Compared with MMPC75 (14%), MMPC77 (7%) showed more remarkable changes in color. Thus, the occurrence of the Maillard reaction in MPC was not linearly proportional to the lactose content. The protein and lactose contents in MMPC77, 77% protein and 7% lactose (a mass ratio of 11:1), facilitated the Maillard reaction.

4 | CONCLUSIONS

In summary, the solubility of all modified MPC powders decreased during storage, and protein cross-linking was the major reason for the solubility decrease in MPC powders during storage. The protein molecules interacted through hydrogen bonding, disulfide bonding, hydrophobic interactions, and nondonsulphide covalent bonding. In addition, the Maillard reaction also decreased solubility during storage. A small amount of lactose in the MPC reacted with proteins or amino acids with free amino groups; this process formed products that promoted protein cross-linking, and in turn, the Maillard reaction influenced protein cross-linking.

ACKNOWLEDGMENTS

This study was financially supported by the National Natural Science Foundation of China (Grant No. 31371805) and the National Science & Technology Pillar Program during the 12th Five-year (2013BAD18B06-03).

CONFLICT OF INTEREST

The authors declare no conflict of interests.

ORCID

Ming Du http://orcid.org/0000-0001-5872-8529

REFERENCES

Anema, S., Pinder, D., Hunter, R., & Hemar, Y. (2006). Effects of storage temperature on the solubility of milk protein concentrate (MPC85). Food Hydrocolloids, 20, 386–393. https://doi.org/10.1016/j.foodhyd.2005.03.015
De Castro-Morel, M., & Harper, W. (2002). Basic functionality of commercial milk protein concentrates. Milchwissenschaft, 57, 367–370.
Dybowska, B. E. (2008). Properties of milk protein concentrate stabilized oil-in-water emulsions. Journal of Food Engineering, 88, 507–513. https://doi.org/10.1016/j.jfoodeng.2008.03.010
Fang, Y., Selomulya, C., Ainsworth, S., Palmer, M., & Chen, X. (2011). On quantifying the dissolution behaviour of milk protein concentrate. Food Hydrocolloids, 25, 503–510. https://doi.org/10.1016/j.foodhyd.2010.07.030

FIGURE 5 Changes in the color of MMPC-L samples during storage. L* represents lightness, and a* and b* represent redness and yellowness, respectively.
Farkye, N. Y., & Yim, B. (2003). Use of dry milk protein concentrate in pizza cheese manufactured by culture or direct acidification. *Journal of Dairy Science*, 86, 3841–3848.

Francolini, S., Locci, F., Ghiglletti, R., Iezzi, R., & Muccetti, G. (2010). Use of milk protein concentrate to standardize milk composition in Italian citric Mozzarella cheese making. *LWT-Food Science and Technology*, 43, 310–314. https://doi.org/10.1016/j.lwt.2009.08.007

Gaiani, C., Scher, J., Ehrhardt, J. J., Linder, M., Schuck, P., Desobry, S., & Banon, S. (2007). Relationships between dairy powder surface composition and wetting properties during storage: Importance of residual lipids. *Journal of Agricultural and Food Chemistry*, 55, 6561–6567. https://doi.org/10.1021/jf070364b

Haque, E., Bhandari, B. R., Gidley, M. J., Deeth, H. C., & Whittaker, A. R. (2009). Protein interactions in milk protein concentrate powder upon aging: Effect on solubility. *Journal of Agricultural and Food Chemistry*, 58, 7748–7755. https://doi.org/10.1021/jf1007055

Haque, E., Bhandari, B. R., Gidley, M. J., Deeth, H. C., & Whittaker, A. K. (2010). Protein conformational modifications and kinetics of water–protein interactions in milk protein concentrate powder during storage. *Journal of the Science of Food and Agriculture*, 91, 2576–2581. https://doi.org/10.1002/jsfa.4478

Havea, P. (2004). Protein interactions in milk protein concentrate powders. *International Dairy Journal*, 16, 415–422. https://doi.org/10.1016/j.idairyj.2005.06.005

Kelly, P. M. (2011). *Milk protein products | membrane-based fractionation*. San Diego, CA: Academic Press. https://doi.org/10.1016/B978-0-12-374407-4.00349-6

Kuo, C., & Harper, W. (2003). Effect of hydration time of milk protein concentrate on cast Feta cheese texture. *Milchwissenschaft*, 58, 283–286.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680–685. https://doi.org/10.1038/227680a0

Le, T. T., Bhandari, B., & Deeth, H. C. (2011). Chemical and physical changes in milk protein concentrate (MPC80) powder during storage. *Journal of Agricultural and Food Chemistry*, 59, 5465–5473. https://doi.org/10.1021/jf2003464

Le, T. T., Bhandari, B., Holland, J. W., & Deeth, H. C. (2011). Maillard reaction and protein cross-linking in relation to the solubility of milk powders. *Journal of Agricultural and Food Chemistry*, 59, 12473–12479. https://doi.org/10.1021/jf204360z

Le, T. T., Deeth, H. C., Bhandari, B., Alewood, P. F., & Holland, J. W. (2012). A proteomic approach to detect lactosylation and other chemical changes in stored milk protein concentrate. *Food Chemistry*, 132, 655–662. https://doi.org/10.1016/j.foodchem.2011.11.012

Le, T. T., Holland, J. W., Bhandari, B., Alewood, P. F., & Deeth, H. C. (2013). Direct evidence for the role of Maillard reaction products in protein cross-linking in milk powder during storage. *International Dairy Journal*, 31, 83–91. https://doi.org/10.1016/j.idairyj.2013.02.013

Mao, X. Y., Tong, P. S., Gualco, S., & Vink, S. (2012). Effect of NaCl addition during diafiltration on the solubility, hydrophobicity, and disulfide bonds of 80% milk protein concentrate powder. *Journal of Dairy Science*, 95, 3481–3488. https://doi.org/10.3168/jds.2011-4691

Mata, J. P., Udabage, P., & Gilbert, E. P. (2011). Structure of casein micelles in milk protein concentrate powders via small angle X-ray scattering. *Soft Matter*, 7, 3837–3843. https://doi.org/10.1039/c0sm01010c

McCarthy, N. A., Kelly, P. M., Maher, P. G., & Fenelon, M. A. (2014). Dissolution of milk protein concentrate (MPC) powders by ultrasonication. *Journal of Food Engineering*, 126, 142–148. https://doi.org/10.1016/j.jfoodeng.2013.11.002

McKenna, A. B. (2000). Effect of processing and storage on the reconstitution properties of whole milk and ultrafiltered skim milk powders: thesis presented in partial fulfilment of the requirement for the degree of Doctor of Philosophy in Food Technology (Doctoral dissertation, Massey University).

Minouni, A., Deeth, H. C., Whittaker, A. K., Gidley, M. J., & Bhandari, B. R. (2009). Rehydration process of milk protein concentrate powder monitored by static light scattering. *Food Hydrocolloids*, 23, 1958–1965. https://doi.org/10.1016/j.foodhyd.2009.01.010

Minouni, A., Deeth, H. C., Whittaker, A. K., Gidley, M. J., & Bhandari, B. R. (2010). Investigation of the microstructure of milk protein concentrate powders during rehydration: Alterations during storage. *Journal of Dairy Science*, 93, 463–472. https://doi.org/10.3168/jds.2009-2369

Morales, F. J., & Van Boekel, M. A. J. S. (1998). A study on advanced Maillard reaction in heated casein/sugar solutions: Colour formation. *International Dairy Journal*, 8, 907–915. https://doi.org/10.1016/S0958-6946(99)00014-X

Mottram, D. S., Wedzicha, B. L., & Dodson, A. T. (2002). Food chemistry: Acrylamide is formed in the Maillard reaction. *Nature*, 419, 448–449. https://doi.org/10.1038/419448a

Sandra, S., & Corredig, M. (2013). Rennet induced gelation of reconstituted milk protein concentrates: The role of calcium and soluble proteins during reconstitution. *International Dairy Journal*, 29, 68–74. https://doi.org/10.1016/j.idairyj.2012.10.011

Sikand, V., Tong, P. S., Roy, S., Rodriguez-Saona, L. E., & Murray, B. A. (2011). Solubility of commercial milk protein concentrates and milk protein isolates. *Journal of Dairy Science*, 94, 6194–6202. https://doi.org/10.3168/jds.2011-4477

Sikand, V., Tong, P. S., & Walker, J. (2013). Effect of adding salt during the diafiltration step of milk protein concentrate powder manufacture on mineral and soluble protein composition. *Dairy Science & Technology*, 93, 401–413. https://doi.org/10.1007/s13594-013-0110-0

Singh, H. (1991). Modification of food proteins by covalent cross-linking. *Trends in Food Science & Technology*, 2, 196–200. https://doi.org/10.1016/0926-6963(91)90683-A

Ye, A. (2011). Functional properties of milk protein concentrates: Emulsifying properties, adsorption and stability of emulsions. *International Dairy Journal*, 21, 14–20. https://doi.org/10.1016/j.idairyj.2010.07.005

How to cite this article: Fan F, Liu M, Shi P, Xu X, Lu W, Wang Z, Du M. Protein cross-linking and the Maillard reaction decrease the solubility of milk protein concentrates. *Food Sci Nutr*. 2018;6:1196–1203. https://doi.org/10.1002/fsn3.657