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Chemical and Physical Changes in Milk Protein Concentrate (MPC80) Powder during Storage

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ABSTRACT: The solubility and chemical changes due to the Maillard reaction were investigated in milk protein concentrate powder containing 80% protein (MPC80) during storage at temperatures and relative humidities in the ranges of 25−40 °C and 44−84%, respectively. The Maillard reaction was studied by measuring furosine (a product of lactosylated protein after digestion with acid) and free hydroxymethylfurfural (HMF) contents by HPLC and L^*, a^*, b^* values with a color-meter. Furosine, free HMF, and browning in MPC80 increased during storage, whereas the solubility decreased. The correlation between the Maillard reaction and solubility loss was explored in modified MPC80 to which glucose was added to enhance the rate of the Maillard reaction. More furosine and brown pigments were observed in the glucose-containing MPC80 than in MPC80 with added lactose. The opposite trend occurred for solubility, suggesting that the Maillard reaction may be a cause of solubility loss in MPC powder.

KEYWORDS: milk protein concentrate, MPC80, glucose-added MPC80, lactose-added MPC80, solubility, Maillard reaction, furosine, hydroxylmethylfurfural, HMF, browning

INTRODUCTION

Milk protein concentrate (MPC) powder is used in many manufactured foods such as beverages, yogurt, and cheese; however, it is still a relatively new dairy ingredient. As a dry product, there are concerns about its stability during storage. Anema et al. found that the solubility of MPC85 powder decreased with increasing storage temperatures.[1] They speculated that the reduction in solubility could be due to covalent cross-linking of the proteins but considered that the interactions could also be via hydrophobic or hydrogen bonds. Havea concluded that an increasing amount of insoluble material in MPC85 powder during storage was due to hydrophobic interaction of casein molecules.[2] In addition, Haque et al. showed by NMR and FTIR that there was protein unfolding, which could lead to protein interaction,[3] and Mimouni et al. used electron microscopy to show the presence of protein strands between casein micelles in stored MPC.[4]

Along with the loss of solubility, protein changes in milk powders during storage over a range of temperature−humidity combinations have been chemically investigated. However, these modifications have not been extensively studied in MPC powder. The Maillard reaction is one of the most deteriorative factors causing discoloration and decrease in nutritive value of milk powders.[5,6] For example, lysine, which is an abundant and valuable amino acid in milk, reacts with lactose to form lactulosyllysine; this product is then degraded via different pathways to produce a range of intermediate compounds followed by brown pigments when the reaction reaches its final stage.[7] The modified lysine, lactulosyllysine, is hardly recognized by gastrointestinal proteases, reducing the digestibility and nutritional value of the milk protein.[8−10] The rate of Maillard reaction in milk products greatly depends on the conditions of heat treatment or storage temperature and humidity and pH, as well as milk composition (e.g., types of sugar).[7,11−13] Although the Maillard reaction itself may not be the main cause of solubility decrease, its advanced products such as glyoxal or methylglyoxal can further react with lysine or arginine, causing cross-linking of proteins.[14,15] Also, conditions that favor the Maillard reaction may also favor cross-linking reactions involving the formation of lysinoalanine (LAL), histidinoalanine, and lanthioalanine linkages in the milk proteins.[16,17]

The aim of this study was to investigate the chemical and physical changes, particularly the Maillard reaction and solubility, of commercial MPC80 powder under selected storage conditions to ascertain whether the Maillard reaction is a cause of solubility loss in MPC. The correlation between the rate of Maillard reaction and solubility change was also investigated in MPC80 powder modified by the addition of glucose or lactose. As glucose reacts more quickly in the Maillard reaction than lactose, which is well-known in model systems,[18,19] glucose-added MPC80 was expected to show more significantly greater Maillard-related changes than lactose-added MPC80.

MATERIALS AND METHODS

Materials. MPC80 powder was obtained from Murray Goulburn Co-op Ltd. (Melbourne, VIC, Australia). Its composition, provided by the manufacturer, was 81.1% protein, 4.2% lactose, 1.4% fat, 6.2% mineral, and 7.1% moisture.

Other materials were obtained as follows: potassium carbonate (K_2CO_3), sodium nitrite ($NaNO_2$), potassium chloride (KCl), glucose, lactose, sodium 1-heptanesulfonate, 5-(hydroxymethyl)furfural, trichloroacetic acid (TCA), and formic acid from Sigma (Sigma-Aldrich, Australia); furosine dihydrochloride from NeoMPS (Strasbourg, France); acetonitrile (HPLC grade), methanol (HPLC grade), and hydrochloric acid (32%) from Labscan; and Sep Pak C18 cartridges from Grace Davison (Alltech Ltd., Australia). HPLC-grade ultrapure water generated by a Milli-R05 coupled to a Milli-Q Water Purification System was used for all aqueous solutions.

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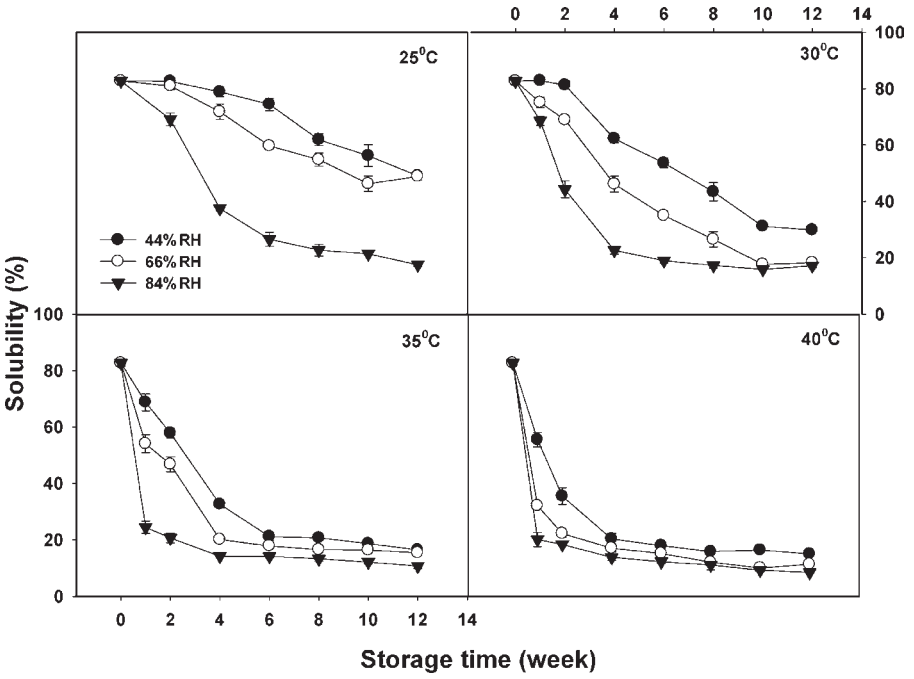

Figure 1. Solubility (%) of MPC80 during storage at different temperatures and humidities. Error bars indicate standard deviation.

Methods. *Milk Powder Storage. (a) Commercial MPC80.* Samples of commercial MPC80 powder were stored in desiccators containing saturated salt solutions (K_2CO_3, $NaNO_2$, and KCl) to achieve relative humidities (RH) of 44, 66, and 84%, respectively. These desiccators were placed in incubators operating at 25, 30, 35, and 40 °C for up to 12 weeks. MPC80 samples were analyzed for water activity, solubility, furosine, hydroxymethylfurfural (HMF), and brown color after 1, 2, 4, 6, 8, 10, and 12 weeks of storage. Control samples were fresh MPC80 stored at −20 °C. The whole trial was performed in duplicate using two different batches of MPC80.

(b) Glucose/Lactose-Added MPC80. Glucose or lactose was added to MPC80 at 2% (w/w). The powder was then mixed with Milli-Q water (to give 5% total solids) with an overhead stirrer at 25 °C for 2 h. The reconstituted MPC80 powders with added glucose or lactose were stored overnight at 4 °C and then stirred for a further 1 h before spray-drying. A sample of MPC80 without the addition of glucose or lactose was processed in the same manner as above and used as a control.

The reconstituted samples were dried in a Mini Spray Dryer B-290 (Buchi Labortechnik AG, Flawil, Switzerland). The inlet temperature was set at 150 °C, and the outlet temperature was between 65 and 76 °C. Pump rate was 20%. The trial was conducted twice using two different batches of MPC80. The modified powder samples were stored at 30 °C and 44% RH for up 12 weeks. Samples were periodically removed for analysis of water activity, solubility, furosine, and brown color; they were stored at −20 °C before analysis. All analyses were performed in duplicate.

Water Activity. All MPC80 samples were analyzed for water activity using an Aqualab water activity meter (Decagon Devices, Pullman, WA). About 0.5 g of the powder sample was put into the Aqua lab, and the a_w was read after a 2 min equilibration period.

Solubility. Solubility was measured by a modification of the methods of Havea and Anema et al.[1,2] MPC80 samples were reconstituted by stirring the powder in Milli-Q water at 30 °C for 30 min (5% MPC80 solution). Aliquots (5 mL) of the solution were centrifuged, and the supernatant was decanted. Samples of the 5% MPC80 solutions (before centrifugation) and the supernatant (after centrifugation) were taken for total solids determination by oven-drying (105 °C) for 24 h. The solubility of MPC80 was calculated as the percentage of total solids in the solution before centrifugation, which remained in the supernatant after centrifugation.

Furosine Analysis. MPC80 powder (50 mg) was hydrolyzed with 10 mL of 8 N hydrochloric acid (HCl) for 24 h at 110 °C. The hydrolysates were filtered through a 0.45 μm Whatman filter paper. A 0.5 mL aliquot of the filtered hydrolysate was purified by a C18 cartridge from which furosine was eluted by 3 mL of 3 N HCl. After evaporation of the HCl under a stream of nitrogen, the residue was redissolved in a mixture of water, acetonitrile, and formic acid (94.5:5:0.5), and 50 μL of the mixture was injected into the HPLC. Furosine was determined by ion-pair RP-HPLC according to the method of Delgado et al.[20] using a Spherisorb ODS2 5 μm column (250 × 4.6 mm i.d.) (Grace Davison, Australia).

Free HMF. Five percent reconstituted MPC80 powder (1 mL) was added to 0.5 mL of 40% trichloroacetic acid (TCA) and mixed with a Vortex mixer for 5 min. After centrifugation, the supernatant was collected and filtered through a 0.45 μm Millipore filter before injection into the HPLC. HMF was determined by RP-HPLC according to the method of Albala-Hurtado et al.[21] using a Spherisorb ODS2 5 μm column (250 × 4.6 mm i.d.) (Grace Davison, Australia).

Browning. The surface color of the milk powder samples was measured using a Hunter *L*, *a*, and *b* tristimulus analyser and the results are expressed as L^*, a^* and b^* values.[22] The b^* value is a good measure of browning in milk powders because it expresses the color change toward yellow and brown.[23] The color values were determined as the mean of three measurements.

RESULTS AND DISCUSSION

Solubility. The solubility of milk protein concentrate (MPC80) decreased during storage and was influenced by both storage temperature and humidity (Figure 1). The decrease in solubility of milk protein concentrate during storage has been observed in previous studies also.[1−3,24] In the first 2 weeks of storage at 44% RH, MPC80 samples stored at 25 and 30 °C showed little change in solubility, but the solubility decreased rapidly when the samples

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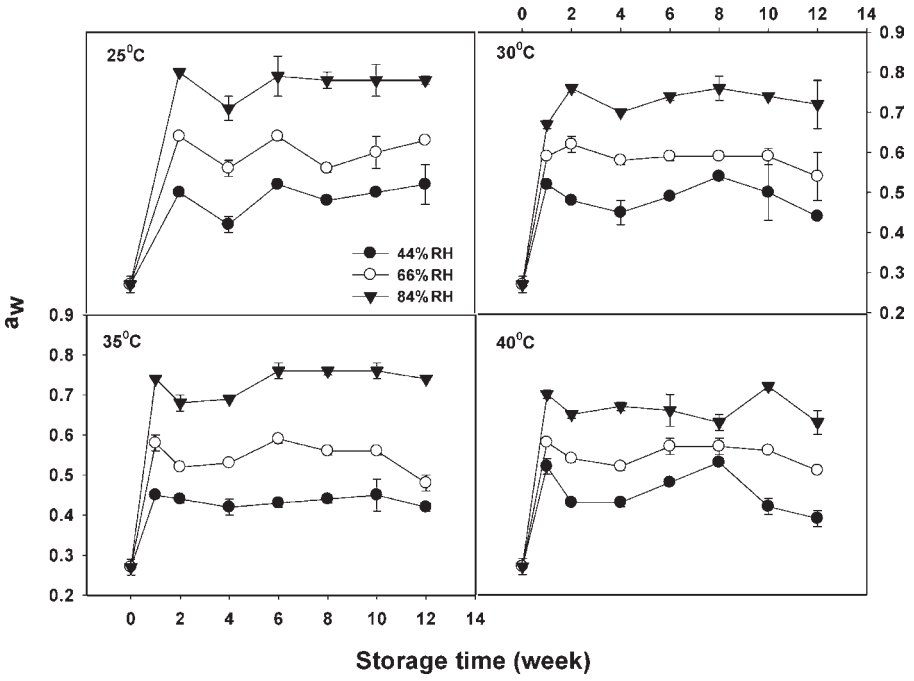

Figure 2. Water activity of MPC80 powder stored at different temperatures and humidities. Error bars indicate standard deviation.

were stored at 35 and 40 °C (Figure 1). These changes in solubility are consistent with those reported by Anema et al. However, the study of Anema et al. did not include the effect of relative humidity on the solubility of milk powder under ambient temperature storage.[1]

At all temperatures, the water activity of MPC80 powder reached its equilibrium within 2 weeks (Figure 2). Humidity has a significant impact on the solubility of MPC80 over time ($P \leq 0.05$). At the same storage temperature of 25 °C, the solubility of MPC80 decreased to 35% after 4 weeks when stored at 84% RH and to approximately 78 and 70% at 44 and 65% RH, respectively (Figure 1). The solubility of MPC80 powder reached a minimum of about 20% when it was stored at 25 °C and 84% RH. Haque et al. also reported the effect of humidity on the solubility of MPC85 powder; the solubility of MPC85 stored at 25 °C and 45 or 85% RH was decreased by 48 and 29% within 4 weeks, respectively.[3]

Overall, the decrease in solubility of MPC80 is dependent on storage time, temperature, and humidity. Anema et al. concluded that the decrease in solubility occurs by a similar mechanism at all temperatures and that the rate of solubility loss increases with increasing storage temperature.[1] From the current results, the solubility reduction of MPC80 during storage is accelerated by humidity as well as temperature. A possible mechanism for the loss of solubility in MPC powder involves protein−protein interaction on the surface of the powder particle, although the type and cause of the interaction are not known. The decrease in solubility of MPC could be related to hydrophobic association of casein micelles on the surface.[1,2] Although the Maillard reaction itself may not cause the decrease in solubility of milk powder after storage, its advanced products could contribute to the formation of a hydrophobic cross-linked protein network. These cross-linked proteins could form a barrier to the permeation of water through the surface of the milk powder particles.[1,2] In addition, the Maillard reaction is considered to be the main factor causing an increase in the surface hydrophobicity of milk protein powders mixed with glucose and lactose.[25]

Maillard Reaction. The Maillard reaction can be divided into three different stages: initial, mid, and late. To investigate the progress of the Maillard reaction in MPC80 during storage, furosine, an indicator of the initial stage, free HMF (mid-stage), and brown pigments (late stage) were measured.[26]

Furosine, an Initial-Stage Maillard Reaction Product. The change in furosine greatly depends on the processing and storage conditions of the milk powder.[27] In this study, the furosine contents of the milk protein concentrate ranged from about 160 to 1580 mg/100 g of protein for powder samples stored at different temperatures (20, 25, 30, 35, and 40 °C) and relative humidities (44, 66, and 84% RH) (Figure 3). The furosine level of fresh powder, which was collected directly from the manufacturer and kept at −20 °C (control samples), was 160 mg/100 g of protein. This level is slightly higher than the furosine content of freshly prepared skim milk powder (about 100−120 mg/100 g of protein) reported by Van Renterghem and De Block.[27] The difference in the furosine levels between MPC80 and those reported for skim milk powder can be explained by the different milk supply sources. A higher furosine content in a milk product indicates a poorer quality product.[28]

The effects of storage temperature on furosine contents were significant ($P \leq 0.05$). Furosine levels dramatically increased from 160 to 1140 mg/100 g of protein for MPC80 samples stored at 40 °C and 44% RH in 12 weeks. Meanwhile, at 25 °C and the same storage time and relative humidity, furosine contents reached only 470 mg/100 g of protein (Figure 3). These results are similar to those of Van Renterghem and De Block's study on skim milk powder, in which temperature had the most significant impact on furosine formation.[27] Likewise, some previous studies on enteral formulas gave similar trends of furosine changes during storage.[29,30]

Relative humidity also had an impact on furosine levels during storage of milk protein concentrate (Figure 3). Even though the humidity effects on furosine formation were not as dominant as those of temperature, humidity still contributed to significant

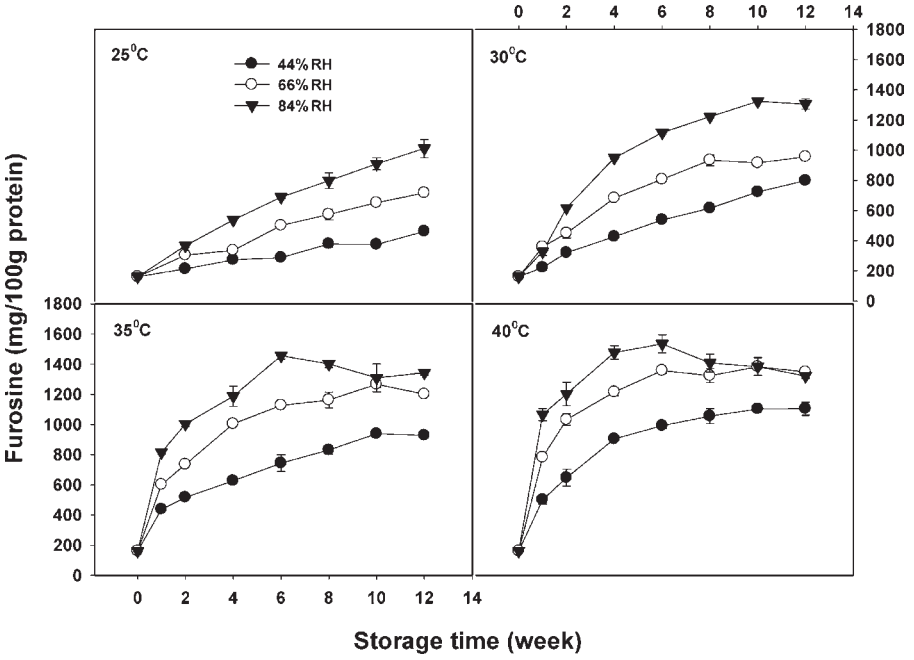

Figure 3. Furosine interactions: storage time−humidity (44, 66, and 84% RH) of MPC80 stored at 25, 30, 35, and 40 °C. Error bars indicate standard deviation.

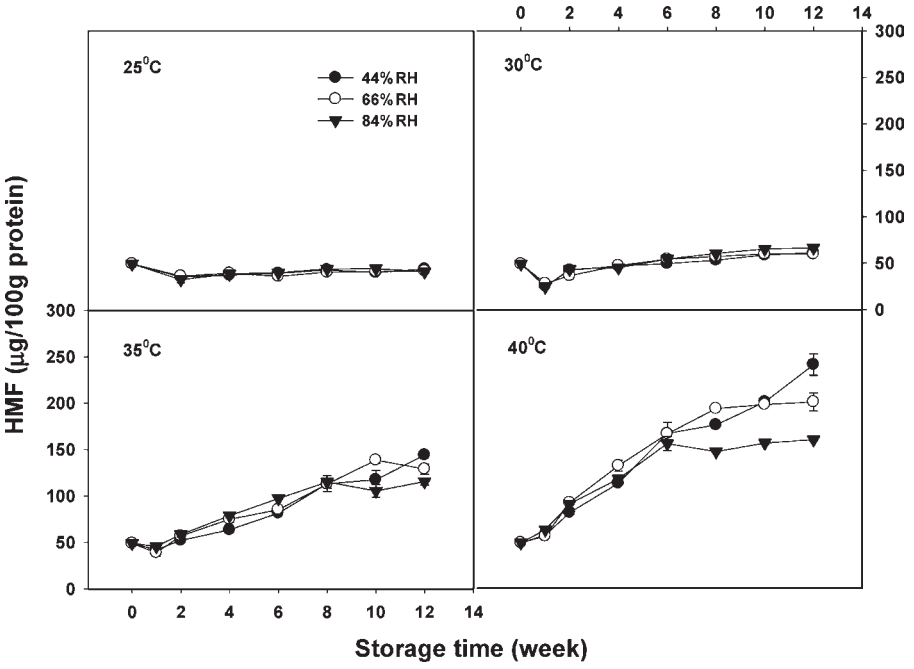

Figure 4. Free hydroxymethylfurfural (HMF) interactions: storage time−temperature (25, 35, and 40 °C) of MPC80 stored at 44% RH. Error bars indicate standard deviation.

changes in furosine contents of MPC80. Of the three selected humidities, 44, 66, and 84%, 84% caused the highest Maillard reaction rate. This result differs from those of the studies on the Maillard reaction in skim milk powder.[31,32] A relative humidity between 60 and 70% has been considered to be a maximal condition for the Maillard reaction.[11,33] When the environmental humidity is >70%, the Maillard reaction rate tends to remain steady because the reactants participating in the Maillard reaction, protein and lactose, become more diluted.

Free HMF, a Mid-Stage Maillard Reaction Product. Free HMF has been used to evaluate the extent of the Maillard reaction during processing and storage as it is produced from the mid-stage Maillard reaction.[34] Similar to the effect of temperature on furosine contents, Figure 4 shows the impact of different storage temperatures and humidities on HMF as a

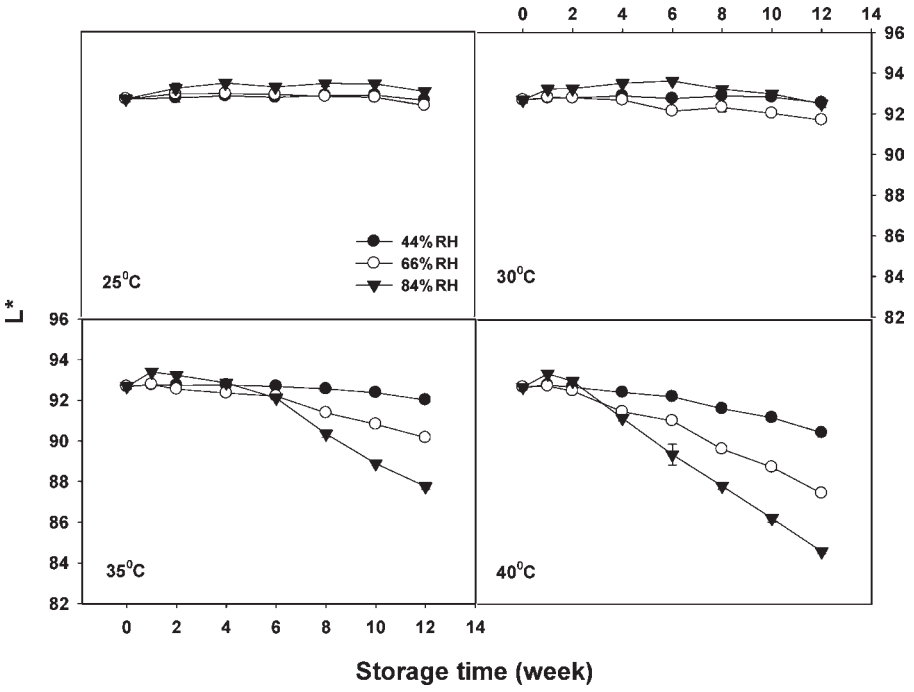

Figure 5. Changes in L^* value during storage of MPC80 at different conditions. Error bars indicate standard deviation.

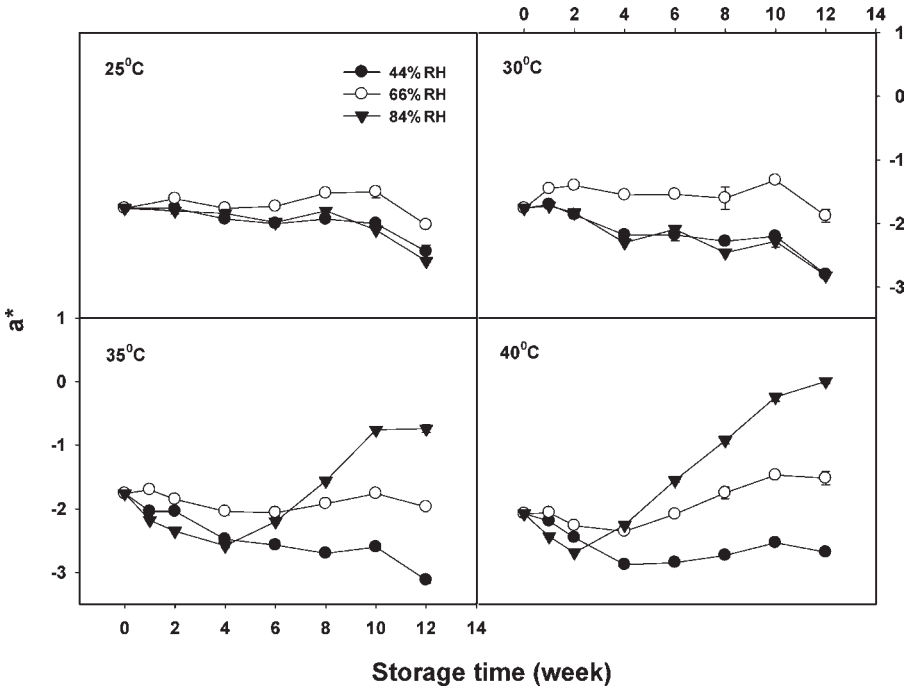

Figure 6. Changes in a^* value during storage of MPC80 at different conditions. Error bars indicate standard deviation.

function of time. The HMF content of MPC80 showed little change when stored at 25 °C and gradually increased with increasing storage temperature. For instance, HMF levels of MPC80 samples stored at 40 °C and 44% RH increased from 49 to 241 μg/100 g of protein over 12 weeks. The increasing trend of HMF followed the progress of the Maillard reaction in which HMF is formed from lactulosyllysine degradation. In Figures 3 and 4, it can be seen that when the furosine remained stable or decreased, there was a significant increase in free HMF level. It seems there is a balance of furosine and HMF contents. Lactulosyllysine, the source of furosine, continues to form during storage of the MPC but at the same time is degraded through the Maillard reaction to free HMF and other advanced Maillard products. However, the absence of a significant effect of humidity on Maillard reaction occurring in MPC80 stored at 44, 66, and 84% RH could not be explained. Progress of the Maillard reaction

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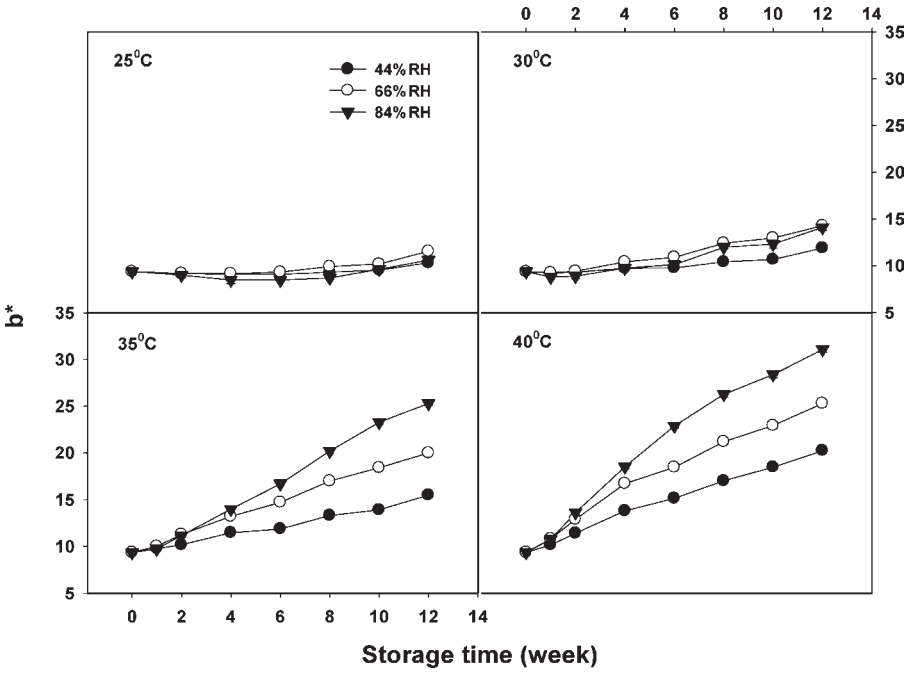

Figure 7. Changes in b^* value during storage of MPC80 at different conditions. Error bars indicate standard deviation.

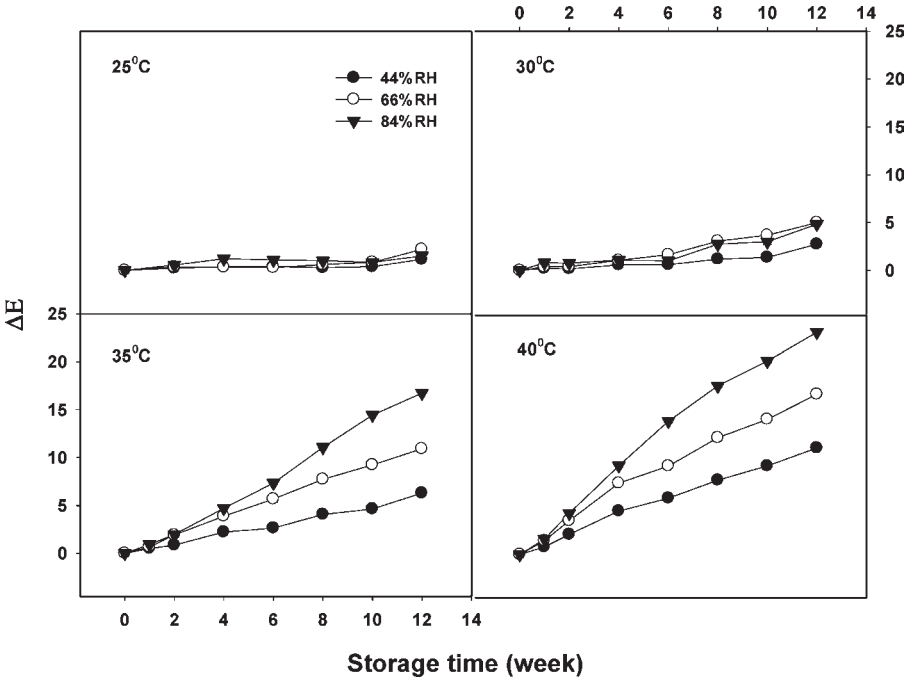

Figure 8. Changes in ΔE value during storage of MPC80 at different conditions. Error bars indicate standard deviation.

to this stage may follow a different mechanism compared to that of the early stage where lactulosyllysine (the furosine precursor) is formed.

Brown Pigments, Late-Stage Maillard Reaction Products. Because furosine and HMF are colorless and because brown pigments are difficult to analyze chemically, L^*, a^*, and b^* values are used to assess the changes in color caused by the Maillard reaction. Figures 5, 6, 7, and 8 show the changes in color of MPC80 during storage at different temperature and humidity conditions. L^* (lightness or whiteness) (Figure 5) and a^* (redness) values (Figure 6) did not show significant changes in powders stored in a low-temperature—low-humidity environment. This matches the HMF trend shown in Figure 4. The decrease in L^* and a^* values became significant with increasing temperature and humidity. The obvious effect of temperature on the whiteness and redness of MPC80 was

Table 1. Average Water Activity of MPC80 Samples: after Spray-Drying (M), 2% Glucose-Added (G), and 2% Lactose-Added (L)[a]

week	M	G	L
0	0.19 ± 0.00	0.27 ± 0.02	0.20 ± 0.01
1	0.48 ± 0.00	0.48 ± 0.00	0.48 ± 0.00
2	0.49 ± 0.00	0.52 ± 0.04	0.46 ± 0.00
4	0.43 ± 0.00	0.43 ± 0.00	0.44 ± 0.02
6	0.42 ± 0.00	0.43 ± 0.00	0.43 ± 0.00
8	0.37 ± 0.01	0.35 ± 0.04	0.38 ± 0.02
10	0.43 ± 0.00	0.43 ± 0.00	0.43 ± 0.00
12	0.41 ± 0.00	0.40 ± 0.00	0.41 ± 0.00

[a] Values are the mean ± SD.

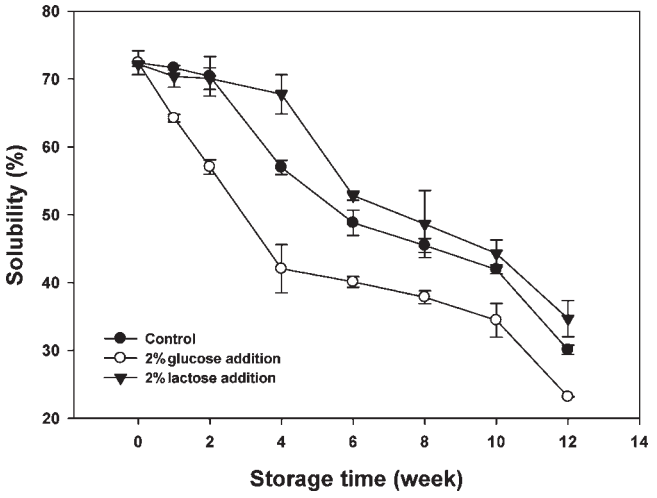

Figure 9. Solubility (%) of MPC80 after spray-drying (control) and 2% glucose- or lactose-added MPC80 during storage at 30 °C and 44% RH. Error bars indicate standard deviation.

seen in samples stored at 35 and 40 °C. Moreover, b^* values (yellowness) (Figure 7) and the color difference index, ΔE (color difference compared with control, $[\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}]^{1/2}$) (Figure 8), showed the same trend with changes of temperature and humidity. Of the color parameters measured, the b^* value and ΔE were the most sensitive indicators of the effects of both temperature and humidity on the progress of the late-stage Maillard reaction (Figures 7 and 8).

The effect of combinations of temperature and humidity on the Maillard reaction in MPC powder has not been previously studied. The browning levels increased with increasing humidity with the maximum occurring at 84% RH, the highest humidity used (Figure 7). The trends in the data for browning and furosine contents were similar in this respect. Although similar results were observed in model systems of sodium caseinate/fructose,[35,36] it has been postulated that at higher RH, the dilution effect ($a_w > 0.7$) reduces the rate of the Maillard reaction in skim milk powder.[31] Therefore, further experiments are required to establish the reason for the different trend observed in this work.

Correlation of Solubility Changes and Maillard Reaction Occurring in MPC80. A number of changes to the milk proteins occur at the same time as solubility loss, especially at high moisture content.[16] Browning caused by the Maillard reaction is one of the key changes. Our study shows a similar trend between

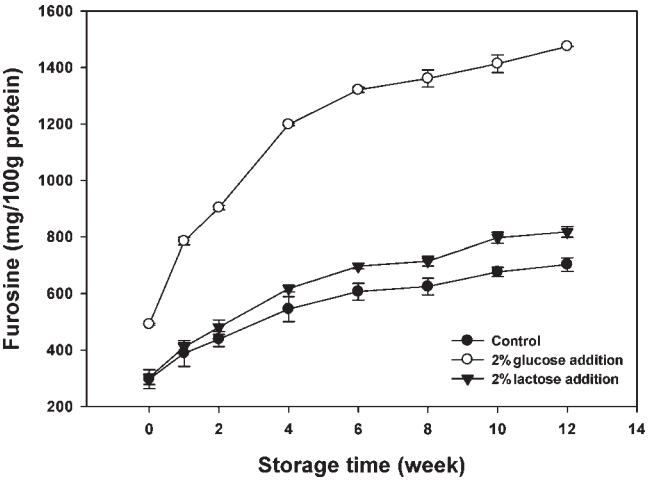

Figure 10. Furosine content of MPC80 (control samples) and MPC80 with added glucose or lactose stored at 30 °C and 44% RH. Error bars indicate standard deviation.

furosine formation and solubility loss of MPC80 under a range of temperature–humidity–time conditions (Figures 1 and 3). As this does not prove a cause and effect relationship, further experiments were carried out on MPC80 powders with added glucose/lactose to provide evidence of whether the Maillard reaction is a cause of solubility decease.

The water activity of all modified MPC80 samples reached equilibrium ($a_w = 0.48$) within 1 week and remained stable for up to 12 weeks at 30 °C (Table 1). These storage conditions were based on the results obtained on commercial MPC80 in which samples stored at 30 °C and 44% RH showed a gradual change of solubility over 12 weeks. Figure 9 shows the solubility changes of MPC80 control samples and samples with added glucose or lactose over the 12 week storage period. The solubility of the control samples and samples with added lactose was stable at about 72% in the first 2 weeks of storage at 30 °C and 44% RH and then decreased for the remainder of the storage time. In contrast, the solubility of the glucose-added MPC80 decreased substantially during the first 4 weeks and then more slowly for the remaining storage time. At all storage times after the first 2 weeks, the order of solubility of the three MPC80 samples was lactose-added ≈ control > glucose-added. From these data, adding glucose to MPC80 decreased the stability of the powder, whereas adding lactose slightly increased the stability.

Figure 10 indicates a significant difference in furosine content between MPC80 samples with added glucose or added lactose ($P \leq 0.05$). The amount of furosine in the control samples was lower at all times than in the samples with the added sugars. The furosine results are consistent with the theory that the Maillard reaction progresses more rapidly with higher concentration of sugars and more rapidly with glucose than with lactose. It has been reported that the rate of Maillard reaction depends on the type of sugar and is in the order pentoses > hexoses > disaccharides.[37] The furosine in the control samples, which were also reconstituted and spray-dried before being stored at 30 °C/44% RH, ranged from 297 to 702 mg/100 g of protein. This is higher than that in commercial MPC80 (160 mg/100 g protein) (Figure 3). This suggests that a second spray-drying accelerated the Maillard reaction in the powder. This was also apparent in the samples with glucose addition, in which the furosine content

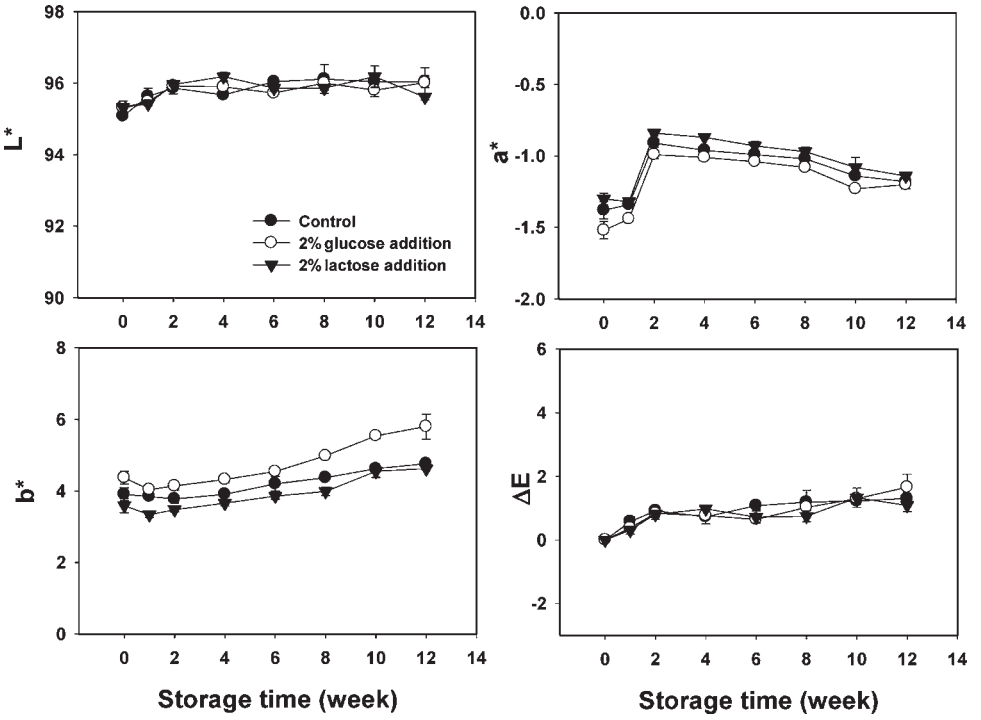

Figure 11. L^*, a^*, b^*, and ΔE of MPC80 stored at 30 °C and 44% RH with or without glucose or lactose addition. Error bars indicate standard deviation.

immediately after spray-drying was 491 mg/100 g protein compared with 297 mg/100 g protein in the control samples; the lactose-added sample was very similar to the control with 303 mg/100 g protein of furosine.

The difference in furosine in the samples with added glucose or lactose during storage is clearly seen in Figure 10. After only 1 week of storage, the increase in the amount of furosine in the glucose-added samples was almost double that in the lactose-added and control samples. The furosine in the lactose-added and control samples slightly increased to 817 or 702 mg/100 g protein, respectively, over 12 weeks, whereas in the glucose-added sample it increased to 1470 mg/100 g protein. Although the furosine in the lactose-added samples was higher than in the control samples, it was significantly lower than in those with added glucose.

Figure 11 indicates changes in color of MPC80 samples with or without added glucose or lactose during storage at 30 °C and 44% RH. L^*, a^*, b^*, and ΔE values were used to quantify differences in whiteness, redness, and yellowness of MPC80 samples. There were no significant changes in whiteness and color difference (ΔE) of the three different samples. However, the increase in a^* and b^* values followed the order lactose-added MPC80 < control MPC80 < glucose-added MPC80, in which the difference between MPC80 with glucose and lactose addition was the more obvious. The result is consistent with the results of the study of Kato et al., which indicated that a glucose−ovalbumin mixture formed a brown color more quickly and more remarkably than a lactose−ovalbumin mixture.[19] Almost no browning was detected in the lactose−ovalbumin mixture during 26 days of storage at 50 °C and 65% RH.[19] The increase in browning (b^* values) started in all samples from week 6 when the furosine formation rate leveled out (Figures 10 and 11). Samples stored for 10 and 12 weeks showed a clear trend of color changes in modified MPC80, whereby browning was accelerated in the glucose-added MPC80 and retarded in the lactose-added MPC80. This result indicates a good correlation with solubility decrease, as shown in Figure 9. However, it is not possible to conclude a cause-and-effect relationship.

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