

Effects of heat treatment on the antigenicity of four milk proteins in milk protein concentrates

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The effects of heat treatment on the antigenicity and potential allergenicity of α -Lactalbumin (α -LA), β -Lactoglobulin (β -LG), α -casein (α -CN), and β -casein (β -CN) in milk protein concentrates (MPCs) were investigated at temperatures in the range of 65–100°C for times from 10 to 30 min. The antigenicity and potential allergenicity of α -LA decreased significantly. The antigenicity of β -LG was sharply increased throughout the heat treatment, while the potential allergenicity decreased significantly only at 85°C and 100°C for 25 min. The antigenicity of α -CN and β -CN showed fluctuated changes, but were all lower than untreated sample. The α -CN potential allergenicity decreased when heated at 65°C and 70°C for 25 min. Compared with untreated sample, the potential allergenicity of β -CN increased sharply after heat treatment. The results showed that different proteins in the MPCs showed different sensitivities under heat treatment.

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Highlights

- (1) Investigated the antigenicity of α -LA, β -LG, α -CN and β -CN in MPCs under different heat treatments.
- (2) Investigated the potential allergenicity of α -LA, β -LG, α -CN, and β -CN in MPCs under different temperatures heating for 25 min.
- (3) Heat treatment decreased the antigenicity and potential allergenicity of α -LA.
- (4) Heat treatment had little effect on β -LG, α -CN, and β -CN.

1. Introduction

Cow milk and milk products are rich in nutrient substance but contain approximately 30 potentially allergenic proteins (Miciński et al., 2013) even at low concentrations (Wal, 2004). It is reported that 66% of milk protein allergies were caused by β -LG, 57% by casein, and significantly less by α -LA and bovine serum albumin (BSA) (18%) (Peñas, Snel, Floris, Préstamo, & Gomez, 2006).

Cow's milk protein allergy (CMPA) is one of the most common food allergies in children under two years of age and can cause serious health problems (Kleber, Weyrich, & Hinrichs, 2006). The incidence of CMPA ranges from 0.3% to 7.5% according to population-based studies in different countries (El-Agamy, 2007). In recent years, some research has shown that the frequencies of sensitization to β -LG, α -LA being decreased at 51%, 19%, and to caseins being increased at 72%. In parallel with the changes of casein and whey protein, sensitization to BSA was increased from 43% to 54% (Tsabouri, Douros, & Priftis, 2014).

Researchers have developed a variety of methods to reduce the antigenicity and allergenicity of milk proteins including heat treatment, glycation reaction, irradiation, high pressure, enzymatic hydrolysis, lactic acid fermentation, microwave irradiation, etc. (Bu, Lu, Zheng, & Luo, 2009; Bu, Luo, Zhang, & Chen, 2010; Bu, Luo, Zheng, & Zheng, 2009; Grar et al., 2009; Hu et al., 2011; Kasera et al., 2012; Peñas et al., 2006; Shi et al., 2014; Yao, Xu, Luo, Shi, & Li, 2015).

Heat treatment is the most common technique used for milk transformation. It may cause immunodominant epitopes to change which, as a result, may influence the allergenic properties of proteins (Besler, Steinhart, & Paschke, 2001). Food processing may lead to the destruction of the epitope structure or the formation of new epitopes (neoallergens), resulting in changes to protein conformation (Miciński et al., 2013). During heating, proteins may germinate physical and chemical reactions, such as denaturation, aggregation, and Maillard reaction and so on. These chemical reactions may modify the structure of proteins, such as the unfolding of secondary structure, cleavage of disulfide bonds, formation of new intra/intermolecular, rearrangements of disulfide bonds and the aggregation of proteins (Davis & Williams, 1998). The molecular basis of alterations in allergenicity is the inactivation or destruction of epitopes, the formation of new epitopes or greater access of hidden epitopes by denaturation of the native allergen (Tsabouri et al., 2014). Thus, thermal processing may alter the antigenicity of food by masking or unmasking antigenic epitope and thereby reducing or enhancing their IgE recognition (Shandilya, Kapila, Haq, Kapila, & Kansal, 2013). Heat treatment can alter protein allergenicity, but it remains controversial in regard to reducing the incidence of immunogenic reactions. Ehn, Ekstrand, Bengtsson, and Ahlstedt (2004) detected only a slight decrease in IgE binding ability of β -LG after heating β -LG solution or milk to 74°C, whereas a more significant decrease was found at 90°C by enzyme linked immunosorbent assay (ELISA) inhibition studies. Some researchers reported that bovine milk retained its allergenicity even when subjected to severe heat treatments (Bu, Lu, et al., 2009).

Milk protein concentrates (MPCs) are spray dried protein powders that are produced by ultrafiltration of skim milk with or without the use of diafiltration. Protein concentrations of MPCs range from 35% (w/w; MPC35) to 90% (w/w; MPC90) in dry matter (Mulvihill & Ennis, 2003). MPCs are commonly used as ingredients in the food and dairy industries, including ice cream, confectionary, high protein bars, yoghurt, cheese, dairy beverages, and other dairy products. In various applications, MPCs are likely to be reconstituted and subjected to heat treatment such as ultra-high temperature processing or retort sterilization. Thus, it is very important to find how heat treatment affects antigenicity and allergenicity of casein and whey protein in MPCs.

However, the effect of heat treatment on the antigenicity and allergenicity of MPCs has not been reported. Therefore, the objectives of our work were to extend the understanding

of the antigenicity and potential allergenicity changes of α -LA, β -LG, α -CN, and β -CN in MPCs by using different time-temperature combinations. The heated MPCs were analyzed using the indirect competitive ELISA, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

2. Materials and methods

2.1. Materials

MPCs used in this work were produced by New Zealand's Dairy Industry Co., Ltd. The powder contained total proteins $\geq 68.5\%$, fat $\leq 2.0\%$, and lactose $\leq 18.2\%$.

2.2. Sera

Thirty-eight sera provided by the Children's Hospital affiliated with Zhejiang University (Zhejiang, China) were selected from CMPA patients displaying various symptoms. All patients had specific IgE antibodies toward cow's milk protein from 0.36 to 2.2 IU mL⁻¹. Six non-allergic sera provided by people who individuals and family members had no allergic history. These six sera were mixed as non-allergic serum pool which was used to examine the IgE binding of allergic serum. The IgE binding (showed by absorbance) of allergic serum and non-allergic serum were compared by indirect ELISA at dual wavelengths of 450 and 630 nm. The IgE specificity of serum for milk proteins was represented by the percentage (%) which were calculated by the following equation:

$$\text{IgE binding} = [(A_a - A_n)/A_n] \times 100\% \quad (1)$$

where A_a was the absorbance obtained with the allergic serum sample, and A_n was the absorbance of non-allergic serum pool. According to the results, the allergenic serum was chosen which specific IgE binding to at least one milk protein allergen was higher than the non-allergic serum for 100% or more. Thirty-one allergic sera were chosen and mixed as allergic serum pool. The allergic serum pool displayed strong IgE binding to all the four milk proteins compared with the non-allergic serum pool. The allergic serum pool and non-allergic serum pool were, respectively, subpackaged in small aliquots and stored at -80°C until used.

2.3. Antigens and antibodies

The first antibody from rabbits which contained polyclonal antibodies corresponding to α -LA, β -LG, α -CN, and β -CN, respectively, was made by our laboratory (Liu, Luo, & Li, 2012). The second antibody (horseradish peroxidase conjugated goat anti-rabbit IgG and goat anti-human IgE) and the standard antigen proteins α -LA, β -LG, α -CN, and β -CN with codes L5385, purity $\geq 85\%$; L3908, approximately 90%; C6780, purity $\geq 70\%$ and C6905, purity $\geq 98\%$, were all purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were analytical grade.

2.4. Heat treatment

A MPCs solution (5.1%, w/v) was prepared with distilled water and stirred for 30 min at 45°C . Five milliliter samples were filled into glass test tubes sealed with rubber stoppers,

and were then heated between 65°C and 100°C for different times (10–30 min). At temperatures below 85°C, the samples were put in a thermostated water bath, and above 85°C, samples were put in a thermostated oil bath. After treated for a certain time, the samples were cooled immediately in an ice-water bath for 15 min to prevent further heating reaction. The unheated sample was also maintained in the ice-water bath for 15 min, serving as the control. The heated and untreated samples were stored at –20°C for further analysis.

2.5. IgE binding assay (potential allergenicity)

The IgE binding ability of the four proteins (α -LA, β -LG, α -CN, and β -CN) in the MPCs was analyzed by the indirect competitive ELISA according to Shi et al. (2014) with a slight modification. Microtiter plates with 96 wells (flat-bottomed; Costar; Corning, New York, USA) were coated with 100 μ L/well of antigen diluted in 50 mmol L^{–1} carbonate buffer (pH 9.8) and incubated overnight at 4°C. The coating concentration of antigens were determined earlier (500, 10, 10, and 50 μ g mL^{–1} for α -LA, β -LG, α -CN, and β -CN, respectively). Whilst the coating was taking place, all MPCs samples from the experiments were diluted in 10 mmol L^{–1} phosphate buffered saline solution (PBS, pH 7.4) (1/3000 for α -LA, 1/800 for β -LG, 1/2000 for α -CN, and 1/1000 for β -CN, v/v) and mixed separately with the selected pool sera (diluted 1/5 for all four main antigens) at a ratio of 1:1 at 4°C overnight. The plates were washed four times the next day with 10 mmol L^{–1} PBS (pH 7.4) containing 0.5 g L^{–1} Tween-20 (PBS-T)). After washing the plates, the wells were blocked with 100 μ L per well of PBS (pH 7.4) containing 10 g L^{–1} BSA and 1 g L^{–1} Tween 20 (PBS-BSA-T) for the purpose of blocking residual free binding sites in wells and incubated for 1 h at 37°C. After each analytical step, the plates were washed four times. One hundred microliters of these mixtures (sample and pool of sera) were added to the wells previously coated with four kinds of antigens and blocked. Plates were incubated at 37°C for 1 h and washed again. 100 μ L second antibody (Anti-human IgE, Epsilon chain specific, peroxidase conjugate developed in goat, Sigma A 9667, 1:500) in PBS-BSA-T were added into each well. After incubation at 37°C for 1 h and washing, micro plates were added 3,3',5,5,3'-tetramethylenbenzidine (Amresco, OH, USA) substrate solutions (100 μ L/well) and then incubated at 37°C for 10 min. Finally, 50 μ L/well 2 mol L^{–1} H₂SO₄ were used to stop the reaction. Uninhibited sera sample (no milk) was used as a control for the maximal absorbance of each antigen.

Absorptions were determined at dual wavelengths of 450 and 630 nm on a Multiskan MK3 ELISA plate reader (Thermo Lab systems, Franklin, MA, USA). The IgE binding was represented by the inhibition percentages (%) which were calculated by the following equation:

$$\text{Inhibition rate (potential allergenicity) (\%)} = [(Abs_a - Abs)/Abs_a] \times 100 \quad (2)$$

where Abs_a was the absorbance obtained with the uninhibited sera sample control, and Abs was the absorbance measured in the presence of different heat treatment samples from the experiments. A low inhibition rate reflects low residual allergenicity of heat treatment MPCs.

2.6. Antigenicity analysis by the indirect competitive ELISA

The indirect competitive ELISA method was used as previously described by Bu, Luo, et al. (2009) to determine the residual antigenicity of heat treatment MPCs. The coating concentration of antigens ($4 \mu\text{g mL}^{-1}$ for α -LA, β -LG, and $2 \mu\text{g mL}^{-1}$ for α -CN, β -CN, respectively) and the dilution of corresponding serum (1: 30,000 for anti- α -LA serum, 1:60,000 for anti- β -LG serum, 1:40,000 for anti- α -CN serum, and 1: 20,000 for anti- β -CN serum) were determined earlier according to the method.

The antigenicity was calculated from standard curves of α -LA, β -LG, α -CN, β -CN, and a linear logarithmic correlation was observed in the range of $0.5\text{--}64 \mu\text{g mL}^{-1}$ for α -LA, $4\text{--}512 \mu\text{g mL}^{-1}$ for β -LG, $1\text{--}128 \mu\text{g mL}^{-1}$ for α -CN and β -CN. The $\text{Ln}[B/(B_0 - B)]$ value was selected as vertical, and the $\text{lg}[\alpha\text{-LA}]$ (or $\text{lg}[\beta\text{-LG}]$, $\text{lg}[\alpha\text{-CN}]$, $\text{lg}[\beta\text{-CN}]$) value was abscissa to draw the standard curve. According to the linear regression equation, and corresponding dilution times, the antigenicity (mg mL^{-1}) of milk proteins were calculated by the following equation (taking α -LA for example):

$$\text{lg}[\text{antigenicity of } (\alpha - \text{LA})] = \frac{\pm b - \ln[B/(B_0 - B)]}{\pm a} \quad (3)$$

where B_0 was the absorbance obtained with uninhibited system (only antigen and anti-serum), and B was the absorbance measured in the presence of different heat treatment samples from the experiments (contained antigen, anti-serum and samples).

2.7. SDS-PAGE analysis

SDS-PAGE analysis was performed according to Li, Luo, and Feng (2011) using electrophoresis apparatus (Bio-Rad Laboratories, Inc, USA) with 15% acrylamide resolving gel and 4% stacking gel. 10 μL of sample dilution was loaded onto gel.

2.8. Statistical analysis

All data were analyzed with SPSS 17.0 (SPSS Inc., Chicago, America) and Microsoft Excel 2010 (Microsoft Corp., Redmond, WA, USA).

3. Results and discussion

3.1. Changes in the antigenicity of four milk proteins in MPCs during heat treatment

The change of α -LA antigenicity is shown in Figure 1. When heated at 65°C , the antigenicity of α -LA had no significant changes compared to control during the first 20 min, but decreased remarkably ($P < .05$) after 20 min. For other heat treatment conditions, the antigenicity of α -LA decreased significantly ($P < .05$) with the temperature and time of heat treatment increasing. The α -LA antigenicity of unheated MPCs was 10.85 mg mL^{-1} and the lowest antigenicity was 1.17 mg mL^{-1} when heated at 100°C for 30 min, which was decreased by 89.22%.

Figure 1(b) shows the effects of heating temperature and time on the antigenicity of β -LG. The antigenicity of β -LG was sharply increased ($P < .05$) by heat treatment. At

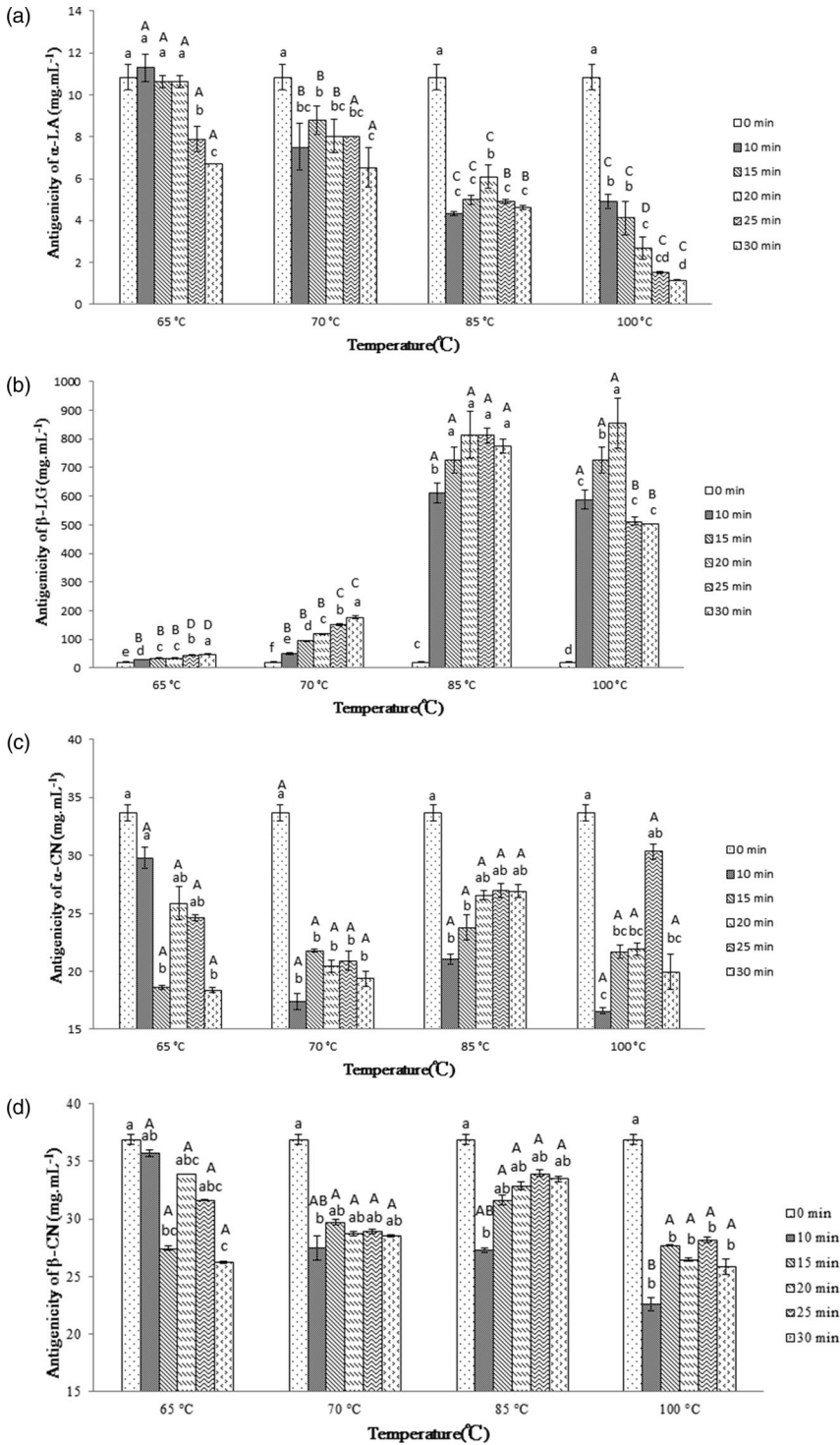


Figure 1. Changes of the antigenicity of α-LA(a), β-LG(b), α-CN(c), and β-CN(d) in MPCs with different heat treatments. Same superscript lowercase letter (a, b, c, and d) in the same heated temperature indicate no significant differences (*P* > .05) among heating times. Same superscript capital letter (A, B, C and D) in the same heating time indicate no significant differences (*P* > .05) among heated temperatures.

100°C, the antigenicity of β -LG increased gradually from a value of 587.16 mg mL⁻¹ (for 10 min) to 854.60 mg mL⁻¹ (for 20 min), and then decreased gradually to 502.64 mg mL⁻¹ (for 30 min) which was nearly 24 times higher than the initial value of the unheated sample. Heating for 25 and 30 min, the antigenicity of β -LG increased with temperature increasing from 65°C to 85°C and decreased at 100°C ($P < .05$). Similar observations were made by Bu, Luo, et al. (2009) who reported that the antigenicity increased initially from 50°C to 90°C and then decreased after 90°C. The fluctuated phenomena of β -LG thermal denaturation are characterized by well-defined temperature thresholds and lead to reversible and irreversible modifications (Dumitraşcu et al., 2013; Kleber, Krause, Illgner, & Hinrichs, 2004). Taheri-Kafrani et al. (2009) suggested that when heating temperatures >60°C it will cause the destabilization of the β -sheets, the unfolding of the β -barrel, the exposition of disulfide bonds and the free cysteine to the solvent. Kleber, Maier, and Hinrichs (2007) also claimed during the heat denaturation of β -LG, firstly the unfolding of conformational structure, the allergenic epitopes which buried inside of the native β -LG molecule may be exposed and in turn increased antigenicity. In addition, the sulfhydryl/disulfide exchange lead to conformational changes and consequently results in more antigenic epitopes being exposed which also enhances the antigenicity (Bu, Lu, et al., 2009; Davis & Williams, 1998). According Sharma, Kumar, Betzel, and Singh (2001) heat-denatured β -LG has been found to have at least one new epitope not found in the native state. All those structure changes may lead to increased antigenicity. The antigenicity of β -LG decreased at 100°C can be attributed to the damage of conformational epitopes or the mask of sequential epitopes (Kleber et al., 2004) by sulfhydryl/disulfide (SH/S-S) exchange and its subsequent aggregation (Bu, Luo, et al., 2009).

MPCs contained total proteins, fat and lactose, and all those components may react with each other by heat treatment. As a result, it may affect the antigenicity of proteins. Claeys, Van Loey, and Hendrickx (2002) claimed that the antigenicity of both β -LG and α -LA not only depends on the structural and conformational particularities of the molecules, but also on the environmental factors. It has been reported that fat and lactose have a positive effect on β -LG denaturation in cow's milk. Due to the protein and lactose germinate Maillard reaction during heating, a stabilization effect of lactose due to an increase of the hydration of protein molecules or/and irreversible aggregation of the whey protein with casein which is sterically hindered by means of protein-lactose complexes formed during heating (Plock, Spiegel, & Kessler, 1998). Because MPCs contains a certain amount of lactose (lactose $\leq 18.2\%$) under the heating condition, the Maillard reaction may lead to the loss of linear epitopes, as a result, reduce the antigenicity (Davis & Williams, 1998).

MPCs also contain a certain amount of fat (fat $\leq 2.0\%$). When heated at sufficiently high temperatures, both β -LG and α -LA interact with the milk fat globule membrane, which would have effects on the antigenicity of both proteins (Ye, Singh, James Oldfield, & Anema, 2004).

Oldfield, Singh, Taylor, and Pearce (2000) declared that α -LA and β -LG form a heat-induced β -LG/ α -LA complex, which then interacts with κ -casein, and the associated extent to the micelle increased with heating time and temperature. The presence of α -LA diminished the proportion of smaller aggregates and increased the number of very large aggregates (Considine, Patel, Anema, Singh, & Creamer, 2007). Heat treatment may cause α -LA and β -LG to aggregate and decrease in solubility. As the treatment

temperature increased, the quantity of native protein steadily decreased and the quantity of higher molecular weight aggregates increased. The aggregates and intermediates are held together via disulfide bridges and non-covalent interactions, mainly hydrophobically driven associations (de la Fuente, Singh, & Hemar, 2002). So, it can be assumed that the decrease of β -LG antigenicity can also be contributed to the decrease in the content of some immunoreactive proteins due to the lower solubility (Bu, Luo, et al., 2009). All of these structural modifications are likely to induce changes in the antigenic character or immunoreactivity of β -LG (Chen et al., 2006).

The α -CN antigenicity by heat treatment is shown in Figure 1(c). The α -CN antigenicity of 33.68 mg mL^{-1} was detected for the unheated samples. No matter how much the heating temperature and time changed, the antigenicities of α -CN were always lower than the control. Compared with the unheated MPCs, the minimum antigenicity of α -CN was 16.57 mg mL^{-1} when heated at 100°C for 10 min which declined by 50.80% ($P < .05$). Heat treatment in proteins may create new allergenic epitopes as well as destroying existing epitopes (Davis & Williams, 1998). These reports suggested that, when heated, whey proteins alone or caseins and whey proteins can form aggregates (Oldfield et al., 2000). As the intensity of the heat treatment increased, the mean diameter of the particles increased due to the aggregation of the heat-denatured whey proteins onto the casein micelle (Dupont et al., 2010).

The antigenicity of β -CN is shown in Figure 1(d). The antigenicity of β -CN fluctuated during the heat treatment, but all below the initial antigenicity. The antigenicity of control was 36.87 mg mL^{-1} and the minimum value was 22.63 mg mL^{-1} when heated at 100°C for 10 min (decreased by 38.62%) ($P < .05$). The antigenicity of β -CN decreased significantly when heated at 100°C , but do not change obviously during the heat treatment from 10 to 30 min at 100°C .

The changes of antigenicity of α -CN and β -CN when heated at 65°C for 10 min were not significant compared with the control. Some research has shown that the cooperative intramolecular forces in proteins could maintain their native state if a protein is heated at a low temperature, so the natural conformational fluctuations in proteins are not enough to overcome free energy barriers which prevent transitions from native to non-native states. As the intensity of the heat treatment increases, these fluctuations in structure become excited and increased in magnitude (Euston, 2013). If the heat temperature is high enough and the free energy barriers are overcome, then the conformations transitions from native to non-native occur. At this point the protein denature and structure slowly starts to unfold, which may cause the antigenicity of α -CN and β -CN to increase with the exposure of hidden epitopes. Finally, as the heat treatment becomes more severe, the antigenicity may decrease the mask of sequential epitopes in the course of aggregation to other protein particles or may damage the conformational epitopes. This explains why the antigenicity of α -CN and β -CN fluctuated with heating temperature and time.

Therefore, in the MPCs samples, the protein heated denaturation could be considered as a multiple-type (including Maillard reaction, proteins aggregation, protein binding to fat surface) and the protein denaturation also a multiple-step process, involving a reversible unfolding of the native structure, followed by the irreversible aggregation of both unfolded monomeric and aggregated (Dumitraşcu et al., 2013; Oldfield et al., 2000).

3.2. Changes in the potential allergenicity (IgE binding inhibition) of four milk proteins in MPCs by heat treatment

Figure 2 shows the variations in the IgE binding inhibition of four milk proteins during heat treatment from 65°C to 100°C for 25 min. The IgE binding inhibitions of the control were 67.99% for α -LA, 52.50% for β -LG, 63.83% for α -CN and 40.99% for β -CN. Compared with unheated samples, the IgE binding inhibition of α -LA in MPCs showed a significant decrease ($P < .05$) and reached the lowest value (25.44%), which reduced by 42.55%. The variation of α -LA in potential allergenicity was in accordance with the antigenicity (Figure 1(a) and Figure 2). Although the IgE binding inhibition of β -LG fluctuated, it declined significantly ($P < .05$) when heated at 85°C and 100°C, and reached minimum value (47.84%) when heated at 85°C. The β -LG IgE binding inhibition was different from the change of antigenicity (Figure 1(b)). As previous studies have shown, heated β -LG is less recognized than native β -LG by IgE from cow milk allergenic patients and the effect become more apparent as heating temperatures increase (Sharma et al., 2001; Taheri-Kafrani et al., 2009). The IgE binding inhibition of α -CN was reduced by 3.34% when heated at 65°C and 70°C for 25 min. Among the tested temperatures, the IgE binding inhibition of β -CN increased sharply ($P < .05$). The highest IgE binding inhibition of β -CN was 66.80% when heated at 100°C, and it was 25.81% higher than the unheated MPCs. The differences in the trend of antigenicity and potential allergenicity (β -LG, α -CN and β -CN) may be due to the different binding sites of proteins in rabbit serum (IgG) and human serum (IgE). Some binding sites of IgE may be destroyed or hidden because of the denaturation and aggregation of proteins during heat treatment. Heating MPCs does not necessarily reduce potential allergenicity to its component proteins.

3.3. SDS-PAGE analysis of MPCs during heat treatment

Throughout the heat treatment, the concentration of four milk proteins showed a clear reduction while the aggregates showed a slight increase (Figure 3). When heated at

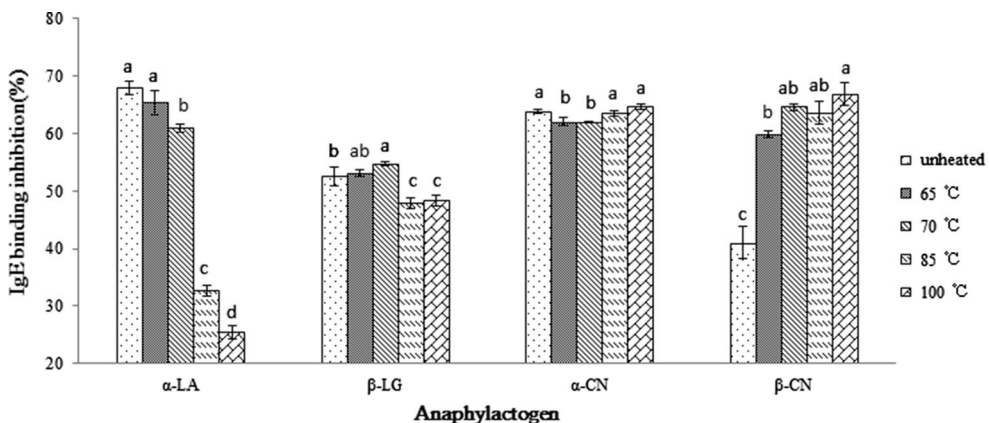


Figure 2. The changes of anaphylactogen IgE binding inhibition in MPCs after heating for 25 min at different temperatures. Same superscript lowercase letter (a, b, c, and d) indicate no significant differences ($P > .05$) among heated temperatures.

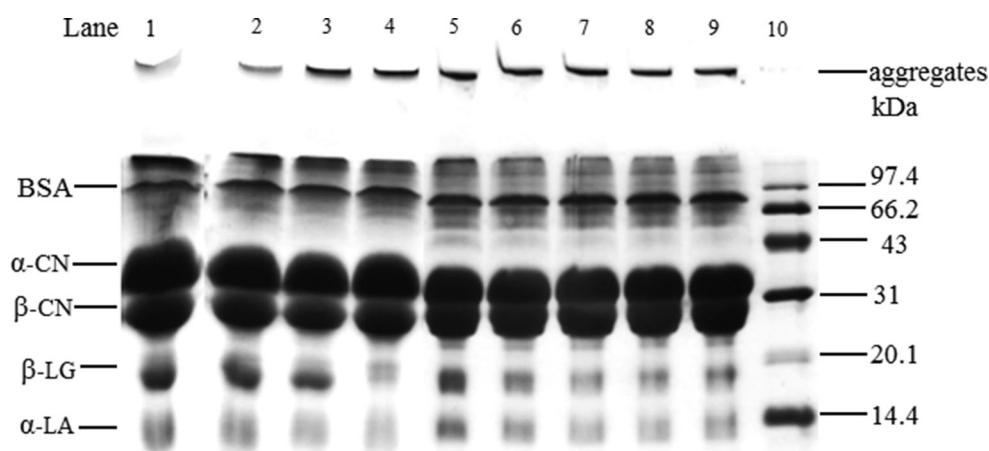


Figure 3. SDS-PAGE analysis of MPCs. Lane 1: unheated MPCs; Lanes 2–4: 65°C, 70°C and 85°C heated for 25 min; Lanes 5–9: heated at 100°C for 10; 15; 20; 25; 30 min; Lane 10: MW, molecular weight marker.

different temperatures for 25 min, there were obvious differences in the intensity of protein bands (Figure 3, Lane 1–4 and Lane 8). The bands of four proteins became shoaled and attenuated clearly from 65°C to 100°C for 25 min. From 65°C to 85°C, the bands of α-LA and β-LG thinned, but the antigenicity of α-LA decreased and β-LG increased. The different changes in the antigenicity of β-LG and α-LA in MPCs may be connected with the different epitopes. The linear epitopes and conformational epitopes are significant for β-LG, while the conformational epitopes play an important role in the antigenicity of α-LA (Ehn et al., 2004).

Figure 3, Lanes 5–9 showed the changes in milk proteins of MPCs when heated at 100°C from 10 to 30 min. The bands of β-LG, α-LA became weaker from 10 to 20 min, and α-CN, β-CN showed no obvious change. It is already well-known that α-LA and β-LG are thermolabile with heat-induced denaturation and aggregation, while α-CN and β-CN were more resistant to heating (Bu, Luo, et al., 2009; Taheri-Kafrani et al., 2009). In addition, by extending the heating time and increasing the temperature, some proteins or aggregates with higher molecule masses did not enter into the gel. It can be assumed that the decrease of antigenicity and potential allergenicity of proteins may be due to the large aggregates of proteins.

4. Conclusion

The antigenicity and potential allergenicity (IgE binding inhibition) of proteins in MPCs were clearly modified by heat treatment. The α-LA antigenicity and potential allergenicity decreased significantly throughout the heat treatment. The antigenicity of β-LG was sharply increased, while the potential allergenicity fluctuated by heat treatment. The potential allergenicity of β-LG decreased significantly when heated at 85°C and 100°C for 25 min. The antigenicity of α-CN and β-CN fluctuated throughout the whole heat treatment and were always lower than the controls. The potential allergenicity of α-CN decreased when heated at 65°C and 70°C for 25 min. The potential allergenicity of

β -CN increased sharply compared with unheated sample under all treatment temperatures. To reduce the antigenicity and potential allergenicity of MPCs, heat treatment was not entirely satisfactory. Thus it is important to study the conjunct effects of heat treatment and other processing technology on the antigenicity and potential allergenicity of MPCs.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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