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Guanhao Bu ^a; Yongkang Luo ^a; Zhe Zheng ^a; Hai Zheng ^a

^a Functional Dairy Lab, College of Food Science & Nutritional Engineering, China Agricultural University, Beijing, P.R. China

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Effect of heat treatment on the antigenicity of bovine α -lactalbumin and β -lactoglobulin in whey protein isolate

Guanhao Bu, Yongkang Luo*, Zhe Zheng and Hai Zheng

Functional Dairy Lab, College of Food Science & Nutritional Engineering, China Agricultural University, Beijing, P.R. China

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The effect of heat treatment on the antigenicity of α -lactalbumin (α -LA) and β -lactoglobulin (β -LG) in whey protein isolate (WPI) was investigated at temperatures in the range of 50–120°C for times from 0.5 to 30 min. The antigenicity was determined by an indirect competitive enzyme-linked immunosorbent assay. The heat denaturation of α -LA and β -LG was revealed by SDS-PAGE electrophoresis. The antigenicity of α -LA and β -LG increased with increasing temperature from 50 to 90°C and the highest antigenicity of α -LA and β -LG was detected at 90°C. However, above 90°C the antigenicity of both proteins showed a remarkable decrease. When treated at 120°C for 20 min, the antigenicity of α -LA decreased below the initial value of the untreated sample. The antigenicity of α -LA and β -LG in WPI can be decreased at certain heat temperature and time.

Keywords: α -lactalbumin; β -lactoglobulin; antigenicity; ELISA; heat treatment

Introduction

In recent years the incidence of food allergies has risen sharply, and food allergy has become a major public health concern around the world. Most common food allergens include cow's milk, eggs, fish, crustacean, peanuts, soy, nuts and wheat (Fritsche, 2003). In a number of epidemiological studies, cow milk protein allergy (CMPA) is reported to be the most prevalent for infants or young children, with serious consequences for infants' health and with an incidence of 2–6% (Hill & Hosking, 1996).

Cow milk contains many proteins that can cause allergic reactions, such as α -lactalbumin (α -LA), β -lactoglobulin (β -LG) and caseins. In addition, bovine serum albumin (BSA) and even lactoferrin (present in trace amounts) are potential allergens. In an investigation, caseins were found to be the most frequent allergens of cow milk. However, other studies revealed that β -LG and α -LA are the main allergens in cow milk (Fritsche, 2003; Sharma, Pravindra, Betzel, & Singh, 2001). β -LG belongs to the lipocalin protein family and has been considered to be the principal milk allergen for a long time. It is the major whey protein in the milk of many mammals. However, it is normally absent from human breast milk (Sawyer & Kontopidis, 2000). Moreover, β -LG can reach the small intestine in its native form

*Corresponding author. Email: luoyongkang@263.net

due to its resistance to pepsin hydrolysis (Wal, 2001). Therefore, milk allergy often occurs in newborns and infants.

β -LG is a major protein in milk (56–60% of total bovine whey proteins). It is predominantly present as a dimer at physiological pH, while it dissociates into native monomers at acidic pH <3.5 or basic pH >7.5 (Mckenzie & Sawyer, 1967). β -LG monomer is a globular protein of subunit molecular weight of about 18 kDa and has two disulfide bridges Cys66–Cys160 and Cys106–Cys119, additionally contains a free sulfhydryl group (–SH group) Cys121, which is supposed to be involved in significant disulfide interchange with –SH group of other whey proteins or of casein (Kleber & Hinrichs, 2007; Sawyer et al., 2002). α -LA is a monomeric globular protein with subunit molecule weight of about 14 kDa and contains four disulfide bonds within its molecule, so the molecular structure of α -LA is more stable than that of β -LG with two disulfide bonds during the heating (Hinrichs & Rademacher, 2005; Wal, 2001).

Heating is an important process in the manufacturing of most dairy products. Different heat treatments, such as homogenisation, concentration, pasteurisation and sterilisation, often are used to modify the functional properties of proteins, to ensure the safety of the product and to provide higher nutritive value (Oldfield, Singh, & Taylor, 2005). Due to heating process, important structure and chemical changes of proteins occur, like denaturation, aggregation and Maillard reaction with other molecules such as sugars. Generally, changes of protein structure is followed by unfolding of secondary structure (55–70°C), cleavage of disulphide bonds (70–80°C), formation of new intra/intermolecular interactions, rearrangements of disulphide bonds (80–90°C) and the formation of aggregates (90–100°C) (Davis & Williams, 1998). The functional properties of whey protein (solubility, foaming and emulsifying properties) can be modified by heating induced the formation of aggregates and gels. In addition, heat treatment can modify the susceptibility of α -LA and β -LG to proteases (Bertrand-Harb, Baday, Dalgalarrrondo, Chobert, & Haertle, 2002). These alterations may have an effect on the antigenicity of whey proteins.

Different studies have been reported about the effect of heat treatment on allergenicity of cow's milk proteins. Baldo (1984) found that heat treatment at 80 and 100°C for 15 min produced a drop in the IgE fixation capacity with β -LG and BSA, but no change in the case of α -LA and caseins. 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. It has also been shown that heat denaturation of β -LG exposes some new epitopes (Davis & Williams, 1998). Rytönen et al. (2002) found that bovine milk retained its allergenicity even when subjected to severe heat treatments.

From the above, these studies focused on the effect of thermal treatment on the antigenicity of β -LG, few studies determined the antigenicity changes of α -LA during heat treatment. In addition, the mechanism of antigenicity alteration of α -LA and β -LG by heat treatment is yet unknown. The purpose of this research is to determine the antigenicity changes of α -LA and β -LG in cow whey proteins with different heat temperatures and holding times, moreover, the mechanism of this modification of β -LG and α -LA antigenicity will be also analysed and explored in this study. The objective is to be able to provide some help for producing hypoallergenic or non-allergenic milk product for consumers, especially infants or babies.

Materials and methods

Materials

Whey protein isolates (WPI) were obtained from Murray Goulburn Co-operative Co. Ltd (Melbourne, Australia). The powder contained dry matter 93.8%, total proteins 85.2% and carbohydrates 0.3%.

The antigen proteins used for sensitisation studies and ELISA were α -LA from bovine milk (L5385; purity $\geq 85\%$; lyophilised power) and β -LG from bovine milk (L3908; purity, 90%; containing β -LG A and B; lyophilised power) purchased from Sigma Chemical Company (St. Louis, MO, USA). The first antibody from rabbit was made by our own laboratory; the second antibody, a conjugate of goat anti-rabbit immunoglobulin with peroxidase, was also purchased from Sigma Chemical Company. All other chemicals used were of analytical grade.

Preparation of rabbit antiserum

For the sensitisation studies, young male New Zealand rabbits were obtained from the Experimental Animal Center of the China Agricultural University. Rabbits were 4–5 months old at the start of the study and weight of rabbits was 2.6–2.7 kg. During the experiment and for at least 3 days prior to the start of the study, the rabbits were housed in stainless steel wire cages in an animal room maintained at $23 \pm 3^\circ\text{C}$ with a relative humidity of 30–70%. They had free access to food and tap water.

Rabbits were sensitised with bovine α -LA (Sigma, L5385) or β -LG (Sigma, L3908) by parenteral administration as shown in Table 1. The titres of antibodies were controlled by the indirect ELISA method taking blood samples from the rabbit marginal vein 2 days before the subsequent scheduled immunisation. Ten days after the fourth sensitisation, blood samples were obtained from the rabbit hearts.

Blood samples were incubated for 1 h at room temperature and left overnight at 4°C , then centrifuged for 20 min at $3000 \times g$ to obtain sera. The sera were stored at -20°C until the following analyses on the antigenicity of α -LA and β -LG by indirect competitive ELISA.

Heat treatment of whey proteins

WPI solution (0.7%, w/v) was prepared with distilled water and was mixed for 3 h. Samples were filled into 10 ml glass test tubes sealed with rubber stoppers, and then were heated from 50 to 120°C with 10 grades. At temperature below 90°C , the

Table 1. Immunisation procedure of New Zealand rabbits.

Sequence	Intervals (weeks)	Antigen sensitisation	Dose (mg protein/kg weight)	Treatment
First	0	Antigen/Freud complete adjuvant	1.0	Crural intramuscular
Second	2	Antigen/Freud incomplete adjuvant	1.0	Dorsal subcutaneous
Third	4	Antigen	1.0	Dorsal subcutaneous
Fourth	6	Antigen	1.0	Otic plexus

samples were put in a thermostated water bath, and above 90°C, were performed in a pressure vessel using saturated vapour. After treated for 0.5, 1, 5, 10, 20 and 30 min, the samples were immediately cooled in ice-water for 1 h to prevent further reaction. Another sample was maintained at room temperature for the untreated one. The heated and untreated samples were stored at -20°C for further analysis.

Indirect competitive ELISA

The antigenicity of α -LA and β -LG in untreated and heated WPI was estimated by the indirect competitive ELISA (Wróblewska & Jedrychowski, 2002; Wróblewska et al., 2004). Microtitre plates with 96 wells (flat-bottomed; Costar, Corning, USA) were coated with 100 μ l/well of antigen diluted in 50 mM carbonate buffer (pH 9.8) with the concentration determined earlier in the indirect ELISA method (2 μ g/ml for α -LA and 0.5 μ g/ml for β -LG) and incubated overnight at 4°C. Solutions of WPI and standard antigens were, respectively, incubated overnight at 4°C in test tubes with an equivalent volume of rabbit anti- α -LA or anti- β -LG serum diluted in 10 mM phosphate-buffered saline (PBS, pH 7.4) containing 1% BSA and 0.1% Tween-20 (PBS-BSA-Tween-20) (1:12,8000 for anti- α -LA serum and 1:6400 for anti- β -LG serum). The plates were washed four times the next day with 10 mM (PBS, pH 7.4 containing 0.05% Tween-20 (PBS-T)). The washing procedure was repeated after each analytical step. After washing the plates, the wells were filled with 100 μ l/well of PBS-BSA-Tween-20 for the purpose of blocking residual free binding sites in wells and incubated for 1 h at 37°C. The plates were washed and then 100 μ l/well of reactive mixtures of samples or standard antigens and polyclonal rabbit antibodies (IgG) were added and incubated for 1 h at 37°C. After washing the plates, the wells were filled with 100 μ l/well of horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG (Sigma A 6154, 1:10,000) in PBS-BSA-Tween-20. After incubation for 1 h at 37°C, the plates were washed again and then 100 μ l/well of 3,3',5,5'-tetramethylbenzidine (TMB, Amresco) solutions were immediately added. The plates were incubated for 15 min at 37°C. Finally, 50 μ l/well of 2 M H₂SO₄ were added to stop the reaction. Absorptions were read spectrophotometrically at dual wavelengths of 450 nm and 630 nm on a Multiskan MK3 ELISA plate reader (Thermo Labsystems, Franklin, MA, USA).

The antigenicity was calculated from standard curves of α -LA and β -LG, and a linear logarithmic correlation was observed in the range: 0.05–200 μ g/ml for α -LA and 0.01–400 μ g/ml for β -LG. All analyses were carried out in triplication and the averaged values were converted to concentration equivalents in milligrams per millilitre.

SDS-PAGE analysis of heated whey protein isolate (WPI)

Native and denaturated WPI was performed on 15% acrylamide plates according to Laemmli (1970). The resolving gel was prepared in 1.5 M Tris-HCl buffer, pH 6.8, and the stacking gel comprising 4% acrylamide was diluted by 0.5 M Tris-HCl buffer, pH 8.8. All the samples containing 8 μ g proteins were prepared in denaturing buffer without β -mercaptoethanol and heated at 100°C for 3 min before electrophoresis.

The gels were run in a Tris-glycine buffer, pH 8.3, and the electrophoresis was carried out at a constant pressure of 80 v for approximately 1 h in the stacking gel, then 100 v for 1.5 h in the resolving gel or until the tracking dye reached the bottom

of the gel. After electrophoresis, total proteins in gels were stained with Coomassie Brilliant Blue R-250.

Statistical analysis

The results were statistically analysed by one-way ANOVA using SAS 8.2 software (SAS Institute Inc., Cary, NC, USA). The significance of the dates was assessed by Duncan's multiple-range test. A p -value of <0.05 was considered statistically significant.

Results

The effect of heating temperatures on the antigenicity of α -lactalbumin (α -LA) and β -lactoglobulin (β -LG)

WPI was heated at temperatures from 50 to 120°C for 20 min. The antigenicity of β -LG for the untreated and heated WPI measured by ELISA is shown in Figure 1A. A β -LG concentration equivalent of 4.8 mg/ml was detected for the untreated samples. When temperature was 50°C, the β -LG antigenicity was 7.8 mg/ml. However, the antigenicity of β -LG in heated WPI was drastically increased with increasing temperature beyond 50°C ($p < 0.05$). The antigenicity increased to maximum level 98.2 mg/ml at 90°C, which was 20 times higher than the initial value of the untreated sample. Above 90°C, the antigenicity significantly declined with increasing heating temperature. When heated to 120°C, the antigenicity of β -LG decreased to 28.7 mg/ml, which was nearly six times higher than the initial value of the untreated sample.

A low antigenicity of 0.4 mg/ml α -LA concentration equivalent was detected for the untreated WPI solution (Figure 1B). Compared to the untreated sample, α -LA antigenicity in WPI solution slowly increased with increasing temperature from 50 to 70°C. However, the antigenicity of α -LA rapidly increased with increasing temperature from 70 to 90°C and reached to the maximum value 22.0 mg/ml at 90°C. Then, similarly to β -LG, the antigenicity of α -LA markedly declined above 90°C. Especially, a considerable decrease was observed from 17.3 mg/ml at 100°C to 1.0 mg/ml at 110°C ($p < 0.05$). When the temperature was up to 120°C, the antigenicity of α -LA was 0.3 mg/ml, which decreased by 25% compared to the initial value of the untreated sample.

The effect of heating times on the antigenicity of α -lactalbumin (α -LA) and β -lactoglobulin (β -LG)

The holding time was varied from 0.5 to 30 min in the temperature range from 60 to 90°C (Table 2). During heating at 60 and 70°C, the antigenicity of β -LG increased with elongation of heating time and reached the highest level after 30 min. At 80°C, the highest antigenicity was 95.3 mg/ml after heating for 20 min. When heated at 90°C for 10 min, the antigenicity was already up to the maximum level 101.2 mg/ml.

The antigenicity changes of α -LA by heat treatment from 60 to 90°C for 0.5 to 30 min were shown in Table 3. The antigenicity of α -LA increased to a lesser extent with extended holding time at 60 and 70°C, when heating for 30 min, the highest value in the

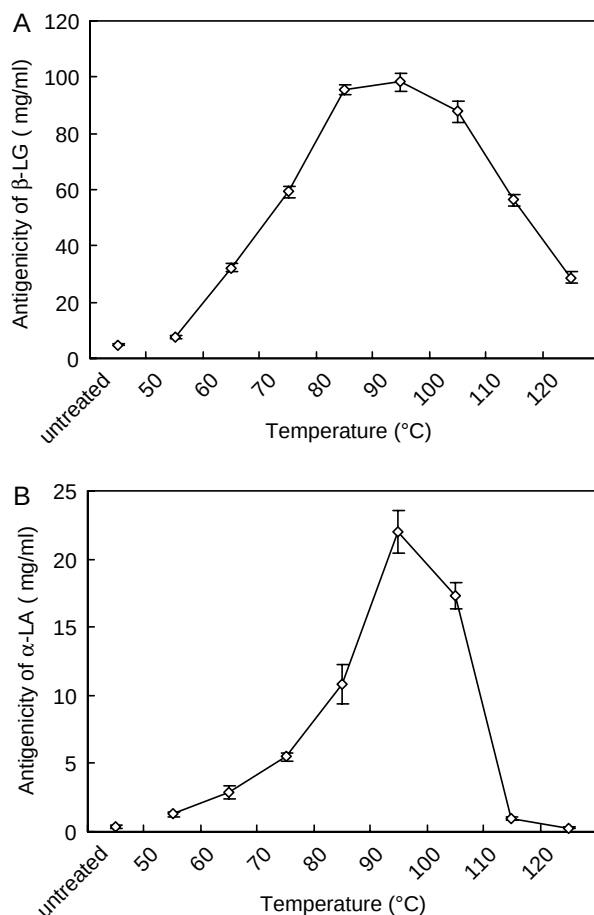


Figure 1. Effect of heating temperature at 50–120 $^{\circ}$ C for 20 min on the antigenicity of β -LG (A) and α -LA (B) in WPI solution.

antigenicity of α -LA was detected as 3.0 mg/ml at 60 $^{\circ}$ C and 6.4 mg/ml at 70 $^{\circ}$ C. When the temperature was 80 $^{\circ}$ C, the highest antigenicity was 14.0 mg/ml after 30 min. However, the antigenicity of α -LA was up to the highest level at 90 $^{\circ}$ C for 20 min.

Heat denaturation of whey protein isolate (WPI) revealed by electrophoresis

SDS-PAGE electrophoresis of heated WPI and untreated WPI solutions was performed to investigate the extent of loss of native β -LG and α -LA due to disulphide bond-mediated aggregation, and the results are shown in Figures 2 and 3. The buffer system of this electrophoresis can disperse all non-covalently linked protein aggregates into monomers, but aggregates linked through disulphide bonds remain intact due to the absence of a disulphide reducing agent like β -mercaptoethanol.

In comparison with known protein standards, SDS-PAGE of unheated WPI solution showed the principal whey proteins: BSA, Immunoglobulin, β -LG and α -LA (Figure 2, Lane 1). It is evident that heat treatment caused gradual decreases in

Table 2. Effect of holding time at 60–90°C on the antigenicity of β -LG in WPI solution.

Holding time (min)	Antigenicity of β -LG (mg/ml)			
	60°C	70°C	80°C	90°C
Untreated	4.8 $\pm$ 0.4 ^d	4.8 $\pm$ 0.4 ^e	4.8 $\pm$ 0.4 ^f	4.8 $\pm$ 0.4 ^e
0.5	6.2 $\pm$ 0.4 ^d	15.3 $\pm$ 1.2 ^d	27.9 $\pm$ 1.3 ^e	49.2 $\pm$ 1.1 ^d
1	7.0 $\pm$ 0.4 ^d	17.1 $\pm$ 0.8 ^d	41.0 $\pm$ 1.4 ^d	67.6 $\pm$ 2.7 ^c
5	16.0 $\pm$ 0.9 ^c	34.6 $\pm$ 2.2 ^c	75.1 $\pm$ 2.1 ^c	82.0 $\pm$ 4.2 ^b
10	20.4 $\pm$ 1.0 ^b	38.5 $\pm$ 1.5 ^c	86.4 $\pm$ 3.0 ^b	101.2 $\pm$ 2.9 ^a
20	32.3 $\pm$ 1.7 ^a	59.2 $\pm$ 2.0 ^b	95.3 $\pm$ 1.7 ^a	98.2 $\pm$ 3.3 ^a
30	33.4 $\pm$ 1.2 ^a	66.3 $\pm$ 1.9 ^a	83.5 $\pm$ 2.4 ^b	95.6 $\pm$ 2.3 ^a

^{a–f}Means $\pm$ SD (standard deviation) for three experiments within a column sharing common small letters were not significantly different ($P > 0.05$).

the monomer bands of β -LG and α -LA (Figure 2, Lanes 2–9), while the intensity of the band corresponding to β -LG dimers had a considerable increase with increasing heat temperature. Above 80°C, a higher quantity of β -LG trimers and polymers were observed owing to self-association of β -LG and co-aggregation of β -LG and α -LA. It was noted that some precipitation of proteins was visible in the well of the stacking gel when temperature was beyond 90°C.

Since the obtained electrophoresis profiles were the same for WPI solution treated at 60 and 70°C for 0.5–30 min, only those results obtained at 70°C for 0.5–30 min are presented in Figure 3(A). No difference of profiles was observed between the untreated sample and the sample treated for 0.5 min, whereas with elongation of heating time, the intensity of the band corresponding to β -LG dimers (approximately 36 kDa) gradually increased. At 80°C (data not shown) and 90°C (Figure 3B), the electrophoresis results of heated WPI are almost identical. With heat treatments beyond 5 min, the intensities of β -LG and α -LA monomer bands were diminished. However, the quantity of β -LG dimers and polymers increased considerably. In addition, with extended heating time, some proteins or aggregates with higher molecule masses did not enter into the gel.

Table 3. Effect of holding time at 60–90°C on the antigenicity of α -LA in WPI solution.

Holding time (min)	Antigenicity of α -LA (mg/ml)			
	60°C	70°C	80°C	90°C
Untreated	0.4 $\pm$ 0.1 ^c	0.4 $\pm$ 0.1 ^d	0.4 $\pm$ 0.1 ^f	0.4 $\pm$ 0.1 ^f
0.5	1.2 $\pm$ 0.1 ^{bc}	1.3 $\pm$ 0.1 ^{cd}	2.2 $\pm$ 0.2 ^{ef}	2.7 $\pm$ 0.2 ^{ef}
1	1.5 $\pm$ 0.2 ^b	1.5 $\pm$ 0.2 ^c	3.1 $\pm$ 0.3 ^{de}	3.7 $\pm$ 0.4 ^c
5	1.7 $\pm$ 0.1 ^b	2.0 $\pm$ 0.3 ^{bc}	5.3 $\pm$ 0.6 ^d	8.6 $\pm$ 0.4 ^d
10	2.7 $\pm$ 0.2 ^a	2.8 $\pm$ 0.4 ^b	8.5 $\pm$ 0.5 ^c	12.6 $\pm$ 0.7 ^c
20	2.9 $\pm$ 0.5 ^a	5.5 $\pm$ 0.3 ^a	10.8 $\pm$ 1.4 ^b	22.0 $\pm$ 1.6 ^a
30	3.0 $\pm$ 0.4 ^a	6.4 $\pm$ 0.6 ^a	14.0 $\pm$ 1.2 ^a	18.3 $\pm$ 1.1 ^b

^{a–f}Means $\pm$ SD (standard deviation) for three experiments within a column sharing common small letters were not significantly different ($P > 0.05$).

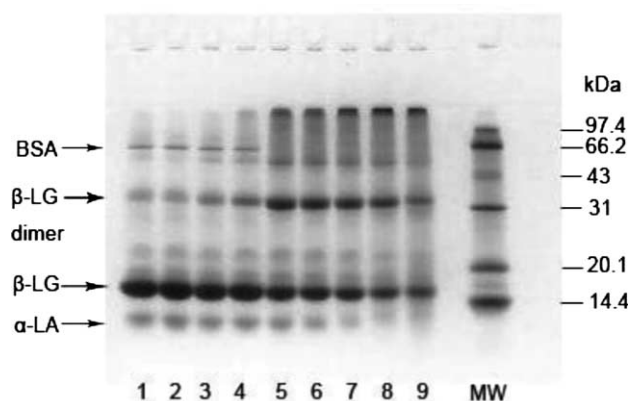


Figure 2. SDS-PAGE analysis of WPI solutions heated at different temperatures for 20 min: Lane 1: untreated; Lane 2: 50°C; Lane 3: 60°C; Lane 4: 70°C; Lane 5: 80°C; Lane 6: 90°C; Lane 7: 100°C; Lane 8: 110°C; and Lane 9: 120°C. MW: molecular weight markers.

Discussion

CMPA is one of the main food allergies, and has 2–6% incidence for infants (Hill & Hosking, 1996). So, to reduce the antigenicity of whey protein becomes a major problem for milk manufactures to solve. In this study, the antigenicity of α -LA and β -LG in WPI had a remarkable change as a result of heat treatment and could be decreased by controlling heat temperature and time.

The antigenicity of β -LG in WPI solution increased markedly from 50 to 90°C (Figure 1A). It can be observed from Figure 2 that the band intensity for β -LG monomer reduced with increasing temperatures, the intensity of β -LG dimer and polymers obviously enhanced, these results indicated irreversible denaturation of β -LG. Heat denaturation of β -LG is described simply as a two-step reaction. First the protein is reversibly unfolded and the hidden SH-groups get exposed, then the irreversible aggregation by intermolecular sulfhydryl/disulfide (SH/S-S) exchange occurs (Oldfield, Singh, Taylor, & Peare, 1998; Roefs & de Kruif, 1994). The increase of β -LG antigenicity from 50 to 90°C may be caused by the exposure of allergenic epitopes buried inside the native molecule owing to the unfolding of conformational structure during the denaturation of β -LG (Kleber & Hinrichs, 2007). In addition, sulfhydryl/disulfide exchange enforces conformational changes and consequently results in more antigenic epitopes being exposed on the surface of the molecule (Davis & Williams, 1998; Rytönen et al., 2002).

However, the antigenicity of β -LG declined remarkably with increasing temperatures from 90 to 120°C. It is observed from Figure 2 that the quantity of polymers evidently increased owing to the further denaturation and aggregation. The decrease in β -LG antigenicity can be attributed to destroy or mask of conformational epitopes being exposed to the surface of the molecule by sulfhydryl/disulfide exchange and subsequent aggregation (Kleber & Hinrichs, 2007). In addition to disulfide-mediated aggregation, under more severe heating conditions, the Maillard reaction may lead to the loss of linear epitopes with the consequence of a reduced antigenic response (Davis & Williams, 1998; Fritsche, 2003). It was found that the formation of bigger and compact particles in sweet whey can reduce the antigenicity

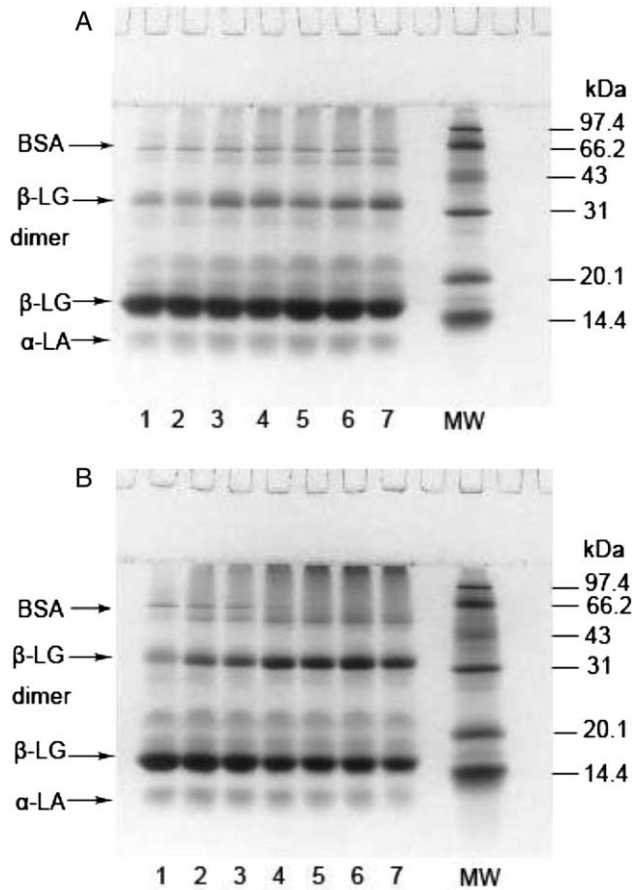


Figure 3. SDS-PAGE analysis of WPI solutions heated at 70°C (A) and 90°C (B) for: Lane 1: untreated; Lane 2: 0.5 min; Lane 3: 1 min; Lane 4: 5 min; Lane 5: 10 min; Lane 6: 20 min; and Lane 7: 30 min. MW: molecular weight markers.

of β -LG (Kleber, Maier, & Hinrichs, 2007). Above 90°C, aggregates with higher molecular weight (>97 kDa) observed from Figure 2 may illuminate the decrease of the β -LG antigenicity. Besides, the precipitates in the well of the stacking gel above 90°C imply the reducing of whey protein solubility. It has been shown that aggregation of proteins and the decrease in solubility of whey proteins occur following heat treatment (de Wit & Klarenbeek, 1984). So, it can be assumed that the decrease of β -LG antigenicity can be also contributed to the decrease in the content of some immunoreactive proteins due to the lower solubility (Merzejewska & Kubicka, 2006).

The changes of the antigenicity of β -LG and α -LA in WPI solution were different at different temperatures. It was detected that β -LG antigenicity changed from 7.8 mg/ml at 50°C to 32.3 mg/ml at 60°C, however, only a small change in α -LA antigenicity was visible from 1.3 mg/ml at 50°C to 2.9 mg/ml at 60°C, which may due to the exposure of less conformational epitopes for α -LA because the molecular structure of

α -LA is more stable than that of β -LG (Wal, 2001). When the temperature is up to 120°C, the antigenicity of α -LA decreased by 25% compared to the value of the untreated sample, while the antigenicity of β -LG was yet higher than the value of the untreated sample (Figure 1A and B). The result may be connected with the difference that apart from the conformational epitopes, the linear epitopes are also significant epitopes for β -LG, while the conformational epitopes play an important role in the antigenicity of α -LA (Ehn et al., 2004; Järvinen, Chatchatee, Bardina, Beyer, & Sampson, 2001; Maynard, Jost, & Wal, 1997). Additionally, due to be devoid of free cysteinyl residues, α -LA can only take part in aggregation if free thiol groups are available from molecules (β -LG, BSA) (Bertrand-Harb et al., 2002; Hinrichs & Rademacher, 2005), whereas β -LG is inclined to self-association (Anema, Lee, & Klostermeyer, 2006). Different aggregate modes may have an influence on the changes of the antigenicity of α -LA and β -LG.

Prolonging heat time could reduce the antigenicity of β -LG and α -LA in WPI solution under certain temperatures. The aggregates with high molecular mass increased gradually with elongation of heating time (Figure 3B), which may mask the antigenic epitopes of the molecular surface (Kleber & Hinrichs, 2007). This may also explain the decrease of β -LG and α -LA antigenicity with extended heating time at 80 and 90°C (Tables 2 and 3). Besides, the Maillard reactions between whey protein and lactose occur due to the long-time heat treatment (Wal, 2003), which may modify the conformational structure and sterically hinder antibodies from binding (Kleber & Hinrichs, 2007).

In conclusion, the present study showed that heat treatment modified evidently the antigenicity of whey protein allergens. It was found that the antigenicity of β -LG and α -LA in WPI solution gradually increased with increasing heat temperature from 50 to 90°C, then presented a remarkable decline with increasing heat temperature from 90 to 120°C, especially the antigenicity of α -LA declined below the initial value of untreated samples when heated at 120°C for 20 min, but the antigenicity of β -LG was yet higher than the initial value of untreated samples when heated to 120°C. To reduce milk protein antigenicity, heat treatment is not entirely satisfactory. Thus, it is of significance to study the conjunct effects of heat treatment and other technological strategies on the antigenicity of whey proteins. The Maillard reaction may be an approach by the addition of reduced sugars into milk proteins at different temperature, which will be investigated in our next work. This will be critical in the development of hypoallergenic milk products.

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