

Effects of Maillard reaction conditions on the antigenicity of α -lactalbumin and β -lactoglobulin in whey protein conjugated with maltose

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Abstract The effects of Maillard reaction conditions (weight ratio of protein to sugar, temperature and time) on the antigenicity of α -lactalbumin (α -LA) and β -lactoglobulin (β -LG) in conjugates of whey protein isolate (WPI) with maltose were investigated. Response surface methodology was used to establish models to predict the antigenicity of α -LA and β -LG and find an optimal reaction condition under which the antigenicity of α -LA and β -LG reduces to minimum value. Conjugating WPI with maltose was an effective way to reduce the antigenicity of α -LA and β -LG. The antigenicity of α -LA decreased from $32.25 \mu\text{g mL}^{-1}$ to $10.91 \mu\text{g mL}^{-1}$. And the antigenicity of β -LG decreased from $272.4 \mu\text{g mL}^{-1}$ to $38.17 \mu\text{g mL}^{-1}$. Temperature had the greatest effect on the antigenicity of α -LA, while weight ratio of WPI to maltose was the most significant factor on the antigenicity of β -LG.

Keywords Antigenicity · WPI · α -Lactalbumin · β -Lactoglobulin · Maltose · Maillard reaction

Introduction

World Health Organization has reported that food allergies are the sixth problem of human health. Cow milk is considered as an important allergen, the main allergens of cow milk to be found were due to the presence of β -lactoglobulin (β -LG), α -lactalbumin (α -LA) and caseins, all of which are the major protein of cow milk [1, 2]. But cow

milk and its products are good nutrient resources for human beings, especially for infants, so it is meaningful to solve the problem of milk allergy.

Previous studies have reported that heating treatment [3], high pressure [3, 4], microwave [5], enzymatic hydrolysis [6], fermentation [7] and glycosylation can reduce the allergenic potential of cow milk proteins. It is well-known that Maillard reaction products have functional or biological activities, such as antioxidant properties and antimutagenic characteristics [8–12]. Glycation reaction, involved in many Maillard reaction products formation, has been proposed as an effective way to change the antigenicity of some proteins. It has been found that the conjugation with trimethyl chitosan can improve the immunogenicity of ovalbumin [13]. van de Lagemaat [14] reported that the antigenicity of soy protein isolate could be reduced by glycation with fructose or fructooligosaccharides. In the concrete case of milk, Bu [15] indicated that conjugation of whey protein isolate with glucose markedly reduced the antigenicity of α -LA, while Kobayashi [16] found that conjugation with carboxymethyl dextran was effective in reducing the immunogenicity of β -LG. Hattori [17] conjugated bovine β -LG with the acidic oligosaccharides, alginic acid oligosaccharide and phosphoryl oligosaccharides by Maillard reaction. And the result showed that the conjugation of β -LG with these acidic oligosaccharides was effective in reducing the immunogenicity of β -LG.

Whey protein, with the main components of α -LA and β -LG, is a widely used high-quality protein. α -LA is a 14.2 kDa Ca-binding protein comprising 123 amino acids with 4 disulfide bonds [18], and β -LG is a 18 kDa small globular protein comprising 162 amino acids [19]. Maltose that can be easily digested and absorbed is a common raw material in food industry. Investigations into the effect of

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maltose on the antigenicity of α -LA and β -LG during Maillard reaction are scarce. The antigenicity of α -LA and β -LG depends on the Maillard reaction condition, such as weight ratio of protein to sugar, temperature and time. This study intended to research the effect of whey protein isolate (WPI) conjugated with maltose at different Maillard reaction conditions (weight ratio of WPI to maltose, temperature, time) on the antigenicity of α -LA and β -LG. Response surface methodology (RSM) was used to establish models to predict the antigenicity of α -LA and β -LG and to determine optimal conditions on which the lowest antigenicity of α -LA and β -LG can be obtained.

Materials

The antigens, α -LA (L5385, purity > 85%) and β -LG (L3908, purity > 90%) were purchased from Sigma Chemical Company (St. Louis, MO, USA). WPI (9410) was obtained from Hilmar (USA). Maltose was purchased from Beijing Aoboxing Biotech Company Ltd (China). All other reagents were analytical grade.

Methods

WPI–maltose system

WPI and maltose mixed as the experimental design in demineralized water. The weight ratios of WPI and maltose were 3.682, 3, 2, 1 and 0.318, respectively. The mixture was frozen at -60°C for 6 h and then freeze-dried for 24 h. The samples were incubated at a relative humidity of 79% in a desiccator (exposed to a saturated KBr solution) at different temperature and reaction for different times according to the experimental design. Then, the samples were carried out and freeze-dried for 24 h.

Brown color development and free amino groups analysis

The samples were dissolved in demineralized water. The protein concentration was 1 mg mL^{-1} . Brown color development was evaluated by the absorbance at 420 nm.

Free amino groups were measured using trinitro-benzene-sulfonic acid (TNBS) (Sigma) method [20] at 420 nm. Then, the value transformed into $\mu\text{mol of Leu mL}^{-1}$ using a calibration curve within the range 0.25–3.5 μmol .

SDS–PAGE analysis

SDS–PAGE was carried out to confirm conjugate formation, using a 12% acrylamide separating gel and a 4%

acrylamide stacking gel. Samples (2 mg mL^{-1}) were dissolved in denaturing buffer with β -mercaptoethanol. The mixture was heated at 100°C for 4 min and $8\text{ }\mu\text{L}$ was loaded onto the gel. A low molecular weight calibration marker (sigma) with protein ranging from 14.4 to 97.0 kDa was applied to the gels. The gels were run in Tris–glycine buffer (pH 8.3). The electrophoresis was carried out at a constant voltage of 80 V in the stacking gel for 1 h and then at 120 V in the resolving gel for 1.5 h. After that, proteins in the gels were stained with Coomassie Brilliant Blue R-250.

Indirect competitive enzyme-linked immunosorbent assay (ELISA)

The antigenicity of the samples was measured by indirect competitive enzyme-linked immunosorbent assay (ELISA). Indirect competitive ELISA was carried out as described by Bu [21]. After preliminary experiments, the appropriate concentrations determined were as follows: rabbit anti- α -LA serum diluted 256000 times, rabbit anti- β -LG serum diluted 128000 times, coating concentrations of α -LA and β -LG were $0.5\text{ }\mu\text{g mL}^{-1}$ and $1\text{ }\mu\text{g mL}^{-1}$, respectively, and the sample was dissolved in PBS at a protein concentration of 0.1 mg mL^{-1} .

Experimental design and statistical analysis

WPI/maltose (WPI/M) weight ratio (X_1), temperature (X_2) and time (X_3) were chosen as independent variables ($k = 3$) in the experimental design. The two dependents variables were to assess the antigenicity of α -LA (Y_1) and β -LG (Y_2) in the conjugates of WPI with maltose. The independent variables were optimized using central composite rotatable design (CCRD) containing five levels coded as -1.682 , -1 , 0 , $+1$ and $+1.682$ for each independent variable. Table 1 showed the coded values and the corresponding actual values of independent variables. The complete central composite design consisted of 23 experiments (Table 2) including 2^3 experiments for a full

Table 1 Coded and uncoded settings of independent variables for Maillard reaction conditions according to central composite rotatable design

Coded level	Independent variables		
	Weight ratio	Temperature ($^{\circ}\text{C}$)	Time(h)
1.682	3.682	76.8	88.4
1	3	70	72
0	2	60	48
-1	1	50	24
-1.682	0.318	43.2	7.6

Table 2 Full factorial central composite design matrix and the antigenicity of α -LA and β -LG in WPI–maltose conjugates

Assay	Independent variables			Dependent variable		OD ₄₂₀	Content of free NH ₂ groups (%)
	Weight ratio	Temperature (°C)	Time (h)	α -LA antigenicity (μ g/mL)	β -LG antigenicity (μ g/mL)		
1	1 (−1)	50 (−1)	24 (−1)	24.77	94.60	0.053	59.87
2	1 (−1)	50 (−1)	72 (1)	12.03	49.37	0.055	32.94
3	1 (−1)	70 (1)	24 (−1)	13.16	39.51	0.091	18.24
4	1 (−1)	70 (1)	72 (1)	16.47	64.96	0.367	19.61
5	3 (1)	50 (−1)	24 (−1)	35.91	117.55	0.052	65.17
6	3 (1)	50 (−1)	72 (1)	27.41	107.26	0.056	44.44
7	3 (1)	70 (1)	24 (−1)	14.13	71.33	0.129	29.93
8	3 (1)	70 (1)	72 (1)	16.64	99.97	0.451	31.29
9	0.318 (−1.682)	60 (0)	48 (0)	14.58	38.35	0.086	35.95
10	3.682 (1.682)	60 (0)	48 (0)	27.79	86.59	0.163	82.97
11	2 (0)	43.2 (−1.682)	48 (0)	31.59	127.38	0.062	48.92
12	2 (0)	76.8 (1.682)	48 (0)	14.5	102.71	0.469	11.76
13	2 (0)	60 (0)	7.6 (−1.682)	35.91	124.42	0.051	59.87
14	2 (0)	60 (0)	88.4 (1.682)	18.27	64.95	0.187	25.28
15	2 (0)	60 (0)	48 (0)	13.81	55.43	0.119	26.46
16	2 (0)	60 (0)	48 (0)	13.73	53.15	0.110	26.24
17	2 (0)	60 (0)	48 (0)	13.68	55.83	0.112	26.32
18	2 (0)	60 (0)	48 (0)	12.42	61.69	0.114	26.51
19	2 (0)	60 (0)	48 (0)	15.04	61.17	0.117	25.94
20	2 (0)	60 (0)	48 (0)	14.15	57.93	0.112	26.73
21	2 (0)	60 (0)	48 (0)	12.34	56.17	0.116	26.02
22	2 (0)	60 (0)	48 (0)	13.92	58.73	0.113	26.36
23	2 (0)	60 (0)	48 (0)	14.04	56.04	0.118	27.13

Content of free NH₂ groups (%) refers to the free NH₂ groups that did not react in the Maillard reaction

factorial design plus 2×3 star experiments and nine centre experiments. The centre experiments were to measure the precision and to verify any change in the estimation procedure. All of the Maillard reaction experiments were run in identical environment.

Experimental data were analyzed by SAS 8.2 (SAS Institute Inc, Cary, NC, USA). Response surface analysis was based on multiple regression. It took into account the main effects, the quadratic effects and the interaction effects, according to the following equation:

$$Y = b_0 + \sum_{i=1}^3 b_i x_i + \sum_{i=1}^3 b_{ii} x_i^2 + \sum_{i < j=2}^3 b_{ij} x_i x_j$$

In the equation, b_0 is constant coefficient of the model, and b_i , b_{ii} and b_{ij} are regression coefficients of the model (b_i for linear terms, b_{ii} for quadratic terms, and b_{ij} for interaction terms). x_i is the independent variable in the coded value. X_i is the independent variable in the uncoded value, and 10 b-coefficients were estimated for three independent variables. The Student's- t test assessed the significance of the b-coefficients for each dependent

variable. The level of significance was defined at $P < 0.05$. The level of highly significance was defined at $P < 0.01$. The goodness of fit of the model was evaluated by the analysis of variance (ANOVA). The three-dimensional response surface plots were drawn using the fit fully quadratic polynomial equations obtained by fixing one of the independent variables at a constant value and varying the other two variables with the experimental range.

Results and discussion

Changes of color

Glycation of proteins occurs by a chemical reaction of reducing sugars with free amino groups in proteins to form the Schiff's base linkage [22]. The complex melanoidins formed in the end of the Maillard reaction. Table 2 shows that when the reaction temperatures were 60 °C and 70 °C, the brown color was deepened with increasing reaction

time and weight ratio of WPI to maltose. But when the reaction temperature was 50 °C, the color did not vary significantly with the change of time and weight ratio of WPI to maltose. WPI control showed a slight brown color (not listed in the table). This is probably because of the Maillard reaction between whey protein and trace amounts of lactose in WPI. While the color of maltose and WPI separately in the same condition of 1–15 groups showed in Table 2 did not change.

Changes of free amino groups

Compared with WPI control, the free amino groups significantly decreased in all glycosylated protein samples (Table 2). After 24 h incubation at 70 °C, free amino groups appeared to decrease slightly. After 48 h incubation at 60 °C (2:1 weight ratio of WPI to maltose), approximately 75% of free amino groups in WPI were blocked by maltose and increased slightly after incubated for 88.4 h. It showed that when glycosylation reaction reached a certain extent, the extension of time would not affect the content of free amino groups. Sun [23] found that after α -LA and D-allose incubation for 24 h, the free amino groups appeared to decrease slightly as incubation time increased. WPI control lost a same quantity of its free amino groups during heat treatment (not list in the table). It could be attributed to the structural modifications of WPI, such as protein polymerization and cross-linking [24, 25]. It might also be attributed to a slight Maillard reaction between whey protein and lactose [26].

SDS–PAGE analysis of WPI–maltose conjugates

The formation of the WPI–maltose was analyzed by SDS–PAGE. When the protein and sugar react, the ϵ -amino residue of the protein and the reducing end of the sugar are expected to attach and form a covalent bond [27]. This reaction led to increase in molecular mass of WPI–maltose conjugates. Figures 1 and 2 show SDS–PAGE patterns of WPI and WPI–maltose conjugates reaction in different conditions. The covalent bond of maltose to the WPI was suggested by the fact that α -LA and β -LG bands had moved up or became shallower. It can be observed that bands in all lanes moved up or were shallower compared with WPI lane. This result proved that maltose bonds to WPI during the reaction. With increasing temperature and reaction time, the components of WPI decreased and higher molecular mass conjugates increased. Figure 2 shows that after maltose and WPI conjugates incubation at 76.8 °C for 48 h, there were no centralized bands in the lane, which suggested the formation of complex mixtures that consist of many different molecular components. The formation of

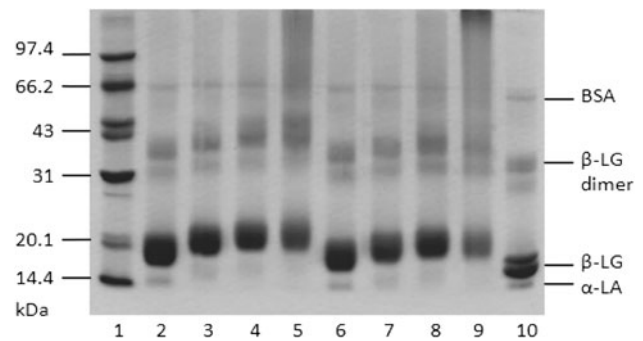


Fig. 1 SDS–PAGE analysis of WPI/maltose (M) conjugates formed at different Maillard reaction conditions: lane 1, molecular weight marker; lane 2, WPI/M (weight ratio 1:1) 50 °C, 24 h; lane 3, WPI/M (weight ratio 1:1) 50 °C, 72 h; lane 4, WPI/M (weight ratio 1:1) 70 °C, 24 h; lane 5, WPI/M (weight ratio 1:1) 70 °C, 72 h; lane 6, WPI/M (weight ratio 3:1) 50 °C, 24 h; lane 7, WPI/M (weight ratio 3:1) 50 °C, 72 h; lane 8, WPI/M (weight ratio 3:1) 70 °C, 24 h; lane 9, WPI/M (weight ratio 3:1) 70 °C, 72 h; lane 10, native WPI

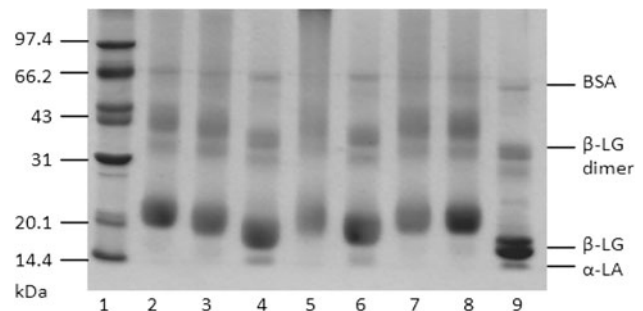


Fig. 2 SDS–PAGE analysis of WPI/maltose (M) conjugates formed at different Maillard reaction conditions: lane 1, molecular weight marker; lane 2, WPI/M (weight ratio 0.318:1) 60 °C, 24 h; lane 3, WPI/M (weight ratio 3.682:1) 60 °C, 24 h; lane 4, WPI/M (weight ratio 2:1) 43.2 °C, 48 h; lane 5, WPI/M (weight ratio 2:1) 76.8 °C, 48 h; lane 6, WPI/M (weight ratio 2:1) 60 °C, 7.6 h; lane 7, WPI/M (weight ratio 2:1) 60 °C, 88.4 h; lane 8, WPI/M (weight ratio 3:1) 60 °C, 48 h; lane 9, native WPI

these compounds reflected changes of α -LA and β -LG, which must be led to the change of antigenicity.

Assessment of models of antigenicity of WPI for three independent variables

The experimental design and the antigenicity of α -LA and β -LG under different reaction conditions are showed in Table 2. The regression analysis on 23 experiments (Table 3, full model) revealed that several terms were not significant ($P > 0.05$). In order to fit the full second-order model, non-significant terms were eliminated step by step from the regression model. After this procedure, there were nine regression terms in the second-order model for α -LA and seven for β -LG (Table 3, fitted model). Models were as follows:

Table 3 Regression coefficients for the linear regression prediction model of the antigenicity of α -LA and β -LG

	Full model				Fitted model			
	α -LA antigenicity		β -LG antigenicity		α -LA antigenicity		β -LG antigenicity	
	b-coefficient	<i>p</i>	b-coefficient	<i>p</i>	b-coefficient	<i>p</i>	b-coefficient	<i>p</i>
Intercept	13.77		57.61		13.77		57.62	
Linear								
Weight ratio	3.65	0.000	16.75	0.000	3.65	0.000	16.75	0.000
Temperature	−5.01	0.000	−9.85	0.008	−5.01	0.000	−9.85	0.005
Time	−3.30	0.001	−7.43	0.036	−3.30	0.001	−7.43	0.026
Quadratic								
Weight ratio ²	1.76	0.029	−0.72	0.811	1.76	0.024		
Temperature ²	2.41	0.005	17.87	0.000	2.41	0.004	17.69	0.000
Time ²	3.84	0.000	10.67	0.003	3.84	0.000	10.49	0.002
Interactions								
Weight ratio × temperature	−3.17	0.007	−1.75	0.680	−3.17	0.006		
Weight ratio × time	0.43	0.677	4.77	0.272				
Temperature × time	3.38	0.005	13.70	0.006	3.38	0.004	13.70	0.003
Other statistics								
<i>R</i> ² (%)	86.50		81.35		87.29		82.97	
<i>F</i>	16.67	0.000	11.66	0.000	19.89	0.000	18.86	0.000

$$Y_1 = 13.77 + 3.65x_1 - 5.01x_2 - 3.30x_3 + 1.76x_1^2 - 3.17x_1x_2 + 2.41x_2^2 + 3.38x_2x_3 + 3.84x_3^2$$

$$Y_2 = 57.62 + 16.75x_1 - 9.85x_2 - 7.43x_3 + 17.69x_2^2 + 13.70x_2x_3 + 10.49x_3^2$$

The *P*-value of fitted model for both α -LA and β -LG was 0.0001. The adjusted *R*² of fitted model for α -LA is 0.8135 and for β -LG is 0.8297. The second-order model was highly significant because of the very small *P*-values and the high adjusted *R*² values. It can show the factual relationship between responses and independent variables.

Effect of Maillard reaction conditions on antigenicity of α -LA in WPI/maltose conjugates

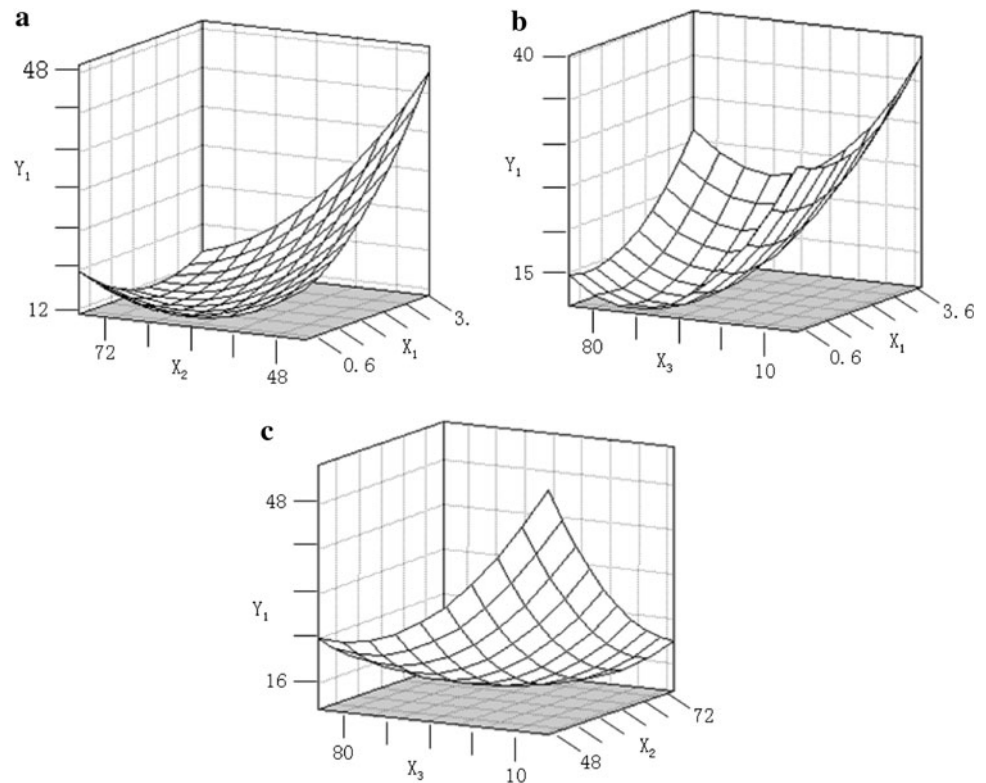
From the analysis of regression coefficients (Table 3), the highly significant (*P*<0.01) effects on the antigenicity were as follows: the linear effects of three independent variables, the quadratic effects of temperature and time, the interactive effect between WPI/maltose (WPI/M) weight ratio and temperature and the interactive effect between temperature and time. The regression coefficient of temperature was the highest among the three independent variables, so the effect of temperature was the most important. The optimal values of three independent variables were as follows: WPI/M weight ratio = 1.1:1, temperature = 61.6 °C and time = 57.6 h. In theory, on these reaction conditions, the antigenicity of α -LA should be the lowest. And the

predicted lowest value should be 10.91 $\mu\text{g mL}^{-1}$. On these reaction conditions, the antigenicity of β -LG should be 38.94 $\mu\text{g mL}^{-1}$. The antigenicity of α -LA and β -LG in WPI was 32.25 $\mu\text{g mL}^{-1}$ and 272.4 $\mu\text{g mL}^{-1}$. So, it can conclude that the antigenicity of α -LA-maltose and β -LG-maltose is lower than that of α -LA and β -LG in WPI, which showed that glycosylation can reduce the antigenicity of WPI effectively.

The effect of WPI/M weight ratio and temperature at an aptotic time of 48 h is shown by the response surface plot in Fig. 3a. The antigenicity of α -LA decreased with the decrease in WPI/M weight ratio within a certain range. However, when maltose fully conjugated with the whey protein, increasing amount of maltose will not affect the antigenicity of α -LA. It was observed that the antigenicity decreased at first and then increased after the temperature reached 60–65 °C. So, the optimal values of temperature were between 60 and 65 °C.

Figure 3b shows the effect of WPI/M weight ratio and time at an aptotic temperature of 60 °C. As revealed by its highly significant quadratic effect, there should exist an optimal value of reaction time. It appeared that the antigenicity was minimal when the reaction time was about 50–60 h, after that the antigenicity increased again. Enomoto [28] compared the antigenicity of α -LA which was glycosylated with maltopentaose through Maillard reaction and subsequently phosphorylated by dry heating in the presence of pyrophosphate for 1 day or 5 days, and he found that the antigenicity of the two conjugates was lower than α -LA but

Fig. 3 Response surfaces of antigenicity of α -LA (Y_1): **a** effect of whey protein isolate/maltose (WPI/M) weight ratio (X_1) and temperature (X_2) on antigenicity at aptotic time of 48 h; **b** effect of WPI/M weight ratio (X_1) and time (X_3) on antigenicity at aptotic temperature of 60 °C; **c** effect of temperature (X_2) and time (X_3) on antigenicity at aptotic WPI/M weight ratio of 2:1



there was no significant difference between them. It shows that to prolong reaction time cannot boundless reduce the antigenicity of α -LA. It is similar to the results of this study. The color of WPI–maltose gradually deepened with the longer reaction time. Thus, in order to obtain conjugates of a lower antigenicity and an acceptable color, it is important to control Maillard reaction time.

The response surface plot in Fig. 3c reveals the effect of temperature and time at an aptotic WPI/M weight ratio of 2:1. As shown in the figure range, with the increasing reaction temperature and time, the antigenicity of α -LA decreased until 60–70 °C and 50–60 h and then gradually increased.

Effect of Maillard reaction conditions on antigenicity of β -LG in WPI/maltose conjugates

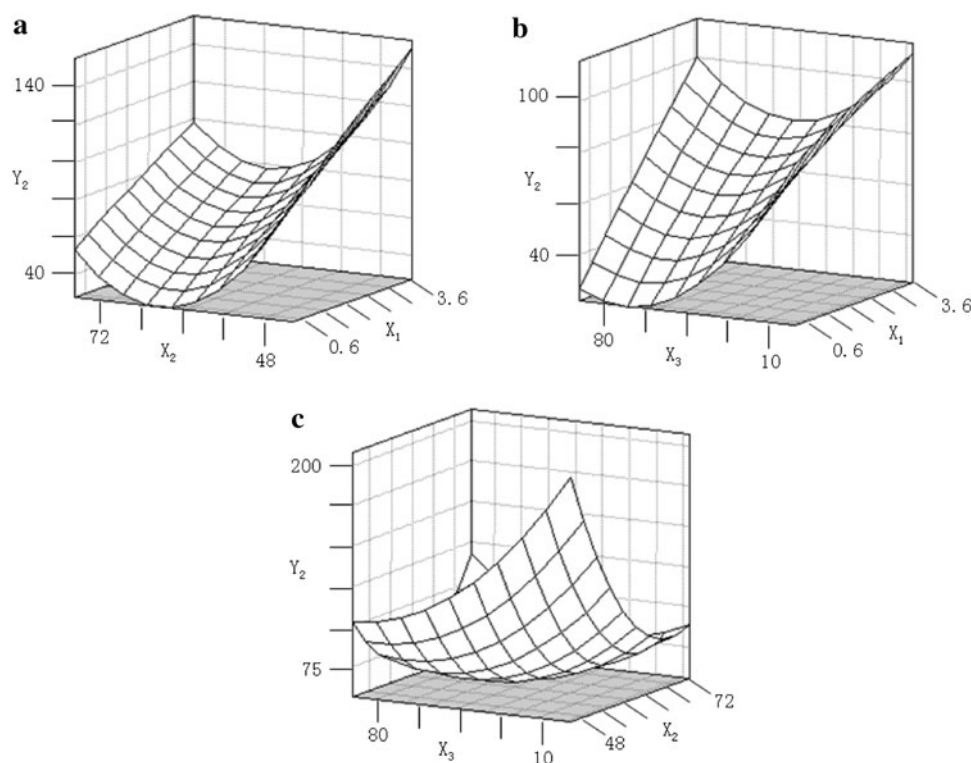
According to the analysis of regression coefficients, the highly significant ($P < 0.01$) effects on the antigenicity were as follows: the linear effect of WPI/M weight ratio and temperature, the quadratic effects of temperature and time and the interactive effect between temperature and time. The effect of WPI/M weight ratio was the most important among the three independent variables. The optimal values of the three independent variables bringing minimum antigenicity of β -LG were as follows: WPI/M weight ratio = 1.04:1, temperature = 60.8 °C and time = 54.3 h. On these reaction conditions, the antigenicity of β -LG was 38.17 $\mu\text{g mL}^{-1}$

and the antigenicity of α -LA was 11.03 $\mu\text{g mL}^{-1}$. The antigenicity of β -LG reduced to about one-sixth of the origin and α -LA retained one-third of the origin. This may be due to the varying glycosylation degrees of α -LA and β -LG with maltose, and this conclusion is consistent with the finding of Jiménez-Castaño [29]. They pointed out that the degree of glycosylation of the whey protein–dextran conjugates was in the order: BSA > β -LG > α -LA.

The effect of WPI/M weight ratio and temperature at an aptotic time of 48 h was shown by the response surface plot in Fig. 4a. This study suggested that from 50 °C to 70 °C, a lower WPI/M weight ratio could induce a lower antigenicity of β -LG. This was similar to the result of van de Lagemaat [14] who glycosylated soy protein with fructooligosaccharides. The antigenicity of β -LG decreased with the increasing temperature until the temperature reached 60–65 °C, and then it increased as the temperature increased. In glycosylation reaction, the masking of the epitope by sugar affected the antigenicity. In addition, heating can change the protein structure, leading some epitope exposed and some epitope hidden. The changes of epitope caused changes in antigenicity. Taheri-Kafrani [30] reported that from 65 °C to 95 °C, the higher the temperature, the faster the antigenicity increased. Therefore, heating and glycosylation combined affected the antigenicity of β -LG.

Figure 4b showed the effect of WPI/M weight ratio and time at an aptotic temperature of 60 °C. The increase in

Fig. 4 Response surfaces of antigenicity of β -LG(Y_2): **a** effect of whey protein isolate/maltose (WPI/M) weight ratio (X_1) and temperature (X_2) on antigenicity at aptotic time of 48 h; **b** effect of WPI/M weight ratio (X_1) and time (X_3) on antigenicity at aptotic temperature of 60 °C; **c** effect of temperature (X_2) and time (X_3) on antigenicity at aptotic WPI/M weight ratio of 2:1



WPI/M weight ratio resulted in increase in antigenicity. The optimal value of time was different when the WPI/M weight ratio was different. The antigenicity of β -LG first raised and then decreased as time expanded, and the time of reaching minimum became shorter when the additive amount of sugar was higher.

The response surface plot in Fig. 4c reveals the effect of temperature and time at an aptotic WPI/M weight ratio of 2:1. It can be seen that the trends between antigenicity and time were not the same when temperature changed. In the research of Corzo-Martínez [31] who glycated β -LG with tagatose, and the trend between antigenicity and time at 40 °C was different with the trend between antigenicity and time at 50 °C. This showed that the reaction time and temperature had an interaction effect on the antigenicity of β -LG. The minimum antigenicity can obtain at a temperature of 60–70 °C and a reaction time of 50–60 h. With an increase in temperature and time in scope of the study, the antigenicity of β -LG decreased at first and then increased as the temperature and time enhanced further.

Conclusions

Models for predicting antigenicity of α -LA and β -LG was established. The antigenicity of α -LA and β -LG was reduced effectively through conjugating with maltose. On the range of conditions studied, temperature had the

greatest effect on the antigenicity of α -LA, while the most important effect on the antigenicity of β -LG was weight ratio. RSM was an effective method to optimize several independent variables of the glycation reaction. The minimum antigenicity of α -LA and β -LG was $10.91 \mu\text{g mL}^{-1}$ and $38.17 \mu\text{g mL}^{-1}$, respectively. Glycation of whey protein with maltose under appropriate conditions was a feasible way to reduce the antigenicity of α -LA and β -LG.

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References

1. Monaci L, Tregoat V, Van Hengel AJ, Anklam E (2006) Milk allergens, their characteristics and their detection in food: A review. *Eur Food Res Technol* 223:149–179
2. Fritsché R (2003) Role for technology in dairy allergy. *Aust J Dairy Technol* 58:89–91
3. Kleber N, Maier S, Hinrichs J (2007) Antigenic response of bovine β -lactoglobulin influenced by ultra-high pressure treatment and temperature. *Innov Food Sci Emerg* 8:39–45
4. Peñas E, Snel H, Floris R, Préstamo G, Gomez R (2006) High pressure can reduce the antigenicity of bovine whey protein hydrolysates. *Int Dairy J* 16:969–975
5. Grar H, Kaddouri H, Gourine H, Negaoui H, Kheroua O, Saïdi D (2009) Microwave irradiation under different pH conditions induced a decrease in β -lactoglobulin antigenicity. *Eur Food Res Technol* 229:779–783

6. Zheng H, Shen X, Bu G, Luo Y (2008) Effects of pH, temperature and enzyme-to-substrate ratio on the antigenicity of whey protein hydrolysates prepared by Alcalase. *Int Dairy J* 18:1028–1033
7. Ehn BM, Allmere T, Teleme E, Bengtsson U, Ekstrand BO (2005) Modification of IgE binding to β -Lactoglobulin by fermentation and proteolysis of cow's milk. *J Agr Food Chem* 53:3743–3748
8. Mulsow BB, Jacob M, Henle T (2009) Studies on the impact of glycation on the denaturation of whey proteins. *Eur Food Res Technol* 228:643–649
9. Hattori M, Numamoto KI, Kobayashi K, Takahashi K (2000) Functional changes in β -lactoglobulin by conjugation with cationic saccharides. *J Agr Food Chem* 48:2050–2056
10. Enomoto H, Nagae S, Hayashi Y, Li CP, Lbrahim HR, Sugimoto Y, Aoki T (2009) Improvement of functional properties of Egg white protein through glycation and phosphorylation by dry-heating. *As Aus J An* 22:591–597
11. Chevalier F, Chobert JM, Pohineau Y, Nicolas MG, Haertlé T (2001) Improvement of functional properties of β -lactoglobulin glycosylated through the Maillard reaction is related to the nature of the sugar. *Int Dairy J* 11:145–152
12. Delgado-Andrade C, Morales FJ (2005) Unraveling the contribution of melanoidins to the antioxidant activity of coffee brews. *J Agr Food Chem* 53:1403–1407
13. Slütter B, Soema PC, Ding Z, Verheul R, Hennink W, Jiskoot W (2010) Conjugation of ovalbumin to trimethyl chitosan improves immunogenicity of the antigen. *J Control Release* 143:207–214
14. van de Lagemaat J, Manuel Silván J, Javier Moreno F, Olano A, Dolores del Castillo M (2007) In vitro glycation and antigenicity of soy protein. *Food Res Int* 40:153–160
15. Bu G, Lu J, Zheng Z, Luo Y (2009) Influence of Maillard reaction conditions on the antigenicity of bovine α -lactalbumin using response surface methodology. *J Sci Food Agr* 89:2428–2434
16. Kobayashi K, Hirano A, Ohta A, Yoshida T, Takahashi K, Hattori M (2001) Reduced immunogenicity of β -lactoglobulin by conjugation with carboxymethyl dextran differing in molecular weight. *J Agr Food Chem* 49:823–831
17. Hattori M, Miyakawa S, Ohama Y, Kawamura H, Yoshida T, To-O K, Kuriki T, Takahashi K (2004) Reduced immunogenicity of β -lactoglobulin by conjugation with acidic oligosaccharides. *J Agr Food Chem* 52:4546–4553
18. Permyakov EA, Berliner LJ (2000) α -lactalbumin: Structure and function. *FEBS Lett* 473:269–274
19. Taheri-Kafrani A, Asgari-Mobarakeh E, Bordbar AK, Haertlé T (2010) Structure-function relationship of β -lactoglobulin in the presence of dodecyltrimethyl ammonium bromide. *Colloid Surfaces B* 75:268–274
20. Adler-Nissen J (1979) Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *J Agr Food Chem* 27:1256–1262
21. Bu G, Luo Y, Lu J, Zhang Y (2010) Reduced antigenicity of β -lactoglobulin by conjugation with glucose through controlled Maillard reaction conditions. *Food Agr Immunol* 21:143–156
22. Singh R, Barden A, Mori T, Beilin L (2001) Advanced glycation end-products: a review. *Diabetologia* 44:129–146
23. Sun Y, Hayakawa S, Puangmanee S, Izumori K (2006) Chemical properties and antioxidative activity of glycated α -lactalbumin with a rare sugar, D-allose, by Maillard reaction. *Food Chem* 95:509–517
24. Chevalier F, Chobert JM, Genot C, Haertlé T (2001) Scavenging of free radicals, antimicrobial, and cytotoxic activities of the Maillard reaction products of β -lactoglobulin glycosylated with several sugars. *J Agr Food Chem* 49:5031–5038
25. Jiménez-Castaño L, Villamiel M, Martín-Álvarez PJ, Olano A, López-Fandiño R (2005) Effect of the dry-heating conditions on the glycosylation of β -lactoglobulin with dextran through the Maillard reaction. *Food Hydrocolloid* 19:831–837
26. Jones AD, Tier CM, Wilkins JPG (1998) Analysis of the Maillard reaction products of β -lactoglobulin and lactose in skimmed milk powder by capillary electrophoresis and electrospray mass spectrometry. *Journal of Chromatogr A* 822:147–154
27. Kato A, Minaki K, Kobayashi K (1993) Improvement of emulsifying properties of egg white proteins by the attachment of polysaccharide of egg white proteins by the attachment of polysaccharide through Maillard reaction in a dry state. *J Agr Food Chem* 41:540–543
28. Enomot H, Hayashi Y, Li CP, Ohki S, Ohtomo H, Shiokawa M, Aoki T (2009) Glycation and phosphorylation of α -lactalbumin by dry heating: Effect on protein structure and physiological functions. *J Dairy Sci* 92:3057–3068
29. Jiménez-Castaño L, Villamiel M, López-Fandiño R (2007) Glycosylation of individual whey proteins by Maillard reaction using dextran of different molecular mass. *Food Hydrocolloid* 21:433–443
30. Taheri-Kafrani A, Gaudin JC, Rabesona H, Nioi C, Agarwal D, Drouet M, Chobert JM, Bordbar AK, Haertlé T (2009) Effects of heating and glycation of β -lactoglobulin on its recognition by ige of sera from cow milk allergy patients. *J Agr Food Chem* 57:4974–4982
31. Corzo-Martínez M, Soria AC, Belloque J, Villamiel M, Moreno FJ (2010) Effect of glycation on the gastrointestinal digestibility and immunoreactivity of bovine β -lactoglobulin. *Int Dairy J* 11:742–752