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Effect of Maillard reaction conditions on antigenicity of β -lactoglobulin and the properties of glycated whey protein during simulated gastric digestion

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Response surface methodology was employed to study the effects of Maillard reaction conditions on the antigenicity of β -lactoglobulin (β -LG) in whey protein isolate (WPI) and to optimise the Maillard reaction conditions of WPI conjugate with oligoisomaltose under which the antigenicity of β -LG reduced to the minimum value. The antigenicity of β -LG and α -lactalbumin (α -LA) in natural and glycated WPI during simulated gastric digestion were investigated. The antigenicity of β -LG was reduced from $272.4 \mu\text{g mL}^{-1}$ to $30.99 \mu\text{g mL}^{-1}$ under the optimal Maillard reaction conditions. After 120 min simulated gastric digestion, the antigenicity of β -LG in natural and glycated WPI were $42.83 \mu\text{g mL}^{-1}$ and $15.66 \mu\text{g mL}^{-1}$, respectively. And the antigenicity of α -LA in natural and glycated WPI were $0.78 \mu\text{g mL}^{-1}$ and $0.03 \mu\text{g mL}^{-1}$, respectively.

Keywords: whey protein; β -lactoglobulin; oligoisomaltose; Maillard; simulated gastric digestion; antigenicity

1. Introduction

Food allergy, which affects 6% of young children and 3–4% of adults, is of great concern (Sicherer & Sampson, 2006). Cow milk allergy is at the top of all lists of epidemiologic data (Sampson, 2004). A higher incidence of milk allergy is usually in neonates and small children. The main whey proteins are β -lactoglobulin (β -LG) and α -lactalbumin (α -LA) which have nutritional and functional properties. β -LG is the most prevalent protein in whey protein and it is the major allergen of bovine milk. Approximately 10% of the total milk protein and 58% of the whey protein is β -LG. It contains 162 amino acids and its molecular weight is approximately 18.4 kDa (Taheri-Kafrani, Asgari-Mobarakeh, Bordbar, & Haertlé, 2010). β -LG consists of two genetic isoforms A and B which are known to possess allergenic potential. β -LG is very stable against peptic digestion under acidic conditions, and this phenomenon is considered to correlate with its high allergenicity (Breiteneder & Mills, 2005; Maier, Okun, Pittner, & Lindner, 2006; Schmidt, Meijer, Slangen, & Van Beresteijn, 1995).

In previous studies, some processing methods have been reported that they can change the allergenic potential of cow milk protein, such as heating treatment (Bu, Luo, & Zheng, 2009a), high-pressure (Bonomi et al., 2003), microwave

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(Grar et al., 2009; Izquierdo, Peñas, Baeza, & Gomez, 2008; Zellal et al., 2011), enzymatic hydrolysis (Liu, Luo, & Li, 2012; Zheng, Shen, Bu, & Luo, 2008), fermentation (Bu, Luo, Zhang, & Chen, 2010a; Ehn, Allmere, Telemo, Bengtsson, & Ekstrand, 2005) and glycosylation (Li, Luo, & Feng, 2011). These processing methods can alter the allergenic properties of protein by hiding, destroying or disclosing allergenic epitopes through conformational changes or by changing digestibility of protein. Chicón, Belloque, Alonso, & López-Fandiño (2008, 2009) reported that high-pressure treatment (400 MPa) on β -LG can increase IgG-binding and promote the hydrolysis of β -LG by pepsin. Kananen et al. (2000) modified the whey protein by sulfitolysis and they pointed out that the antigenicity of β -LG decreased markedly during pepsin hydrolysis. Maier et al. (2006) reported that fermentation of milk products increases the susceptibility of β -LG towards peptic digestion and the immunoreactive β -LG content of fermented products is reduced. Some studies had been reported that glycosylation was an effective way to change the antigenicity of some potentially allergenic proteins. It had been found that conjugation with sugars can change the antigenicity of ovalbumin (Slütter et al., 2010), soy protein (van de Lagemaat, Manuel Silván, Javier Moreno, Olano, & Dolores del Castillo, 2007), peanut (Gruber, Becker, & Hofmann, 2005) and wheat protein (András et al., 2009). In the case of milk, glucose (Bu, Lu, Zheng, & Luo, 2009b; Bu, Luo, Lu, & Zhang, 2010b), maltopentaose (Enomot et al., 2009), lactose (Taheri-Kafrani et al., 2009), chitosan (Aoki, Iskandar, Yoshida, Takahashi, & Hattori, 2006), carboxymethyl dextran (Kobayashi et al., 2001), acidic oligosaccharides (Hattori et al., 2004) and other sugars had been reported can change the antigenicity of whey protein.

The impacts of conjugation with different sugars on the antigenicity were different. Investigations into the effect of oligoisomaltose on the antigenicity of β -LG during Maillard reaction were scarce. Oligoisomaltose can effectively promote the growth and reproduction of bifidobacterium in body. Moreover, studies about the antigenicity changes of whey protein isolate (WPI) and glycated WPI during simulated gastric digestion were also lacking. β -LG is the main allergen of cow milk. The aim of this study was to investigate the effects of Maillard reaction conditions on the antigenicity of β -LG, and use response surface methodology to optimise Maillard reaction conditions in order to obtain the lowest antigenicity of β -LG. This study also performed on the simulated gastric digestion of natural and glycated whey protein and explored the antigenicity changes of α -LA and β -LG during simulated gastric digestion.

2. Materials and methods

2.1. Materials

α -Lactalbumin (L5385, purity >85%) and β -LG (L3908, purity >90%) used in enzyme-linked immunosorbent assay (ELISA) were purchased from Sigma Chemical Company (St. Louis, MO, USA). WPI (9410) was obtained from Hilmar (USA). Oligoisomaltose was purchased from Baolingbao Biotechnology Co., Ltd (China). Pepsin was purchased from Amresco (USA). Other reagents were analytical grade.

2.2. Sample preparation: Maillard reaction between WPI and oligoisomaltose

WPI and oligoisomaltose were mixed in demineralised water in order to evenly mix them, and then the mixture was freeze-dried to powder. The weight ratios of oligoisomaltose and WPI (oligoisomaltose/WPI weight ratio) were different. The powder was incubated in a desiccator which was exposed to a saturated KBr solution (relative humidity of 79%) at different reaction conditions according to the experimental design. At last samples were carried out from the desiccators and dissolved and then freeze-dried before analysis.

2.3. Indirect competitive enzyme-linked immunosorbent assay

Indirect competitive ELISA was carried out as described by Bu et al. (2010b) to measure the antigenicity of α -LA and β -LG in the samples. After preliminary experiments, the determined ELISA test conditions were as follows: coating concentration of α -LA and β -LG were $0.5 \mu\text{g mL}^{-1}$ and $1 \mu\text{g mL}^{-1}$, respectively, rabbit anti- α -LA serum diluted 2,56,000 times, rabbit anti- β -LG serum diluted 1,28,000 times, sample was dissolved in PBS at a protein concentration of 0.1 mg mL^{-1} . The antigenicity was calculated from standard curve. For α -LA, a linear logarithmic correlation was observed in the range: 2–256 $\mu\text{g mL}^{-1}$. For β -LG, a linear logarithmic correlation was observed in the range: 0.5–256 $\mu\text{g mL}^{-1}$.

2.4. Experimental design and statistical analysis

Oligoisomaltose/WPI weight ratio (X_1), temperature (X_2) and time (X_3) were chosen as independent variables ($k=3$) in the experimental design. The dependent variable was the antigenicity of β -LG (Y) in the conjugates of WPI with oligoisomaltose. The independent variables were optimised using central composite rotatable design which contains 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 the three independent variables. The complete central composite design consisted of 23 experiments (Table 2). And the 23 experiments included a full factorial design plus 2×3 star experiments and nine centre experiments. The centre experiments were to measure the accuracy and to verify changes in the estimation procedure. In addition, all of the 23 experiments were run in identical environment. Experimental data were analysed by SAS 8.2 (SAS Institute Inc, Cary, NC, USA) and carried out as described by Li et al. (2011).

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	7.36	76.8	44.2
1	6	70	36
0	4	60	24
-1	2	50	12
-1.682	0.64	43.2	3.8

Table 2. Full factorial central composite design matrix and the antigenicity of β -LG in WPI-oligoisomaltose conjugates.

Assay	Independent variables ^a			Dependent variable
	Weight ratio	Temperature (°C)	Time (h)	β -LG antigenicity ($\mu\text{g/mL}$)
1	2 (−1)	50 (−1)	12 (−1)	141.1
2	2 (−1)	50 (−1)	36 (1)	105.7
3	2 (−1)	70 (1)	12 (−1)	64.1
4	2 (−1)	70 (1)	36 (1)	36.4
5	6 (1)	50 (−1)	12 (−1)	172.6
6	6 (1)	50 (−1)	36 (1)	88.6
7	6 (1)	70 (1)	12 (−1)	51.0
8	6 (1)	70 (1)	36 (1)	33.2
9	0.64 (−1.682)	60 (0)	24 (0)	143.6
10	7.36 (1.682)	60 (0)	24 (0)	70.1
11	4 (0)	43.2 (−1.682)	24 (0)	192.3
12	4 (0)	76.8 (1.682)	24 (0)	39.7
13	4 (0)	60 (0)	3.8 (−1.682)	141.9
14	4 (0)	60 (0)	44.2 (1.682)	62.5
15	4 (0)	60 (0)	24 (0)	68.0
16	4 (0)	60 (0)	24 (0)	63.2
17	4 (0)	60 (0)	24 (0)	66.2
18	4 (0)	60 (0)	24 (0)	65.1
19	4 (0)	60 (0)	24 (0)	66.9
20	4 (0)	60 (0)	24 (0)	64.0
21	4 (0)	60 (0)	24 (0)	65.1
22	4 (0)	60 (0)	24 (0)	65.0
23	4 (0)	60 (0)	24 (0)	65.4

^aValues in parentheses are the coded levels of independent variables.

2.5. Simulated gastric digestion

Simulated gastric digestion was carried out as Moreno, Mellon, Wickham, Bottrill, and Mills (2005) described. The simulated gastric fluid (SGF) was 0.15 M NaCl solution whose pH was adjusted to 2.5 with 1 M HCl. WPI and WPI-oligoisomaltose were dissolved in SGF (3 mg mL^{-1}). After incubation at 37°C for 15 min, pepsin (Amresco, activity: 3000 U/mg) was dissolved in SGF (0.32%) and then added to SGF at an approximately physiological ratio of enzyme/substrate (1:20, w/w). The digestion was performed at 37°C for 2 h. Samples were taken at 0, 2.5, 5, 10, 20, 30, 60 and 120 min for further analysis.

2.6. Free amino acids analysis

Free amino groups were measured using trinitrobenzene sulfonic acid (TNBS) method (Adler-Nissen, 1979). The value transformed into μmol of Leu mL^{-1} using a calibration curve within the range 0.25–3.5 μmol .

3. Results and discussion

3.1. Assessment on models of antigenicity of β -LG for three independent variables

WPI and oligoisomaltose occurred Maillard reaction under the conditions as the experiment design (Table 2). The antigenicity of β -LG in WPI-oligoisomaltose was shown in Table 2. From regression analysis on 23 experiments (Table 3, full model), the results revealed that several terms were not significant ($P > 0.05$). The non-significant terms were eliminated step by step from the regression model in the procedure of fit the full second-order model. After this procedure, there were six regression terms in the second-order model for β -LG (Table 3, fitted model). The model of predicting the antigenicity of β -LG under different Maillard reaction conditions was as follow:

$$Y = 70.73 - 9.20X_1 - 42.51X_2 - 21.84X_3 + 9.53X_1^2 + 12.83X_2^2$$

The P value of fitted model was 0.0001 and the adjusted R^2 of fitted model was 81.66%. The P value was very small and the adjusted R^2 was high, so the second-model was highly significant. The conclusion was that the relationship between response and independent variables was factual.

3.2. Effect of Maillard reaction conditions on antigenicity of β -LG in WPI-oligoisomaltose conjugates

The regression coefficients were shown in Table 3. The highly significant ($P < 0.01$) effects on the antigenicity were temperature and time of linear effects. The significance ($P < 0.05$) effect on the antigenicity was temperature² of quadratic effects. Temperature was the most important effect on the antigenicity because of

Table 3. Regression coefficients for the regression prediction model of the antigenicity of β -LG.

	Full model		Fitted model	
	b-coefficient	p	b-coefficient	p
Intercept	65.96		70.73	
Linear				
Weight ratio	-9.20	0.075	-9.20	0.088
Temperture	-42.51	0.000	-42.51	0.000
Time	-21.84	0.001	-21.84	0.000
Quadratic				
Weight ratio ²	9.58	0.049	9.53	0.059
Temperture ²	12.89	0.012	12.83	0.015
Time ²	7.93	0.095		
Interactions				
Weight ratio $\times$ temperature	-3.83	0.548		
Weight ratio $\times$ time	-4.83	0.451		
Temperature $\times$ time	9.24	0.161		
Other statistics				
R^2	83.95%		81.66%	
F	13.79	0.0001	20.59	0.0001

its highest regression coefficient among the three independent variables. The optimal values of three independent variables were as follows: oligoisomaltose/WPI weight ratio = 4.7, reaction temperature = 68.4 °C, reaction time = 29.0 h. In theory, on the optimal reaction conditions, the antigenicity of β -LG should be the lowest. The predicted lowest value was 30.99 $\mu\text{g mL}^{-1}$. The antigenicity of β -LG in WPI was 272.4 $\mu\text{g mL}^{-1}$. So it could be proved that glycosylated WPI with oligoisomaltose was an effectively method to reduce the antigenicity of β -LG.

The effects of Maillard reaction conditions on the antigenicity of β -LG were shown by the response surface plots in Figure 1. Within the scope of this study, the antigenicity of β -LG decreased at first and then increased with the oligoisomaltose/WPI weight ratio increasing. The impact of weight ratio on the antigenicity was weaker than the other two reaction conditions. As the reaction temperature changed in the range showed in Figure 1a and c, the higher the temperature, the lower the antigenicity. When the temperature was high enough, increasing the temperature did not significantly reduce the antigenicity. The antigenicity of β -LG first decreased and then changed little as time expanded in the range showed in Figure 1b and c. van de Lagemaat et al. (2007) reported that heating for longer than 1h of soy protein isolate with fructooligosaccharides did not significantly reduce the antigenic response. It was similar to the results of this study.

During the glycosylation process, some factors could cause changes in antigenic epitopes, thus affected the antigenicity of the protein. Glycation of proteins occurred by reducing sugars with free amino groups in proteins to form the Schiff's base linkage (Singh, Barden, Mori, & Beilin, 2001) and this chemical reaction could cause reduction of antigenic epitopes. Heat treatment could affect the structure of the protein, such as exposure of hidden SH-groups, polymerisation and cross-linking (Oldfield, Singh, Taylor, & Pearce, 1998), which resulted in changes of antigenic epitopes. Thereby increasing the reaction time and temperature might promote binding of WPI and oligoisomaltose, but it might also promote changes in protein structure. By analysing the effects of reaction conditions on the antigenicity of β -LG, it could be concluded that with the increment of oligoisomaltose/WPI weight

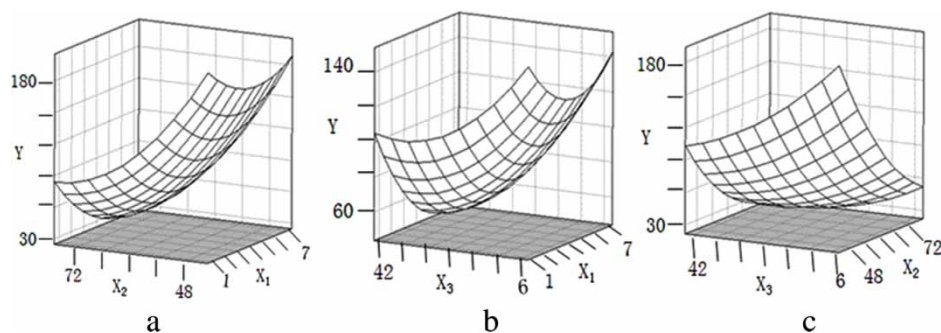


Figure 1. Response surfaces of the antigenicity of β -LG (Y): (a) effect of oligoisomaltose/whey protein isolate (O/WPI) weight ratio (X_1) and temperature (X_2) on antigenicity at fixed time of 24 h; (b) effect of O/WPI weight ratio (X_1) and time (X_3) on antigenicity at fixed temperature of 60 °C; (c) effect of temperature (X_2) and time (X_3) on antigenicity at fixed O/WPI weight ratio of 4:1.

ratio, reaction temperature and time, the antigenicity would not reduced infinitely. Therefore, optimisation of reaction conditions was necessary.

3.3. The free amino acid in natural and glycated WPI during simulated gastric digestion

Table 4 showed the free amino acid changes of WPI and WPI-oligoisomaltose which obtained under the optimal Maillard reaction conditions during simulated gastric digestion. The free amino acid increased within 20 min in the beginning and then changed little from 20 min to 120 min. After 120 min of pepsin hydrolysis, the free amino acid of WPI increased from $1.568 \text{ mmol L}^{-1}$ to $1.943 \text{ mmol L}^{-1}$, with an increment of $0.375 \text{ mmol L}^{-1}$, and the free amino acid of WPI-oligoisomaltose increased from $0.573 \text{ mmol L}^{-1}$ to $0.996 \text{ mmol L}^{-1}$, with an increment of $0.423 \text{ mmol L}^{-1}$. Compared with natural WPI, WPI-oligoisomaltose was more susceptible to pepsin hydrolysis. But Chevalier, Chobert, Dalgalarondo, Choiset, and Haertlé (2002) stated that β -LG became more resistant to digestive enzymes as a result of modifications evoked by Maillard reaction and it was contrast to the conclusions of this study. Perhaps the reason was that the sugars used in Maillard reaction were different. Marciniak-Darmochwal and Kostyra (2009) reported that glycosylation of pea extract changed the susceptibility of it towards peptic digestion. The degree of hydrolysis of raw pea extract was lower than pea extract glycated by fructose and pea extract glycated by glucosamine, but it was higher than pea extract glycated by glucose and pea extract glycated by lactose.

3.4. The antigenicity of α -LA and β -LG in natural and glycated WPI during simulated gastric digestion

The antigenicity of α -LA and β -LG in the hydrolysates during simulated gastric digestion (Table 4) were determined. The antigenicity of α -LA in digested natural WPI was decreased from 26.73 to $0.78 \text{ } \mu\text{g mL}^{-1}$. Moreover, the antigenicity of α -LA in digested WPI-oligoisomaltose almost decreased to zero. Although the antigenicity of β -LG in digested natural WPI was significantly lower than that of undigested, the former still exhibited a clear antigenicity of $42.83 \text{ } \mu\text{g mL}^{-1}$. After the 120 min pepsin hydrolysis, the antigenicity of β -LG in digested WPI-oligoisomaltose was $15.66 \text{ } \mu\text{g mL}^{-1}$. Therefore, highly allergic patients would still react to WPI-oligoisomaltose, but glycosylation might be a primary strategy for reducing the antigenicity of β -LG. The antigenicity of α -LA and β -LG in digested WPI and WPI-oligoisomaltose were all decreased fast in 20 min and then changed little from 20 min to 120 min. This was consistent with the trend of the changes of free amino acid during digestion. It might be because the epitopes were destroyed during pepsin hydrolysis, which could result in reduction of antigenicity.

4. Conclusion

Under the optimal Maillard reaction conditions (oligoisomaltose/WPI weight ratio = 4.7, temperature = 68.4°C , time = 29.0 h), the antigenicity of β -LG was efficiently reduced about 88.6% by glycosylation with oligoisomaltose. Compared with natural WPI, WPI-oligoisomaltose was more susceptible to pepsin hydrolysis.

Table 4. The free amino acid and the antigenicity of β -LG and α -LA in WPI and WPI-oligoisomaltose during simulated gastric digestion.

Time/min	Free amino acid (mmol L ⁻¹)		Antigenicity of β -LG (μ g mL ⁻¹)		Antigenicity of α -LA (μ g mL ⁻¹)	
	WPI	WPI-oligoisomaltose	WPI	WPI-oligoisomaltose	WPI	WPI-oligoisomaltose
0	1.568 $\pm$ 0.136 ^c	0.573 $\pm$ 0.032 ^f	241.68 $\pm$ 6.83 ^a	36.55 $\pm$ 0.04 ^a	26.73 $\pm$ 1.54 ^a	4.71 $\pm$ 0.9 ^a
2.5	1.569 $\pm$ 0.016 ^c	0.707 $\pm$ 0.010 ^e	106.88 $\pm$ 1.85 ^b	33.84 $\pm$ 0.20 ^b	25.37 $\pm$ 0.07 ^b	2.22 $\pm$ 0.25 ^b
5	1.618 $\pm$ 0.017 ^{bc}	0.780 $\pm$ 0.029 ^d	82.28 $\pm$ 3.95 ^c	25.49 $\pm$ 1.85 ^c	23.83 $\pm$ 0.31 ^c	1.92 $\pm$ 0.14 ^b
10	1.667 $\pm$ 0.014 ^b	0.857 $\pm$ 0.039 ^c	61.77 $\pm$ 1.15 ^d	20.84 $\pm$ 1.42 ^d	14.29 $\pm$ 0.91 ^d	0.72 $\pm$ 0.09 ^c
20	1.859 $\pm$ 0.027 ^a	0.933 $\pm$ 0.024 ^b	52.61 $\pm$ 2.82 ^e	19.68 $\pm$ 0.05 ^d	2.87 $\pm$ 0.04 ^e	0.16 $\pm$ 0.04 ^{cd}
30	1.874 $\pm$ 0.008 ^a	0.943 $\pm$ 0.022 ^b	51.00 $\pm$ 0.08 ^e	19.73 $\pm$ 0.95 ^d	2.63 $\pm$ 0.05 ^e	0.14 $\pm$ 0.04 ^{cd}
60	1.904 $\pm$ 0.002 ^a	0.970 $\pm$ 0.005 ^{ab}	49.85 $\pm$ 1.70 ^e	17.08 $\pm$ 1.17 ^e	2.30 $\pm$ 0.09 ^e	0.14 $\pm$ 0.03 ^{cd}
120	1.943 $\pm$ 0.038 ^a	0.996 $\pm$ 0.014 ^a	42.83 $\pm$ 4.40 ^f	15.66 $\pm$ 1.68 ^e	0.78 $\pm$ 0.07 ^f	0.03 $\pm$ 0.02 ^d

Values with common letters are not significantly different ($P > 0.05$).

After 120 min simulated gastric digestion, the antigenicity of α -LA and β -LG in glycated WPI were reduced to $0.03 \mu\text{g mL}^{-1}$ and $15.66 \mu\text{g mL}^{-1}$, respectively.

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