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REVIEW PAPER

Milk processing as a tool to reduce cow's milk allergenicity: a mini-review

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Abstract Milk processing technologies for the control of cow's milk protein allergens are reviewed in this paper. Cow's milk is a high nutritious food; however, it is also one of the most common food allergens. The major allergens from cow's milk have been found to be β-lactoglobulin, α-lactalbumin and caseins. Strategies for destroying or modifying these allergens to eliminate milk allergy are being sought by scientists all over the world. In this paper, the main processing technologies used to prevent and eliminate cow's milk allergy are presented and discussed, including heat treatment, glycation reaction, high pressure, enzymatic hydrolysis and lactic acid fermentation. Additionally, how regulating and optimizing the processing conditions can help reduce cow's milk protein allergenicity is being investigated. These strategies should provide valuable support for the development of hypoallergenic milk products in the future.

Keywords Cow's milk protein · Allergen · Milk processing · Control technologies

1 Introduction

Food allergy is a major public health concern worldwide. It has been estimated to affect around 1% to 2% of the adult population and 5% to 8% of children below the age of 3 (Eggesbo et al. 2001; Halmerbauer et al. 2002; Helm and Burks 2000). The most common food allergens are contained in eight foods, including cow's milk, eggs, fish, crustacean/shellfish, peanuts, soy, nuts and wheat, which account for over 90% of the occurrence of all serious allergic reactions to foods worldwide (Fritsche

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2003). Epidemiological studies have reported that cow's milk protein allergy (CMPA) was the most prevalent allergy for infants or young children, with an incidence of about 2% to 7.5% in population-based studies in different countries (Fiocchi et al. 2010).

Cow's milk proteins represent the first source of antigens encountered in large quantities in infancy (Isolauri and Turjanmaa 1996). Among these antigens, β-lactoglobulin (β-LG), α-lactalbumin (α-LA) and caseins are the main allergens in cow's milk; other proteins, such as bovine serum albumin (BSA) and even lactoferrin (present in trace amounts) are also potential allergens (Fritsche 2003; Sharma et al. 2001). The differences in the protein composition between cow's milk and human milk may be one of the reasons that induce cow's milk allergy of infants (Fox and McSweeney 1998). Moreover, the content of immunological materials is higher in human milk than in cow's milk, which can enhance the immune functions of infants and prevent milk allergy (El-Agamy 2007; Fox and McSweeney 1998; Guo 2001).

Cow's milk allergy is clinically an abnormal adverse reaction to cow's milk proteins regulated by immunological mechanisms. Many infants present skin, gastro-intestinal, respiratory and systemic anaphylactic symptoms of CMPA. More than two symptoms occur in the majority of infants with CMPA, and the severity of which varies from mild to life-threatening (El-Agamy 2007; Exl and Fritsche 2001). Although this allergy can be outgrown in the first year of life, 15% of the affected children remain allergic.

Current measures of prevention and management of milk allergy rely on complete elimination of milk consumption. However, complete avoidance of cow's milk proteins is difficult because they are often present in many processed foods. Moreover, the elimination of cow's milk proteins will cause a nutritional inadequacy and may influence the growth of infants and children. To date, measures of controlling milk allergy by antigen elimination have not been satisfactory. Finding new and effective processing technologies to reduce the allergen content of cow's milk is therefore important for controlling milk allergy. The purpose of this article is to discuss the processing methods currently in use to control cow's milk allergy, and consequently provide some help for the development of hypoallergenic milk products for patients with CMPA.

2 Heat treatment

Heating is an important process in the manufacturing of most dairy products. During the heating processes, important structural and chemical changes in proteins occur, such as denaturation, aggregation and the Maillard reaction with other molecules. These alterations may have significant impacts on the antigenicity of milk protein allergens.

Among cow's milk proteins, casein is the most heat stable; however, the globular whey proteins are heat sensitive in the order of immunoglobulins (Ig) < bovine serum albumin (BSA) < β-LG < α-LA (Kleber and Hinrichs 2007). Heating milk at 120 °C for 15 min did not affect the antigenicity of bovine casein, but BSA and Igs lose their antigenicity at 70 to 80 or 100 °C (Fiocchi et al. 1998; Hanson and Mansson 1961). 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; however, no change was observed

for α-LA and caseins. Ehn et al. (2004) detected only a slight decrease in the IgE-binding ability of β-LG after heating β-LG solution or milk at 74 °C, whereas significant decrease was found at 90 °C by enzyme-linked immunosorbent assay (ELISA) inhibition studies.

Bu et al. (2009b) studied the effects of heat treatment on the antigenicity of α-LA and β-LG in whey protein isolate (WPI) via in vitro competitive ELISA inhibition tests with rabbit serum. They found that the antigenicity of α-LA and β-LG increased with increasing temperature from 50 to 90 °C. However, antigenicity of both proteins decreased remarkably above 90 °C. When treated at 120 °C for 20 min, the antigenicity of α-LA decreased by 25% compared with the initial value of the untreated sample. The increase in whey protein antigenicity from 50 to 90 °C may be caused by exposure of allergenic epitopes buried inside the native molecule due to the unfolding of conformational structure during heat denaturation (Kleber and Hinrichs 2007). It has also been shown that heat-denatured β-LG presents some new epitopes, which is not found in the native state (Davis and Williams 1998). The antigenicity decrease at above 90 °C can be attributed to the destruction or masking of conformational epitopes exposed to the surface of the molecule by sulfhydryl/disulfide exchange and subsequent aggregation (Kleber and Hinrichs 2007). Under more severe heating conditions, the Maillard reaction may lead to loss of linear epitopes and consequently reduced antigenic response (Davis and Williams 1998; Fritsche 2003). Kleber and Hinrichs (2007) reported that the antigenicity of β-LG in skim milk and sweet whey increased with increasing heating temperature from 50 to 80 °C, but declined above 90 °C with increasing heating temperature, which was determined by an indirect competitive ELISA.

In addition, the effect of heating on the allergenicity of milk proteins has been investigated in several in vivo studies. Rytkönen et al. (2002) showed that heat-denatured β-LG (heated at 90 °C for 30 min) induced more intensive local immunologic reaction in the gastrointestinal mucosa of rats than native β-LG. High heat (baking) reduces the allergenicity of several food proteins, presumably by altering the conformation of heat-labile proteins and thus destroying the allergenic epitopes (Thomas et al. 2007). Nowak-Wegrzyn et al. (2008) evaluated whether patients with milk allergy can tolerate extensively heated (baked) milk products. In their study, 100 children with milk allergy underwent heated milk challenges. Sixty-eight children (68%) tolerated the extensively heated milk, 23 reacted to the heated milk and 9 tolerated both the heated and the unheated milk. Heated milk-tolerant subjects had significantly smaller skin prick test wheals, lower milk-specific and casein specific IgE and lower IgE/IgG4 ratios to casein and β-lactoglobulin compared with the heated milk-reactive subjects.

The modification of milk protein allergens by thermal processing is influenced by many factors, including the composition of the whole milk, processing conditions, circumstances of exposure to the consumer and the genetic make-up of the individual (Davis et al. 2001). Therefore, the proper thermal processing conditions need to be controlled for developing the hypoallergenic milk products.

3 Glycation reaction

Conjugation with reducing sugars through the Maillard reaction is an effective means of improving the functional properties of proteins. Besides, it also seems to be a

promising method for masking food protein allergenicity (Babiker et al. 1998; Nakamura et al. 2008; van de Lagemaat et al. 2007). The conjugation of β-LG with carboxymethyl dextran (CMD) improved emulsifying properties, enhanced thermal stability and reduced immunogenicity of this protein (Hattori et al. 1994; Nagasawa et al. 1996). Conjugation with CMD was thought to induce the exposure of hydrophobic region(s) and enhanced flexibility of β-LG molecule, which would facilitate the interaction with oleic acid at the time of emulsification, thus the emulsifying activity of β-LG at neutral pH was much improved by conjugation with CMD (Hattori et al. 1994). Hattori et al. (2004) also found that β-LG–acidic oligosaccharide conjugates exhibited enhanced thermal stability and reduced immunogenicity. Their findings indicated that one of the mechanisms responsible for the reduced immunogenicity was the shielding of the B cell epitopes because of the conjugation. Furthermore, another study suggested that the suppressive effect on the generation of T cell epitopes by conjugation with CMD played an important role in the mechanism for the reduced immunogenicity of β-LG (Kobayashi et al. 2003).

Reduction of milk protein allergenicity by the Maillard reaction depends on the amount of saccharides conjugated to proteins and the molecular weights of the saccharides. Hattori et al. (2000a) found that β-LG–CMD conjugates with higher saccharide content showed marked low immunogenicity through the evaluation of a noncompetitive ELISA using antiserum from mice. Kobayashi et al. (2001) estimated the immunogenicity of β-LG–CMD conjugates with similar saccharide contents using CMD of different molecular weights. It was concluded that conjugation with CMD of higher molecular weight is effective in reducing the immunogenicity of β-LG, and the masking of epitopes by CMD is responsible for the decreased immunogenicity. Similar observations were made by Hattori et al. (2000b). They prepared three β-LG–cationic saccharide conjugates using the Maillard reaction and found that conjugation with high molecular weight cationic saccharides was effective in achieving multiple functional improvements of proteins, including reduced allergenicity (Hattori et al. 2000b).

Modification in the antigenicity of milk proteins is also associated with the reaction conditions and the degree of the Maillard reaction. The effects of the Maillard reaction conditions on the antigenicity of β-LG and α-LA in the conjugates of WPI with glucose were investigated using response surface methodology (Bu et al. 2009a, 2010a). The results showed that the conjugation of WPI with glucose effectively reduced the antigenicity of β-LG and α-LA via the in vitro the detection of competitive ELISA with rabbit serum. This reduction of antigenicity can be controlled by regulating three independent variables (weight ratio of protein to sugar, temperature and reaction time). The model for optimal reaction conditions of a lower antigenicity of β-LG and α-LA was established. Inhibition rates of the antigenicity of both whey proteins were over 90% under optimal reaction condition.

The allergenicity of milk proteins can be significantly reduced by conjugation with saccharides through controlled Maillard reaction. Further investigations should be conducted to elucidate the structural characteristics of conjugates and the mechanism of reduced immunogenicity. However, new epitopes may have emerged as a result of exposure of the hydrophobic region, buried in the native state because of the conjugation (Hattori et al. 2000a, b). Hattori et al. (2000b) reported that the emergence of novel immunogenicity was observed in the case of both β-LG–glucosamine (β-LG–GlcN)

and β-LG–chitopentaose (β-LG–CPO). Conformational changes upon conjugation with GlcN or CPO might have induced the emergence of novel epitopes in these conjugates.

4 High pressure

High pressure is one of novel processing techniques in food production. High-pressure treatment can give rise to structural changes in milk proteins, such as denaturation and formation of aggregates (Iametti et al. 1997). These changes may also influence the allergenic potential of milk proteins.

Kleber et al. (2007) showed that high-pressure treatment (200 to 600 MPa) at different temperatures (30–68 °C) could enhance the antigenicity of β-LG in the WPI-solution, sweet whey and skim milk. Another study indicated that high-pressure treatment of β-LG and WPI at 200 and 400 MPa increased the binding to β-LG-specific rabbit IgG and did not affect the binding to IgE from allergic patients (Chicón et al. 2008a). The increase in antigenicity may be associated with exposure of epitopes buried in the native protein molecule and becoming accessible for the antibodies due to pressure-induced unfolding and aggregation (Kleber et al. 2007).

Meanwhile, the changes in the conformation of proteins induced by high pressure may make enzymatic digestion easier. β-lactoglobulin can be efficiently hydrolyzed by various enzymes under high pressure (Chicón et al. 2006a, 2006b). The hydrolysates obtained via the enzymatic treatment of β-LG under high pressure may exhibit reduced antigenicity and IgE binding (Bonomi et al. 2003; Fritsche 2003; Peñas et al. 2006a). The effect of high-pressure treatment (100 to 300 MPa) on the hydrolysis of dairy whey proteins by trypsin, chymotrypsin and pepsin was studied (Peñas et al. 2006a). The results indicated that high pressure enhanced whey protein hydrolysis, and reduced the residual antigenicity of the hydrolysates, depending upon the choice of enzymes. An important decrease in immunoreactivity was found for pepsin and trypsin hydrolysates obtained under high pressure (200 or 300 MPa) compared with enzymatic treatment alone. Another study showed that the antigenicity of bovine whey protein hydrolysates obtained with Corolase PN-L and Neutrase was significantly reduced when combined with high-pressure treatment (300 MPa) prior to or during enzymatic hydrolysis, respectively (Peñas et al. 2006b).

Recently, López-Expósito et al. (2012) evaluated the in vivo allergenicity of β-LG hydrolysates obtained with chymotrypsin under atmospheric pressure or high-pressure conditions using a β-LG allergy mouse model. They showed that the tested hydrolysates lost allergenicity, as revealed by the absence of anaphylactic symptoms and a decrease in body temperature. In addition, they demonstrated that the peptides present in the hydrolysates lost their ability to cross-link 2 human IgE antibodies to induce mast cell degranulation, indicating that most of the peptides formed retained only one relevant IgE-binding epitope. Other studies have also reported that application of high pressure during enzymatic hydrolysis can effectively reduce the antigenicity and serum IgE-binding properties of milk protein hydrolysates (Beran et al. 2009; Chicón et al. 2008b; Peñas et al. 2006a, c). This reduction can be explained by an increase of accessibility of potentially immunogenic hydrophobic regions to the enzyme, thereby resulting in an improved hydrolysis (Bonomi et al. 2003). Therefore, using high pressure during

hydrolysis of milk proteins may be an efficient strategy for producing hypoallergenic whey hydrolysates.

5 Enzymatic hydrolysis

Proteolysis offers an efficient way to destroy allergenic epitopes (Heyman 1999). To reduce their allergenicity, proteins can be broken down by enzyme hydrolysis into small peptide molecules and amino acids. Proteolytic enzymes are widely distributed in animal, plant and microbial organisms. Some food-grade proteinases have been used to manufacture whey protein hydrolysates with reduced antigenicity.

In the process of hydrolysis, the differences in the types of enzyme, hydrolysis model and the degree of hydrolysis may result in some discrepancies in peptide composition and residual antigenicity of the hydrolysate, as well as the taste. Pahud et al. (1985) indicated that the antigenicity of whey protein can be decreased by hydrolysis with trypsin. Nakamura et al. (1993) showed that using papain and neutrase or alcalase, protease S, proleathe combination in hydrolysis was more effective in reducing the allergenicity of whey protein compared with single enzyme. In another study, the 'two-step' alcalase-papain hydrolysis process was the most effective in reducing the immunoreactivity of cows' milk whey protein although allergenic epitopes were still present. Addition of papain to whey protein concentrate (WPC) hydrolysates prepared with alcalase improved the sensory properties of the product obtained, especially reduced bitterness (Wróblewska et al. 2004). In addition, the antigenicity of WPC hydrolysates obtained with Alcalase can be effectively reduced by optimizing the hydrolysis conditions (pH, temperature and enzyme-to-substrate ratio) (Zheng et al. 2008).

The enzymatic digestion of protein may generate new antigenic substances. Haddad et al. (1979) detected serum IgE from allergic patients using radioallergosorbent tests with a total tryptic hydrolysate of β-LG even when no IgE response could be detected with native β-LG. Schmidt et al. (1995) determined the degree of hydrolysis by pepsin of bovine α-LA, β-LG, BSA and bovine immunoglobulin G (B-IgG) in the pH range 2–4 as well as the antigenic properties of the resulting hydrolysates. No differences were found in the antigenic properties of the hydrolysates at pH 2 or 3, however, at pH 4 a decrease in pepsin hydrolysis resulted in enhancement of antigenicity of all proteins except β-LG. Ena et al. (1995) showed that enzymatic proteolysis may also increase protein antigenicity by exposing more antigenic sites during hydrolysis with Corolase 7092 as observed for BSA and B-IgG. In another study (Selo et al. 1999), it was shown that tryptic hydrolysis retained and even enhanced the allergenicity of β-LG, because the derived peptides were capable of a specificity to bind human IgE by ELISA assays. Their results also indicated that numerous epitopes are widely scattered all along the β-LG molecule. They may be located in hydrophobic parts of the molecule, inaccessible for IgE antibodies in the native conformation of the protein but become bio-available after digestive processes (Selo et al. 1999).

The molecular weight of the peptides obtained after hydrolysis is often analyzed for investigating the residual allergenicity. The allergenicity of peptides of molecular weight 3,000–5,000 Da has been described by Van Beresteijn et al. (1994). For peptides of molecular weight smaller than 3,000 Da, there is no agreement about

their allergenic character. The components of the whey in the hypoallergenic formulas (HF) were fractioned according to their molecular weight and analyzed by an enzyme-linked immunoaffinity chromatography method, showing that peptides of molecular weight less than 3,000 Da were antigenic and are probably allergenic (Puerta et al. 2006). Van Beresteijn et al. (1994) showed that the 3,000 Da permeate of a Corolase 7092 WPC hydrolysate did not elicit an IgE-mediated allergic reaction. Van Hoeyveld et al. (1998) reported that an ultrafiltrated whey hydrolysate was fractionated in different molecular weight fractions using fast protein liquid chromatography. In addition, peptides with molecular masses above 2,600 Da elicited a clearly positive skin response and inhibited IgE-binding, whereas peptides below 1,400 Da did not provoke any positive skin response, but were still able to partly inhibit IgE-binding to the hydrolysate. In another study (Calvo and Gómez 2002), peptides of molecular weight 500 Da of two HF reacted with the sera of babies allergic to milk proteins by immunodotting assays. The disagreement found in the literature may be due to the hydrolysis process of proteins used, and to the sensitivity of the patient against the allergen (Puerta et al. 2006).

Other studies indicated that the specificity of enzyme, rather than the degree of hydrolysis or molecular mass distribution of hydrolysates, determines the residual antigenicity of whey protein (Ena et al. 1995; Svenning et al. 2000). Ena et al. (1995) found that a filter with molecular mass cut-off 3,000 Da decreased residual antigenicity of the Corolase 7092 WPC hydrolysate, but had little effect on the pepsin/carolase PP hydrolysate. Peptides still have allergenicity if epitopes exist, although their molecular masses may be very low (Wal 2001). This result suggests that it is crucial to choose the appropriate enzyme having specificities in antigenic epitopes to effectively decrease the allergenicity of proteins.

It has been shown that combining of enzymatic hydrolysis with preceding heat treatment considerably enhanced the tryptic and peptic hydrolysis of the major milk protein (α-LA and β-LG) and thereby reduced the allergenicity of milk (Bertrand-Harb et al. 2002; Peyron et al. 2006). This result can be explained by the possible exposure of cleavage sites resulting from thermal denaturation and the increase in the susceptibility of protein to proteolysis. In addition, application of microwaves combined with enzymatic hydrolysis has been reported in several studies (El Mecherfi et al. 2011; Izquierdo et al. 2007, 2008). El Mecherfi et al. (2011) investigated the effect of combined microwave and enzymatic hydrolysis on the human immunoglobulin E (IgE)-binding properties of β-lactoglobulin and bovine whey proteins. They found that microwave treatment enhanced the hydrolysis rates of β-lactoglobulin and bovine whey proteins compared with the same proteolytic treatment realized under conventional heating. Microwave treatment at 200 W enhanced the hydrolysis of β-lactoglobulin by pepsin in 3 min and significantly decreased its immunoreactivity.

Whey proteins and peptides derived from the enzymatic proteolysis of whey proteins can modulate a variety of immune functions, including lymphocyte activation and proliferation, cytokine secretion, antibody production, phagocytic activity, and granulocyte and natural killer cell activity (Gauthier et al. 2006; Saint-Sauveur et al. 2008). Duan et al. (2012) reported that mice sensitized by tryptic hydrolysates of β-LG showed a significantly lower spleen lymphocyte proliferation level than intact β-LG. Moreover, the hydrolysates of β-LG significantly up-regulated IFN-γ and IL-10 production and down-regulated IL-4 and IL-5 secretions by murine splenocytes. These results suggested

that the enzymatic hydrolysis could partly reduce the allergenicity of β-LG. Prioult et al. (2005) studied the allergenicity of acidic peptides from bovine β-LG by hydrolysis with *Bifidobacterium lactis* NCC362 enzymes, and their results indicated that the peptide fragments significantly up-regulated IFN-γ and IL-10 production and down-regulated IL-4 secretion by murine splenocytes. The β-LG-derived peptides released after *B. lactis* hydrolysis could therefore be used as supplement in hypoallergenic infant formulas to stimulate oral tolerance induction to β-LG in babies at risk of allergy.

The hydrolysate formula was developed to reduce the allergenicity of cow's milk proteins. The first of the partially/moderately hydrolyzed formulae (Beba HA, Good Start, NAN HA, Nestlé) was introduced in 1985 (Exl 2001). To date, many hypoallergenic formulas are available on the market in the form of partially and extensively hydrolyzed whey or casein, as well as amino acid-derived preparations. The extensively hydrolyzed casein-based formula can be safely used for feeding children with IgE-mediated cow's milk allergy (Terheggen-Lagro et al. 2002). According to the results of a randomized controlled study, only an extensively hydrolyzed formula, and not a partially hydrolyzed formula, significantly decreased the prevalence of cow's milk allergy (Businco et al. 1999). However, partially hydrolyzed formulas may be useful in the primary prevention of cow's milk allergy in high-risk infants (Chan et al. 2002) because their taste and nutritional value may be better than those of an extensively hydrolyzed formula (Blecker 1997; Exl 2001).

6 Lactic acid fermentation

Proteolytic enzymes can be produced during fermentation by lactic acid bacteria (LAB). The proteolytic systems of LAB are complex and are composed of proteinases, peptidases and transport systems (El-Ghaish et al. 2011a). The hydrolysis of milk proteins by *Lactobacillus* fermentation may have important effects on milk digestibility and the production of bioactive peptides. Proteolysis can cause some epitopes to break and may decrease milk allergenicity (Bertrand-Harb et al. 2003; Cross et al. 2001). Moreover, probiotics have beneficial effects on immune-mediated diseases, including stimulating the immune system and decreasing the prevalence of atopy (Cross et al. 2001; Kalliomäki et al. 2003). Clinical reports have suggested that dietary consumption of fermented foods, such as yogurt, can alleviate some of the symptoms of atopy and might also reduce the development of allergies, possibly via a mechanism of immune regulation. Controlled studies indicated that consumption of fermented milk cultures containing LAB can enhance production of Type I and Type II interferons at the systemic level (Cross et al. 2001). Probiotic bacteria such as *Lactobacillus* GG may promote endogenous barrier mechanisms in patients with atopic dermatitis and food allergy, and may act as a useful tool in the treatment of food allergy by alleviating intestinal inflammation (Majamaa and Isolauri 1997).

Many studies have demonstrated that *Lactobacillus* fermentation can induce degradation of milk allergens. Twenty-one *Lactobacillus* strains from traditional Bulgarian yogurts have been reported to display different proteolytic activities toward α-LA and β-LG based on electrophoresis and RP-HPLC analysis (Tzvetkova et al. 2007). Phromraksa et al. (2008) identified nine proteolytic bacteria from Thai traditional fermented foods, and found that the concentrated crude enzyme of *Bacillus*

subtilis DB can digest β-LG and reduce the allergenicity of β-LG by means of SDS-PAGE and immunoblotting analysis. Several recent studies have revealed that different LAB strains can reduce the antigenic properties of milk proteins. Pescuma et al. (2011) showed that *Lactobacillus delbrueckii* subsp. *bulgaricus* CRL 656 degraded pure β-LG and its epitopes, thus reducing their recognition by the IgE of allergic children using ELISA competitive test. Similarly, IgE binding of α_{S1}-casein and β-casein can be significantly reduced by proteolytic activity of *Lactobacillus fermentum* IFO3956 and *Lactobacillus helveticus* A75 (El-Ghaish et al. 2011b; Ahmadova et al. 2012).

Changes of milk protein antigenicity and allergenicity depend on the species of LAB and conditions of fermentation. Kleber et al. (2006) found that lactic acid fermentation can attenuate β-LG antigenicity in skim milk and sweet whey. More than 70% and 90% reduction in antigenicity of sweet whey and skim milk, respectively, compared with untreated samples were detected. Synergism in the reduction of the antigenicity was observed using 1:1 mixtures of lactic acid bacteria with *Streptococcus thermophilus* subsp. *salivarius* strains. Bu et al. (2010b) indicated that the fermentation with lactic acid bacteria can significantly reduce the antigenicity of α-LA and β-LG in skim milk by the indirect competitive ELISA using rabbit serum. Combined strains of *L. helveticus* and *S. thermophilus* were the most effective in reducing the antigenicity of α-LA (inhibition rate 87%) and β-LG (inhibition rate 95%). Jedrychowski and Wroblewska (1999) reported that antigenicity of whey proteins in sterilized cow's milk was reduced by over 99% compared with raw milk after fermentation with selected LAB. However, their allergenicity in skin tests was not eliminated, only slightly attenuate. In another study (Ehn et al. 2005), fermentation with lactobacilli did not give a significant decrease in the IgE binding ability, even though chromatography data showed a gradual degradation of β-LG. This suggests that the extracellular proteolytic activity in the fermentation process did not extensively degrade the IgE epitopes. The degradation might only be partial, leaving peptides long enough to bind the antibodies. It is also possible that there is a greater access to some buried epitopes (Ehn et al. 2005).

The reduction in antigenicity suggested that during the fermentation process, some epitopes of milk proteins were destroyed because of the hydrolysis of proteolytic enzymes from *Lactobacillus*. The difference of antigenicity reduction from the aforementioned studies may be due to the diversity in the hydrolytic ability and the specificity of the proteinases from different LAB strains. These results are very useful for the preparation of new fermented milk products with reduced antigenic properties.

7 Conclusions

Cow's milk is a high nutritious food, especially for infants. However, cow's milk proteins are also a major food allergen, and tend to induce allergic reactions in infants. Prevention of milk allergy is an urgent problem all over the world and needs to be resolved through combined efforts of nutritionists, food scientists and physicians. Some processing technologies (glycation, enzymatic hydrolysis and lactic acid fermentation) can be used to effectively reduce the allergenicity of milk proteins by controlling and optimizing the processing conditions. However, a combination of different technologies may be crucial for reducing cow's milk allergy. Considering the risk of appearance of

some new epitopes during processing, attention should be paid during modification of milk proteins. Moreover, animal and human tests should be carried out to further detect the residual allergenicity of proteins and ensure the edible safety of milk products obtained by processing technologies in the future. These strategies should provide valuable support for the development of the hypoallergenic milk products.

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