Cow’s Milk: a Food and a Potential Source of Allergens

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ABSTRACT

Cow’s milk is an exclusive food of newborn infants when breast-feeding is not possible. It is also considered to be an important dietary source for adult humans. Because of its richness in essential amino acids it can be of significant nutritional value. In addition, the hydrolysis of certain compounds releases bioactive peptides which have important biological properties. However, in some subjects, these peptides can be potentially allergenic and cause an adverse immunologic response, known as cow’s milk allergy (CMA). Pathophysiological mechanisms involved in CMA are still not yet completely understood. A possible solution can be obtained by the use of extensively hydrolyzed formulae (enzymatic hydrolysis and/or heating and ultrafiltration) or by other technological methods (bacterial fermentation, microwave heating and gamma irradiation). These formulae show a reduced but never complete abolishment of antigenicity/allergenicity. This article attempts to present a review on main nutritional characteristics of cow’s milk and impact of various technological means on antigenicity/allergenicity properties.

Keywords: antigenicity/allergenicity, bacterial fermentation, cow’s milk proteins, microwave, gamma radiation

Abbreviations: α-La, α-lactalbumin; β-Lg, β-lactoglobulin; AAP, American Academy of Paediatrics; ACE, Angiotensin I converting enzyme; CMA, cow’s milk allergy; CMP, Cow’s milk proteins

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INTRODUCTION

Mammals produce milk which ensures nursing and the survival of newborn offspring. In most, lactation is vital for reproductive success as well as for the production of gametes. However, humans are able to go without lactation and consume the milk of many species. Many animals such as cows, goats, sheep, buffaloes, camels and mares are exploited to produce milk for human consumption. The cow is the most important of all these animals as a source of milk. Bovine milk is a part of the adult diet and a protein source commonly used for the manufacture of infant formula. It is the most commonly used milk due its ready availability. However, bovine milk consumption is not without risk because it may be one of the common causes of childhood allergy. This paper provides an overview of current areas of research with regard to the biological and allergenic properties of bovine milk and focuses on strategies used to modify milk proteins’ allergenicity.

COW’S MILK

Milk is a complex nutritious product that contains typically water (87.3%), fat (3.9%), proteins (3.2%), carbohydrates (4.6%) and quantitatively minor components. The composition of milk varies considerably with the breed of cow, stage of lactation, feed, season of the year and many other factors. Milk fat is a source of energy, essential fatty acids and fat
soluble vitamins. It is present in milk in small globules suspended in water. Each globule is surrounded by native biological native membrane composed mainly of phospholipids, proteins, cholesterol, glycoproteins and vitamins. Milk fats are composed mainly of triglycerol which accounts for 98.3% of milk fat, 0.2-1% phospholipids, 0.2-0.4% free sterols, traces of free fatty acids and a varying amount of fat-soluble vitamins A, D, E and K. Milk is an excellent source of water-soluble vitamins such as thiamin, riboflavin, pantothenic acid and vitamins B_6 and B_12.

Milk proteins are a principal source of nitrogen and essential amino acids. Caseins and whey are the main groups in milk. Caseins (80%) are isolated from milk by acid or by rennet precipitation. Their isoelectric precipitation is performed at pH 4.6 where whey remains soluble. The principal carbohydrate in milk is lactose which accounts for about 45% of milk calories which depends mostly on its fat content. Whole milk provides approximately 701 Kcal/L. Milk minerals account for approximately 0.7% of total milk powder and include major minerals such as calcium, phosphorus and magnesium which are needed at 100 mg or more daily and trace elements such as iron, zinc, iodine, copper, manganese, selenium and fluoride which are needed at a few milligrams daily. Moreover, it is a source of salts or electrolytes (<0.1 μg) such as chloride (103 mg/100 ml), potassium (138 mg/100 ml) and sodium (58 mg/100 ml) (Gaucheron 2005).

**COW’S MILK PROTEINS (CMP)**

**Caseins**

Caseins exist primarily as calcium phosphate stabilised micellar complexes. The casein fraction comprises predominately four proteins (α_s1, α_s2, β, and κ-caseins) with little primary structure homology. Caseins contain no disulphide bonds and possess an unusually high content of proline residues which cause particular ‘bending’ of proteins chains and inhibit the formation of a close-packed, ordered secondary structure. Since they do not contain any tertiary structure there is considerable exposure of hydrophobic residues. Casein micelles function as a carrier for a large amount of highly insoluble Ca (more than 85%) and P and enhance their bioavailability in intestinal absorption by keeping them soluble and preventing precipitation (Hoffman and Falvo 2004).

**Whey proteins**

Whey proteins, which comprise approximately 20% of the total milk proteins, are a heterogeneous group of essentially globular proteins. The globular proteins have a high level of secondary (α helix and β-sheeted sheets), tertiary and quaternary structure. These globular proteins are more water soluble than caseins. Whey proteins are made up of major and minor proteins. β-lactoglobulin (β-Lg), α-lactalbumin (α-La), bovine serum albumin and immunoglobulins are major constituents. Minor proteins include lactoperoxidase, lactoferrin, lysozyme, β-microglobulin and other small proteins. β-Lg, which is absent from human milk is considered to be the most prevalent protein in bovine whey. It comprises about 50% of the whey protein. It occurs naturally in the form of a dimer with 36 kDa. Each sub-unit contains 162 amino acids. There are 7 genetic variants and the most known are A and B. B. β-Lg possess two disulphide linkages and is a free sulphhydryl group. β-Lg belongs to lipocarnitins and functions as a transport molecule. It binds fat-soluble vitamins making them more available to the body. It is also an excellent source of essential amino acids and branched chain amino acids (BCAA) which are involved in the prevention of muscle breakdown. α-La is the second most prevalent protein. It consists of 123 amino acids and has a molecular weight of 14,146 kDa. The molecule contains 4 disulphide linkages and no phosphate groups. It has a highly ordered secondary structure and a compact spherical tertiary structure. α-La of most other mammals shows a high degree of homology. It is an important source of essential amino acids: lysine, leucine, threonine, tryptophan and cystine. It exhibits a high affinity to metal ions, calcium in particular. It is a component of the lactose synthetase complex. α-La binds to galactosyl transferase promoting glucose bonding and facilitating the synthesis of lactose in lactating mammary gland (Pernyakov and Berliner 2000).

**Nutritional value**

Many animals are exploited to produce milk for human consumption. However, cow’s milk represents one of the greatest contributions to human nutrition. Milk produced for human consumption is commercially available as fluid milk or used in the manufacture of dairy products such as cheese and yogurt. A large variety of products result due to the availability of bovine milk (Hinrichs 2004). Milk has a high nutritional value; it is an important source of energy, high-quality protein, calcium, and riboflavin. Milk proteins have a high nutritional value compared to other proteins. However, the nutritive value of whey may be superior to that of caseins, because it presents amino acid profile superior to caseins (Séverin and Wen-shui 2006). Whey proteins provide exceptional nutritional quality because of their relatively high content of essential amino acids and good digestibility as quantified by the measure of protein efficiency (PER = 3.6), protein digestibility corrected amino acid score (PD-CAAS = 1) and biological value (BV = 104) (Bos et al. 2000). They have high lysine, tryptophan and cysteine content. Only methionine is slightly limiting when compared to the adult estimated requirement of essential amino acids.

Breast milk is the best nutrition of young. When it is not available it is often replaced by cow’s milk. However, because the total protein level, the ratio between casein and whey protein, and the level of individual proteins differ markedly between human milk and cow’s milk. In infant formulas the composition of cow’s milk proteins must be adapted to ensure adequate requirement of young. Compared to cow’s milk, breast milk is less rich on proteins (8-12 g/l) and percentage in casein is weaker (40% of total breast milk proteins) (Hamosh 2001).

**Functional properties**

Beyond its nutritional value, cow’s milk is a source of peptides with various biological effects. Indeed, major milk proteins (caseins, β-Lg, α-La and immunoglobulins) contain many biologically active peptides that may affect human health. These bioactive peptides can be generated in vivo through enzymatic hydrolysis during gastrointestinal process or released in vitro by a hydrolysis reaction using digestive, bacterial or fungal enzymes. Many of these peptides reveal multifunctional properties. They are potential modulators of gastrointestinal, nervous, cardiovascular and immune systems (Korhonen and Pihlanto 2006). Moreover, their preventive and therapeutic roles have been demonstrated by numerous studies (Yalcin 2006).

Caseins are an important source of bioactive peptides. The main effects reported are antihypertensive, anti-thrombotic and agonist and antagonist opioid activity. Angiotensin I converting enzyme (ACE) inhibitory peptides and calcium binding peptides have been identified in bovine α_s1 casein and κ casein hydrolysed by digestive enzymes. In addition, β and κ caseins generated ACE inhibitory peptides: Val-pro (vpp) and (Ipp) when fermented with Lb heveticus (Yalın 2001). The major opioid peptides are fragments of β-casein called β-casomorphin which exert antiserotony (antidiarrheal) action by enhancing water and electrolyte absorption and reducing intestinal motility. In addition, this bioactive peptide regulates appetite and has important effects on amino acid uptake (Martinez Augustin and Martinez de Victoria Muñoz 2006). Caseinophosphopeptides (CPP) prevent osteoporosis, anemia, dental caries and hypertension because they can bind minerals.
years, β-lg and α-la have also been shown to contain bioactive peptides. Opioid peptides (α and β-lactorphin) are generated from pepsin-hydrolysed α-La and from pepsin and trypsin-hydrolysed β-Lg, respectively (Pihlanto-Lepppala et al. 2000). Bioactive peptides derived from β-Lg using pepsin and chymotrypsin are f(15-20), f(102-105) and f(142-148).

Other peptides can also be obtained by hydrolysis of bovine β-Lg with thermolysin under non-denaturing and denaturing conditions. Among them, there are peptides f(58-61) and f(47-149) (Hernandez-Ledesma et al. 2006). These peptides show ACE inhibitory, opioid and hypcholesterolemic activities. Functional properties of minor proteins are also reported. Lactoferrin (Lf) has been observed to possess numerous bioactive properties. It exhibits antibacterial, antifungal, antiviral and antitumoral activities (Pan et al. 2006). Lactoperoxidase and lysosome cooperate with Lf to exhibit antibacterial activity. In addition, milk contains cytokines and growth factors which possess biological activity that influence both cell growth and immune function (Michaelidou and Steijs 2006). Folic acids, vitamins B_{12} and B_{12} have a protective role against cardiovascular and neurologic diseases and child birth defects. Nucleotides exhibit an anticarcinogenic effect by inhibition of cell proliferation and the activation of apoptosis.

Cow's milk allergens

Although β-Lg and caseins are the major cause of cow's milk allergies other proteins like α-La, bovine serum albumin and even lactoferrin present in trace amounts are potent allergens (Wal 2001). The allergenic properties of cow's milk protein have been studied and several immunoreactive epitopes have also been studied and characterised. Allergens contain both B cell and T cell epitopes. B cell epitopes are mostly conformational peptides and bind IgE antibodies. T cell epitopes which are linear peptides are able to induce type 2 lymphocyte response (Toseland et al. 2005). B cell and T cell epitopes of milk proteins were investigated by using tryptic and synthetic peptides and cyanoen bromide digested peptides. IgE epitopes of bovine milk proteins which have been studied by several groups have been usually analysed by using sera from allergic patients and also with the help of animal sera (Table 1). Major IgE epitopes of β-Lg have been identified in (41-60), (102-124) and (149-162) sequences (Sélo et al. 1999). The most allergenic epitope in α-La has been identified on the amino acid sequence (59-94) (Maynard et al. 1997). For caseins, the major allergenic epitopes are located in sequences (63-79) in casein αs1, (1-20) and (55-70) in casein αs2 and (14-23) in β-casein (Spuergin et al. 1996).

Cow's milk allergy

In some subjects, ingestion of milk can cause adverse reaction because their tolerance mechanism has failed. Adverse reactions to milk include cow milk intolerance and cow’s milk allergy (CMA). Intolerance is due to lactase deficiency. Cow’s milk allergy is defined as hypersensitivity reactions to milk proteins. Several cow’s milk substitutes have been investigated for feeding babies with cow’s milk allergy. Alternatives to milk include soy, rice, corn, and even lactoferrin present in trace amounts are potent milk allergies other proteins like /g302-La, bovine serum albumin and even lactoferrin present in trace amounts are potent protein antigens from the diet of the lacting mother can be given for non-sensitized at risk infants. Avoidance of cow’s milk with exclusive breast feeding has been suggested for infants at risk of allergy. Breast feeding is certainly the best way for preventing sensitization of babies to cow’s milk proteins, as induced oral tolerance and enhances IgA production (Lomeland 2003). It enhances natural defences and promotes immunoregulation (Zeiger 2007). However, its allergy protective role is controversial. Human milk can be an important source of dietary antigens in exclusively breast-fed infants. It has been shown that several protein antigens from the diet of the lacting mother pass to human milk. Some studies have shown that limiting the diets of the lacting mother may be successful in decreasing the allergic symptoms of the suckling infants (Chandra et al. 1989; Hattevig and Kjellman 1996). Based on these studies, the nutritional Committee from the American Academy of Paediatrics (AAP) and European Committees recommended exclusive and prolonged breast feeding in the first 6 months of life. When breast feeding is insufficient or not possible it is generally recommended to administer so-called “hypoallergenic infant formula”. In primary prevention, partially hydrolysed formula can be given for non-sensitized at risk infants. These formulas seemed to be better at inducing oral tolerance (Zuercher et al. 2006). Whereas, in secondary prevention extensively hydrolysed formula and amino acids formula are recommended for sensitized infants. Overall, it seems that extensively hydrolysed formula reduces allergic manifestations in early life in children at high risk.

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Epitopes’ sequences</th>
<th>Reference</th>
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<tbody>
<tr>
<td>β-Lg</td>
<td>97-108, 124-134</td>
<td>Ball et al. 1994</td>
</tr>
<tr>
<td></td>
<td>41-60, 102-124, 149-162</td>
<td>Adams et al. 1991</td>
</tr>
<tr>
<td>α-La</td>
<td>5-18</td>
<td>Adams et al. 1991</td>
</tr>
<tr>
<td></td>
<td>91-96</td>
<td>Hopp and Woods 1982</td>
</tr>
<tr>
<td>Casein</td>
<td>59-94</td>
<td>Maynard et al. 1997</td>
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Table 1 Some examples of cow’s milk epitopes.

Prevention of cow’s milk allergy

Avoidance of cow’s milk with exclusive breast feeding has been suggested for infants at risk of allergy. Breast feeding is certainly the best way for preventing sensitization of babies to cow’s milk proteins, as induced oral tolerance and enhances IgA production (Lomeland 2003). It enhances natural defences and promotes immunoregulation (Zeiger 2007). However, its allergy protective role is controversial. Human milk can be an important source of dietary antigens in exclusively breast-fed infants. It has been shown that several protein antigens from the diet of the lacting mother pass to human milk. Some studies have shown that limiting the diets of the lacting mother may be successful in decreasing the allergic symptoms of the suckling infants (Chandra et al. 1989; Hattevig and Kjellman 1996). Based on these studies, the nutritional Committee from the American Academy of Paediatrics (AAP) and European Committees recommended exclusive and prolonged breast feeding in the first 6 months of life. When breast feeding is insufficient or not possible it is generally recommended to administer so-called “hypoallergenic infant formula”. In primary prevention, partially hydrolysed formula can be given for non-sensitized at risk infants. These formulas seemed to be better at inducing oral tolerance (Zuercher et al. 2006). Whereas, in secondary prevention extensively hydrolysed formula and amino acids formula are recommended for sensitized infants. Overall, it seems that extensively hydrolysed formula reduces allergic manifestations in early life in children at high risk.

Treatment of cow’s milk allergy

The treatment of choice is complete avoidance of cow’s milk proteins. Several cow’s milk substitutes have been investigated for feeding babies with cow’s milk allergy. Alternative food includes milk from different animal species,
sized that are also allergenic. As shown by a recent study, these allergenic peptides may be hydrolysed by intestinal microbiota (Prioult et al. 2005). The reduction of antigenicity may be due to enzymes produced by different strains. Reduction in the antigenicity of preheated milk fermented by selected lactic acid bacteria was observed (Jedrychosw and Wroblewski 1999). Moreover, Lactobacillus bulgaricus and Streptococcus thermophilus seemed to be very effective regarding the reduction of β-Lg antigenicity in fermented sweet whey and skim milk (Kleber et al. 2006). As shown by recent work, the intestinal microbiota may reduce milk proteins antigenicity not only by hydrolysis of allergenic peptides but also by stimulating the immune system (Prioult et al. 2005). Thus, Lactobacillus rhamnosus prevents cow’s milk allergy probably by hydrolysis of specific allergens and/or by releasing peptides able to modulate immune system (Sütas et al., 1996). To inhibit isomeration of lactose, maillard reaction and protein denaturation in higher proportion compared to conventional heating, microwave heating is effective in reduction of milk antigenicity (Davis et al. 2005). Thus, microwave heating on milk antigenicity has been the subject of recent article. From this work it can be concluded that microwave heating is effective in reduction of milk antigenicity (Kaddouri et al. 2006) as well as the allergenicity.

**Hydrolysed infant formulas**

Hydrolysed formulas commercially available are generally obtained by caseins or whey enzymatic hydrolysis and heat processing and/or ultrafiltration. In these formulas, proteins can be extensively or partially hydrolysed depending on the extent of hydrolysis and ultrafiltration to which they are subjected to. Partially hydrolysed formula which is recommended for high risk infants seemed to have preventive effects against allergy. Extensively hydrolysed formulas should be used to avoid reactions in highly sensitized infants. However, residual allergenic potential can be found in partially and extensively hydrolysed whey or casein which may lead to anaphylactic reactions in some cases (Calvo and Gomez 2002).

**Effect of processing on cow’s milk allergens**

Numerous strategies addressing the problem of allergy include total or partial destruction of allergens in milk. Among these methods there are heating, enzymatic hydrolysis, high pressure and irradiation (Husband et al. 2001). These technical procedures mainly influence milk proteins and thus may affect protein antigenicity (Wal 2003). Milk processing can reduce antigenicity by destroying the epitopes (Davis et al. 2001). Nevertheless, its can enhance or have no effect on milk protein antigenicity. The effects of processing on milk antigenicity are strongly influenced by the kind and the degree of processing, condition of its environment and the protein.

**Effects of enzymatic hydrolysis**

Enzymatic hydrolysis is usually used to improve nutritional, functional and technological properties of foods. In dairy processing, it is mainly used in infant formula manufacture in order to reduce milk protein antigenicity. Commercially available proteases are of animal, vegetable or microbial origin. During protein proteolysis, amide bonds are cleaved and peptides and/or free amino acids are released. Conformational structure of protein is one of the factors that can influence its antigenicity. Caseins that have open and flexible structure are very susceptible to enzymatic hydrolysis. However, whey globular proteins are less susceptible to hydrolysis because their compact structure ensures limited access for protease. β-Lg is very stable against peptic digestion under acidic conditions, reflecting its high stability to gastric digestion (Breiteneder and Mills 2005). However, milk proteins i.e. β-Lg (Seló et al. 1999), α-La (Maynard et al. 1997) and caseins (Spurga et al. 1996) are very susceptible to trypsin and chymotrypsin. Proteolysis help eliminate certain epitopes (conformational and linear), depending on the enzyme specificity and the susceptibility of epitope to enzyme. Some work has shown that hydrolysis of milk proteins by trypsin and/or chymotrypsin decrease milk protein allergenicity (Kananen et al. 2000). However, when antigenicity is reduced it is always limited. The higher stability of milk allergens was described (Astwood et al. 1996). It is shown that hydrolysis generated peptides with different

**Effects of microwave heating**

Microwave heating is used as an alternative method for conventional heat processing of foods. It results from the conversion of microwave energy into heat by friction of vibrating water molecules due to rapid fluctuation in the electromagnetic field. This method provides significant advantages such as energy saving and the speed of heating with high nutritional quality. Several studies have been carried out on the effects of microwave treatment on milk vitamins, lactose, lipids, enzyme and whey proteins (Villamiel et al. 1996a; Valero et al. 2000). It was shown that microwaved milk exhibited isomeration of lactose, maillard reaction and protein denaturation in higher proportion compared to conventionally heated milks (Villamiel et al. 1996b). The effect of microwave heating on milk antigenicity has been the subject of recent article. From this work it can be concluded that microwave heating is effective in reduction of milk antigenicity (Kaddouri et al. 2006) as well as the allergenicity.
Effects of gamma irradiation

Gamma irradiation is used in food industry to improve the safety of food through the reduction of pathogenic bacteria and other microorganism and parasites. In practice, the dose used varies from 1-10 KGY according to the type of food and desired effect. This dose is safe for human consumption; it is without any nutritional or toxicological effects. The radiation energy used in food processing induces chemical changes. Effects of irradiation on proteins which are influenced by numerous factors (condition of irradiation, nature of protein) have been the subject of several studies. It has been shown that gamma-irradiation influences the ordered structure of protein molecules and their physicochemical properties (Gaber 2005). Chemical changes include fragmentation, cross-linking and aggregation. As shown by several studies, these structural changes may affect the antigenic and allergenic properties of proteins (Kum and Matsuda 1995; Byum et al. 2000). The studies which mainly focused on isolating milk allergens show a reduction in milk antigenicity and allergenicity due to the destruction of the binding epitopes of β-Lg and casein (Lee 2001). However, irradiation effects on the food matrix are more complex and may affect allergenicity differently. As shown by several studies, matrix food allergen seemed not to be reduced but rather to be enhanced by irradiation (Leszczynska et al. 2003). Gamma irradiation seemed to be ineffective in reducing milk allergenicity. This may be due to the fact that epitopes of milk protein were not likely altered. In addition, at higher doses (10 KGY), gamma irradiation may enhance milk allergenicity by unmasking or creating new epitopes.

Effect of combined processes

Milk proteins seem to be resistant to processing which may reduce but not abolish allergenicy. Intense treatment may rather enhance allergenicity and affect milk quality. A combination of proteolysis or hydrolysis with physical processes, such as heat treatment, microwaving, or high pressure, is used to improve the impact of processing on milk quality and allergenicity. It was shown that high pressure enhances β-Lg susceptibility to pepsin, trypsin and chymotrypsin (Iametti et al. 2002). Similar observations were obtained with the use of heat treatment combined with trypsin and chymotrypsin hydrolysis. However, both enzymes lose their activity at higher temperature (up to 65°C). Another study has reported that mettrypsin hydrolysis. However, both enzymes lose their activity at higher temperature (up to 65°C). Another study has reported that mettrypsin hydrolysis with thermolysin under denaturing temperature on the reduction of milk allergenicity. This may be due to the fact that epitopes of milk protein were not likely altered. In addition, at higher doses (10 KGY), gamma irradiation may enhance milk allergenicity by unmasking or creating new epitopes.

CONCLUSION

Cow’s milk is a complete nutritious food with exceptional nutritional and functional properties. It constitutes a useful substitute for breastfeeding when is insufficient or not possible. However, it can also be a potential source of biologically immune peptides for allergic infants. In this case, milk proteins must be avoided and use of so-called hypoallergenic formulas is recommended. However, none of the so far available formulas are completely safe. This reflects the stability of milk allergens and difficulties met in technological separation of residual allergens. A better knowledge of the basic structure of proteins can lead to a better understanding of their allergenicity. Moreover, probiotic use and a combination of processes may open novel ways to improve approach of allergenicity.

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