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Review Article

Characteristics of cow's milk proteins including allergenic properties and methods for its reduction

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ABSTRACT

Introduction: Composition and hygienic quality of milk determine its nutritional value and processing suitability. However, biological quality of milk depends on the content of bioactive components, which due to its health promoting properties have a positive effect on human health. Biologically active substances present in cow's milk include proteins, peptides, amino acids, sugars, vitamins, enzymes, sterols, phospholipids and fatty acids. Among these components, several proteins (lactoferrin, β-lactoglobulin, bovine serum albumin and casein) that inhibit cancer cell growth, deserve special attention. However, cow's milk contains also approximately 30 potentially allergenic proteins. The most common bovine milk allergens are casein fractions and β-lactoglobulin naturally not present in human breast milk.

Aim: The aim of this study was to analyze the available literature on the characteristics of cow's milk proteins as allergens that may cause food allergies and identify methods of reducing their immunogenicity.

Material and methods: On the basis of the available literature characteristics of cow's milk proteins and their effect on the occurrence of food allergy in human are presented. A review of the available methods of modification of animal proteins that may reduce its allergenicity was also conducted.

Discussion: It is possible to reduce cow's milk allergenicity in the production of dairy products by thermal, enzymatic and biotechnological techniques. The majority of subjects that demonstrate intolerance of bovine milk may safely consume fermented dairy products.

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Conclusions: Prevalence of food allergy is so high that it may be considered a disease of civilization. Therefore, attention should be paid to food technological processes that may eliminate or reduce allergenicity of cow's milk proteins.

1. Introduction

1.1. *Significance of human milk proteins*

Due to its nutritional value, human milk is considered the best nourishment for infants.[32] Composition of breast milk varies over the course of lactation. In the period of early lactation colostrum, that contains a lot more proteins than mature milk, is being produced; content of proteins in milk decreases with each month of lactation. Colostrum and then mature milk are the most important and difficult to replace nourishment for infants, perfect source of nutrients, such as protein, fat, calcium, phosphorus, magnesium, fat soluble and water soluble vitamins. It provides infants with all components necessary for their growth and development. It is also a source of bioactive proteins, i.e. lactoferrin, lysozyme, secretory immunoglobulin (IgA), vitamin B_{12}, lactalbumin, bile salt, κ-casein and β-casein, whose role is to increase the absorption of nutrients in gastrointestinal tract and stimulate immune system and defense mechanisms against pathogens. In gastrointestinal tract these proteins increase digestion and absorption of nutrients through inhibitors, such as trypsin inhibitor or other active enzymes. In addition, they are involved in stimulation of the immune response against pathogens. It is a complex set of defense mechanisms against bacteria and viruses through prebiotic effect that consists of creating an environment to promote growth of beneficial bacteria in the intestines, i.e. *Lactobacillus* or *Bifidobacteria*, and inhibiting growth of pathogenic intestinal flora, i.e. *Streptococcus mutans*, *S. pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*.[21]

Cellular immune factors found in breast milk include

- B cells, which increase concentration of antibodies against specific microorganisms,
- macrophages, which destroy microbes directly in child's intestines, produce lysozyme and activate other elements of the immune system,
- neutrophils, which may act as phagocytes by consuming bacteria in the gastrointestinal tract,
- T cells, which directly destroy infected cells or produce chemical transmitters that stimulate other elements of the immune system, proliferate in the presence of microorganisms that cause serious disease of a child, produce immune response-enhancing factors,
- secretory immunoglobulin A (SIgA), which binds to pathogens in the gastrointestinal tract and prevents its penetration through intestine to the tissues,
- vitamin B_{12}-binding protein, which reduces the amount of this vitamin required for bacterial growth,
- bifidogenic factor, which stimulates growth of *Lactobacillus bifidus* in child's intestines, contributing to displacement of pathogenic bacteria,
- fatty acids, which damage membranes of some viruses and destroy them,
- fibronectin, which increases macrophage activity against bacteria, facilitates regeneration of tissues damaged by intestinal immune response,
- gamma interferon, which increases the activity of immune cells,
- hormones and growth factors, which accelerate maturation of the intestinal epithelium, making it "tight" and resistant to penetration of pathogens and allergens,
- lactoferrin, which binds iron – trace element required for bacterial growth; reduced availability of iron inhibits growth of pathogens,
- lysozyme, which kills bacteria by damaging its cell walls,
- mucous substances, which adhere to bacteria and viruses and prevent contact with mucous membranes, and
- oligosaccharides, which bind with microorganisms and prevent them from contact with mucous membrane.[24]

1.2. *Characteristics of cow's milk proteins*

Similar to human milk, the main component of bovine milk that determines its nutritional value is protein (Table 1). Cow's milk proteins are a heterogeneous mixture and can

Table 1 – Main cow's milk proteins given by Bernatowicz and Reklewska.[3]

Protein	Content in milk (g/L)	Functions
Casein	28	Precursor of bioactive peptides, carrier of Ca, PO_4, Fe, Zn, Cu ions
β-Lactoglobulin	6.30	Allergenic protein, carrier of retinol, fatty acids, antioxidant
α-Lactalbumin	3.20	Immunomodulatory, antineoplastic, carrier of Ca, Zn, Mn, Co ions; involved in the synthesis of lactose
Lactoferrin	0.10	Antibacterial, antioxidant, antineoplastic, immunomodulatory, Fe absorption
Lactoperoxidase	0.03	Antibacterial
Lysozyme	0.0004	Antibacterial
Glycomacropeptide	1.20	Antiviral

be divided into two basic groups, i.e. caseins and whey proteins.[11,12,23]

1.2.1. *Caseins*

Caseins are the most abundant proteins of cow's milk. Their content varies in the range of 2.6%–2.8%, which represents approximately 79% of total milk proteins. Casein fractions differ in concentration, contents of phosphorus, amino acid composition, molecular weight and isoelectric point.[17] Casein from bovine milk may induce inflammatory reactions in mucous membrane of patients with celiac disease, which may be diagnosed by the presence of anti-prolamin antibodies. Therefore, casein epitopes might be responsible for clinical symptoms. There are five casein fractions in cow's milk: αs_1, αs_2, β, κ, and γ, which constitute 30%, 9%, 28%, 10% and 2% of the total amount of casein, respectively.[7,12]

αs_1-Casein – is a phosphoprotein with a molecular weight of 23 kDa and is present in milk in the amount of 12 –15 g/dm. In breast milk there is no structural and functional homolog of animal αs_1-casein. This is a major cause of immunogenicity of this protein in human and occurrence of cow's milk allergy.[12]

αs_2-Casein – its content in milk is relatively low (3 –4 g/dm^3). There are four known isomers with different amount of phosphoryl groups (from 10 to 13). It is composed of 207 amino acid residues and has a molecular weight of 25 kDa. In cattle there are four genetic variants, i.e. A, B, C and D, while the A variant is the most common.[12,39]

β-Casein – is a phosphoprotein (occurring in cattle) with a molecular weight of 24 kDa, composed of 209 amino acid residues and 5 phosphate residues. There are 12 genetic variants, while the most common are A_1, A_2 and B. In breast milk, homolog protein with similar structure and chemical properties as bovine β-casein was found. It is a particle consisting of 212 amino acid residues, i.e. 3 more than β-casein of animal origin.[12,29]

κ-Casein is the least phosphorylated and the only glycosylated casein in mammals' milk. In cattle, in its structure it contains galactose, *N*-acetylogalactosamine and *N*-acetyloneuraminic acid. It exhibits anticoagulant properties, prevents platelet agglomeration and serotonin secretion. Glicomacropeptide, released during its hydrolysis, reduces secretion of gastric acid and serum gastrin.[3]

γ-Casein – in bovine milk $\gamma_{1,}$ $\gamma_{2,}$ and γ_3 casein fractions have been identified, that are plasmin-induced β-casein products.[12]

1.2.2. *Whey proteins*

They constitute approximately 0.6% of milk composition. Whey proteins represent an important group with high nutritional and functional properties that have a positive effect on human body.[9,18,22,48] Whey proteins include β-lactoglobulin, α-lactalbumin, lactoferrin, lactoperoxidase, lysozyme, bovine serum albumin, immunoglobulins, transferrin, proteose–peptones.

β-Lactoglobulin (β-Lg). Allergenic protein present is the highest proportion among whey proteins (51% of total whey proteins). In 100 mL cow's milk there is 420 mg of it. It is a chain polypeptide comprising of 162 amino acid residues, including methionine. Molecular weight was estimated to be 18 kDa. Owing to the high sulfur content it has antineoplastic properties. It is a multifunctional protein, retinol carrier, binds Cu^{2+} and Fe^{2+} ions and inhibits autooxidation of fats. Food allergies associated with this protein may present even in 80% of population.[3]

α-Lactalbumin (α-La). It is a less allergenic protein than β-lactoglobulin and constitutes 22% of total whey protein. It is an albumin well soluble in water. Chain is built of 123 amino acid residues and has a molecular weight of 142 kDa. It also contains high amounts of cystine, lysine and particularly tryptophan, which is a precursor of serotonin. Serotonin, on the other hand, exerts influence on the central nervous system, improves mood and reduces stress. α-Lactalbumin participates in the formation of lactose and also binds metals, such as cobalt, magnesium and zinc. Serum albumins are characterized by high contents of sulfur amino acids, which positively affect cell regeneration and growth regulation processes.[2] Chemical composition of bovine and human α-lactalbumin bears a strong resemblance.[44]

Lactoferrin is a protein synthesized by secretory epithelial cells of the mammary gland. The main function of this protein is binding iron and transporting it to the intestinal vascular system. It is of particular importance in subjects with busy lifestyle and pregnant women. Lactoferrin supports immune functions, detoxification processes, and above all, has an antineoplastic effect by inhibiting the attachment of tumor growth factors.[24,44]

Lactoperoxidase and **lysozyme** are biologically active enzymes with antibiotic-like activity. Lactoperoxidase is an oxidoreductase with antibacterial function, viral growth inhibitor and antineoplastic agent. Lysozyme lyses bacteria in milk, particularly gram-positive bacteria. It has antiviral and anti-inflammatory properties.[43]

Bovine serum albumin (BSA) – similar to αs_1-, αs_2- and β-casein and α-lactalbumin it may also be a milk allergen. In 100 mL of cow's milk there is usually 20 mg bovine serum albumin, which has a pI of 4.7. This protein is inactivated at a temperature of 70°C–80°C. Among all cow's milk proteins probably only bovine serum albumin remains immunoreactive after heat treatment.[29,38] In cow's milk there are more than 100 proteins, each of which may theoretically cause hypersensitivity, known as cow's milk protein allergy (CMPA).

Immunoglobulins are antibodies produced in response to viruses, bacteria and animal antigens. They are directed against different antigens and occur in five classes: A, G, M, E and D, in serum, intestinal lumen and intestinal wall. They constitute approximately 90% of total milk globulin and play an important role in passive immunization of mammalian offspring. These are easily denatured proteins.

Transferrin is a glycoprotein similar to lactoglobulin. It has an iron-binding capacity. It transports iron from serum to tissues. In muscles, Iron is used for biosynthesis of cytochromes and myoglobin, whereas bone narrow uses it in the production of hemoglobin.

Proteose–peptones. They are present in minor amounts as polypeptides. They are the products of enzymatic degradation of casein and may be present as dipeptides. Protein fraction includes glutamylcysteine, which participates in synthesis of glutathione that has antioxidant properties and thus repairs and protects damaged cells.[25]

In milk, there are also fat soluble vitamins A, D, E and K. These vitamins stimulate immune response, regulate growth and vision. One L of milk provides 25% of the daily value for β-carotene and vitamin A, and 10% for vitamins D and E.[27]

1.3. Cow's milk protein allergy

Cow's milk contains approximately 30 potentially allergenic proteins. 1 dm^3 of milk includes about 30–35 g of it.[42,45] Even 66% of milk allergy is caused by β-lactoglobulin, 57% by casein, and significantly less by α-lactalbumin and bovine serum albumin (18%).[10,30] Antibodies do not react with the entire antigenic molecule but only with its part called epitope. One antigen may have several epitopes, which might be the same or different. Epitopes of allergenic molecules include, above all, immunodominant epitopes, which are the main targets of immune response. Allergy may be caused by direct contact between allergen and skin or mucous membrane or through bloodstream by absorption of allergen.[10]

Cow's milk allergy constitutes 10%–40% of food allergies. Food allergy is thought to result from disorders of immune response to food protein and develop due to the defect in oral tolerance. This problem concerns primarily children, less frequently adults. A change in diet that involves replacing breast milk with cow's milk is a risk factor for the development of milk protein allergy. This results in inhibition of the protective barrier of immunoglobulin A in infants, who do not yet produce it and receive it with mother's milk. Thus, breastfeeding protects against allergy only in infants with no genetic burden of this disease.[8,20,28,29,34,40,49]

2. Aim

The aim of this research was to gather the available literature related to the characteristics and evaluation of cow's milk proteins with particular reference to their allergenic properties that cause food allergies, and a review of the available methods of animal proteins modification that may reduce its allergenic properties.

3. Material and methods

This work is a monograph. In order to achieve this objective, scientific literature was gathered, categorized and properly used.

4. Discussion

4.1. Diagnosis of cow's milk allergy

Diagnosis of cow's milk allergy may involve skin and blood tests. In children, the majority of milk allergies does not present immediately but with some delay, which in turn rarely gives positive test results.[10] Specialized diagnostic tests for determining allergenicity of foods and its ingredients include skin prick tests (SPT), radioallergosorbent tests (RAST), immunoenzymatic tests (ELISA) and elimination/challenge tests.[10,48]

Skin prick test is particularly accurate in children. It diagnoses an IgE-mediated skin reaction in response to the administrated allergen, e.g. milk proteins. Immunoglobulin E is located on the surface of mast cells (mastocytes) present in the skin. Small drops of milk are placed on patients forearm to expose mastocytes on milk proteins. A wheal and flare reaction after 15 min may indicate allergic reaction. Skin tests may be considered reliable only in case of visible strong reaction to allergen. These tests might be used both in infants and older children.[10]

Blood tests for diagnosis of cow's milk allergy include radioallergosorbent test (RAST) and enzyme-linked immunosorbent assay (ELISA). Both tests are based on measurement of the level of IgE in serum. In RAST, milk allergen is attached to a solid phase, such as cellulose plate, and then incubated with radiolabeled anti-IgE, which enables measuring the total serum IgE. In ELISA, instead of radioisotopes, anti-IgE is linked with enzymes, activity of which is proportional to the amount of antigens present in the sample. Both tests are often used in parallel in order to receive more reliable results.[37,10]

Elimination/challenge tests contain gradual elimination of milk and dairy products from diet until resolution of symptoms. They are eliminated for 7–14 days prior planned challenge test. The purpose is to relieve symptoms, which at the next stage allows objective assessment of the results. Challenge test is the reintroduction of previously eliminated products and may be conducted only after asymptomatic state was reached and upon completion of response altering treatment (glucocorticoids and antihistamines).[19]

4.2. Cow's milk allergy accompanying symptoms

In the majority of cases symptoms of allergy are non-specific and difficult to diagnose. Many infants present with gastrointestinal symptoms or skin lesions (approximately 50%–60%), while respiratory symptoms occur in 30% of cases. In the majority of infants with diagnosed cow's milk allergy (75%–92%), symptoms include more than two organ systems.[47] Predominant symptoms of allergy involve:

- in the skin (immunodependent – atopic dermatitis, acute or chronic urticaria, angioedema; immuno-independent – rash),
- in gastrointestinal tract (immunodependent – nausea and vomiting, colic, diarrhea; immuno-independent – inflammatory bowel disease, eosinophilic gastroenteritis, transient enteropathy imitating celiac disease or protein-losing enteropathy), and
- in respiratory system (immunodependent – catarrh, asthma, laryngeal edema; immuno-independent – otitis media, pulmonary hemosiderosis).

Other symptoms may include systemic anaphylaxis, headache, anemia, thrombocytopenia, proteinuria or irritability.[14,47]

4.3. Effect of heat treatment on the allergenicity of milk proteins

Heating of food and food ingredients may cause changes in immunodominant epitopes, which in turn influences allergenic properties of proteins.[5] Food processing may lead to destruction of epitope structure or formation of new epitopes (neoallergens), resulting in changes in protein conformation. However, more often food processing is associated with the reduction of allergenic properties of allergens or lack of influence on its allergenicity.[35] Most frequently food processing involves thermal processes. Heat treatment uses hot air (e.g. baking, roasting) or aqueous conditions (e.g. cooking, blanching).[35] Thermal processes include two major categories: pasteurization and sterilization.

According to Wróblewska and Jędrychowski[46] the basic process used in dairy industry is heat treatment of milk, which has a significant influence on properties and structure of proteins by reduction of allergenicity and increased microbiological quality (Table 2). It was observed that heat treatment of milk may change allergenic potential of proteins, which leads to increased, reduced or similar allergenicity. Heat treatment has a different effect on various proteins found in cow's milk.[26] Jost et al.[16] observed that heating whey proteins at a temperature of 80°C and 90°C for 30 minutes may reduce contents of immunoglobulins with allergenic activity. Therefore, some manufacturers use denatured whey proteins for the production of hypoallergenic infant formulas.

4.4. *Changes in allergenicity of hydrolyzed proteins*

First enzymatic hydrolysates were prepared with the use of digestive enzymes: trypsin, pepsin, chymotrypsin, task of which was to imitate potential digestion processes and reduce intestinal activity and activity of enzymatic system in newborns.[26] It was proved that hydrolysis of β-lactoglobulin by trypsin alone or in combination with chymotrypsin and pepsin reduces its allergenicity, not eliminating it though. On the contrary, when using the combined method (enzymatic hydrolysis and heat treatment), allergenicity of β-lactoglobulin is significantly reduced.[4,6,26]

α- and β-Caseins also show sensitivity to trypsin (unlike immunoglobulins and serum albumin). Pepsin and α-chymotrypsin are considered the most effective combination of enzymes used for the reduction of allergenicity and act by selective proteolysis of both α-lactalbumin and β-lactoglobulin. Degree of hydrolysis of hydrolysates obtained in this way was 1%–20% and depended on enzyme combination and incubation time.[26]

Commercialization of milk protein hydrolysates led to the use of new types of enzymes, e.g. bacterial or fungal, which are more specific. Currently, production of hydrolysates involves the use of enzymatic preparations containing endo- and exo-peptidases, which improve taste by reducing bitterness and reduce allergenicity through hydrolysis of hydrophobic peptides responsible for the allergenic potential of proteins. Attempt was also made to increase the effectiveness of enzymatic hydrolysis by means of reactor systems, in which hydrolysis of proteins is continuous with the use of immobilized enzymes (endo- and exo-peptidases), instead of conventional batch reactors.[33,47]

An innovative technique of preparing hypoallergenic formulas involves combining hydrolysates with probiotics, which reduces allergic symptoms – such products are allergy

Table 2 – Efficacy of modification methods of properties and immunoreactive milk proteins according to Wróblewska and Jędrychowski.[46]

Type of technological process	Operating conditions	Immunoreactivity of raw milk, %		Efficacy of the process	Utility of the process in food industry
		α-Lactalbumin	β-Lactalbumin		
Thermal processes					
Pasteurization 1	90°C/15 s	46.12	30.44	low	v. high
Pasteurization 2	90°C/15 min	12.72	18.74	low	v. high
Microwave	98°C/2 min	1.37	12.86	medium	low
Ultrasound	52°C/60 min	0.88	6.42	high	low
Enzymatic hydrolysis with					
Alcalase	55°C/24 h	0.62	>100	low	low
Pepsin	37°C/24 h	10.25	4.78	medium	medium
Trypsin	55°C/24 h	15.47	50.20	low	low
α-Chymotrypsin	37°C/24 h	11.34	>100	low	medium
Rennet	55°C/24 h	1.09	25.11	medium	medium
Chemical modifications					
Conjugation with polyethylene glycol (PEG)	–	0.08	0.05	v. high	low
Use of lactic acid fermentation process					
Lactococcus lactis ssp. *lactis* 136	–	0.10	3.36	high	v. high
Lactococcus lactis ssp. *cremoris* 8F6	–	0.48	0.79	v. high	v. high
Lactobacillus delbruecki ssp. *bulgaricus* S11	–	0.11	3.30	high	v. high
Lactobacillus casei 2	–	0.56	2.18	high	v. high
Lactobacillus acisophilus 67L	–	0.09	1.46	v. high	v. high

treatment and prevention method.[41] Probiotics, such as *Lactococcus lactis*, *Lactobacillus rhamnosus* or *Bifidobacterium lactis* significantly reduce the severity of atopic dermatitis in breast-fed infants after 2 months of treatment. Probiotics probably participate in mucosal degradation of macromolecules, leading to reduced allergenicity.[1,31,15]

Effect of high pressure on the efficacy of changes in allergenicity of enzymatic hydrolysates was also a subject of research. It was demonstrated that significantly higher degree of hydrolysis is obtained in high pressure (600 MPa), in comparison to atmospheric pressure. This phenomenon is explained by increased enzyme availability of immunogenic hydrophobic areas, which as a result intensifies hydrolysis.[6,28,36]

Sazhinov et al.[36] obtained enzymatic hydrolysates from skimmed milk and whey, which demonstrated 30%–40% lower allergenicity compared with substrates. Ziajka and Dzwolak[48] state that modification of milk by production of enzymatic hydrolysates that have advantageous immunoreactive properties, must be performed in the appropriate conditions. If protein degradation is too deep, peptides formed are characterized by unpleasant, bitter taste.

4.5. *Changes in allergenicity of homogenized proteins*

There are cases where children tolerate pasteurized milk, while unpasteurized homogenized milk causes allergic reactions. It is explained by the fact that during homogenization process milk fat significantly increases its surface, which allergenic milk proteins can be absorbed to. In unprocessed milk numerous allergenic proteins are located inside casein micelles, hence, in homogenized milk exposition of these allergens is increased. Nevertheless, clinical studies show that in terms of allergy, there are no significant differences between homogenized and unhomogenized milk, since unprocessed milk contains sufficient amount of proteins and exposure to cause allergic reactions.[13,22,47]

It seems that in animal models homogenization promotes food hypersensitivity. The use of homogenization and pasteurization increases humoral immune response in rats, in which milk was administered intraperitoneally. Homogenized milk administered orally to sensitize mice causes anaphylactic shock, increases production of specific IgE and weight of intestinal segment and degranulation of mast cells. In addition, allergenicity of homogenized milk in mice increases with fat content. Unhomogenized milk usually induces only several symptoms or does not cause any allergic reactions. In contrast, when administered intravenously or subcutaneously, the same symptoms and reactions are observed as in case of raw milk.

4.6. *Other processes changing allergenicity of proteins*

It is believed that irradiation of milk may be a potential method of reducing allergenicity. It was suggested that milk allergenic epitopes (α-casein and β-lactoglobulin) change its structure under the action of γ radiation. This effect is associated with reduced solubility of proteins due to agglomeration.[26]

The process of lactic acid fermentation may also influence potential allergenicity of cow's milk proteins. Researches in this field were conducted with the use of numerous bacterial strains (meso- and thermophilic) used in the production of fermented dairy products. This process did not significantly reduce allergenic properties, since in vitro antigenicity was highly reduced but it was not reflected in vivo. Potential reduction of allergenicity was demonstrated only for lactic acid bacteria – *Lactobacillus casei*, which are defined as probiotics.[26] It was proved that *L. rhamnosus* GG strain has the ability to reduce or even eliminate phagocytosis stimulated by milk allergens. At the same time, it blocks receptors involved in phagocytosis on monocytes and neutrophils. It can also modify clinical symptoms in children with eczema and dermatitis.[47]

An important method to reduce milk protein allergenicity is chemical modification by acetylation, succinylation, citrinilation, decitrinilation, phosphorylation and methylation which modify properties of milk proteins.[46]

Researches of Wróblewska and Jędrychowski[46] demonstrate that acetylation and succinylation significantly reduce immunoreactivity of β-lactoglobulin and α-lactalbumin. It was found that aforementioned processes reduce the ability to bind antibodies specific for whey proteins by more than 99%.

5. Conclusions

In addition to numerous positive effects of consumption of cow's milk and dairy products, it should be noticed that milk contains many proteins that may cause food allergies, which are recently becoming more common. The proper conduct of technological process of food preparation, which may weaken allergenic properties of cow's milk proteins should be followed. Increasingly important in diet is the use of probiotics containing strains of *Lactobacillus* and *Bifidobacterium*, which may fully eliminate allergy or reduce allergenic properties of proteins, particularly β-lactoglobulin and α-lactalbumin.

Conflict of interest

None declared.

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