Cow's Milk Proteins Immunoreactivity and Allergenicity in Processed Food

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Abstract

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The immunoreactivity and allergenicity of proteins present in processed food (UHT milk, yoghurt, hard cheese, cottage cheese, biscuit, and sausage intended for children) were determined in this study. Proteins were characterised by SDS-PAGE electrophoresis. The changes in immunoreactivity were compared by enzyme-linked immunosorbent assay (ELISA) using polyclonal rabbit antibodies specific to α-lactalbumin (α-la), β-lactoglobulin (β-lg), α-, β-, and κ-casein. The allergenicity was determined with human pooled sera from CMA allergic patients by ELISA and immunoblot. The results have shown that the allergenicity of the food products is mainly correlated with bovine serum albumin (BSA), lactofferin (LF), and α-casein or the products of non-specific reactions between carbohydrate and proteins (e.g. lactosylation).

Keywords: biscuit; cottage cheese; hard cheese; sausage intended for children; technological processes; yoghurt

The gastric system daily obtains about 1–1.5 kg of food. Most people tolerate their diets well, however, there are quite a few of more sensitive nature whose immune systems do not recognise all proteins as neutral and thus interpret them as allergens. In the course of allergic sensitisation, the production of IgE specific antibodies can lead to the onset of allergic disorders such as urticaria, asthma, or atopic dermatitis.

Approximately 2% of adults and about 5% of infants and young children in the USA suffer from food allergies according to the Experts of Food and Drug Administration (FDA) in the United States. Each year about 30 000 individuals require emergency room treatment and 150 individuals die due to severe allergic reactions to food. Eight major foods or food groups (milk, eggs, fish, Crustacean shellfish, nuts, peanuts, cereals containing gluten, and soybeans and products thereof) account for 90% of food allergies (http://www.fda.gov). The European list is more comprehensive and includes other allergens that may induce severe adverse reactions: celery, mustard, sesame seeds, lupin, molluscs and products thereof, as well as sulphur dioxide and sulphites at concentrations of more than 10 mg/kg or 10 mg/l expressed as SO_2 (http://eur-lex.europa.eu; Monaci & Visconti 2009). For the time being, there is no effective cure for food allergy and the only way to avoid it is to exclude from the diet the food labelled as containing components exhibiting allergenic properties. The know-how on detailed composition and technol-

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ogy of food production is not available to a wide population of consumers. Protein contamination during food processing or packaging, formulation errors, and substitution of the ingredients are common causes of the hidden allergen presence (Hefle 2001; Puglisi & Frieri 2007). The literature describes cases of unsuspected lupin allergens found in samples of cookies, chicken boullion cubes, or powdered chicken soup (Rojas-Hijazo *et al*. 2006) or traces of hazelnut and almond proteins in chocolate (Scheibe *et al*. 2001). Spanish researchers claim that hidden allergens were the cause of one fourth of all allergic reactions. The most frequently involved was the *Anisakis simplex* larvae present in fish and shellfish. Fruits and nuts were not listed as common hidden allergens (Añíbarro *et al*. 2007).

Individual food components influence the total allergenic potential of food ingested by human gastric system. A study on peanut showed that the fat-rich food matrices may affect the kinetics of allergen release enhancement (Grimshaw *et al.* 2003), and hydrolysis can decrease or increase cow's milk proteins immunoreactivity as it is dependent on specific activities of different enzymes (Wróblewska & Jędrychowski 2002; Wal 2003). Milk proteins are often used as components in different kinds of foods of non-dairy origin, and they can retain their allergenic character even after technological processes. It was observed that thermal processing can reduce the allergenicity of some milk proteins, but the changes in the proteins depended on the temperature applied, duration of the processing, intrinsic characteristics of the protein, and physico-chemical conditions of the environment (e.g. pH), (Davis & Williams 1998). The reactions within food matrix leading to the formation of covalent bonds, advanced glycation products, Maillard reaction, and cross-linking are able to form new immunological reactive structures.

The aim of this work was to estimate immunoreactive and allergenic properties of some food proteins extracted from dairy and non dairy products used for children at infancy (UHT milk, yoghurt, cottage cheese, hard cheese, biscuit, and sausage declared as designed for children).

MATERIAL AND METHODS

Patient sera. Sera were obtained from 15 patients of the ward of Pediatric Pneumonology and Allergy (Medical University of Warsaw, Warsaw, Poland) who were allergic to cow's milk proteins and had previously demonstrated milk specific IgE-dependent allergy proved by UniCAP IgE FEIA system (Phadia AB, Uppsala, Sweden). The study was approved by the Medical Research Ethics Committee, Warsaw, Poland (Nr 30/2009).

Production of polyclonal antibodies. Antibodies were obtained from two white rabbits for each antigen (α-la, β-lg, α-, β-, and κ-casein). Immunogen prepared for the first immunisation contained 0.5 mg of protein in phosphate buffer solution (PBS), pH 7.4, emulsified with equivalent volume of Freunds complete adjuvant. Next, three immunisations were made at 14-day intervals in the presence of incomplete adjuvant. The production of antibodies and the increases in their titres were controlled by ELISA method taking blood samples from the rabbit marginal vein 2–3 days prior to the subsequent scheduled immunisation. A week after the last immunisation, the rabbits were exsanguinated. Blood was incubated at 37°C for 1 h and left overnight at 4°C. Following centrifugation (1500× g/20 min), serum IgG antibodies were obtained at 20% saturation with sodium sulphate (Boehringer Mannheim Instruction 1991/1992). After centrifugation (1500 × *g* for 30 min), the residue was dissolved in phosphate buffer, pH 8.0, and dialysed at 4°C for 15 h against PBS, which was changed four times. IgG fraction thus obtained was lyophilised.

Products samples. Individual samples of UHT milk, yoghurt, cottage cheese, hard cheese, biscuit, and sausage bought in a local market were used in this study. Solid products, three items of one type, in an amount of 5 g were ground in a mortar with 10 ml of phosphate-buffered saline, pH 7.4 (PBS), then homogenised in ULTRA-TURRAX T25 (IKA-Labortechnik, Starfen, Germany) and incubated at the temperature of 4°C for 12 hours. The extracts obtained were centrifuged at 10 000× *g* for 20 min, filtered, and lyophilised. Milk and yoghurt were homogenised with PBS as mentioned above, and also lyophilised. Protein content in the samples was determined by the Kjeldahl method.

Separation of food extract proteins by SDS-PAGE. The extracts of samples were diluted to the same proteins content and in the amount of 0.5 mg of protein/ml of sample, 12 µl/well, were applied onto 12% polyacrylamide gel (SDS-PAGE) (Laemmli 1970). Before electrophoresis, all protein samples were heated by boiling for 3 min with SDS

(3% w/v) and 2-mercaptoethanol (0.1% v/v). High molecular markers (Sigma) ranging from 6.5 kDa to 200 kDa were used as standards. The samples were run in a Tris-glycine buffer, pH 8.3, and the proteins were stained with Coomassie Blue and silver. The separation was conducted at 200 V for approx. 70 min at room temperature. The proteins were determined with Bio-Rad PDQuest 2D software (Warsaw, Poland).

Immunoblotting of food extract proteins. Proteins separated by SDS-PAGE were transferred onto a nitrocellulose membrane by blotting overnight at room temperature using a Tris-glycine buffer with methanol, pH 8.3 (192 mmol/l glycine, 25 mmol/l Tris, and 20% (v/v) methanol). The membranes were incubated overnight at 4°C in pooled sera from patients with CMA. Antigen-antibody complexes were stained on the membranes by placing them in the solution of species-specific antibodies, horseradish peroxidase conjugated goat anti-human IgE. The reaction of the enzyme with the substrate (H_2O_2/4-chloro-1-naphtol) produced navy blue bands at the site of the conjugated antibodies. For the estimation of electrophoretic protein separation and electrotransfer efficiency, gels were stained with Coomassie Brilliant Blue R-250.

Immunoreactivity of food extract proteins-competitive ELISA. Microplates (Nunc, Roskilde, Denmark) were coated in triplicate with the antigen (α-casein, Sigma C-6780, β-casein, Sigma C-6905, κ-casein, Sigma C-0406, α-la, Sigma L-6010, β-lg, Sigma L-6879; Sigma, Poznan, Poland) at the concentration of 5 μg/ml in a 9mM carbonate buffer solution, pH 9.6, in the amount of 100 μl/well. The microplates with the antigen were incubated for 18 h at 4°C, then rinsed 3 times with 10mM phosphate-buffered saline, pH 7.4, containing 0.5% Tween 20. This procedure was repeated after each step of this experiment. The residual free binding sites were blocked with 150 μl/well of 1.5% gelatine solution and the plates were incubated at 25°C for 30 minutes. Afterwards, the wells were filled with equal amounts of the samples or standard proteins in the amount of 50 μl adequately diluted to 0.001, 0.01, 0.1, 1.0, 10, 500, and 50 μl of polyclonal rabbit antibodies diluted for each antigen as follows: for α-casein 1:6000, β-casein 1: 8000, κ-casein 1:10 000, α-la 1:20 000, β-lg 1:25 000. Then incubation continued for 1 h at 37°C. Subsequently, the anti-rabbit immunoglobulin conjugated with peroxidase produced in goat (Sigma A-6154) was added and the incubation continued at 37°C for another hour. After rinsing the microplates, the substrate, i.e. 3,3'5,5'-tetramethylbenzidine (Sigma 5525) (TMB-solution in citrate buffer, pH 5.0), was added and after 30 min the process was terminated with 50 μl 2M H_2SO_4 solution. The absorbance was read at 450 nm using the Sunrise (Tecan, Grödig, Austria). The results obtained were processed by means of ImmunofitTM EIA/RIA software by Beckman (Fullerton, USA).

Immunoreactivity of proteins was estimated as 50% inhibition of antigen binding to the standard protein.

Allergenicity of food extract proteins – IgE ELISA inhibition. The assessment of the total IgE in human sera was carried out using ELISA (Wróblewska *et al.* 2005). The microplates were coated with antigen extract and standard proteins at the concentration of 0.001–200 μg/ml, diluted with a 9mM carbonate buffer solution, pH 9.6, in the amount of 100 μl per well. The microplates with the antigens were incubated at 4°C for 18 h, then rinsed 4 times with PBS-T. This procedure was repeated after each step of the experiment. The areas of the microplates that were not saturated with the antigen were filled with 1.5% gelatine solution of 150 μl/well and incubated at 37°C for 30 minutes. After rinsing the microplates, the wells were filled with patients' sera diluted 1:1 and incubated at 37°C for 2 hours. Then biotin-conjugated IgE fraction of polyclonal goat antiserum to human (1:5000) (Nordic Immunology) was used, and the next step involved the addition of 100 μl horseradish-conjugated streptavidin diluted at 1:1000 (Sigma, Poznan, Poland). After rinsing the microplate, the substrate was added (TMB) and after 30 min the process was stopped with 50 μl 2M H_2SO_4 solution. The absorbance was determined by the Sunrise, Tecan, at 450 nm wavelength.

RESULTS AND DISCUSSION

Protein composition in food extracts

The protein contents in lyophilised extracts were determined to be 3.6% (UHT milk), 4.9% (yoghurt), 20% (cottage cheese), 12.3% (biscuit), 27.3% (hard cheese), and 14.7% (sausage). No significant differences were found in the protein content between the samples.

The results of SDS-PAGE indicated the presence of characteristic proteins in the analysed samples (Figures 1 and 2). More detailed electrophoretic

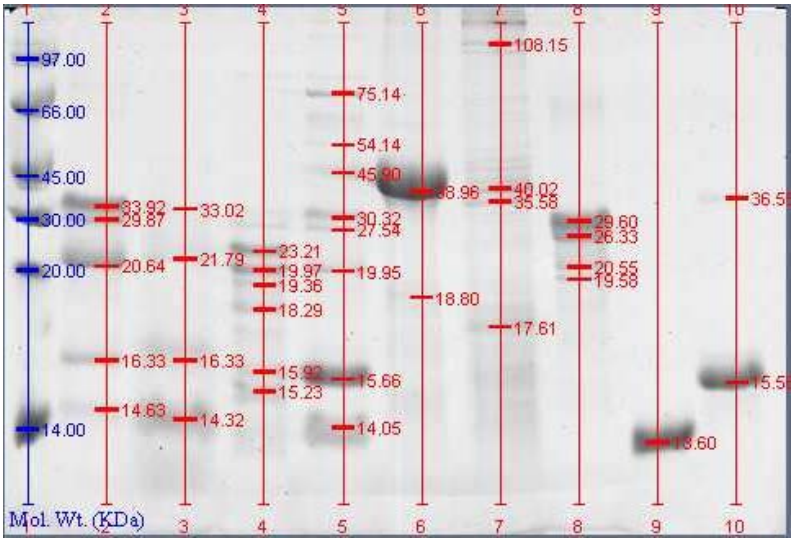

Figure 1. SDS-PAGE Coomassie Blue stained – detailed definition of molecular mass analysed food extracts: (1) molecular weight marker (Sigma); food extract proteins from: (2) UHT milk, (3) yoghurt, (4) cottage cheese, (5) hard cheese, (6) biscuit, (7) sausage, (8) casein, (9) α-la, (10) β-lg

analysis was obtained with silver staining (Figure 2). Line 2 showed UHT cow's milk proteins with two distinct bands corresponding to whey proteins (α-la = 14 kDa, β-lg = 18 kDa) and casein fractions of about 22–26 kDa. Line 3 showed that casein fractions predominated in whey proteins found in yoghurt extract. Line 4 depicted hard cheese protein extract which was a mixture of eight dominant proteins with the molecular weights lower than 30 kDa, which could be the result of deep milk proteins proteolysis during the technological process of cheese production. A number of proteins were also visualised as narrow bands in the range of 30–70 kDa. The cottage cheese protein extract (line 5) was rich in proteins over the whole range of molecular weights analysed, with the most distinct protein bands corresponding to 14, 18, 20, 27, 30, 45, 54, 66, and 75 kDa. Line 6 showed the extract of proteins obtained from biscuit where one very distinct band was observed, corresponding to the protein of about 38–45 kDa, and another, a weak one, of about 20 kDa. The most electrophoregram representing sausage proteins extract was showed on line 7. Characteristic numerous bands appeared in the range of molecular weights of 15–20 kDa and of 28–36 kDa, and two bands could be observed at about 66 and 100 kDa.

The silver staining method, regarded as 10 times more sensitive as compared to Coomassie blue staining, showed two protein bands of molecular weights in the range of 60–80 kDa corresponding to bovine serum albumin (66 kDa) and lactoferrin (75–80 kDa), in all dairy products. This observation is of particular importance if the elecrophoretic results are correlated with immunoblot. Generally, it is known that peptides and low molecular weight proteins should cross-link with mast cells in order to elicit an IgE reaction. Also larger protein fragments or intact proteins are able to induce an allergy reactions (Thomas *et al*. 2007). BSA is relatively resistant to digestion. Sequential

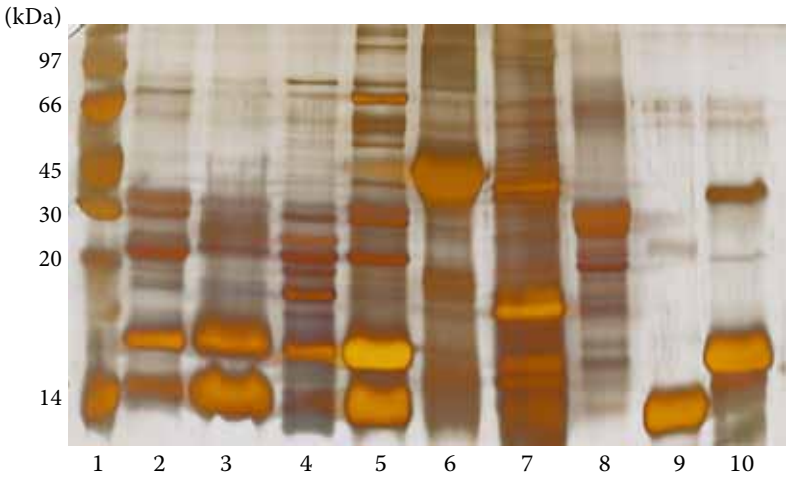

Figure 2. SDS-PAGE silver stained: (1) molecular weight marker (Sigma); food extract proteins from: (2) UHT milk, (3) yoghurt, (4) cottage cheese, (5) hard cheese, (6) biscuit, (7) sausage, (8) casein, (9) α-la, (10) β-lg

epitopes were resistant for 60 min when BSA was digested *in vitro* with pepsin (E/S = 1:120, w/w), and at least one sequential epitope (AA: 524–542) was still active as an allergenic site (Beretta *et al.* 2001). Immunoblotting with the allergic children's serum showed that circulating IgEs reacted with BSA even after the reducing treatment (Restani *et al.* 2004). Some authors emphasise the fact of physical transformations and chemical modifications of the proteins which may occur at high temperatures during food processing. These may involve the formation of covalent bonds between lysine residues of a protein and other constituents of the food matrix leading to various adducts with higher molecular weights, which may contribute to the formation of new immunologically reactive structures (Wal 2003). In our study, we focused on the high molecular weight proteins (BSA and LF) as described below.

Immunoreactivity of food extract proteins

The immunoreactivity of the individual food extract measured by ELISA with the use of rabbit polyclonal antibodies directed to α-la, β-lg, α-, β-, and κ-casein (Table 1). During dairy technological processing, cow's milk proteins undergo a plenty of changes as a result of normalisation, homogenisation, standardisation, pasteurisation, cooling, and enzymatic hydrolysis among others. The impact of these processes on whey proteins and casein in UHT milk, yoghurt, hard cheese, and cottage cheese were varied while immunoreactive properties were still detected. The immunoreactivity of milk resulting from the presence of α-la epitopes was 22% in milk extract and 18% in hard cheese, but in the products with higher contents of whey proteins (yoghurt and cottage cheese), β-la immunoreactivity was much higher, up to 205% and 154%, respectively. The extract of sausage was characterised by 5% immunoreactivity corresponding to α-la epitopes, in spite of the fact that this protein had not been listed on the label. In the experimental conditions, the level of β-lg immunoreactivity was rather low. In UHT milk and yoghurt extract samples, the result of 3% was observed and in the cottage cheese extract – 5%. Considering casein immunoreactivity, it was noted than the extracts of all products reacted with α- and β-casein antibodies which, in turn, was contributed to the presence of epitopes characteristics for these proteins, or indicated a possibility of positive cross reactions imitating the presence of milk proteins in the product. α-Casein immunoreactivity detected in dairy products was in the range of 171–333% and in biscuit and sausage extracts it was 5% and 3%, respectively. The same tendency was observed with β-casein. In dairy products, immunoreactivity of β-casein was noted between 50–500%, and in biscuit and sausage extracts, it was on a low but still measurable level of 0.1%. The results obtained demonstrated a high reactivity of cow's milk proteins which was confirmed by immunoreactivity higher than 100%. The results proved that some products, e.g. sausage, contained trace amounts of milk proteins as hidden allergens or accidental contamination. However, the producer of sausages declared that this product consisted mainly of homogenised pork, however, other products are processed in the factory, namely such which are made with milk proteins. The unexpected level of

Table 1. Immunoreactivity of analysed products compared to standard proteins (determined as 100%) – ELISA results with different rabbit antibodies

Product	Level of reaction with a specific rabbit antibody (in %)									
	anti-α-lactalbumin		anti-β-lactoglobulin		anti-α-casein		anti-β-casein		anti-κ-casein	
	residual immuno-reactivity	*CV*	residual immuno-reactivity	*CV*	residual immuno-reactivity	*CV*	residual immuno-reactivity	*CV*	residual immuno-reactivity	*CV*
UHT milk	22	0.46	3	1.66	214	2.57	50	2.24	14	3.58
Yoghurt	205	0.89	3	1.37	171	0.33	183	5.92	2	1.28
Hard cheese	18	0.71	0.56	1.53	333	0.39	500	2.53	0	3.31
Cottage cheese	154	1.70	5	1.47	193	0.13	175	8.96	3	0.33
Biscuit	0.0016	1.84	0	0.46	5	1.79	0,1	6.41	0	2.92
Sausage	5	0.13	0.0023	0.77	3	0.60	0,1	1.71	0	0.94

immunoreactive properties found in food could be the reason of the protein structure similarity, expressed as cross reactivity. Some cross-reactivity was suggested between α- and β- caseins (Bernard *et al.* 2000a) and α-la and β-lg (Adams *et al.* 1991). The research into human and bovine β-caseins as well as derived peptides demonstrated that one or several common epitopes located in different parts of the molecule were shared by the two homologous proteins (Bernard *et al.* 2000b). It was also pointed out that polyclonal anti-β-lactoglobulin reacted with human lactoferrin, β-casein, and α-lactalbumin (Bertino *et al.* 1996). Such information emphasises the fact that ELISA technique is not an unequivocal method of analysing the allergenicity of food. De Luis *et al.* (2008) showed diversified results obtained during determining allergenic properties of β-lactoglobulin and ovomucoid in model processed foods using two formats of ELISA (indirect competitive and sandwich). It was concluded that sandwich ELISA was more effective in detecting β-lg, and the indirect competitive ELISA was better suited for ovomucoid analysis. The intensity of heat processing causes denaturation and aggregation of proteins resulting in the decrease of the immunnoreactive level. There is also information on the increase of the allergenic potential of food as a result of peptides re-associating to aggregates or unmasking existing epitopes (Thomas *et al.* 2007). It is obvious that during proper estimation of protein immunoreactivity, other kinds of analysis should also be taken into consideration, for instance immunoblotting.

The coefficient of variation (*CV*%) estimated for all samples by ImmunoFit Software EIA/RIA Analysis (Beckman, Fullerton, USA) was in the range of 0.13–8.96%. This statistical information allowed us to compare the data obtained for different units. In this research, in accordance with The National Committee for Clinical Laboratory Standards recommendations, *in vitro* test was characterised by a coefficient of variation lower than 15% (http://www.worldallergy.org). Some of the immunoreactive results were on a very low level, but when estimating antigenicity, even trace amounts of allergens must be taken into consideration since they can still initiate an allergic reaction.

Allergnicity of food extract proteins

Immunoblotting allowed to find out that two proteins present in the extract of dairy products (UHT milk, yoghurt, hard cheese, and cottage cheese) with molecular weights of 66 and 80 kDa reacted with pooled CMA patient sera (Figure 3). The results obtained with immunoblot proved the active potential allergenicity of BSA and LF, revealed after the technological processes. Whey proteins present in dairy products (α-la and β-lg) were denatured and modified so that they did not react with human sera. At the moment there is no sufficient data in the literature on the protein detected in electrophoresis and characterised as BSA without confirming the data by immunoblot (Sletten *et al.* 2008), and BSA or LF as potential allergens (Bu *et al.* 2009).

In our study, another characteristic protein band corresponding to α-casein was detected. α-Casein was still found in UHT milk after the technological processes. Weaker α-casein spots were also found in cottage cheese and hard cheese extracts. The detailed literature data on immunoreactions between CMA human sera and milk proteins showed that

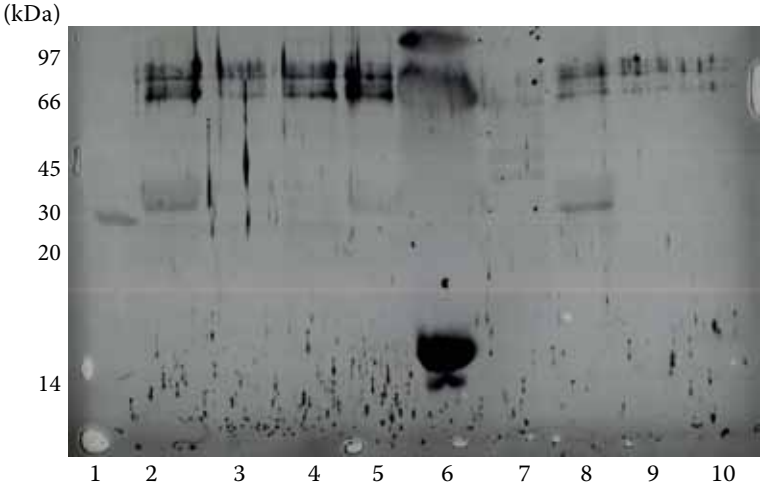

Figure 3. Immunoblot with human sera: (1) molecular weight marker (Sigma); food extract proteins from: (2) UHT milk, (3) yoghurt, (4) cottage cheese, (5) hard cheese, (6) biscuit, (7) sausage, (8) casein, (9) α-la, (10) β-lg

50% of 20 sera from cow's milk allergic patients aged between 4 months to 14 months contained IgE against lactoferrin, 45% BSA, 95% IgG-heavy chain, α_{s1}-casein (55%), α_{s2}-casein (90%), β-casein (15%), κ-casein (50%), and β-lactoglobulin (45%) and 0% α-lactalbumin (Natale *et al.* 2004). Another information regarding the role of high molecular weight milk proteins in inducing anaphylaxis was also published (Abe *et al.* 2010). The authors reported that 75 kDa protein found in dairy products, together with aspirin, was a trigger which could cause anaphylaxis. A similar reaction was also observed after cheese digestion without aspirin intake.

Immunoblot of biscuit proteins revealed the presence of immunoreactive protein in the molecular weight range of 14–18 kDa, but due to a big visible spot precise identification was difficult. Other spots in biscuit proteins were observed at 70–80 kDa and 100 kDa. The immunoblot of sausage extract proteins revealed reactions between specific human IgE antibodies and proteins presented at 30–45 kDa, and a weak reaction with 66–80 kDa proteins. Some authors observed that the sera of children suffering from milk allergy possess IgE specific antibodies against bovine immunoglobulins, and they characterised immunoreactive proteins with molecular weights of 29–60 kDa as heavy and light chains of immunoglobulins (Bernhisel-Broadbent *et al.* 1991). On the other hand, Ayuso *et al.* (2000) pointed out that no IgE reactivity to IgG was detected in cooked meat. IgE to serum albumin was reduced in the cooked meat extract whereas the raw meat extract showed a stronger reaction.

The ELISA method with pooled patients' sera with high levels of milk specific IgE analysed by UniCAP was used to determine allergenic properties of processed food extracts. Klubal *et al.* (2009) indicated the possible increase of cow's milk immunoreactivity as a result of milk products processing. This resulted in a higher level of IgE patients sera as compared with native milk protein. In our study, all analysed sample extracts exhibited a strong reaction with human cow milk specific antibodies. The reaction with UHT milk extract was established as 100% of allergenicity and other results were determined in relation to this data (Table 2). None of the food extracts was free of the residual allergenicity still observed after technological processes and detected on a high level in food matrix. Yoghurt extract was characterised by lower allergenicity than UHT milk and hard cheese, but on SDS-PAGE (Figure 2) it was

Table 2. ELISA results of reaction of food protein extracts with IgE antibodies obtained from pooled human sera

Product	Level of protein extract reaction with a human pooled sera (%)
UHT milk	100.00
Yoghurt	65.00
Hard cheese	108.00
Cottage cheese	75.00
Biscuit	98.00
Sausage	56.00

demonstrated that yoghurt, which contained all milk proteins, was characterised by a significant amount of whey proteins. Perhaps this was due to the enriching of the dry weight with milk proteins which is necessary during the technological process of yoghurt production. Hard cheese exhibited even higher allergenicity than UHT milk, which was probably the result of proteolytic changes occurring in milk proteins during cheese production. All fractions of casein were present and visible on SDS-PAGE (Figure 2) and on immunoblot (Figure 3). Binding between human specific IgE and α-casein was noted after harsh technological processes. The protein extract from biscuit was found to have 98% of UHT milk allergenicty, which correlated with immunoblot, where a very strong immune reaction was observed. This observation is of great importance as biscuits are often recommended as the first solid food for children. CMA usually appear together with other form of food allergy and the sera contain more than one type of antibodies. It is possible that the patient sera obtained from hospital were rich in antibodies directed against different proteins, hence the result of the immunoblot. The protein extract from sausage showed a lower allergenicity of 56%. Still, these results indicated the possibility of the cross reaction between meat and cow's milk proteins, a fact that had been mentioned in Mamikoglu (2005) study where a very strong correlation between milk and beef and also between milk and pork meat was noted. Therefore, it was concluded that the patients who are allergic to milk should be tested for meat allergies. Literature describes cases of children (13–92%) with beef allergy who exhibited milk allergy symptoms as well (Martelli *et al.* 2002). Our findings confirm the need to carry out careful testing for allergenicity among CMA patients.

CONCLUSION

In conclusion, it was observed that severe technological processes did not destroy the total immunoreactivity and allergenicity of cow milk protein epitopes present in dairy products and also in non dairy products such as biscuit and sausage. Hard cheese extract proteins were characterised with a higher immune reactivity with human IgE as compared to UHT milk. Other samples extracts (yoghurt, cottage cheese, biscuit and sausage) showed a lower relative allergenicity, although it was still detectable. The results obtained prove that the overall allergenicity of food products was mostly related to high weight molecular proteins (70–80 kDa), bovine serum albumin (BSA), lactofferin (LF), and α-casein. The CMA patients sera did not react with α-la and β-lg. Their immunoreactive potential may have been destroyed during technological processes. The technological modification of proteins in food matrix caused that the proteins with high molecular weights, e.g. BSA and LF, were noted as the main factors of allergenicity. BSA, LF, and α-casein are resistant to high temperature and bacterial enzymes used during the processes, therefore, they were still immunoreactive, which was proven on immunoblot with human sera. Probably also due to the chemical modification, e.g. reaction of proteins with carbohydrates at high temperature, caused the changes and enhanced the immune protein properties of these two. Allergic patients with severe immune reactions against cow milk should be tested for meat protein allergenicity because of the proteins cross reactivity. A more profound understanding of how food processing changes the protein structure would help allergic patients to select the most beneficial kinds of food.

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