Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 841 (2006) 160–167
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Changes in peptic digestibility of bovine β-lactoglobulin as a result of food processing studied by capillary electrophoresis and immunochemical methods☆

Irene Maier[b], Vadim M. Okun[a], Fritz Pittner[b], Wolfgang Lindner[a,*]

[a] *Institute of Analytical Chemistry and Food Chemistry, University of Vienna, Währingerstraße 38, 1090 Vienna, Austria*
[b] *Max F. Perutz Laboratories, Department of Biochemistry, Dr.-Bohr-Gasse 9, 1030 Vienna, Austria*

Received 8 February 2006; accepted 26 June 2006
Available online 28 July 2006

Abstract

Digestion studies constitute a functional tool for allergen characterisation. This strategy for investigating allergenic proteins relates to the observation of increased proteolytic resistance of some proteins recognised to exhibit allergenic potential. β-Lactoglobulin (βLG) is one of the major whey proteins, a potent milk allergen and shows a high stability against peptic hydrolysis in its native form. In order to study the impact of milk fermentation process on its digestibility, two complementary analytical methods were applied: capillary zone electrophoresis (CZE) to quantitatively study proteolytic degradation of βLG isolated from different fermented bovine milk products, and enzyme linked immunosorbent assay (ELISA) to assess differences in immunoreactivity. βLG, isolated from either raw or pasteurised cow's milk (CM), as expected, showed only minimal digestibility (less than 10% in 2 h). However, when raw milk or pasteurised milk was fermented, the rate of peptic digestion of the protein significantly increased (up to 45% in 2 h). In accordance with changes in digestibility, the immunochemical response for all fermented samples was lower than that of non-fermented references. Raw and pasteurised milk "naturally" fermented in our laboratory only resulted in a slight reduction (βLG detected is still in the range of milligrams per gram sample), whereas the industrially manufactured sour milk as well as the "Acidophilus milk" reflected a remarkably lower level of immunoreactivity (55–56 μg/g sample).

Keywords: β-Lactoglobulin; Milk; Fermentation; Allergenicity; Peptic digestibility; Complementary analytical methods

1. Introduction

In consideration of public health, allergenicity of processed food has become a research topic of highest interest for the scientific community, as well as a top-ranging issue for regulatory authorities [1]. Food allergies are most prevalent during infancy, affecting up to 6% of young children. With approximately 2.5% of newborns suffering from allergic reactions, cow's milk (CM) is at the top of all lists of epidemiologic data [2]. One approach to characterise the allergenicity of a food protein refers to the complementary investigation at different levels: in terms of its durability during food processing, structural characteristics and digestibility, and immunochemical properties [3].

The principle of digestive stability as a criterion for protein allergenicity stems from the observation that many food allergens exhibit proteolytic stability, whereas nutritionally desirable proteins are more rapidly digested by the human gastrointestinal fluids [4–6]. The main focus of this study was to investigate changes in peptic digestibility of the major allergenic protein cow's milk, β-lactoglobulin (βLG), due to fermentation processes by two complementary analytical methodologies: capillary zone electrophoresis (CZE) and enzyme linked immunosorbent assay (ELISA).

Bovine β-lactoglobulin consists of two genetic isoforms A and B, which differ only at positions 64 (Asp → Gly) and 118 (Val → Ala), and both are known to possess allergenic potential [7]. In the following, however, we mostly speak about the total quantity of both isoforms and averaged characteristics. Native

☆ This paper is part of a special volume entitled "Analysis of proteins, peptides and glycanes by capillary (electromigration) techniques", dedicated to Zdenek Deyl, guest edited by I. Miksik.

* Corresponding author. Tel.: +43 1 4277 52300/1; fax: +43 1 3151826.
E-mail address: wolfgang.lindner@univie.ac.at (W. Lindner).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.06.040

bovine βLG is very stabile against peptic digestion under acidic conditions, which corresponds to the situation in the stomach of humans. This phenomenon is considered to correlate with its high allergenicity [8,9].

A number of publications investigated the conformational and linear epitopes of βLG [10–12]. Tertiary structure turned out to be an inherent factor in immunoreactivity of native βLG. An intense study on discontinuous epitopes by monoclonal antibodies succeeded in the determination of 12 antigenic sites [13]. There may be short fragments located in hydrophobic parts of the molecule that comprise highly conserved sequences responsible for IgE cross-reactivity with corresponding milk proteins of other mammals, including humans. Therefore, clinical investigations on the allergenic potential of proteins must be aware of both a decrease and a possible increase of antibody binding capacities as a result of protein unfolding upon processing. In vivo proteolysis might eliminate epitopic sites but also increases their susceptibility.

Digestion assays came up to be a predictive tool for protein allergenicity assessment, as several food allergens were claimed to be stable to conditions simulating human gastrointestinal digestion. Astwood et al. observed higher stability for a selected group of egg, milk and soybean allergens, if compared to non-allergenic proteins, and demonstrated the formation of stable peptide fragments in a standard simulated gastric fluid [14]. Fu et al. resumed the discussion on a comparable work (simulated gastric and intestinal fluid) between digestibility of allergens and non-allergenic proteins. However, they could not confirm any clear relationship between in vitro digestibility and protein allergenicity [15]. Nevertheless, the information whether proteolytic degradation can be achieved for stable allergens due to technological treatments can be seen as a promising base for the management of food allergy.

Increased proteolytic degradation was observed upon heat treatment [19–21], in contrast to the pronounced reduction of immunoreactivity of the partially hydrolysed allergens [18]. Controversial results can be found in literature in this context. In order to give full information about the allergenic content in food, in vitro analysis must detect the native protein, digestion-derived peptides, and the immunoreactivity of both. Indeed, recent publications have started distinguishing between these two aspects—protein degradation on the one hand, and studying immunochemical properties on the other hand, but an advanced correlation of the results is still missing [19–21].

Considerable efforts have been put forth to decrease allergenicity of cow's milk products. It was shown that various manufacturing processes such as heating, chemical treatment, high pressure, fermentation, etc. can alter the allergenic potential of food [16,17]. Combined physical and proteolytic treatment offered a promising way for reduction of protein allergenicity [22,23]. Lactic acid fermentation of milk products is associated with proteolytic activity [24,25]. In some geographical regions (e.g. Caucasus republics), it is an old tradition to feed infants in their first years of life only with fermented milk products to prevent the risk of allergic reactions. The co-administration of lactobacilli with cow's milk was also shown to improve disorders because of gut mucosal permeability and to stimulate IgG response in suckling rats [26].

Even though digestibility of βLG upon processing has already been studied by different analytical and biochemical methods (SDS-PAGE, RP-HPLC, chemical characterisation), no quantitative data were given about the proteolytic degradation or kinetics of the digestion assay [27–29].

The present study applied CZE to quantitatively monitor peptic hydrolysis of βLG isolated from different bovine milk products, and to bridge these data with information from immunochemical measurements. The overall goal was to study the impact of fermentation on the peptic digestibility of bovine βLG.

2. Experimental

2.1. Materials

Raw milk was obtained from a farmer in Styria (Austria). The fresh milk was collected and worked up within 3–5 h. "Naturally fermented" products were obtained by performing fermentation in an uncontrolled way without adding a certain starter culture by storing the samples at room temperature for two days. Afterwards, the samples were cooled to 4 °C, and the isolation procedure started immediately. Commercially available products were purchased at local food stores. All chemicals used for the preparation of buffers, sample work-up and subsequent analysis were purchased from Sigma (Vienna, Austria) or Fluka (Buchs, Switzerland) unless stated otherwise and were of analytical grade or better.

2.2. Sample preparation: bovine milk and dairy products

Whey fractions and purified βLG for peptic digestion were prepared from the following differently processed bovine milk and dairy products: raw milk, pasteurised and homogenised milk, "naturally fermented" raw and pasteurised milk, industrially manufactured sour milk (NÖM AG, Austria), and "Acidophilus milk" (Lactobacillus acidophilus as an additive to normal pasteurised milk, Kärntnermilch, Austria).

The isolation of the βLG fraction was performed with modifications according to the protocol previously described by Neyestani et al. [30] A typical work flow diagram is outlined in Fig. 1. Sample preparation procedures for subsequently applied investigations by electrophoresis and ELISA were developed separately for each analytical method.

2.3. Gel-electophoresis

SDS-PAGE analysis was performed essentially according to Laemmli (1970) using a BioRad Mini Protean II system (BioRad, Hercules, CA, USA) with discontinuous acrylamide gels (15% resolving gel and 4% stacking gel). Pre-stained molecular weight marker with molecular weights of 14.4, 18.4, 25.0, 35.0, 45.0, 66.2 and 116.0 kDa was used as reference (MBI Fermentas).

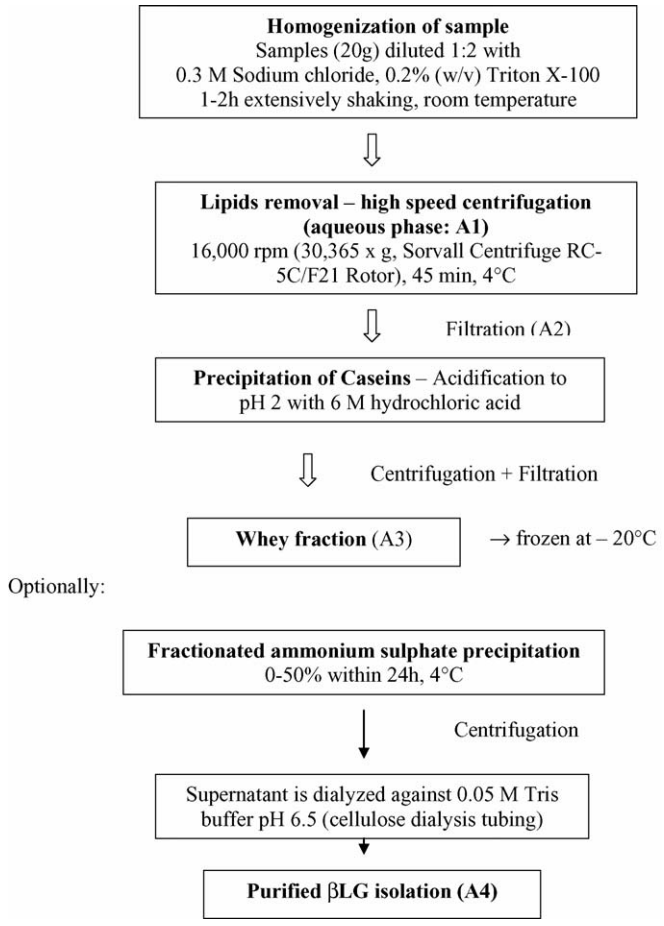

Fig. 1. Schematic description of the isolation procedure of βLG from bovine milk matrices.

2.4. Capillary electrophoresis

A HP3D Capillary Electrophoresis System (Agilent, Waldbronn, Germany) with a diode-array detector was used to carry out all CZE experiments. Untreated fused silica capillaries with 50 μm ID were purchased from Polymicro Technologies (Phoenix, AZ). Total capillary length was 39.0 cm and the effective length was 30.5 cm. The capillary was thermostated at 20 °C.

Injection was accomplished by application of 50 mbar pressure to the inlet vial for 5 s. The positive polarity mode with a voltage of 25 kV was used. The current generated was typically about 18.5 μA. UV adsorption was recorded at 205 nm. In some cases the fast spectral scanning mode was used to aid peak identification. Data were processed using the Hewlett-Packard Chemstation Software.

The background electrolyte (BGE) was 100 mM boric acid adjusted to pH 8.3 with 1 M NaOH and contained 0.05% Tween 20 as an additive. Unless otherwise stated, all samples were prepared in 25 mM borate buffer pH 8.3, supplemented with 25 μg/mL of *o*-phtalic acid (Sigma, Steinheim, Germany) as internal standard (sample buffer). Prior to CZE analyses, all samples were 1:5 diluted with sample buffer.

A new capillary was conditioned by flushing with 100 mmol/L HCl, followed by water, 1 mol/L NaOH, and water for 10 min each. Between each analysis run, the capillary was flushed with 100 mmol/L NaOH, water and BGE for 2 min each.

2.5. Peptic digestion

Prior to digestion experiments, CZE was performed to determine the amounts of βLG and its isoforms βLG A and B, respectively, in all studied samples. A calibration line was constructed in the range of 25–400 μg/mL βLG (Sigma, Steinheim, Germany). Every sample was measured at least twice.

For peptic digestion to 400 μL of the βLG containing samples 10 μL of internal standard (*o*-phtalic acid, 3 mg/mL) were added and the solutions acidified to pH 1.5 with 8 μL of 0.1 M HCl. 10 μL of these solutions were taken as blank samples in CZE analysis. To the rest of the mixtures, 5 μL of pepsin solution in water was added to gain a final enzyme/substrate ratio of 1:20 (w/w, pepsin from hog stomach, 3348 U/mg, Fluka, Buchs, Switzerland). The solutions were incubated at 37 °C and aliquots were taken at the time periods indicated in the text. Prior to CZE analysis all aliquots were diluted 1:4 in a sample buffer void of internal standard.

2.6. Enzyme linked immunosorbent assay

Microtiter plates (Nunc-Immuno Plate MaxiSorp, 96-well) were coated with 100 μL per well βLG-standards (100–700 ng/mL) and samples diluted 1:10,000 in 50 mM carbonate/bicarbonate buffer, pH 9.6, or with lower dilution factors to obtain concentrations within the range of the standards. The plate was covered and incubated overnight at 4 °C. The βLG-coated plate was washed once with washing buffer composed of 0.01 M phosphate buffered saline (PBS), pH 7.4 containing 0.5% (w/v) Tween 20. Remaining sites for protein binding on the microtiter plate were saturated with 2% (w/v) HSA (incubation for 2 h at room temperature). Next, the plate was washed three to four times with washing buffer, as also, after all of the following incubation steps with first and second antibody. One hundred microliters of the first antibody solution composed of rabbit anti-bovine βLG IgG (Bethyl Laboratories, Montgomery, TX) in 0.01 M PBS pH 7.4 containing 0.5% Tween 20 and 0.5% HSA (1/10,000 dilution) was added to the wells. Incubation was performed overnight at 4 °C. For detection HRP-conjugated anti rabbit IgG (Sigma, Steinheim, Germany) was used (1/10,000 dilution) and incubated for 1–2 h at room temperature. After the final washing step, 100 μL of TMB substrate (3,3′,5,5′-tetramethylbenzidine solution, Sciotec, Vienna, Austria) was added per well. The reaction was stopped after an appropriate time of about 10–15 min at room temperature, by adding 50 μL of 3 M sulphuric acid per well. The absorbance was read at 450 nm.

Peptic digestion for ELISA, monitoring changes of the proteins regarding changes of antibody binding properties due to proteolysis was tested with the whey protein fractions of "Acidophilus milk" and sour milk. Pepsin was added in the same enzyme/substrate ratio 1:20 as for the CZE analysis. Incubation was carried out at 37 °C. Aliquots were taken before adding pepsin and after several increasing time intervals over 90 min. To

stop the digestion, the pH was adjusted to 6.0 with a minimum of volume of 1 M NaOH, and the samples were cooled to 4 °C.

3. Results and discussion

3.1. Isolation of βLG fraction for peptic hydrolysis

As outlined in Fig. 1, sample preparation included a defatting step by high-speed centrifugation and acidification of the milk samples with hydrochloric acid to precipitate caseins. The so obtained whey fractions were used for immunochemical assaying of the undigested βLG content and to monitor changes in antibody binding capacity upon digestion. Strong importance was given on to apply non-destructive procedures as not to influence the target protein (βLG) by sample preparation or by physico-chemical conditions used for running the analytical detection method. The impacts of both the acidic pH and the peptic hydrolysis on whey proteins, corresponding to conditions in the human stomach, were studied as a characterising tool for modified stability of βLG based on the fact that native βLG is highly resistant to peptic digestion. Fig. 2 visualizes the progress of purification steps of βLG fraction from milk products.

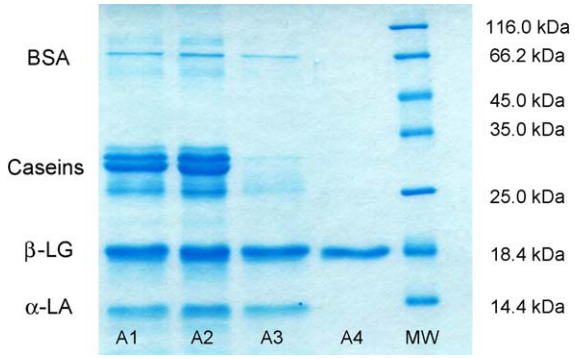

Fig. 2. SDS-PAGE (15% PAA, coomassie staining): showing different isolation steps of βLG from pasteurised and homogenised milk. A1: supernatant after initial centrifugation, A2: filtrated supernatant, A3: after precipitation of caseins, A4: after fractionated ammonium sulphate precipitation; MW: protein molecular weight marker.

Capillary electrophoresis was applied to prove the purity of isolated βLG from various milk and dairy products in addition to the SDS-PAGE approach. To diminish possible protein sorption on the capillary wall, a mild detergent, Tween-20, was added to the background electrolyte. Fig. 3(A) shows a typical CZE analysis of aliquots from the commercially available pasteurised milk, that represent two consecutive steps in the isolation protocol—whey fraction (A3) and purified βLG isolation (A4). The upper trace shows the separation of whey, where major whey proteins, namely α-lactalbumin and βLG of both major isoforms A and B are clearly distinguished. The lower trace shows CZE separation of the final isolate, where only βLG isoforms A and B are present. Peak identification was done by spiking the sample with standards and by spectral scanning. The latter enables clear differentiation of βLG from α-lactalbumin, which possesses a characteristic local maximum at 224 nm (Fig. 3(B)). This separation also sets a stage for the quantitative determination of both major isoforms of βLG.

3.2. Peptic digestibility of βLG isolated from different milk products

Because of its compact globular structure, βLG is a very poor substrate for proteolytic enzymes, especially for pepsin, the most important proteolytic enzyme in the human stomach.

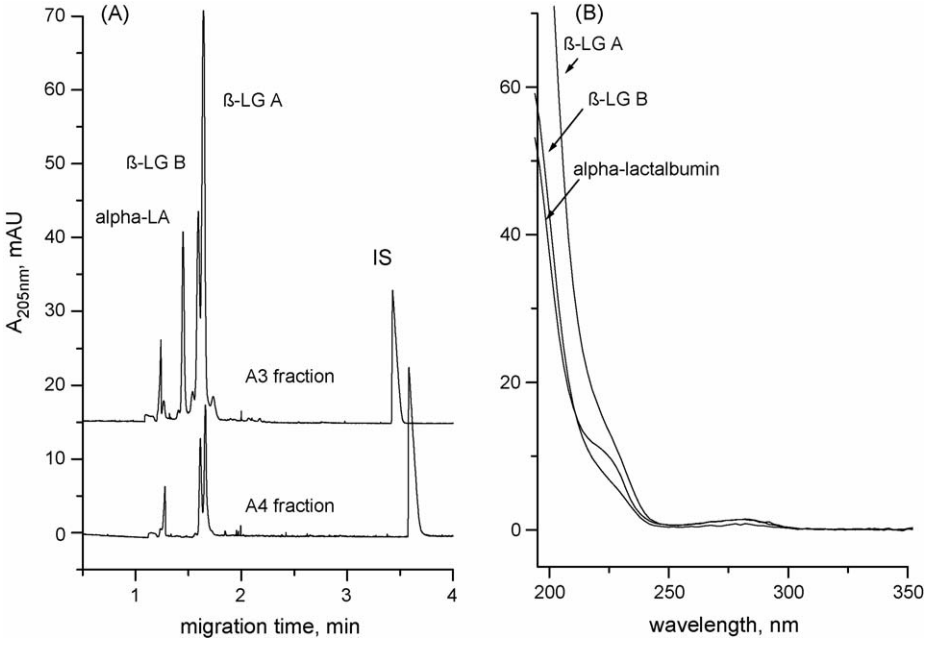

Fig. 3. (A) CZE separation of whey, corresponding to isolation step A3 in Fig. 2 (upper trace) and sample from the final isolation step, corresponding to A4 in Fig. 2 (bottom trace). CZE conditions: fused silica capillary 50 μm ID × 30.5 cm effective length; buffer: 0.1 M borate, pH 8.3 with 0.05% Tween 20; voltage 25 kV; IS, internal standard, *o*-phtalic acid 30 μg/mL. (B) Normalized UV spectra derived from the peak apexes of α-lactalbumin and βLG A and B as shown in (A).

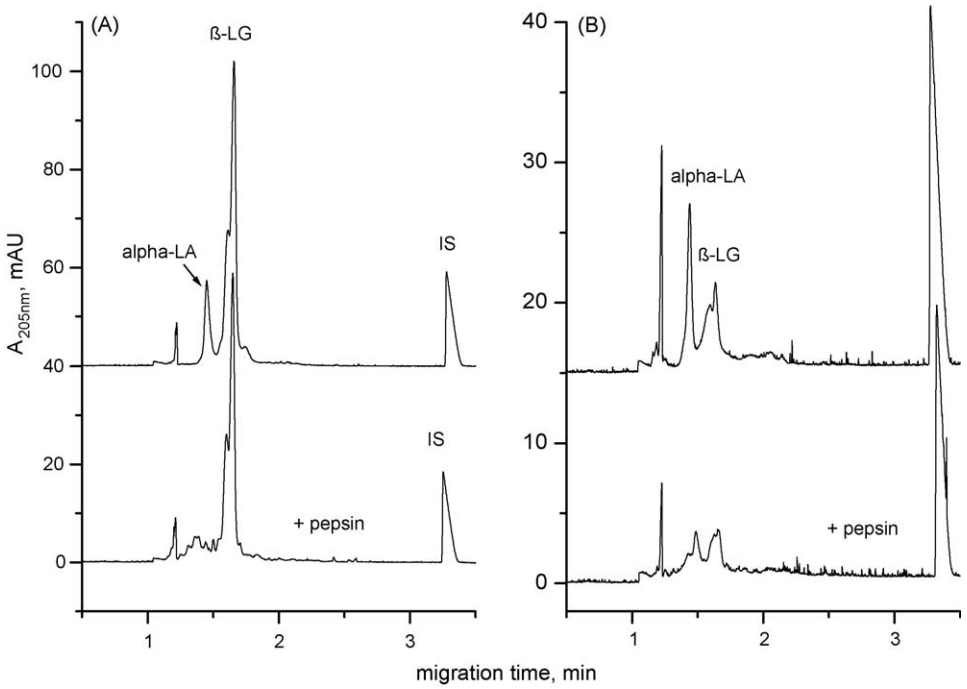

Fig. 4. Peptic digestion of whey proteins. (A), a whey sample (upper trace) was treated with pepsin and analyzed by CZE after 30 min of incubation (lower trace). (B), whey sample was pre-heated at 95 °C for 10 min (upper trace), pepsin was added and the sample was analyzed by CZE after 30 min of incubation (lower trace). CZE conditions as in Fig. 3.

Peptic digestibility can be affected by the processing conditions the milk products have undergone, e.g. heat treatment, fermentation, etc. Heating is known to increase the digestibility of βLG. Fig. 4(A and B) demonstrates how CZE can monitor digestion of whey proteins. Addition of pepsin rapidly degrades α-lactalbumin whereas βLG remains intact (Fig. 4(A), lower trace). Extensive heating (95 °C for 10 min) of the whey sample drastically lowers the amount of βLG in solution leading to its precipitation (Fig. 4(B), upper trace), whereas α-lactalbumin remains soluble. Pepsin, added to whey treated that way, hydrolysed both proteins within minutes, as shown on the electropherogram in Fig. 4(B), lower trace.

Based on the fact that extensive heat treatment will lead to increased digestibility of βLG or even degrade it without the influence of a protease, additional heat treatment was banned for the whole analysis. This should ensure that only fermentation affected βLG and its digestion behaviour. In contrast to our approach, most proteolytic studies of "unfolded" βLG addressed the products of severe thermal treatment [28,29].

We investigated the kinetics of peptic digestion to assess whether proteolytic degradation upon fermentation processes has pronounced impacts on structural and hence immunochemical properties of βLG. Raw and completely unprocessed milk was fermented at room temperature. βLG was isolated from fermented milk, pepsin was added, and aliquots were analyzed by CZE at different incubation times. The upper trace of Fig. 5 shows the βLG isolate before the addition of pepsin, and the lower trace demonstrates the decrease of the βLG concentration in this sample (approximately 25%) after 1 h of incubation with pepsin. Several other milk products such as raw milk, pasteurised milk and naturally fermented pasteurised milk were analyzed

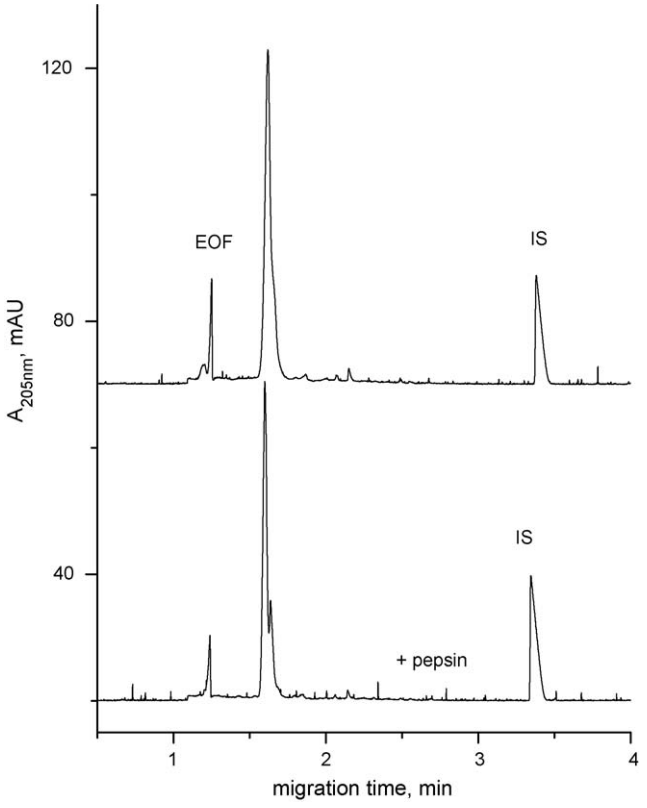

Fig. 5. Peptic digestion of βLG isolated from naturally fermented raw milk. βLG isolate (upper trace) was incubated with pepsin for 1 h and analyzed by CZE (lower trace). CZE conditions as in Fig. 3.

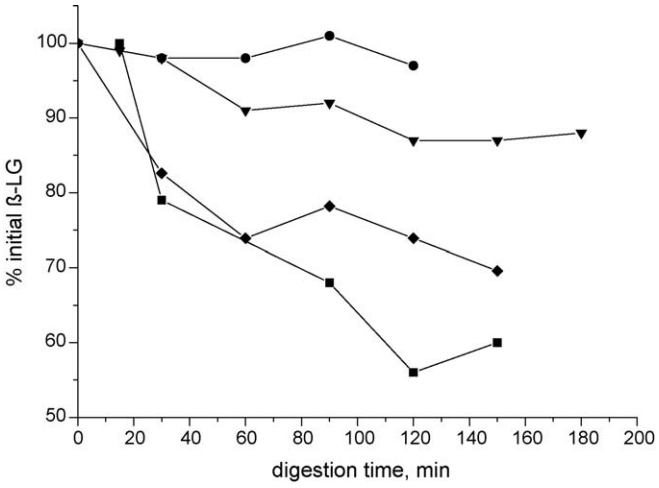

Fig. 6. Comparison of peptic digestion of βLG isolated from different milk products ((●) raw milk; (▲) pasteurised milk; (♦) naturally fermented pasteurised milk; (■) naturally fermented raw milk). Each βLG sample was incubated with pepsin for the time periods indicated and analyzed by CZE. The peak area of the protein was normalized to that of the IS and the initial value before addition of pepsin was set to 100%. Each data point represents the mean of two independent measurements. CZE conditions as in Fig. 3.

under these conditions. To provide similar conditions for all samples, the pepsin/βLG ratio was kept constant at 1:20 (w/w). For this purpose the concentration of βLG in the various samples was determined by CE.

Fig. 6 represents the amount of the remaining non-digested βLG relative to its initial concentration plotted against the digestion time of all milk products studied. It shows that pasteurisation itself does not have any noticeable impact on βLG digestibility. Only about 10% of βLG isolated from pasteurised milk was digested after 2 h. However, when milk was naturally fermented, βLG isolated from these products showed a significantly higher rate of digestion. After 2 h of peptic digestion, about 40% of the protein had been hydrolysed. This indicates that fermentation significantly changes the properties of the protein.

3.3. Immunochemistry

An indirect, non-competitive ELISA with polyclonal rabbit anti-βLG antibodies was constructed for assaying the immunoreactive βLG content in whey fractions of acidified and fermented bovine milk products. Immunochemical data of samples corresponding to those analyzed by CZE are collected in Table 1. The ELISA responses relate to equal quantities (mass) of products used for each sample preparation. The results for fermented samples were all lower than for non-fermented references. Raw and pasteurised milk "naturally" fermented in our lab only resulted in a slight reduction (βLG detected is still in the range of milligrams per gram sample), whereas the industrially manufactured sour milk, as well as the "Acidophilus milk" reflected a remarkably lower level of immunoreactivity (55–56 μg/g sample). Compared with CE findings that clearly demonstrated an increase of peptic digestibility of βLG for "naturally" fermented milk samples, the equivalent βLG content detected by ELISA was still quite high for these samples. This

Table 1
Immunochemical data of whey fractions of milk references and fermented dairy products measured in ELISA

Milk product		ELISA response—βLG [mg/g sample][a]
Reference samples	Raw milk	3.71
	Pasteurised milk	2.77
Fermented samples	Naturally fermented raw milk	3.16
	Naturally fermented pasteurised milk	2.50
	Sour milk	0.056
	Acidophilus milk	0.055

[a] Mean values of duplicate analyses of two independent sample preparations.

might indicate the formation of intermediate fragments upon proteolysis, which are instable to acidic hydrolysis but contribute to the polyclonal immuno response gained by ELISA [31]. The ELISA gives no specific information about the concentration of native protein. The immunoreactivity measured by ELISA is a mixture of quite different epitopic sites recognised at the level of the intact protein, as well as of peptide fragments. Haddad et al. claimed the ability of enzymatically hydrolysed βLG (peptic and peptic-tryptic digestion) to elicit IgE antibodies even to a higher extent than undigested βLG, whereas peptides obtained by tryptic hydrolysis showed lower in vitro immunoreactivity [31,32].

Therefore, the immunochemical results show that – despite a reduction of native βLG determined by CZE – the naturally fermented milk products still possess a high level of immunoreactivity which presumably results in part from preserved and newly exposed epitopes on peptide fragments resulting from the fermentation process.

The absence of heat treatment in the "naturally" fermented samples (except for the pasteurisation process that was shown to have no influence on the kinetics of peptic digestion), might explain the differences compared to the sour milk and the Acidophilus milk. Industrially manufactured fermentation products need to be treated in an elaborated process for food safety by a combination of multiple heat treatment and fermentation steps.

3.4. Immunochemical monitoring of peptic digestion

In the next step, peptic digestion of whey fractions from the industrially fermented products was carried out. Aliquots were taken before adding pepsin and after several time intervals. All aliquots were assayed for their βLG contents. Milk samples freshly purchased from the store and prepared for peptic digestion at the same day showed no reduction of immunochemial responses upon digestion (within 90 min of incubation with pepsin), while aging of the milk in presence of Lactobacillus acidophilus, sour milk, and whey fractions of both samples, respectively, facilitated the impact of the acid and enzymatic hydrolysis (pepsin) on the stability of βLG in these fermented samples enormously (reduction to approximately 30% of the initial immunoreactive content). Fig. 7 compares the immunore-

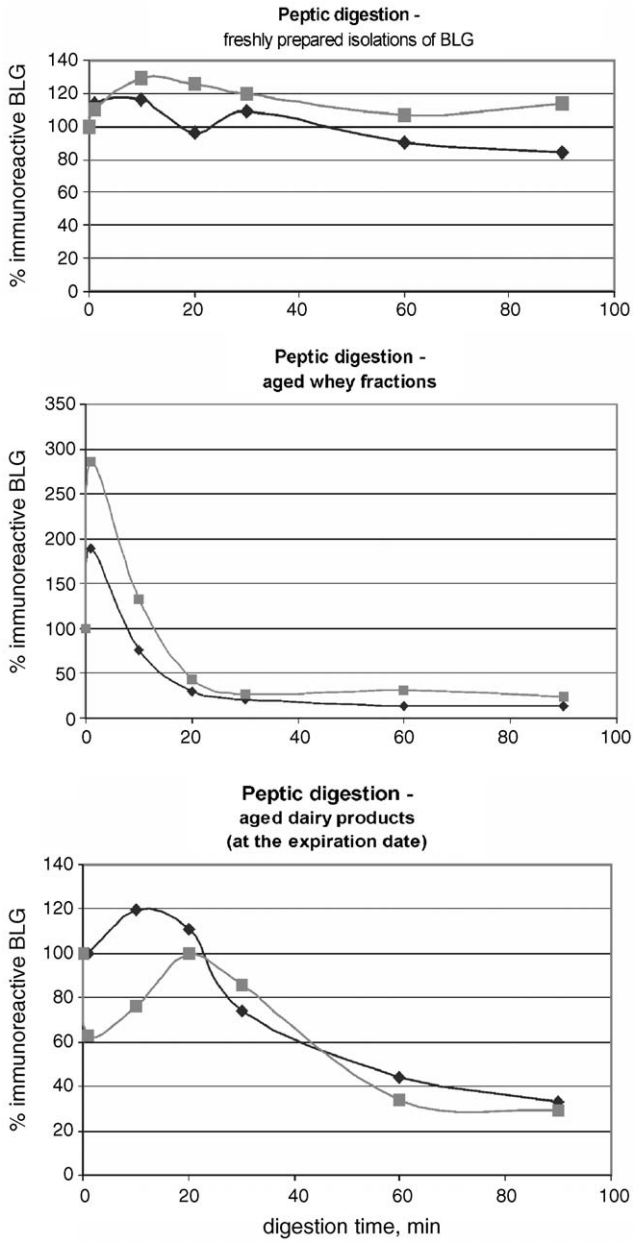

Fig. 7. Comparison of fresh with aged fermented dairy products ((♦) sour milk; (■) "Acidophilus milk"). Whey fractions were incubated with pepsin and several aliquots measured in ELISA at time points given in the figure.

activity of the samples upon peptic digestion measured by ELISA. For these investigations not only fresh samples, but also aged dairy products, as well as whey fractions were digested and subsequently screened for their different antibody binding capacities. The samples were stored at 4 °C and investigated two weeks later once more (at the expiration date). The addition of pepsin significantly modified the immunochemical response and induced a short but strong increase of the ELISA signal shortly after starting the digestion.

All non-fermented reference samples exhibited high stability during sample preparation, storage, digestion and ELISA set up. No reduction in immunoreactivity was observed. Acidification itself had no impact on the antibody binding properties of βLG or its digestibility, as this was tested with a sample of pasteurised milk in two aliquots before and after acidic casein precipitation. The concentration of βLG detected was the same for both aliquots where the first one was at pH 7 and the second one at pH 2. Thus, βLG remained stable.

4. Conclusions

Fermentation of milk products increases the susceptibility of βLG towards peptic digestion and results in highly reduced stability of this allergenic protein. As a consequence, the immunoreactive βLG content of fermented products is reduced. Relative immuno response alterations were measured and could be correlated with the changes in peptic digestibility of βLG due to the fermentation process of bovine milk products. Increased digestibility might offer a promising way for reducing allergenicity. Due to the fact, that only a reduction of βLG allergenicity could be observed, the possibility of the remaining βLG content to elicit immune response in individuals is still given. Therefore, highly allergic patients will still react to fermented products, but it might be a strategy for primary prevention in infants.

CZE enabled to separate, identify, and quantify allergens under non-destructive conditions for monitoring the proteolytic degradation. To achieve improved correlation of immunological antigen–antibody reaction with characteristics of electrophoresis, further investigations will include the hybrid type given by Affinity-CZE which involves an antibody–antigen capture system to be integrated in the total analysis and separation workflow.

Acknowledgments

The present work was carried out within the EU-funded project—Reduced Allergenicity of Processed Foods (Containing Animal Allergens), REDALL QLK1-CT-2002-02687.

We cordially thank Christoph Czerwenka and Gisela Pittner for constructive discussions and critical proofreading of the manuscript.

References

[1] H. Breiteneder, E.N. Mills, J. Allergy Clin. Immunol. 115 (2005) 14.
[2] H.A. Sampson, J. Allergy Clin. Immunol. 113 (2004) 805.
[3] R. Bredehorst, K. David, J. Chromatogr. B Biomed. Sci. Appl. 756 (2001) 33.
[4] D.D. Metcalfe, J.D. Astwood, R. Townsend, H.A. Sampson, S.L. Taylor, R.L. Fuchs, Crit. Rev. Food Sci. Nutr. 36 (Suppl.) (1996) S165.
[5] B.B. Buchanan, C. Adamidi, R.M. Lozano, B.C. Yee, M. Momma, K. Kobrehel, R. Ermel, O.L. Frick, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 5372.
[6] G. del Val, B.C. Yee, R.M. Lozano, B.B. Buchanan, R.W. Ermel, Y.M. Lee, O.L. Frick, J. Allergy Clin. Immunol. 103 (1999) 690.
[7] J.M. Wal, Ann. Allergy Asthma Immunol. 93 (2004) S2.
[8] S. Sharma, P. Kumar, C. Betzel, T.P. Singh, J. Chromatogr. B Biomed. Sci. Appl. 756 (2001) 183.
[9] A. Kananen, J. Savolainen, J. Mäkinen, U. Perttilä, L. Myllykoski, A. Pihlanto Leppala, Int. Dairy J. 10 (2000) 691.
[10] L. Negroni, H. Bernard, G. Clement, J.M. Chatel, P. Brune, Y. Frobert, J.M. Wal, J. Grassi, J. Immunol. Methods 220 (1998) 25.

[11] I. Selo, G. Clement, H. Bernard, J. Chatel, C. Creminon, G. Peltre, J. Wal, Clin. Exp. Allergy 29 (1999) 1055.
[12] I. Selo, C. Creminon, J. Grassi, J.Y. Couraud, Immunol. Lett. 80 (2002) 133.
[13] G. Clement, D. Boquet, Y. Frobert, H. Bernard, L. Negroni, J.M. Chatel, K. Adel-Patient, C. Creminon, J.M. Wal, J. Grassi, J. Immunol. Methods 266 (2002) 67.
[14] J.D. Astwood, J.N. Leach, R.L. Fuchs, Nat. Biotechnol. 14 (1996) 1269.
[15] T.J. Fu, U.R. Abbott, C. Hatzos, J. Agric. Food Chem. 50 (2002) 7154.
[16] M. Besler, H. Steinhart, A. Paschke, J. Chromatogr. B Biomed. Sci. Appl. 756 (2001) 207.
[17] A. Paschke, M. Besler, Ann. Allergy Asthma Immunol. 89 (2002) 16.
[18] E.B. Boso, E.P. Brestel, Allergy 42 (1987) 151.
[19] B.M. Ehn, B. Ekstrand, U. Bengtsson, S. Ahlstedt, J. Agric. Food Chem. 52 (2004) 1398.
[20] B.M. Ehn, T. Allmere, E. Telemo, U. Bengtsson, B. Ekstrand, J. Agric. Food Chem. 53 (2005) 3743.
[21] S. Iametti, P. Rasmussen, H. Frokiaer, P. Ferranti, F. Addeo, F. Bonomi, Eur. J. Biochem. 269 (2002) 1362.
[22] R. Fritsche, Australian, J. Dairy Technol. 58 (2003) 89.
[23] F. Bonomi, A. Fiocchi, H. Frokiaer, A. Gaiaschi, S. Iametti, C. Poiesi, P. Rasmussen, P. Restani, P. Rovere, J. Dairy Res. 70 (2003) 51.
[24] L. Jedrychowski, B. Wróblewska, Food Agric. Immunol. 11 (1999) 91.
[25] I. Ivanova, S. Antonova-Nikolova, I. Iliev, Biol. Wet. 66 (2001) 585.
[26] E. Isolauri, H. Majamaa, T. Arvola, I. Rantala, E. Virtanen, H. Arvilommi, Gastroenterology 105 (1993) 1643.
[27] K. El-Zahar, J.M. Chobert, M. Sitohy, M. Dalgalarrondo, T. Haertle, Nahrung 47 (2003) 199.
[28] N. Kitabatake, Y. Kinekawa, J. Agric. Food Chem. 46 (1998) 4917.
[29] K. Takagi, R. Teshima, H. Okunuki, J. Sawada, Biol. Pharm. Bull. 26 (2003) 969.
[30] T. Neyestani, et al., Protein Expr. Purif. 29 (2003) 202.
[31] Z.H. Haddad, V. Kalra, S. Verma, Ann. Allergy 42 (1979) 368.
[32] S. Pecquet, L. Bovetto, F. Maynard, R. Fritsche, J. Allergy Clin. Immunol. 105 (2000) 514.