

Development of Two Immunoassay Formats To Detect β -Lactoglobulin: Influence of Heat Treatment on β -Lactoglobulin Immunoreactivity and Assay Applicability in Processed Food

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ABSTRACT

Milk proteins are commonly used as ingredients in the food industry because of their functional properties, but they can cause severe reactions in milk-allergic individuals. In this work, two enzyme-linked immunosorbent assay (ELISA) formats were developed to detect bovine β -lactoglobulin. The indirect competitive ELISA involved the use of anti- β -lactoglobulin antisera, and the sandwich ELISA involved the use of specific antibodies isolated using a β -lactoglobulin immunosorbent material. The effect of heat treatment on immunoreactivity of the protein in buffer and in milk was determined with both assays. The amount of immunoreactive protein in buffer and in milk decreased as determined by the sandwich ELISA, whereas the amount increased when measuring with the competitive ELISA. Several food products, including meat, bakery products, sauces, and snacks, were analyzed. With both assays, 10 of 11 products in which the ingredient list included the terms “powdered milk” or “milk proteins” contained β -lactoglobulin. However, the β -lactoglobulin concentration in these products obtained with the competitive ELISA were much higher than those obtained with the sandwich ELISA. These differences could be explained by the fact that the determination of β -lactoglobulin concentration by immunoassay is influenced by differences in antibody recognition of the protein present in highly processed foods. Therefore, the antigen-binding properties of antibodies used in a particular immunoassay are important for a correct interpretation of results obtained in food processed at high temperature.

In the past few years, consumers and the food industry have been facing the challenge of a rapid and substantial increase in food allergies characterized by an increase in the prevalence and severity of clinical manifestations. Food allergies have become a major public health concern with important socioeconomic consequences (10). Eight foods (peanuts, soybeans, wheat, tree nuts, milk, eggs, fish, and crustaceans) are responsible for almost 90% of all food allergies. The allergens in these foods are naturally occurring proteins.

The only effective treatment for food allergy is total avoidance of the offending allergen. Thus, food processors should implement an allergen prevention plan and inform consumers about the presence of food allergens in their products (4). Recently published European legislation about food labeling, such as Directive 2000/13/EC and amendments (8, 9), makes obligatory the inclusion on the label of all ingredients intentionally added and an explicit declaration of those liable to cause allergic reactions or intolerances.

Major attention must be focused on the problem of hidden allergens, which make their way into the food via unintended routes such as misformulation, improper clean-up, or cross-contamination in the processing system. Thus, reliable methods for detecting food allergens are necessary to ensure compliance with food labeling legislation and to

improve consumer protection (22). However, the detection of allergens in food products is sometimes a difficult task because these proteins often are present in trace amounts or could be masked by the food matrix or by technological treatments applied during manufacture (26).

Milk is one of the major allergenic foods, probably because it is the earliest food eaten by humans. Cow's milk allergy affects 2.5% of children younger than 2 years, but about 60 to 80% become clinically tolerant within the first 3 years of life, although severe allergy may persist into adulthood (15). Symptoms of cow's milk allergy are the normal symptoms of many food allergy reactions, mainly urticaria, atopic dermatitis, and vomiting appearing in less than 2 h (27). In recent studies, researchers have tried to determine the threshold doses of milk allergens that produce clinical reactions. The smallest amount that provoked symptoms in oral challenge studies ranged from 0.2 to 5 ml, corresponding to 0.6 and 150 mg of protein, respectively (14).

Cow's milk proteins are present in infant formulas, dairy products, and nondairy foods in which they are added because of their useful technological properties. Therefore, milk proteins are found in a large variety of processed food, including confections, margarine, pies, cookies, pudding, sausage, sauces, and soups (5, 26).

The major cow's milk allergens are caseins and whey proteins such as β -lactoglobulin and α -lactalbumin (2). β -Lactoglobulin is the major protein of bovine whey, at a

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concentration of 4 mg/ml (23), and it has no homologous counterpart in human milk (11). It is a fatty acid-binding protein whose biological function is to participate in the digestion of milk lipids in newborn calves (24).

In the last few years, several immunochemical techniques have been developed to detect bovine milk proteins in food products. These techniques mainly are based on the interaction between the allergenic protein and specific antibodies against it (13, 20, 26).

The type of immunoassay is important with respect to the way an allergen is presented to the specific antibodies, e.g., in a competitive assay the accessibility of determinants for an adsorbed protein might differ from the protein in solution. The allergen-binding properties of the antibody population that is used in a particular assay also are crucial for a correct interpretation of the results. Because most foods are subjected to heating during processing, the antibodies should bind the allergen via heat-stable epitopes; however, these epitopes often are not heat stable (31).

In this study, two enzyme-linked immunosorbent assays (ELISAs), sandwich and indirect competitive, were developed to quantify bovine β -lactoglobulin. The effect of heat treatment on the immunoreactivity of the protein in buffer and in milk was determined with both formats. The β -lactoglobulin concentration also was determined with both ELISAs in several commercial food products (meat and bakery products, sauces, and snacks) in which powdered milk or milk proteins were included or not included on the label.

MATERIALS AND METHODS

Antisera. Antisera to bovine β -lactoglobulin (Sigma Chemical, Poole, UK) were obtained from three New Zealand White rabbits. About 1 mg of β -lactoglobulin was dissolved in 0.5 ml of 0.15 M NaCl and 10 mM potassium phosphate buffer, pH 7.4 (SSF), emulsified in an equal volume of Freund's complete adjuvant (Sigma), and administered in several subcutaneous injections to the back of each rabbit. After 21 days, booster injections prepared as described but using Freund's incomplete adjuvant (Sigma) were administered. Blood was collected from each rabbit from the marginal vein 15 days after booster injections. This blood was allowed to coagulate spontaneously at room temperature, centrifuged at $500 \times g$ for 15 min, and stored at -20°C until use. Titers were determined with a noncompetitive assay and defined as the log reciprocal of the midlinear portion of the dilution curves from plates coated with 1 $\mu\text{g/ml}$ β -lactoglobulin (13). Antiserum with the highest titer was used. The specificity of the antiserum was checked by immunodotting with α -lactalbumin and serum albumin, and no cross-reaction with these whey proteins was observed (results not shown).

Isolation and conjugation of anti- β -lactoglobulin antibodies. Bovine β -lactoglobulin was insolubilized in BrCN-activated Sepharose 4B (Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's instructions, 20 ml of antiserum from immunized rabbits was applied to the immunosorbent, and the column was washed with SSF. Retained antibodies were eluted with 0.5 M NaCl and 0.1 M glycine-HCl buffer (pH 2.8) and immediately neutralized with 0.5 M Tris buffer (pH 8.0). The antibodies obtained were about 95% immunoglobulin G (IgG), as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 4 to 15% acrylamide gradient gels (Pharmacia Bio-

tech). The concentration of purified IgG was determined by spectrophotometry with the extinction coefficient at 280 nm $E_{1\%}^{1\text{cm}} = 14$.

Anti- β -lactoglobulin IgG was conjugated with horseradish peroxidase (250 to 503 U/mg; Sigma) using the periodate method as previously described (3). The conjugate obtained was dialyzed against SSF at 4°C overnight and stored at -20°C until use.

Preparation of food samples. Meat and bakery products, sauces, and snacks were purchased from local retailers. A 5.0 ± 0.1 -g sample was added to 15 ml of SSF and homogenized with an Ultra Turrax homogenizer (Janke & Kunkel IKA Labor Technik, Staufen, Germany) until all particulate material was dispersed. Samples were then centrifuged at $3,000 \times g$ for 15 min, and the supernatant was filtered through fiberglass and stored at -20°C until use.

Heat treatment. A 20- μl volume of raw skim milk or a solution of 200 $\mu\text{g/ml}$ pure β -lactoglobulin in 8 mM Na_2HPO_4 , 3 mM KCl, 0.14 M NaCl, and 1.5 mM potassium phosphate buffer, pH 7.4 (PBS), was introduced into glass capillary tubes (1.5 mm outside diameter and 1.1 mm inside diameter) that were then flame sealed. Samples were heated in two ways: at various temperatures for 10 min and at 80°C for various periods of time. Samples were treated by immersion of capillary tubes in a temperature-controlled water bath ($\pm 0.1^\circ\text{C}$). Heated samples (in duplicate) were removed from the bath at different intervals and immediately cooled by immersion in an ice-water bath. The concentration of immunoreactive β -lactoglobulin was measured by sandwich and indirect competitive ELISAs following the procedure described below.

Indirect competitive ELISA. Each well of a Maxisorp microtitration plate (Nunc, Roskilde, Denmark) was coated with 100 μl of a 5- $\mu\text{g/ml}$ β -lactoglobulin solution in 0.05 M sodium carbonate buffer (pH 9.6). After incubation overnight at 4°C , the wells were washed three times with distilled water. Wells then were coated with 300 μl of 3% (wt/vol) ovalbumin in PBS and incubated for 2 h at 37°C . After being washed three times with distilled water, plates were allowed to dry in air and stored at 4°C until use. β -Lactoglobulin standards were prepared in 1% (wt/vol) ovalbumin in PBS containing 0.05% thimerosal to give concentrations of 1.5 $\mu\text{g/ml}$ to 5 mg/ml, and aliquots were kept frozen until use. Fifty microliters of β -lactoglobulin standards (diluted 1/100) or appropriate dilutions of samples in PBS and 50 μl of rabbit anti- β -lactoglobulin antiserum (diluted 1:6,000 in PBS) were added to the wells. After incubation for 1 h at 37°C , plates were washed five times with distilled water and incubated with 100 μl of horseradish peroxidase-labeled anti-rabbit IgG (diluted 1:4,000 in PBS). Plates were then incubated for 1 h at 37°C and washed five times with distilled water. Tetramethylbenzidine substrate (TMB; ZEU-Immunotec, Zaragoza, Spain) was then added at 100 μl per well and incubated for 30 min at 37°C . The enzyme reaction was stopped by adding 50 μl of 2 M H_2SO_4 per well, and the absorbance was determined at 450 nm with a microplate reader (Multiskan, Labsystem, Helsinki, Finland). All samples were assayed in triplicate in at least three independent experiments.

Sandwich ELISA. A sandwich ELISA was performed in 96-well immunoassay Maxisorp microtitration plates. Each well was coated with 100 μl of anti- β -lactoglobulin IgG (5 $\mu\text{g/ml}$) in 0.05 M sodium carbonate buffer (pH 9.6). After incubation overnight at 4°C , the wells were washed three times with 300 μl of distilled water per well. Each well was then blocked with 300 μl of 3% (wt/vol) ovalbumin in PBS, and the plates were incubated for 2

h at 37°C. After washing three times with distilled water, plates were allowed to dry in air and stored at 4°C. β -Lactoglobulin standards were prepared in 1% (wt/vol) ovalbumin in PBS containing 0.05% thimerosal to give concentrations of 0.5 to 10 μ g/ml, and aliquots were kept frozen until use. One hundred microliters of β -lactoglobulin standards (diluted 1/100) or appropriate dilutions of food samples in PBS were added to the wells, and the plates were incubated for 1 h at 37°C. After incubation, the plates were washed five times with distilled water and incubated with 100 μ l of horseradish peroxidase-labeled anti- β -lactoglobulin IgG (diluted 1:4,000 in PBS) for 1 h at 37°C. After plates were washed five times with distilled water, they were incubated with 100 μ l of TMB substrate per well for 30 min at 37°C. The enzyme reaction was stopped by adding 50 μ l of 2 M H_2SO_4 per well, and the absorbance was determined at 450 nm. All samples were assayed in triplicate in at least three independent experiments.

RESULTS AND DISCUSSION

In this work, two ELISAs were developed and used to determine bovine β -lactoglobulin in food products. The indirect competitive immunoassay is based on the competition between pure β -lactoglobulin bound to the plate and β -lactoglobulin contained in the standards or samples for binding to anti- β -lactoglobulin antibodies present in the rabbit antiserum. The sandwich immunoassay involves the capture of β -lactoglobulin contained in standards or samples by anti- β -lactoglobulin antibodies that have been isolated by immunoabsorption and coated onto the plate. Binding is detected by anti- β -lactoglobulin antibodies conjugated with peroxidase, which bind to the protein bound to the first antibody, forming a sandwich. Calibration curves were obtained using the relationship between the values of absorbance and the logarithm of the concentration of β -lactoglobulin standards dissolved in PBS; the relationship was linear between 15 ng/ml and 50 μ g/ml ($r^2 > 0.99$) for the competitive assay and between 5 and 100 ng/ml for the sandwich assay ($r^2 > 0.98$) (Fig. 1a and 1b). The standard deviation of the absorbance for each of the standard points was less than 0.01. The detection limit of the assays, which was defined as the protein concentration corresponding to the mean absorbance of a negative control minus (for the competitive assay) or plus (for the sandwich assay) three times the value of its standard deviation, was 12 and 2 ng/ml for the competitive and sandwich ELISAs, respectively.

To develop reliable immunoassays to determine the presence of an allergenic protein in food products, it is essential to understand how changes produced during processing affect antibody reactivity (1). Considering that most food products are subjected to heating during processing, the effect of heat treatment on the determination of β -lactoglobulin has been studied using both types of ELISA. Immunoreactivity (IR) refers to the relative changes in the binding of the antibody to the antigen and is calculated as a percentage using the following equation:

$$\text{IR (\%)} = C_h/C_n \times 100$$

where C_n is the concentration of β -lactoglobulin in the untreated sample and C_h is the apparent concentration of the protein in the heat-treated sample. Thus, the immunoreac-

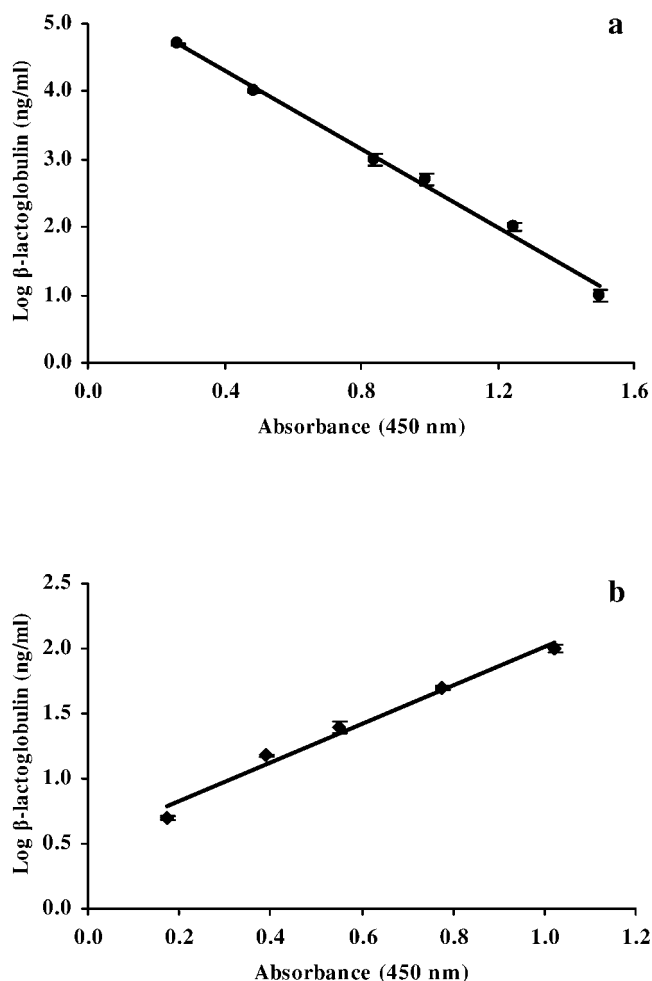


FIGURE 1. Calibration curves obtained for indirect competitive (a) and sandwich (b) ELISAs for determination of β -lactoglobulin concentration in standard solutions of pure β -lactoglobulin in PBS.

tivity of β -lactoglobulin in heated samples will be affected by both the amount of protein and the way in which the antibody population recognizes the thermally denatured protein in a specific assay (17).

Results obtained for the immunoreactivity of β -lactoglobulin in PBS and in raw skim milk after heat treatment are presented in Figures 2a, 2b, 3a, and 3b. Samples were heated in two ways: at various temperatures for 10 min and at 80°C for various periods of time. The graphs show results of three independent experiments, each performed in triplicate.

With the sandwich ELISA, the amount of immunoreactive protein heated at 80°C in buffer or in milk fell sharply within the 4 min of treatment to about 40 and 30% of the initial value, respectively. Afterward, it decreased slowly to reach stable levels of 25% of the initial value after 10 min of treatment in both media (Fig. 2a). In heating experiments performed at different temperatures for 10 min, the immunoreactivity of β -lactoglobulin in buffer and in milk decreased markedly above 70°C. At this temperature, immunoreactive protein was about 50% of the amount of untreated protein. At higher temperatures, immunoreactiv-

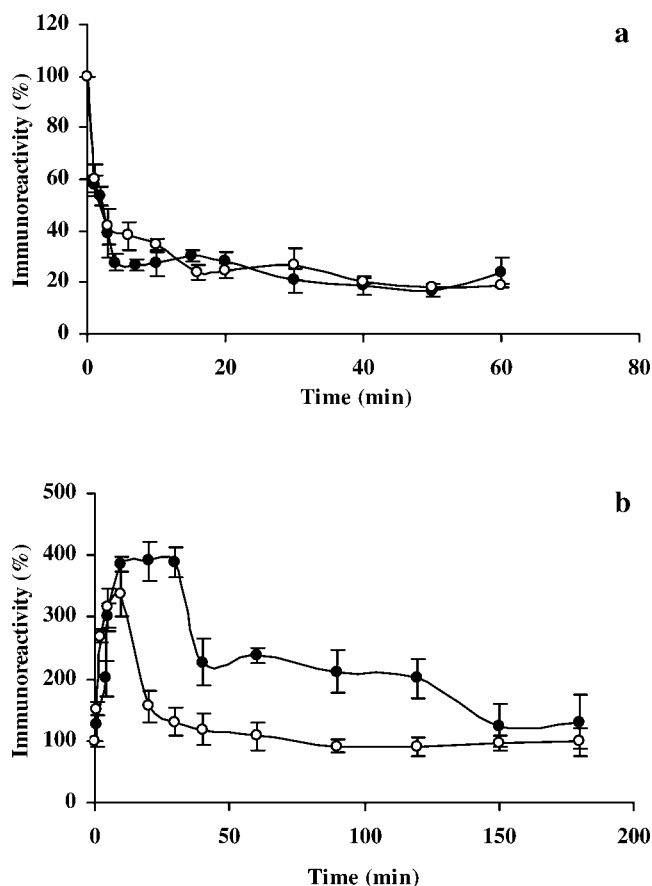


FIGURE 2. Effect of heating at 80°C on β -lactoglobulin immunoreactivity as determined with sandwich (a) and indirect competitive (b) ELISAs. Purified β -lactoglobulin (200 μ g/ml) was analyzed in PBS (●) or skim milk (○). Immunoreactivity is expressed as a percentage of native protein (100%).

ity decreased slowly, maintaining at about 20% after 10 min at 95°C (Fig. 3a).

Similar behavior has been observed by others. Lyster (19) determined by radial immunodiffusion that the amount of immunoreactive β -lactoglobulin decreased to 55% of the initial value after 10 min of heating milk samples at 78°C and to stable values of 15% at 35 min of treatment. Negroni et al. (21), using an immunometric assay and monoclonal antibodies recognizing native β -lactoglobulin, found that about 20% of the initial reactivity of β -lactoglobulin was preserved in skim milk after 5 min at 85°C, and this level was maintained even after treatment at 100°C for 5 min.

The detection of β -lactoglobulin after prolonged heating reported in this and other works indicates that the protein maintains a low but significant immunoreactivity with the antibodies probably because some antibodies recognize continuous linear epitopes of the protein structure and thus can detect partly denatured protein (18, 19, 21).

In contrast, with the competitive ELISA the amount of immunoreactive protein heated in buffer at 80°C increased to about 380% of the initial value within 20 min of treatment. Afterward, immunoreactivity was maintained and started to decline after 40 min of heating, reaching about 200% at 2 h (Fig. 2b). Likewise, the immunoreactivity of the protein in skim milk samples also increased with time

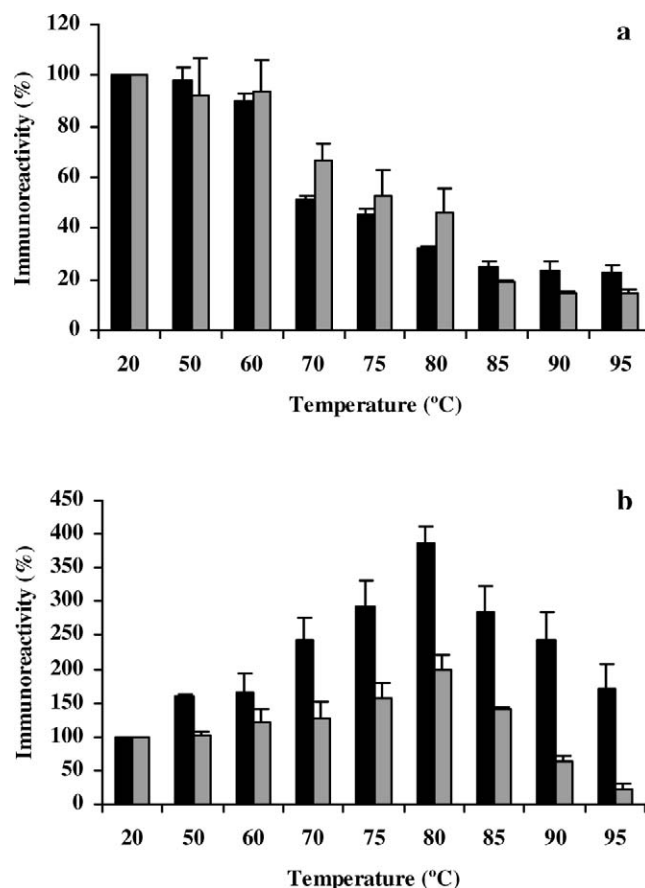


FIGURE 3. Effect of heating at various temperatures for 10 min on β -lactoglobulin immunoreactivity as determined with sandwich (a) and indirect competitive (b) ELISAs. Purified β -lactoglobulin (200 μ g/ml) was analyzed in PBS (solid bars) or skim milk (shaded bars). Immunoreactivity was expressed as a percentage of native protein (100%).

(Figs. 2b and 3b), reaching about 300% of the initial value after 5 min of heating at 80°C and then decreasing quickly to a value of about 120% at 2 h (Fig. 2b). These results indicate that the increase in the immunoreactivity of β -lactoglobulin is higher in buffer than in milk probably because interactions with other milk proteins hamper immunoreactivity (Figs. 2b and 3b).

These results are in close agreement with those reported by Karamonova et al. (17), who used polyclonal antibodies and observed a marked increase in immunoreactivity of β -lactoglobulin variants A and B when the proteins were heated. The immunoreactivity of both variants began to increase at 50 to 60°C, with a marked increase to about 800 and 1,100%, respectively, when heated to 70°C for 15 min. Negroni et al. (21), using monoclonal antibodies that recognized specifically denatured β -lactoglobulin, found that the native form of the protein did not react with the antibodies until the protein was heated to about 72°C, with maximum reactivity at 85°C.

The majority of protein epitopes recognized by antibodies are considered conformational (28). These epitopes depend on the structure of the protein and are displayed on the surface areas of the molecule. For this reason, antibody-binding reactions may be perturbed by changes in the con-

formational structure of a protein, such as those produced during heat denaturation. Therefore, immunological techniques offer a way to determine the thermal behavior of a protein, because loss of immunoreactivity is related to loss of structure (6, 30).

However, antibodies against β -lactoglobulin that were obtained in this study seemed to recognize both native and denatured protein in spite of having been raised against the native form of the protein, as also was observed by Karamonova et al. (17). Similar findings were obtained by other groups (21, 29) with monoclonal antibodies against native β -lactoglobulin. Some monoclonal antibodies recognized native protein whereas others recognized specifically denatured β -lactoglobulin in spite of immunization with a native protein preparation. All these results suggest that a single immunization procedure performed with native β -lactoglobulin can lead to the production of antibodies with different specificities against native and denatured forms of the protein. This result be caused by emulsification of β -lactoglobulin in mineral oil prior to immunization, because this protein undergoes a certain degree of unfolding when adsorbed to the oil-water interface (16).

The way in which anti- β -lactoglobulin antibodies were purified to be used in the sandwich ELISA also may influence the ability of these antibodies to recognize the protein molecule. In the competitive ELISA, whole antiserum was used and seemed to contain antibodies that recognized both native and denatured forms of the protein. However, in the sandwich ELISA, the antibodies employed were purified with an immunosorbent material that contained the native protein thus enabling the isolated antibodies to recognize preferentially that form of the protein. Therefore, the denaturation degree of β -lactoglobulin present in processed food could differentially affect its determination depending on the immunoassay format applied, as has been observed in this study.

This difference between the two ELISAs is important when considering food proteins that retain their allergenicity following heat processing, as is the case of milk proteins (14). Under those circumstances, the apparent reduced protein content estimated by a particular assay does not reflect a reduction in the hazard to the allergic patient. Thus, it is necessary to understand the pitfalls of any assay before it is used to generate data that will be used in risk assessment.

The sandwich ELISA requires antibodies directed against two or more distinct epitopes, whereas the competitive inhibition assay uses a one-epitope approach for the antibody to recognize the protein in a sample (31). To determine the applicability of these two immunoassays for detecting β -lactoglobulin in foods, we tested several commercial products, bakery and cooked meat products, sauces, and snacks, using the two ELISAs.

Some ingredients commonly used in food manufacture, including pasteurized and boiled egg, bovine meat, soy, wheat and pea proteins, sugar, olive oil, and wheat flour, were analyzed previously. Some of these ingredients caused a small decrease or increase of the background level for the competitive and sandwich ELISAs, respectively, indicating interference (results not shown). To overcome this problem,

TABLE 1. Determination of β -lactoglobulin in food products by indirect competitive and sandwich ELISAs

Sample no. ^a	β -lactoglobulin concn (ppm) ^b			
	Competitive ELISA		Sandwich ELISA	
	Mean	SD	Mean	SD
1	35.15	8.38	1.06	0.27
2	8.52	2.45	0.20	0.05
3	37.59	13.21	0.15	0.03
4	45.00	15.72	0.46	0.15
5	7.48	1.91	0.38	0.15
6	22.27	4.01	0.28	0.11
7	495.16	95.00	0.95	0.20
8	709.23	71.52	0.68	0.22
9	1.61	0.61	0.29	0.08
10	0.87	0.31	0.37	0.14
11	0.25	0.10	0.05	0.01
12	0.13	0.04	0.04	0.01
13	0.14	0.05	0.04	0.01
14	0.06	0.02	0.03	0.01
15	0.61	0.08	0.15	0.04
16	0.10	0.03	0.04	0.01
17	0.08	0.03	0.03	0.01
18	0.10	0.03	0.03	0.01

^a Samples were from food products in which the presence of powdered milk or milk proteins was included (samples 1 through 11) or not included (samples 12 through 18) on the label. Samples 1 and 13, snacks; samples 2, 14, and 15, sauces; samples 3 through 6, 9, 10, 12, 17, and 18, cooked meat products; samples 7, 8, 11, and 16, bakery products.

^b Values are the mean and standard deviation (SD) of three independent experiments, each performed in triplicate.

we considered the mean value of the concentration of those ingredients plus three times the value of the standard deviation as the threshold or concentration limit above which a food product would be considered to contain β -lactoglobulin and, thus, milk proteins. These threshold concentrations were 0.20 and 0.06 ppm for the competitive and the sandwich ELISAs, respectively.

The sample survey was performed using 18 food products. The presence of powdered milk or milk proteins was stated in the ingredient list of 11 products but was not stated in 7 products. All samples were analyzed in triplicate in at least three independent experiments. Table 1 shows the results obtained with the competitive and sandwich ELISAs.

Ten products whose labels included the terms "powdered milk" or "milk proteins" contained β -lactoglobulin at concentrations of 0.87 to 700 ppm as determined with the indirect competitive ELISA and of 0.15 to 1.10 ppm as determined with the sandwich ELISA. However, one bakery product with milk listed on the label failed to provide a positive result in either assay. This false-negative result could be due to the lack of sensitivity of the assay for the amount of β -lactoglobulin present or to the addition of a casein fraction instead of whey proteins. Because baking involves higher temperatures than those tested in this study, those higher temperatures might have denatured the protein, creating the false-negative result. The browning reaction

that happens when sugars and proteins are reduced during baking might also alter protein epitopes.

For the products without milk proteins, only one sauce gave a β -lactoglobulin concentration above the detection limit established for the two ELISAs. This presence of this protein could have resulted from the addition of dairy ingredients intentionally without including them on the label. Likewise, the product could have been contaminated with milk constituents during storage or processing from carry-over due to inadequate cleaning of shared processing equipment or through rework of milk-containing products.

Currently, there are no reliable data on the frequency of contamination of processed foods with undeclared allergens. According to the report on control of foodstuffs by the Standard Committee for Foodstuffs of the EU (1, 7), 2.3% of 838 "milk-free" food samples from six EU members states contained undeclared milk proteins.

The concentration of β -lactoglobulin in all food products in this work analyzed with the competitive ELISA was much higher than that obtained with the sandwich ELISA, and the concentration data obtained with both assays were not correlated ($r^2 < 0.37$). However, in raw milk, in which the protein is in its native form, the concentrations of β -lactoglobulin determined with both assays were around 4 mg/ml, similar to the concentration commonly found in milk (23). These facts could be explained in part by assuming that the degree of denaturation of β -lactoglobulin during heat processing may differentially affect the concentration determination by the antibody population used in each ELISA. Assuming a different detection limit for each assay, both formats developed in this work discriminated in a similar way between positive and negative samples. These results indicate the need to use standard reference materials to establish a threshold for detection and thus to make the results of different assays comparable (12, 25).

Several immunochemical methods for the detection of milk proteins in food products have become available in kits during the past few years. Some of these assays are based on determination of β -lactoglobulin and employ different standards, formats, and antibodies (5, 25). As a result, quantitative data obtained from identical samples may differ significantly when these samples are analyzed with different commercial kits, as has been observed in a quality control assay of peanut products in food samples analyzed with different commercial kits (25, 31). These findings may be explained in part by the fact that the determination of allergic proteins is influenced by differences in antibody recognition of allergenic proteins from highly processed foods. In this sense, results derived from this study indicate that the antigen-binding properties of the antibody population used in a particular immunoassay are important for correct interpretation of results obtained.

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