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Effects of Thermal Processing on the Enzyme-Linked Immunosorbent Assay (ELISA) Detection of Milk Residues in a Model Food Matrix

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Food products and ingredients are frequently tested for the presence of undeclared allergenic food residues (including milk) using commercial enzyme-linked immunosorbent assays (ELISAs). However, little is understood about the efficacy of these kits with thermally processed foods. This study evaluated the performance of three milk ELISA kits with a model food processed by several methods. A model food (pastry dough squares) was spiked with nonfat dry milk at several concentrations. The pastry squares were processed by boiling (100 °C for 2 min), baking (190 °C for 30 min), frying (190 °C for 2 min), and retorting (121 °C for 20 min with 17 psi overpressure). Samples were analyzed with three commercial ELISA kits: Neogen Veratox Total Milk, ELISA Systems β-lactoglobulin, and ELISA Systems casein. The detection of milk residues depended upon the type of processing applied to the food and the specific milk analyte targeted by the ELISA kit. Poor recoveries were obtained in all processed samples (2−10% of expected values) using the β-lactoglobulin kit. Better recoveries were obtained in boiled samples (44 and 59%, respectively) using the total milk and casein kits. However, these kits performed poorly with baked (9 and 21%) and fried (7 and 18%) samples. Moderate recoveries were observed in retorted samples (23 and 28%). The decreased detection in processed samples is likely due to protein modifications, including aggregation and Maillard reactions, which affect the solubility and immunoreactivity of the antigens detected by the ELISA methods. The observed decreases in ELISA detection of milk are dramatic enough to affect risk-assessment decisions. However, a lower detection of milk residues does not necessarily indicate decreased allergenicity. These ELISA kits are not acceptable for all applications, and users should understand the strengths and limitations of each method.

KEYWORDS: Food allergy; milk; ELISA; thermal processing; allergen detection methods

INTRODUCTION

For millions of individuals, the diagnosis of a food allergy has an enormous impact on their lives. The task of completely avoiding certain foods can prove to be challenging, tedious, and confusing. The impact of food allergies reaches far beyond those diagnosed with a food allergy. From caregivers and family members to healthcare professionals to food manufacturers, a myriad of individuals and groups are impacted by food allergies.

In the United States, 3.5−4.0% of the general population and 5−8% of children under the age of 3 suffer from food allergies (*1*). The most common food allergy in children is cow's milk allergy (CMA), which affects 2−3% of young children (*2*−*5*). Many of the children affected by CMA will eventually outgrow the disease. However, the rate of tolerance development is less certain. Original estimates (*2*) indicated a recovery rate of 87% by age 3. A more recent study (*5*) indicated that only 5−19% of children outgrew CMA by age 4 and 55−79% outgrew CMA by age 16.

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The symptoms of an allergic reaction to cow's milk can range from mild to life threatening and can include gastrointestinal, cutaneous, respiratory, and generalized symptoms (*1*). In severe cases, potentially fatal anaphylactic reactions can occur (*6*−*10*). As with other food allergies, the only definite way to prevent reactions is to completely remove the offending food, in this case milk, from the diet (*1*).

The most common form of CMA is an abnormal, heightened IgE-mediated immune response to proteins present in milk (*1*,*3*,*4*). Cow's milk contains numerous proteins, many of which have been shown to be capable of sensitizing susceptible individuals (*3*). However, the major allergens (those proteins against which at least 50% of allergic individuals have specific IgE) are whole casein (CN) and the whey proteins β-lactoglobulin (BLG) and α-lactalbumin (ALA) (*1*, *3*, *11*).

Typical bovine milk contains 3.0−3.5% protein, and milk proteins can be divided into two categories based on their solubility at pH 4.6 (*4*, *12*). Whey proteins, which are soluble at pH 4.6, account for approximately 20% of the total milk protein fraction and include specific proteins, such as BLG and ALA.

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Caseins represent the remaining 80% of milk proteins and are insoluble at pH 4.6 (*4*, *13*). The casein fraction consists of α_{s1}-, α_{s2}-, β-, and κ-caseins (*13*).

The prevalence of cow's milk allergy and the potential severity of allergic reactions to cow's milk have warranted the required labeling of milk and milk-derived ingredients in food products in the United States, Canada, and Europe (*14*−*16*). Because of the shared nature of most food-processing facilities, food manufacturers often need reliable testing methods to ensure that products do not contain residues of undeclared allergenic foods.

Enzyme-linked immunosorbent assays (ELISAs) are commonly used to detect allergenic foods, and a number of commercial ELISA methods are available for the detection of milk residues. The commercial ELISAs have variations in format, specificity, sensitivity, and reporting units. Both sandwich and competitive ELISA formats are available for various milk proteins, and dependent upon the manufacturer, the kits can be specific for whey proteins, casein proteins, or a combination of both. In addition, the assays express results based on one of several materials, including nonfat dry milk (NFDM), whole casein, and BLG.

While ELISA techniques have a number of advantages for detecting allergenic foods (e.g., specificity, sensitivity, and simplicity), these methods are not necessarily validated with processed food matrices. In particular, thermal processing can have an impact on the detection of milk residues. Because antibodies recognize specific epitopes on a protein, processes that alter the protein structure have the potential to alter antibody binding (*17*). In addition, heating can result in the formation of insoluble protein aggregates, which are undetectable by many ELISA methods (*18*−*20*). Despite the potential detection issues associated with heated products containing milk, studies investigating the effects of thermal processing on ELISA detection of milk residues in food matrices are very few in number.

The objective of this study was to determine how adequately commercial ELISA methods are able to detect milk residues in a thermally processed food matrix.

MATERIALS AND METHODS

Materials. The following ELISA kits were purchased from their respective manufacturers: Neogen Veratox Total Milk (Lansing, MI), ELISA Systems BLG, and ELISA Systems casein (Windsor, Queensland, Australia). Gold Medal unbleached all-purpose wheat flour, Crisco all-vegetable shortening, Morton iodized salt, and Nestle Carnation Instant NFDM were obtained from a local grocery retailer.

Preparation of the Model Food: Pastry Dough Squares. The formulation for the pastry dough was as follows: 57.1% unbleached all-purpose wheat flour, 19.5% vegetable shortening, 1.5% salt, and 21.9% type-I reagent-grade water. NFDM was incorporated into the model food by first producing a concentrated spiking material (10 000 ppm NFDM in flour). Flour (396.0 g) and NFDM (4.0 g) were placed in the bowl of a food processor fitted with a blade attachment and thoroughly blended. To produce the desired concentrations of NFDM in the model food, this spiking material replaced an appropriate amount of the flour in the pastry dough formulation.

Pastry dough was prepared with the following concentrations of NFDM: 0, 10, 25, 50, 100, 250, 500, 1000, and 2500 ppm on a wet basis (μg/g of total mass). The flour (and concentrated spiking material when appropriate) was blended in the bowl of a food processor. Salt, shortening, and water were added sequentially with appropriate mixing. The resulting dough was allowed to rest in the refrigerator (4 °C) for 2 h. The dough was then rolled to an even thickness (3 mm) using a pasta roller and cut into 2 × 2 cm squares, which were kept frozen (−15 °C) until further use.

Thermal Processing. The pastry dough squares were boiled, baked, fried, and retorted according to the procedures below.

Boiling, baking, and frying were performed on the following dough samples: 0, 10, 25, 50, 100, and 250 ppm NFDM. For each concentration, approximately 75 g of frozen dough squares was boiled in 1.0 L of water (100 °C) for 2 min, fried in 1.0 L of soybean oil (190 °C) for 2 min, or baked in a conventional oven (190 °C) for 30 min. After heating, the squares were drained briefly (boiling and frying), transferred to a blender jar, allowed to return to room temperature, and blended until homogeneous. In addition, the cooking water from boiling was allowed to cool to 60 °C, and two subsamples were taken for analysis.

Retort processing used the following batches of dough: 0, 100, 250, 500, 1000, and 2500 ppm NFDM. To simulate the fluid-based products often associated with retorting, the pastry dough samples were placed in 1 quart glass canning jars and diluted 10-fold in type-I reagent-grade water. Canning lids and rims were sealed onto the jars in a vacuum oven for approximately 10 min. The samples were processed in a still retort at 121 °C for 20 min with 17 psi of overpressure. The retort was allowed to cool for 30 min before the jars were removed. The canning lid was removed from each jar and replaced with a blender blade assembly, and the entire contents of the jar were homogenized. Unheated jar controls were prepared in a similar manner to the retorted samples.

ELISA Analysis. All of the processed samples and unheated controls were analyzed on the following kits: Neogen Veratox Total Milk, ELISA Systems BLG, and ELISA Systems casein. All three kits used sandwich ELISA formats. The kit manufacturers supplied all buffers, reagents, and other assay components. For each commercial kit, two independent extracts were prepared and each extract was analyzed in triplicate using the respective kits. Extraction and analysis were performed according to procedures provided by each kit manufacturer. Briefly, for the total milk assay, 5.0 g of homogenized sample was extracted in 125 mL of buffer with one scoop of extraction additive for 15 min in a 60 °C shaking water bath. The samples were diluted as necessary and analyzed. The absorbance values of the standards and samples were measured at 650 nm. The quantitative results were calculated from the standard curve using the software provided by the manufacturer. The total milk kit expresses results in parts per million (ppm) NFDM and has a limit of quantitation (LOQ) of 2.5 ppm NFDM.

The assays for casein and BLG both used the same extraction protocol, which consisted of extracting 5.0 g of sample in 50 mL of extraction buffer for 15 min in a 60 °C water bath, with mixing every 5 min. The samples were diluted when necessary and analyzed. The quantitative results were determined using GraphPad Prism 4 software by analyzing the standard curve absorbance values with a cubic spline function and interpolating the sample results. The casein kit expressed results on a ppm NFDM basis, while the BLG kit used ppm BLG. The LOQ for the casein kit is 1.0 ppm NFDM, and the LOQ for the BLG kit is 0.1 ppm BLG.

Moisture Content Determination. The moisture contents of the unheated, boiled, baked, and fried samples were determined using an OHAUS MB200 infrared moisture balance (Auto Dry, 110 °C, change in weight less than 0.01 g in 60 s). The determination was performed in duplicate. The moisture content data were used to compensate for any moisture loss or gain that occurred during processing. Results were converted to dry basis values with the following formula: [(result on a wet basis/solids content) = result on a dry basis].

Standard Solution Analysis. A series of NFDM solutions was prepared and analyzed on the three kits discussed above to establish expected values for each of the different assays. A solution of 1000 ppm NFDM in type-I reagent-grade water was prepared using a 100 mL volumetric flask, and five solutions (10, 25, 50, 100, and 250 ppm NFDM) were prepared from this stock solution. The samples were extracted and analyzed in triplicate using the kits listed previously, according to the instructions of each manufacturer.

Statistical Analysis. The results from each ELISA kit were analyzed by two-way analysis of variation (ANOVA) using the SAS 9.2 software package, with the NFDM concentration and heat treatment as the two factors. The dough samples (unheated, boiled, baked, and fried) were analyzed separately from the jar samples (unheated jar and retorted) because the former was expressed on a dry basis and the latter was expressed on a wet basis. The simple effect differences between thermally processed samples and the unheated control at each NFDM concentration were determined with a significance level of $\alpha = 0.05$. The percent of unheated and percent of expected values were also analyzed by two-way ANOVA, with the kit and heat treatment as the two factors. The simple effect differences among kits were determined for each thermal treatment ($\alpha = 0.05$).

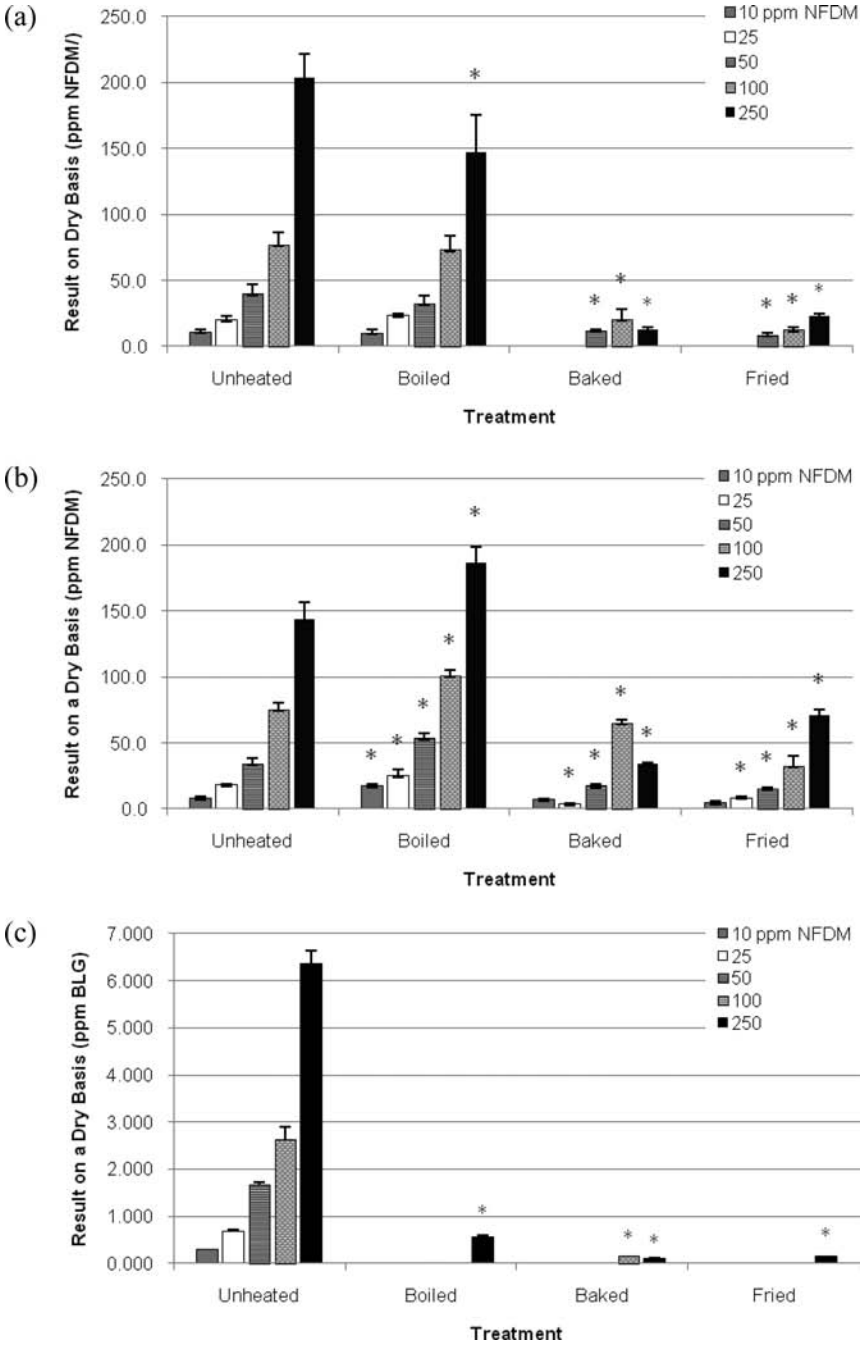

Figure 1. Results of ELISA analysis of unheated and processed dough samples: (**a**) Neogen Total Milk, (**b**) ELISA Systems casein, and (**c**) ELISA Systems BLG. The bars within each treatment represent dough samples with different levels of incurred NFDM (10, 25, 50, 100, or 250 ppm). Dough samples without a value shown were BLQ. (∗) Samples that are significantly different ($\alpha = 0.05$) from the respective unheated sample.

RESULTS AND DISCUSSION

This study evaluated the ability of commercial milk ELISAs to detect milk present in thermally processed food matrices. The detection of milk residues in the pastry dough model food system was dependent upon both the type of processing applied to the dough and the assay used to analyze the products. The results of the analysis can be seen in **Figures 1** and **2**. Boiling did not have a tremendously negative impact on detection using either the total milk or casein kit (panels **a** and **b** of **Figure 1**, respectively). In the case of the total milk assay, only one NFDM concentration (250 ppm) was significantly lower than the unheated control ($p < 0.05$). When analyzed with the casein assay, the boiled samples surprisingly delivered significantly higher results than the unheated dough. One could speculate that this observed increase was due to changes in the solubility or structure of casein or matrix components after boiling. In contrast to the total milk and casein kits, the BLG assay exhibited very poor detection with the boiled samples, only detecting BLG in the dough with the highest NFDM concentration (**Figure 1c**). The BLG signal in this sample was also significantly less than the unheated control. The levels of milk proteins in the cooking water samples were below the limit of quantitation (BLQ) in all kits.

Unlike boiling, baking and frying resulted in dramatically lower detection in all three kits (**Figure 1**). The casein assay perhaps performed the best of the three, detecting milk residues in all of the samples but at significantly lower levels than the unheated control (except for the 10 ppm NFDM sample). The total milk assay only detected NFDM in the 50, 100, and 250 ppm

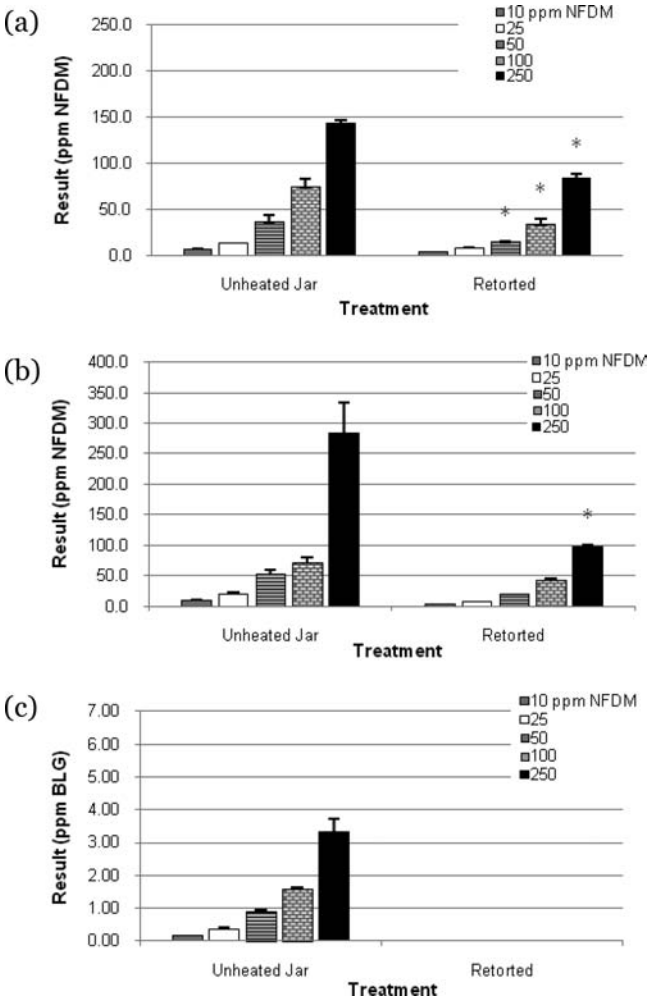

Figure 2. Results of ELISA analysis of unheated and retorted jar samples: (**a**) Neogen Total Milk, (**b**) ELISA Systems casein, and (**c**) ELISA Systems BLG. The bars within each treatment represent dough samples with different levels of incurred NFDM (10, 25, 50, 100, or 250 ppm). Dough samples without a value shown were BLQ. (∗) Samples that are significantly different ($\alpha = 0.05$) from the respective unheated sample.

samples that had been baked or fried. The level of total milk detected after baking or frying was significantly less than detected in the unheated dough. Milk residues were only detected in one fried sample (250 ppm NFDM) and two baked samples (100 and 250 ppm NFDM) using the BLG kit. As indicated in **Figure 1c**, the level of BLG detection was again significantly less than for the unheated control.

Retorting the dough and water mixtures similarly had disparate effects on the detection with the BLG kit versus the total milk or casein kits (**Figure 2**). Milk was detected in all of the samples with the total milk kit, although the signal was significantly decreased in comparison to the unheated jar controls in the 50, 100, and 250 ppm samples (**Figure 2a**). Likewise, analysis with the casein kit resulted in lower values for the retorted samples, but only one NFDM concentration (250 ppm) was significantly decreased from the unheated control (**Figure 2b**). However, milk residues could not be detected in any of the retorted samples with the BLG assay (**Figure 2c**).

Thermal processing affected the ability to detect milk residues in this model food using the commercial ELISA methods. The BLG kit performed very poorly in all of the processed products, detecting milk residues at only the highest spike levels (100 and/or 250 ppm NFDM). Even when BLG was detected, the apparent concentration of analyte estimated by the assay was dramatically and significantly lower than in the unheated control. In contrast, milk residues were detected reasonably well in the boiled and retorted products using the casein and total milk kits, but significantly poorer results were obtained in the baked and fried products. The total milk kit, which according to the manufacturer targets both casein and whey proteins, generally yielded lower results than the casein kit. If thermal processing decreases the detection of whey proteins by the total milk kit as highly as was observed with the BLG kit, the total milk assay could be detecting predominantly casein in the heat-processed products. This difference in detection of the various milk protein fractions would account, at least in part, for the differences observed between the total milk and casein kits.

Diaz-Amigo documented similar decreases in the detection of NFDM after thermal processing in a baked cookie system (*21*). Peanut butter cookies were produced with 500 ppm NFDM, baked, and analyzed with five ELISA kits detecting total milk, casein, and BLG. After 15 min of baking, the detected level of the analytes of the respective kits had decreased to 11−62% of the unheated signal, depending upon the kit manufacturer and specificity.

The decreased detection of milk proteins observed after the thermal processes implemented in this study could be due to two main factors. Milk protein extractability is critically important in ELISAs because only the proteins present in the sample extract have the possibility of being detected by the antibodies in the test. Upon heating, milk proteins have been well-documented to form insoluble aggregates (*22*−*24*). Additionally, thermal processing could induce interactions between milk proteins and other matrix proteins, particularly wheat gluten proteins. Researchers working with egg white proteins have reported decreased extraction and immunoreactivity of ovomucoid when it is processed in a wheat gluten-containing matrix (*18*−*20*).

Alterations in protein structure and conformation could also contribute to the decreased detection of milk residues in heated products. Antibodies recognize specific conformational epitopes on a protein. Thus, any process that alters the structure of a milk protein has the potential to change the ability of an antibody to bind to that specific antigen (*25*). Milk proteins have varying susceptibilities to thermal denaturation, which could explain the differing effects of heating on the detection of BLG and casein. BLG has well-defined secondary and tertiary structures, which unfold fairly readily upon exposure to high temperatures (*26*). Caseins, on the other hand, lack a formal secondary structure and are very resistant to thermal processing (*13*, *27*).

The type of thermal processing applied to the product also impacted the detection of the various milk protein fractions using these ELISA methods. In the case of the kits detecting casein or total milk, detection was much poorer after dry-processing methods (i.e., baking and frying) than wet-processing methods (i.e., boiling and retorting). Without further investigation, the reason for these dissimilar results cannot be explained. The pastry squares should have achieved higher temperatures with the dry-processing methods after most of the water has evaporated from the system. These elevated temperatures could have a more substantial impact on the structure and extractability of milk proteins. In addition, different types of protein modifications can take place at lower water activities. In particular, Maillard browning occurs more rapidly in dry-processing systems with intermediate water activities and high temperatures (*28*). Maillard modifications have been shown to change antibody−antigen binding for a number of allergenic foods, including milk (*29*, *30*).

An important distinction must be made between the decreased detection of milk residues and decreased allergenicity.

Table 1. Results of ELISA Analyses of Standard NFDM Solutions[a]

	Total Milk		casein		BLG	
NFDM	standard solution result	expected value on a dry basis	standard solution result	expected value on a dry basis	standard solution result	expected value on a dry basis
10	12.6	16.9	13.6	18.2	0.19	0.25
25	37.9	50.6	36.6	48.9	0.43	0.58
50	71.5	95.5	71.1	94.9	1.04	1.38
100	126.2	168.5	150.5	201.0	1.84	2.46
250	332.3	444.0	347.5	464.2	4.27	5.70

[a] The standard solution result columns indicate the signal obtained when the standard solutions of NFDM in water were analyzed with each kit. The expected value on a dry basis columns indicate the standard solution results converted to a dry basis based on the mean solids content of the unheated dough (μg of standard material/g of dry mass).

The diminished detection of milk after thermal processing, while dramatic, does not necessarily indicate a reduced risk to milk-allergic consumers. In human challenge trials with milk-allergic individuals, some subjects were able to consume milk-containing products that had undergone baking but other patients had anaphylactic reactions (some severe) to these same products (*31*). Because the differences between these two groups of patients have yet to be well-defined, it would be prudent for food manufacturers to consider any milk residues as potentially hazardous to allergic consumers. In addition, insoluble aggregates of milk proteins not detected by ELISA methods could be resolubilized by digestion and retain their ability to elicit allergic reactions.

The results from this study illustrate another important consideration in the selection and development of ELISA allergen kits. Even under the best of circumstances, direct quantitative comparisons between kits are quite difficult. The numerous milk ELISA kits on the market have different antibody specificities, different standard curves with various reporting units, and different LOQs. Even with just the three kits used for this study, the assays had antibodies recognizing three different milk proteins or mixtures of proteins (BLG, α_s-casein, or whey and casein), two different standard curve materials (BLG or NFDM), and three different LOQs. The use of theoretical conversion factors based on the typical protein profiles of milk may not be appropriate for general use because of the abundance of different commercially available milk sources (milk powders, whey protein isolates, whey protein concentrates, caseinates, etc). Theoretical conversions also do not account for important factors, such as protein solubility, processing modifications, and seasonal or breed variations, in the levels of specific proteins in milk.

One possible method for making direct comparisons between ELISA kits and their performance to thermally processed foods is to calculate the results as a "percent of unheated control". This type of evaluation can indicate the relative effects of heating on the detection capacities of the kits and would deliver information similar to that displayed in **Figures 1** and **2**. While using "percent of unheated control" to compare the performance of various kits in a thermally processed food matrix has value, a more comprehensive approach would be to use "percent of expected value" for such comparisons. Analyzing the source of milk formulated into the product (in this case, the specific NFDM) in each of the kits, one can develop experimental expected values for the detection of a particular milk ingredient, irrespective of the food matrix. The "percent of expected" values can be calculated with the following formula: [(result/experimental expected value) $\times$ 100 = percent of expected value]. The advantage of using this type of calculation is that it can convey the effects of both the food matrix itself and the processing applied to the food.

Table 1 provides the experimental expected values from the analysis of standard solutions of NFDM in water. In addition, **Table 1** includes expected values expressed on a dry basis because the dough samples were spiked on a wet basis but treated samples were compared on a dry basis. The standard solution results represent the apparent analyte values in the following conversion equation: [apparent concentration of analyte spiked into unheated dough on a wet basis/solids content of unheated dough = expected value on a dry basis].

Both the total milk and casein kits delivered results for the standard solutions that were higher than the theoretical values, indicating that the NFDM used in the standard solutions was different from the NFDM present in the standards of the manufacturers. While these types of differences are not entirely unexpected because of the inherent variability of cow's milk (e.g., breed, seasonal, and individual variations), the results emphasize the difficulties with developing a standardized yet relevant calibration standard.

In contrast to the other kits, the BLG kit delivered much lower results for the standard solutions than predicted from the conversions supplied by the kit manufacturer. These theoretical conversions indicated that the 10, 25, 50, 100, and 250 ppm NFDM concentrations would be equivalent to 0.32, 0.80, 1.6, 3.2, and 8.0 ppm BLG, respectively. When the standard solutions of NFDM were analyzed with the BLG kit, the apparent concentrations were 0.19, 0.43, 1.04, 1.84, and 4.27 ppm BLG. The conversion table from the manufacturer seems to be based on the typical protein composition of NFDM, with total protein accounting for 32% of NFDM and BLG comprising 10% of the total protein fraction (*13*, *26*). However, this observation is purely an assumption, because the kit manufacturer gives no indication as to how the conversion table values were developed.

When the results from this study are analyzed as percents of the expected values, a number of interesting features of kit performance can be observed (**Table 2**). The results calculated for the unheated dough samples indicate that significantly lower percents of expected values were obtained with the total milk and casein kits by comparison to the BLG kit, indicating that interactions with the food matrix itself had an effect on the detection in these two kits. Perhaps BLG is inherently more extractable in its native form than the caseins. Similar results were seen with the unheated jar samples.

Because of the nature of ELISA methods, one will probably never be able to obtain the "right" answer from every food matrix. However, kit manufacturers could improve the comparability between kits by adopting a common reference material for the standard curves and then expressing the assay results in the same units. The use of units such as ppm BLG or ppm casein makes interpretation of results quite difficult for both allergic consumers and food manufacturers. Most milk thresholds are reported as milligrams of milk and not milligrams of BLG or casein (*32*). Thus, expression of results in ppm NFDM seems more clinically relevant and was used as the unit of measurement in two of the three kits compared here. Furthermore, purified BLG is not an ingredient used by the food industry. Thus, the expression of results on the basis of BLG content would require unit conversion to some more relevant unit of measurement.

Table 2. ELISA Analysis Results Expressed as a Mean Percent of the Expected Value[a]

	kit		
treatment	Total Milk (%)	casein (%)	BLG (%)
unheated dough	48 ± 11.4 a	37 ± 5.8 b	116 ± 6.9 c
boiled	44 ± 12.7 a	59 ± 20.6 b	10 ± 0.5 c
baked	9 ± 5.4 a	21 ± 13.4 b	4 ± 2.8 a
fried	7 ± 2 a	18 ± 4.9 b	3 ± 0.5 a
unheated jar	47 ± 10.1 a	69 ± 15.6 b	84 ± 6.5 c
retorted	23 ± 3.2 a	28 ± 6.2 a	N/A

[a] Values within each row followed by different letters are significantly different ($\alpha = 0.05$).

While theoretical conversion factors based on typical milk protein profiles can be developed to convert between specific protein units, they may not accurately reflect the amount of milk in the sample. The post-analysis conversion of results does not take into account factors such as the relative antigen-binding abilities and solubilities of the various proteins, because the protein profiles are usually determined by methods other than immunochemical analysis. An ideal reference standard would be one that is applicable to both allergic consumers and the food industry. The material should also be readily available and consistent in composition. Traditional NFDM seems to be the most obvious choice for a standard curve material because it fits most of these criteria. In addition, it contains all of the potentially allergenic milk proteins, i.e., both whey and casein fractions. Some of the natural variation in the composition of specific milk proteins in NFDM could be controlled using NDFM from a single manufacturing process and a diverse pool of milk to produce a standard calibration or reference material. While the use of purified proteins in standard curves may provide more precise information about the specific dynamics of a food system, a single protein is not representative of all milk allergens and would not provide useful information to industry, consumers, or regulators.

The use of a common reference material by itself still may not give the kit user all of the desired information. This set of experiments used NFDM as the source of hypothetical milk contamination. However, the food industry commonly uses a number of different milk-containing ingredients, including caseinates, whey protein isolates, and whey protein concentrates. If the source of milk primarily contains proteins from one main fraction (casein or whey), it would be logical to use a kit that specifically detects the proteins found in the fraction in question. For example, if a whey protein isolate was the source of potential contamination, it would make sense to use a kit that detects BLG or ALA. However, as illustrated in this study, thermal processing can have differing effects on the various milk proteins. If whey protein isolate was present in a thermally processed product, it might not be detectable by any immunochemical method. Users of milk ELISA kits are advised to consider the nature of the milk ingredient, the antigenic specificity of the kit, and the effect of processing on the antigen in selecting the best ELISA for a particular purpose.

In light of the decreased detection of milk residues in heated model foods, the use of different extraction and/or detection methods might be required. Several groups have had success in extracting thermally processed proteins of various allergenic foods using combinations of reducing and disaggregating agents (*33*−*37*). If, however, attempts to modify immunochemical methods prove unsuccessful, other detection methods, such as mass spectrometry, might be required for the detection of certain milk residues in heated food products.

The application of various thermal processes to a milk-containing food matrix can result in dramatically reduced detection of milk residues by commercial ELISA. The level of decrease is dependent upon both the type of processing applied to the food and the target protein(s) of the assay. The observed decreases in detection are certainly substantial enough to affect risk assessment decisions by food industry professionals and public health authorities. Users of commercial ELISA kits should carefully consider the strengths and weaknesses of the various assays before selecting one for a particular use.

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