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ARTICLE

Development and Validation of an Enzyme-Linked Immunosorbent Assay (ELISA) for Quantification of Lysozyme in Cheese

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A competitive enzyme-linked immunosorbent assay (ELISA) was developed to quantify the amount of the preservative and potential allergen lysozyme in cheese using a commercially available monoclonal antibody against hen egg white lysozyme. The limit of detection for lysozyme in a cheese matrix amounted to 2.73 ng/mL, and the working range comprises 3.125−800 ng/mL. Intra- and interassay coefficients of variation were lower than 12%. Neither cross-reactivity with α-lactalbumin and human lysozyme nor unspecific interference with matrix components was observed. The recovery of lysozyme-spiked cheese ranged from 87.4 to 93.6% at four concentrations (50, 100, 200, and 400 mg/kg). The ELISA method was also compared to a high-performance liquid chromatography (HPLC) method, confirming the reliability and accuracy of the ELISA. A total of 21 commercially available cheese samples produced with and without lysozyme were analyzed with ELISA as well as HPLC. Both methods showed good agreement with a correlation index of R^2 = 0.990.

KEYWORDS: Cheese; lysozyme; ELISA; allergen; monoclonal antibody

INTRODUCTION

Lysozyme (muramidase, EC 3.2.1.17) is used as a preservative, mainly during the production of ripened cheese. Its antimicrobial effect is based on the catalytic activity of lysozyme to hydrolyze the cell wall of Gram-positive bacteria, such as *Clostridium tyrobutyricum* (*1*), which cause late gas blowing in cheese. The use of lysozyme as a preservative in cheese is legal in the European Union (EU) but must be declared (European Parliament and Council Directive 95/2/EC; Codex Alimentarius Codex Standard 283-1978).

With a concentration of 3.5%, lysozyme is one of the major components of hen egg white (HEW) (*2*). Therefore, HEW is used as the raw material to produce lysozyme for application in the food industry. Egg products, however, count among the most common causes of food allergies, with the estimated prevalence of egg allergies being 1.6% in children (*3*) and 0.4% in adults (*4*). Lysozyme is considered to be one of the allergens in HEW (*5*−*7*). According to the joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Codex Alimentarius Commission, egg and egg products shall therefore always be declared on prepackaged foods (Codex Standard 1-1985). In Europe, the use of egg and egg products used as ingredients or food additives must be labeled according to the Directive 2000/13/EC, Annex IIIa of the European Parliament.

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Several methods have been developed for the identification and quantification of lysozyme. Some assays determine the lytic effect of lysozyme on the cell wall of *Micrococcus luteus* (*Micrococcus lysodeicticus*) (*8*, *9*) by turbidimetric analysis. Furthermore, the presence of lysozyme can be detected by sodium dodecyl sulfate (SDS) gel electrophoresis (*10*), chromatography by either high-performance liquid chromatography (HPLC) (*11*, *12*) or liquid chromatography−mass spectrometry (LC−MS) (*13*), capillary zone electrophoresis (*14*), or immunoassays. Recently, a rapid but very reliable method to detect lysozyme in cheese was described using immunocapture mass spectrometry (*15*). The enzyme-linked immunosorbent assay (ELISA) technique provides high sensitivity and specificity without demanding sophisticated equipment. Several ELISA methods are described in the literature for the detection and quantification of HEW lysozyme in foods (*16*−*18*), wine (*19*), and the hen egg white itself (*20*). However, in these studies, polyclonal, mostly lab-made antibodies were used, which are not readily available for a broad application in routine analysis and food control. Furthermore, polyclonal antibodies often show high batch−batch variation of the antibody properties. This requires new optimization and rigorous validation for each batch of antiserum and limits its application in an official standard method. To our knowledge, no commercial ELISA designed for the detection of lysozyme in cheese is currently available. It was shown that a commercial ELISA intended for the detection of HEW proteins in pasta, salad dressing, sausages, wine, and ice cream was not reliable for the detection of lysozyme in cheese (*21*).

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Therefore, the aim of the present study was to develop an ELISA for the quantification of lysozyme in cheese, which can be readily applied as a standard method in food industry and food inspection. For this purpose, a commercially available monoclonal antibody against lysozyme was used. The application of a monoclonal antibody theoretically guarantees an unlimited supply of constant quality and avoids time-consuming revalidation.

MATERIALS AND METHODS

Chemicals and Buffers. HEW lysozyme (L6876), polyoxyethylene sorbitan monolaurate (Tween 20, ≥40%), 3,3′,5,5′-tetramethylbenzidine dihydrochloride hydrate (TMB, ≥98%), trifluoroacetic acid (TFA, ≥98%), bovine serum albumin (BSA, ≥98%), and sheep anti-mouse IgG peroxidase antibody (A6782) were supplied by Sigma-Aldrich (Taufkirchen, Germany). Potassium sorbate (≥99.0%) was from Fluka (Taufkirchen, Germany). The monoclonal mouse anti-chicken lysozyme antibody was obtained from Biotrend (Cologne, Germany).

Phosphate-buffered saline (PBS) consisted of 10 mM KH_2PO_4, 70 mM K_2HPO_4, and 145 mM NaCl (pH 7.4). The washing solution was made up of 1 mM KH_2PO_4, 7 mM K_2HPO_4, 15 mM NaCl, 0.05% Tween 20 (v/v), and 0.02 mM potassium sorbate. The dilution buffer consisted of 0.2% BSA and 0.05% (v/v) Tween 20 in PBS. The TMB solution was freshly prepared by mixing 300 μL of TMB (52 mM in 83.3% methanol/16.7% dimethyl sulfoxide) with 34 μL of H_2O_2 (3% in water) and 18 mL of substrate buffer [200 mM potassium dihydrogen citrate and 0.01% (m/v) potassium sorbate in water].

Instruments. The microplate washer Atlantis was obtained from Asys (Eugendorf, Austria), and the microplate spectrophotometer μQuant was obtained from Biotek (Bad Friedrichshall, Germany). The plate shaker MTS 4 and the T18 basic Ultra Turrax were supplied by IKA (Staufen, Germany).

Cheese Samples. A lysozyme-free Parmigiano Reggiano from a local supermarket was used for the spiking experiments. A total of 21 different commercially available ripened cheeses from cow, sheep, and goat with and without declared lysozyme were employed for the comparison to the HPLC method.

Extraction of HEW Lysozyme from Cheese. The cheese was extracted as described before (*15*). Briefly, 2 g of grated cheese and 20 mL of sodium chloride solution (1 mol/L in water) were homogenized with an Ultra Turrax. The Ultra Turrax was washed with additional 20 mL of sodium chloride solution. The suspensions were combined and adjusted to pH 6.0 with sodium hydroxide solution (1 mol/L). After stirring for 1 h at room temperature, the pH was adjusted to pH 4.3 with hydrochloric acid (1 mol/L). Then, the suspension was filled with sodium chloride to a total volume of 50 mL. After standing for 15 min, the suspension was filtered through a fluted paper filter.

Preparation of Spiked Cheese. A quantity of 2 g of a lysozyme-free grated cheese was weighed into a 100 mL flask, and 100, 200, 400, and 800 μL of the standard lysozyme solution (1 mg/mL lysozyme in 1 mol/L sodium chloride in water) were added, respectively. The spiked cheese samples corresponded to concentrations of 50, 100, 200, and 400 mg of lysozyme/kg of cheese, respectively. Afterward, 20 mL of sodium chloride solution was added to the grated cheese. The suspension was homogenized with an Ultra Turrax, and the cheese samples were extracted as described above.

Preparation of the Samples and Standards. For the preparation of the samples, 100 μL of the cheese extracts were mixed with 100 μL of a phosphate buffer (100 mM KH_2PO_4, 700 mM K_2HPO_4, and 450 mM sodium chloride at pH 7.4) and 800 μL of water. Further dilutions were performed with dilution buffer.

For the standards, a stock solution with 2 mg/mL lysozyme in water was prepared and diluted 1:50 with water. Further dilutions were made with dilution buffer. The concentrations of the standards were 800, 400, 200, 100, 50, 25, 12.5, 6.25, and 3.125 ng/mL.

ELISA Procedure. For the competitive ELISA, a polystyrene microtiter plate (Maxisorp F96, Nunc, Langenselbold, Germany) was coated with 100 μL/well of 0.75 μg/mL lysozyme in 50 mM sodium carbonate buffer (pH 9.6) at 4 °C overnight. The plate was washed 3 times with 300 μL/well of washing solution. Subsequently, free binding sites of the wells were blocked with 200 μL/well of 1% BSA in PBST [0.05% Tween 20 in PBS (v/v)] for 2 h at room temperature under agitation to prevent unspecific bindings of the antibodies to the wells. Then, the wells were washed again 3 times with 300 μL/well of the washing solution. The standard and sample solutions were pre-pipetted in PCR-Stripes (VWR, Darmstadt, Germany), and 50 μL/well of standard or sample solution was transferred to the wells of the ELISA plate with a multichannel pipet. An aliquot of 50 μL/well primary antibody solution (1:1000 in dilution buffer) was added to each well. After 1.5 h of incubation at room temperature under shaking, the plate was washed 3 times with 300 μL/well washing solution. Thereafter, 100 μL/well of peroxidase-labeled second antibody (1:4000 in dilution buffer) was added and incubated for 1 h on the shaker at room temperature. After the wells were washed 3 times with 300 μL/well of washing solution, 100 μL/well of the TMB solution was added and incubated for 15 min under agitation at room temperature in the dark. The reaction was stopped by adding 25 μL/well of 1 mol/L sulfuric acid, and the absorbance was measured at 450 nm. All measurements were performed in triplicate.

ELISA Validation. The linearity limits of the assay were determined by five assays, run on 5 different days. The limit of detection (LOD) was defined as the lowest lysozyme concentration that could be distinguished from a sample containing no analyte and calculated with the formula LOD $= B_0 - 3\mathrm{SD}_{B_0}$, where B_0 is the average zero reading, SD_{B_0} is the standard deviation of B_0. The LOD was calculated using the results of four different assays with lysozyme-free cheese samples diluted 1:2, with dilution buffer as the zero calibrant. Each assay had 30 zero calibrants applied on the plate.

Intra-assay reproducibility was estimated by examining a low, medium, and high concentration of lysozyme. For the samples, lysozyme-free cheese was spiked with 25, 100, and 800 mg/kg of lysozyme and diluted 1:8 with dilution buffer after extraction. Each sample was checked in 18 replicates on the same microplate. To calculate the interassay reproducibility, the assay was run on five different days in triplicate with independent extractions of cheese samples spiked with four different concentrations of lysozyme (50, 100, 200, and 400 mg/kg). The accuracy of the assay was assessed by determining the recoveries of lysozyme from the spiked cheese samples. Unspecific interference of matrix components was tested by measuring different types of lysozyme-free cheese. For this purpose, typical representatives of ripened cheese were chosen: hard and semi-hard cheese made of cow milk and hard cheese made of sheep milk. Further specificity of the assay was investigated by checking the cross-reactivity of lysozyme with α-lactalbumin and human lysozyme. Both proteins were employed in the concentrations of 800, 400, 200, 100, 50, 25, 12.5, 6.25, and 3.125 μg/mL.

HPLC Analysis. HPLC analysis was performed according to Pellegrino and Tirelli, with some modifications (*12*). Lysozyme was quantified in the extracts using a reversed-phase polymeric column (PLRP-S, 250 × 4.6 mm, 300 Å pore size, 5 μm) from Polymer Laboratories (Darmstadt, Germany). Eluting solvents were as follows: solution A, water/0.1% trifluoroacetic acid (TFA, v/v); and solution B, acetonitrile/0.1% TFA (v/v). Elution conditions were expressed as a proportion of solvent B: 0−12 min, 30−37%; 12−13 min, 37−70%; 13−14 min, 70%; 14−15 min, 70−30%, 15−25 min, 30%. The flow rate was 0.8 mL/min. The injection volume was 50 μL. The HPLC apparatus was autosampler AS-2057, HPLC pump PU-1580, ternary gradient unit LG-1580-02, 3-line-degasser DG-1580-53, and fluorescence detector FP-920 (Jasco, Gross-Umstadt, Germany). Fluorescence was recorded at 280 nm excitation and at 340 nm emission. The area of the lysozyme peak was measured using the valley−valley integration mode and quantified by an external calibration curve. For external calibration, standard solutions of lysozyme (in 1 mol/L sodium chloride in water) containing 1, 2, 4, 8, and 12 μg/mL were prepared from a stock solution of 1 mg/mL lysozyme (in 1 mol/L sodium chloride).

Data Processing and Statistics. For the ELISA, fitting of the curves was performed with the Gen5 software (Biotek, Bad Friedrichshall, Germany) using the four-parameter log-logistic model [$Y = (A - D)/(1 + (X/C)^B) + D$]. HPLC data were recorded and processed using the Borwin software package (Jasco, Gross-Umstadt, Germany). Statistical data evaluation was carried out with Microsoft Office 2003.

Table 1. Optimized Assay Conditions as Determined by Checkerboard Titration and Variation of Assay Parameters

washing	washing solution, 300 μL
coating	0.75 μg/mL lysozyme in carbonate buffer, pH 9.6, 100 μL overnight, 4 °C, static conditions
blocking	1% BSA−PBST 2 h, room temperature, agitation
standards	3.125−800 ng/mL lysozyme in dilution buffer, 50 μL
AB1 dilution	1:1000 in dilution buffer, 50 μL 1.5 h, room temperature, agitation
AB2 dilution	1:4000 in dilution buffer, 100 μL 1 h, room temperature, agitation
color development	TMB solution, 100 μL 15 min, agitation 1 M H_2SO_4, 25 μL
reaction measurement	450 nm

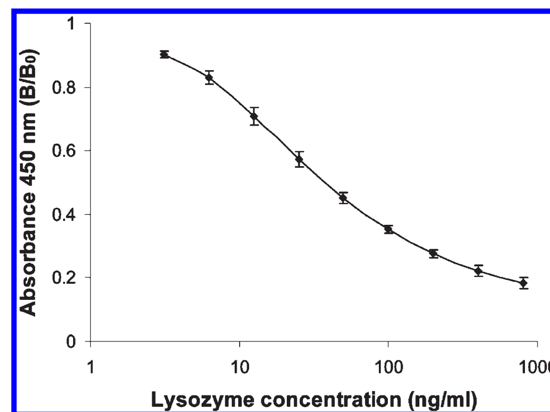

Figure 1. Calibration curve of the optimized lysozyme ELISA. The data show the mean ± standard deviation of five assays run on 5 different days. The curves were prepared using triplicates for each standard concentration, with $R^2 \geq 0.999$. B = absorbance at 450 nm. B_0 = absorbance of the positive control containing only dilution buffer at 450 nm.

RESULTS AND DISCUSSION

ELISA Optimization. First, the lysozyme concentration for coating and the dilution of the monoclonal mouse anti-chicken lysozyme antibody, used as a primary antibody (AB1), and the sheep anti-mouse IgG peroxidase antibody, used as a second antibody (AB2), were optimized. For this purpose, checkerboard titrations were performed, taking into account the specific conditions necessary for an indirect competitive ELISA (*22*). For optimization of the coating conditions, the lysozyme concentrations ranged from 0.04 to 40 μg/mL. As coating buffers, sodium carbonate buffer (pH 9.6) and PBS (pH 7.4) were tested. Because of the high pI of lysozyme, the carbonate buffer was chosen for coating. However, the PBS buffer showed similar results. The AB1 dilutions ranged from 1:125 to 1:8000, and the AB2 dilutions ranged from 1:250 to 1:16000. The blocking agents tested for the blocking step were 3% nonfat dry milk in water, 1 and 3% BSA−PBS, 1% BSA−PBST (PBS with 0.05% Tween 20), 1% gelatin−PBS, and 0.25% Tween 20−PBS. Among the different agents tested, 1% BSA−PBST was chosen as the blocking agent, because it yielded the highest sensitivity and the smallest variations of triplicate analysis. Different incubation times were tested for coating, antibody reactions, and the TMB reaction. The coating was performed overnight at 4 °C under static conditions. The coating, however, could also be performed within 2 h at room temperature under agitation, yielding similar results. The optimized concentrations and incubation times are shown in **Table 1**. Between the different incubation steps, the wells were washed 3 times with washing solution.

Pre-pipetting of the Samples. When the lysozyme standards were measured, identical lysozyme concentrations showed considerable variations, when applied on different areas of the microtiter plate. An effect of the microtiter plate itself could be ruled out, but it was obvious that the time span between application of the samples to the coated plate and further analysis had the major influence on the precision. A long time span prior to pipetting resulted in lower absorbance. This effect is most likely caused by surface denaturation of the coated lysozyme as a consequence of drying of the wells (*23*). Hence, the standards and the samples were pre-pipetted. The solutions were then transferred to the microtiter plate with the help of a multichannel pipet. Thus, the time required for applying the standards and samples to the wells was reduced from about 20 min to less than 2 min, resulting in acceptable intra-assay variation.

Sample Extraction and Matrix Interferences. In cheese making, the transfer of lysozyme to the curd is due to the association of the enzyme with the caseins (*24*). Additionally, interactions of lysozyme with the whey proteins α-lactalbumin and β-lactoglobulin have been reported (*25*). The temperature hardly influences binding of lysozyme to the milk proteins. Therefore, it was suggested that electrostatic rather than hydrophobic interactions are responsible for the binding (*26*). High concentrations of electrolytes must, therefore, be applied to overcome the electrostatic interactions of lysozyme with the milk proteins and to achieve complete desorption. To avoid interference with the ELISA, however, the electrolyte concentrations must be reduced prior to ELISA analysis. Different approaches were tested to optimize sample preparation. The extraction of lysozyme with 10-fold concentrated PBS and subsequent dilution of water to 1-fold concentrated PBS prior to ELISA led to a recovery of about 50−70% lysozyme only. As an alternative, the extraction was performed with 1 M sodium chloride solution. The extraction of lysozyme from cheese with 1 M sodium chloride has been successfully applied before for HPLC and immunocapture−MS analysis (*12*, *15*). After the cheese had been extracted with sodium chloride, the pH was adjusted to pH 4.3 with hydrochloric acid to reprecipitate caseins. The resulting extract was then diluted with PBS containing reduced amounts of sodium chloride, so that the samples were finally dissolved in regular PBS for ELISA analysis. With this method, a recovery of about 90% lysozyme was achieved for Parmigiano Reggiano cheese. Because an influence of specific factors on the extraction cannot be fully excluded, the recovery rate should be verified when other cheese types are analyzed. All competitive immunoassays applied for food analysis share a common challenge. Matrix interference may cause false-positive results by reducing the color development. Interferents in the sample extract can hinder the interaction between antigen and antibody, or the peroxidase activity can be inhibited. The reduction of the matrix interference can be achieved by further dilution of the sample or cleanup procedures. To minimize matrix interference, the caseins were reprecipitated from the extracts by adjusting the pH value to 4.3. Additionally, the cheese extracts were diluted at least 1:2 with dilution buffer.

ELISA Validation. *Standard Curves and Sensitivity*. The working range of the calibration curve was 3.125−800 ng/mL. The curve was generated from a four-parameter log-logistic model and showed a correlation index of $R^2 \geq 0.999$ (**Figure 1**). The LOD, defined as the lowest lysozyme concentration outside the range of three standard deviations over a lysozyme-free cheese sample (*27*), was 2.73 ± 0.77 ng/mL lysozyme. The value measured for lysozyme in all examined lysozyme-free cheese samples was below the lowest concentration of the lysozyme standard. Cheese extracts with results less than the lowest

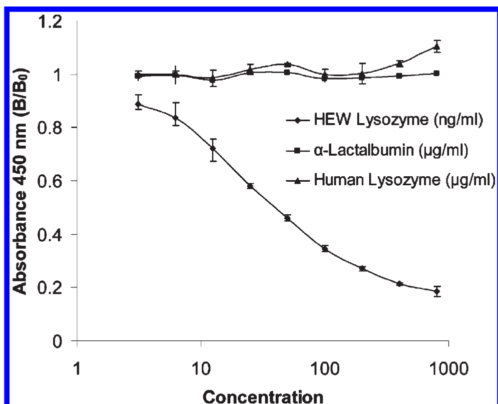

Figure 2. Test for cross-reactivity of the HEW lysozyme ELISA with α-lactalbumin and human lysozyme. The data show the mean absorbance of the lysozyme standard curve (n = 4) and α-lactalbumin and human lysozyme standard curves (3.125−800 µg/mL, n = 2). Error bars indicate the range of measured values. B = absorbance at 450 nm. B_0 = absorbance of the positive control containing only dilution buffer at 450 nm.

standard (3.125 ng/mL) were therefore considered to be free of lysozyme. This corresponds to a LOD in cheese samples of 1.6 mg of lysozyme/kg of cheese, if the cheese extracts have been diluted 1:2 with dilution buffer. Because the technological relevant concentration of lysozyme in cheese ranges between 50 and 350 mg/kg, the assay seems to be sensitive enough to reliably detect the application of lysozyme for manufacturing of cheese.

Assay Specificity. To test assay specificity, specific cross-reactivity with proteins that show sequence homology to the antigen as well as unspecific interference of matrix components were determined.

Specific cross-reactivity must be considered for the whey protein α-lactalbumin and human lysozyme. Both proteins show a sequence homology with HEW lysozyme of about 60% (*28*, *29*). The concentrations of both possible cross-reactants were 1000-fold higher than the lysozyme standard concentrations. No cross-reactivity was detected for both proteins (**Figure 2**).

Furthermore, unspecific interference of matrix components, such as caseins, was tested. For this purpose, typical representatives of hard and semi-hard cheeses made from cow milk and also hard cheeses made from sheep milk were chosen. For all of these lysozyme-free samples, values lower than the LOD were detected. Thus, detectable interference of the matrix components can be excluded.

Precision. Assay precision was determined by its intra- and interassay reproducibility. For the intra-assay reproducibility, lysozyme-free cheese was spiked with a low, medium, and high concentration level (25, 100, and 800 mg of lysozyme/kg of cheese) of lysozyme. A total of 18 replicates of each spiked cheese extract were measured with the optimized ELISA method. Thus, the intra-assay reproducibility with variation coefficients between 8.3 and 10.6% was determined. For the interassay reproducibility, a lysozyme-free cheese was spiked with four different concentrations of lysozyme (50, 100, 200, and 400 mg of lysozyme/kg of cheese). The spiking was performed in five independent experiments on 5 different days. The interassay variation ranged between 8.0 and 11.9%.

Accuracy. First, it was tested if the results of the assay are independent from the sample dilution. For that purpose, lysozyme-free cheese was spiked with four different levels of lysozyme (50, 100, 200, and 400 mg of lysozyme/kg of cheese). After lysozyme extraction, the samples were diluted 1:2, 1:4, 1:8, 1:16, and 1:32 with dilution buffer. The results were plotted as the measured mean concentration against the dilution factor (**Figure 3**).

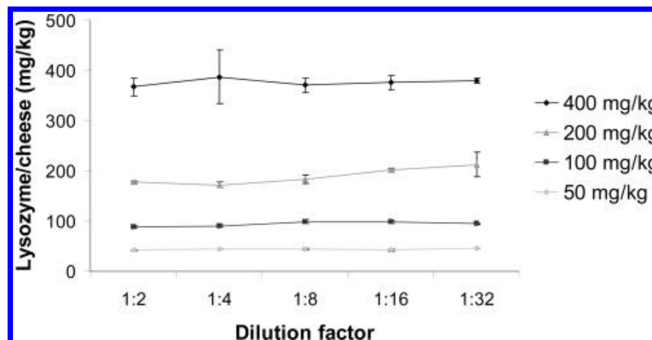

Figure 3. Determination of the linear dilution range. Plots of the mean measured concentrations of lysozyme against the dilution factor of the cheese extracts are shown (n = 2). Error bars indicate the range of measured concentrations. The spiked cheese samples contained 50, 100, 200, and 400 mg/kg of lysozyme.

Table 2. Validation Parameters of the Optimized Lysozyme ELISA[a]

parameter	value
intra-assay variability (n = 18), CV (%)	8.3−10.6
interassay variability (n = 18), CV (%)	8.0−11.9
recovery (50 mg/kg of lysozyme, n = 5) (%)	87.4 ± 10.2
recovery (100 mg/kg of lysozyme, n = 5) (%)	93.6 ± 8.6
recovery (200 mg/kg of lysozyme, n = 5) (%)	89.1 ± 8.0
recovery (400 mg/kg of lysozyme, n = 5) (%)	87.0 ± 11.9
limit of detection (n = 4) (ng/mL)	2.73 ± 0.77

[a] Recovery values are expressed as mean ± coefficient of variation (CV).

The recovery varied between 83.6 and 106.3%. Thus, it was shown that the assay was linear over the whole dilution range.

To determine lysozyme recovery, a lysozyme-free cheese was spiked on 5 different days with four different concentrations of lysozyme (50, 100, 200, and 400 mg of lysozyme/kg of cheese). The samples were diluted 1:8, as described in the standard protocol. The recovery of lysozyme ranged from 87.0 to 93.6%. These results are in good agreement with a previous study, in which recovery rates for lysozyme from cheese samples between 85.0 and 97.5% were obtained using an ELISA with a polyclonal antibody (*16*). **Table 2** gives a summary of the validation results.

Comparison with a Reference Method. To verify the reliance and accuracy of the new ELISA system, the results were compared to those obtained with an HPLC method. For this purpose, the method described by Pellegrino and Tirelli was used with some modifications (*12*). Lysozyme-free cheese was spiked with four different concentrations of lysozyme (50, 100, 200, and 400 mg/kg). The results obtained by ELISA and HPLC are plotted against each other in **Figure 4**. The correlation index R^2 was very good (0.999), with a linear regression curve of $y = 0.8994x + 1.18$. These results confirm those of the validation experiments. The findings indicate that reliable results can be obtained over the whole concentration range and that the ELISA provides slightly lower values than the HPLC method.

Application to Commercial Cheese Samples. Finally, lysozyme was quantified in commercially available cheese samples (n = 21) by ELISA and HPLC. In seven of these samples, lysozyme was detected by neither ELISA nor HPLC. In the other 14 cheese samples, lysozyme was measured with both methods. In **Figure 5**, the HPLC results are plotted against the ELISA results. The correlation index R^2 was 0.990. The slope of the trend line was 0.9372, which indicates that the recovery rates obtained by ELISA tend to be a little lower than those obtained by HPLC. At a cutoff level of 3.125 ng/mL, the ELISA did not render any false-negative or false-positive results.

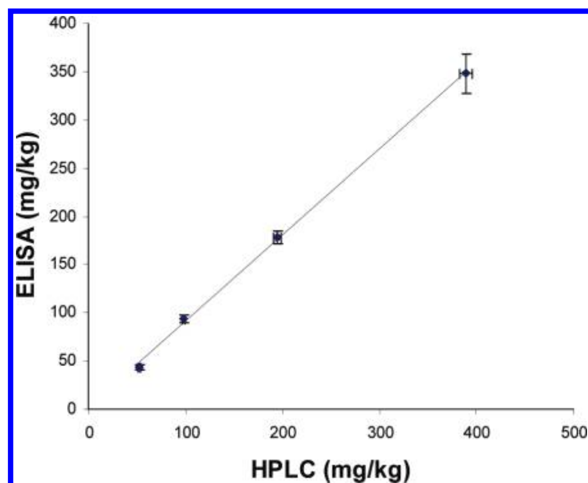

Figure 4. Correlation of the lysozyme concentrations of spiked cheese samples measured by ELISA and HPLC. The cheese samples were spiked with 50, 100, 200, and 400 mg/kg of lysozyme. The linear regression is given by $y = 0.8994x + 1.18$, with $R^2 = 0.999$. The data show the mean ± standard deviation of five independent experiments.

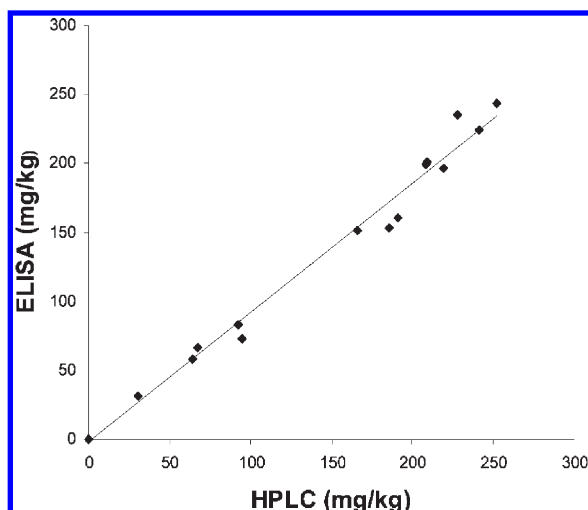

Figure 5. Correlation of the lysozyme concentrations in 21 samples of commercially available ripened cheese obtained by ELISA and HPLC. The linear regression is given by $y = 0.9372x + 1.47$, with $R^2 = 0.990$. The data show the mean of two independent experiments.

In this study, a competitive ELISA was developed for the quantification of lysozyme used as preservative in ripened cheese. The assay showed very good specificity and sensitivity, as well as good accuracy with an acceptable precision. The agreement of the ELISA results with those obtained with a standardized HPLC method was very good for both spiked cheese samples and commercially available products. Because a commercially available monoclonal HEW lysozyme antibody has been used, the assay can be readily and generally applied for food control.

ABBREVIATIONS USED

ELISA, enzyme-linked immunosorbent assay; SDS, sodium dodecyl sulfate; HEW, hen egg white; Tween 20, polyoxyethylene sorbitan monolaurate; TMB, 3,3′,5,5′-tetramethylbenzidine dihydrochloride hydrate; BSA, bovine serum albumin; PBS, phosphate-buffered saline; PBST, phosphate-buffered saline with 0.05% Tween; LOD, limit of detection; TFA, trifluoroacetic acid; AB, antibody; CV, coefficient of variation.

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